Santo Davide Ferrara *Editor*

P5 Medicine and Justice

Innovation, Unitariness and Evidence

Springer

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Foreword

Medicine and law have their practical functions, health care, and justice, accompanied by related activities, on the basis of each scientific realm, medical science, and jurisprudence, respectively, to maintain and improve the quality of human life and community cooperatively under domestic and partly international legislation in civilized communities. However, medicine globally shows steady progress with expanding innovation in technology against a traditional background, while stability and continuity are mandatory for legal systems of individual communities in the historical process. Meanwhile, medicine and law have been involved in multifaceted mutual interactive influences; health care should be provided under medical care and related acts, while up-to-date medical knowledge should be considered on the enactment or revision of laws involving items related to the human mind and body, and enforcement; accelerated progress in medicine has led to the relevant modification and revision of law, such as the renewed definition of human death in relation to organ transplantation, updated drug control laws, and altered healthcare law as the consequences of the revised criteria of mental disorders, and modified concept and practice of healthcare management following the advances in medical sciences and technology, as well as the emergence of notable issues in healthcare service, which often involve aspects of medical ethics.

The aforementioned overlaps between medicine and law immediately correspond to the academic concept of legal medicine involving the practical function on the medical side and medical law as an academic discipline on the legal side, while forensic medicine is predominantly concerned with issues particularly relevant to justice. With respect to this, medicolegal and forensic evidence should have an adequate relevance to clinical observations. Both medicolegal/forensic medicine and clinical medicine are applied fields of medicine but they have different tasks with consequent obligations, respectively: the task of clinical medicine is health care, while that of legal/forensic medicine is medicolegal/forensic assessment, forming a bridge between medicine and law. However, their common objective is to maintain and improve the quality of human life, although there may be potential conflicts regarding medicolegal issues presented in clinical managements. While their diagnostic approaches also differ markedly, medicolegal evidence should have adequate relevance to clinical observations, collected by the full application of up-to-date medical sciences; unitary and integrated approaches in practices of legal/forensic and clinical medicine will contribute to a better understanding of medical evidence in justice.

From the global viewpoint of academic performance, medicolegal and forensic sciences are making great strides in individual specialties for innovation to provide sophisticated medical evidence relevant to judicial assessment, legislation, and legal management; thus, biomedical approaches have become an important part of legal and forensic medicine. However, developed technologies and observations do not appear to have been integrated or effectively applied in routine casework in the context of systematic data collection and assessment. Against the aforementioned background, Prof. Ferrara, as President of the International Academy of Legal Medicine (IALM), organized the IALM Symposium in Venice, June 21–24, 2016, following previous serial symposiums, to discuss the present and future evolutions of bio-medicolegal sciences in the post-genomic framework of personalized medicine, in terms of innovation, unitariness, and evidence, in the promotion of a personalized justice. The Symposium comprised forensic pathology and anthropology regarding deceased persons, and clinical legal and forensic medicine for living persons, as well as medicolegal laboratories including genetics, molecular pathology, and toxicology. This monograph P5 Medicine and Justice takes its inspiration from the Symposium in Venice, based on the contribution of the leading authors of the aforementioned wide-ranging specialties from various regions including Europe, the USA, Australia, and Japan.

In the clinical setting, P5 medicine is an extension of P4 medicine, which was proposed based on traditional personalized medicine. On the basis of biology and computation as a challenge to establish "systems biology", the P4 medicine approach was primarily introduced for clinical trials in cancer medicine, involving predictive, personalized, preventive, and participatory aspects for understanding the biological complexity and better patient care to enhance the voluntary participation of patients. P5 medicine has been supplemented with psycho-cognitive aspects to aid patients with personal actions to prevent, cope with, and react to illness, decide about therapeutic options, and adhere to treatment, enhancing the interaction of patients with healthcare staff. The doctor–patient relationship can be a crucial element to ensure patients' quality of lives.

Despite the aforementioned principal difference in the tasks and obligations between legal/forensic medicine and clinical medicine, the concept of P5 medicine can potentially be introduced into medicolegal and forensic casework from the viewpoint of personalization with clinical relevance to living persons under healthcare management. In medicolegal and forensic investigations on deceased persons, medicolegal/forensic autopsy laboratory systems involving data processing, established by the full application of up-to-date medical sciences including genetics, molecular pathology, toxicology, and imaging techniques, is essential for the "visualisation", i.e., objective demonstration, of postmortem evidence relevant to clinical observations. In this process, it is particularly important to consider how to learn about life from the deceased, aided by bio-medicolegal sciences involving biomolecular approaches. Such innovation in medicolegal/forensic casework will enable the unitary and integrated collection of personalized objective datasets and computation, involving ethnic and demographic features, to be used to upgrade medicolegal/forensic assessment, leading to juridical (legal and judicial) proof with confidence. Interaction with the persons concerned may involve conflict in forensic casework in terms of objectivity in the justice system; however, it is important in the care of victims or their relatives, and criminals in the court process, as well as peoples' participation in the justice system from the participatory and psycho-cognitive aspects of "P5 legal/forensic medicine". Furthermore, the computation of massive data, so-called big data, will contribute to the prediction and prevention of hazardous events.

This monograph project, consisting of 40 unique chapters detailed and discussed by respective specialists in the context of the present statuses and future perspectives, will have a significant impact on medicolegal and forensic casework involving the care of victims and criminals, as well as peoples' participation in the justice system, from the perspective of "personalized justice". Thereby, it is expected to promote the innovation of medicolegal and forensic practices, as well as medical education and training, for the contribution to global harmonization in legal and forensic medicine in the future. Thus, this is an important, challenging proposal for the innovation of legal and forensic medicine from the University of Padova, which is one of the oldest universities and has the first stable anatomical theater in the world as a basis of modern medicine, founded by scholars and professors who moved in their search for more academic freedom from Bologna, where the earliest historical document of medicolegal dissection in 1302 was found.

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Preface

The reason for wanting to involve a significant number of eminent authors of varying biomedical, legal, and forensic standing from diverse continents resides in the rational premise and scientific objective of the monograph, both entailed by the neologism proposed by us and that characterizes its title, “P5 Medicine and Justice”, to which a few words of clarification and explanations must be dedicated.

The established and shared concept of “P4 Holistic and Personalized Medicine” is essentially a return to the past. A rediscovery of the immense humanitarian value of the analogical binomial “Doctor–Patient” relationship, to which the well-being of the person is entrusted.

The P5 extension, in reiterating the value of the past and adding the value of the “Protection” of the freedom and dignity of the person, looks to the future through the finalization of said “Protection” for the attainment of “Justice”, which calls for a “Personalization” combined and consonant with that of “Personalized Medicine”.

As explained by Zaccaria,[1] “Personalized Justice” is a seeming “Paradox” of a plurimillenial historical parabola that concludes with the current definitive affirmation of “personal and group identity”, the “entitlement to rights to differences”, and the coexistence of anthropological and juridical “pluralism”, in guaranteeing “protection”, equal and without differences, of the “freedom, dignity and defense” of the person, also taking the form of a “just trial”.

A just trial in which, according to Forti[2] and Tarfusser,[3] <<science and law are divided in their objectives>>, <<the criminal law must stick to and lag Science>> <<not lead it>> and <<the Judge must be a consumer not a producer of scientific knowledge>>, <<in order to comply with the principle of legality and human rights>> and << to build legal foundations sound in science as well in law>>.

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[1]*See* Zaccaria G, Epistemological and Ethical Implication of the P4 Revolution, Chap. 4.

[2]*See* Forti G, From Scientific Evidence to Juridical Proof, Chap. 6.

[3]*See* Tarfusser CJ, Scientific Evidence and Proof. Toward a Personalized Justice, Chap. 1.

Where, again, according to Dettmeyer,[4] beyond reaffirming the human rights of the Universal Declaration of 1948 and, in particular, the right <<to life, liberty security and presumed innocence>>, the right to protection from <<torture, cruel, degrading treatment or punishment>>, it is necessary to preserve the <<freedom and independence of forensic medical experts>> and to establish <<additional provisions relating to the collection of evidence>>.

And as Comandè[5] emphasizes, also in order to <<recognize compensation for personal injury damages and for non-pecuniary harm, characterizing new interests in every legal order>> where <<there are adequate resources to dedicate to their protection>>.

In such a context, with this aim of civilization, and in line with tradition and with the view outlined by Pollak[6], we have always held that <<To overcome Future Challenges one has to be aware of the past>>, and that from the past we grasp <<the danger of a fragmentation of bio—medico—legal disciplines into separate entities>>, as well as the consequent indispensability of <<unitariness>> from which naturally arise Transdisciplinary Innovation and Forensic Evidence, based on heeding other biomedical sciences and, in particular, the omic and imaging sciences, upon which Parts I–X of the monograph are focused.

An innovative role for the bio-medicolegal sciences, perhaps equal to that of Genomics, will probably be taken by "Omics" (Chaps. 2–3) and by "Living and Post-Mortem Cross Sectional Imaging", (Chaps. 34–38) which entail significant ethical-epistemological reflections (Chap. 4).

From the past we also grasp that "*Forensic Evidence*" cannot but be derived from the combination of "Research and Casuistry" and "Interpretation and Criticism". And it is for this that the Monograph has been organized into "Parts", with the final aim of tracing the *State of the Art of the Bio-Medicolegal disciplines*, (Chap. 5), and related Innovation and Evidence accompanied by juridical and epistemic reflections (Chaps. 1, 6), specifically concerning: *Forensic Pathology* and *Anthropology* (Chaps. 7–11); *Clinical Legal and Forensic Medicine* in the living person, with reference to "Violence", "Criminogenesis" and "Humanitarian Forensic Action" related to "Antisocial Behaviour", "Personal Injury", "Sexual Assault", "Torture" and "Asylum" (Chaps. 12–17); "Personal Damage" (Chaps. 19–21); "Malpractice and Medical Liability" under civil and penal law (Chaps. 22–24); "Personal Identification" and "Age estimation" (Chaps. 25–27); *Forensic Genetics* and *Genomics* (Chap. 28); and *Toxicology* (Chaps. 29–33).

At the conclusion of the monograph, the scenarios of transdisciplinary innovation of the bio-medicolegal sciences and, in particular, their levels of evidence have been partly defined (Chap. 39) in a perspective of further scientific development aimed at meeting the demands of evidence and proof, for a better mutual

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[4]*See* Dettmeyer R, Current and Future Evidence in Personal Injury Ascertainment under Criminal Law, Chap. 14.

[5]*See* Comandé G, International Juridical Overview on Personal Injury Compensation, Chap. 19.

[6]*See* Pollak S, Forensic Pathology. Historical Roots and Modern Evolution, Chap. 7.

understanding of Scientists and Judges in relation to a Personalized Justice, extensively inclusive of the value of the “Protection” of the freedom and dignity of the person.

The *Reader* is warmly invited to communicate to the editor any observations, comments, and critical issues aimed at the improvement of the work and of the scientific research carried out by the bio-medicolegal community.

To the *Authors* of the Various chapters,

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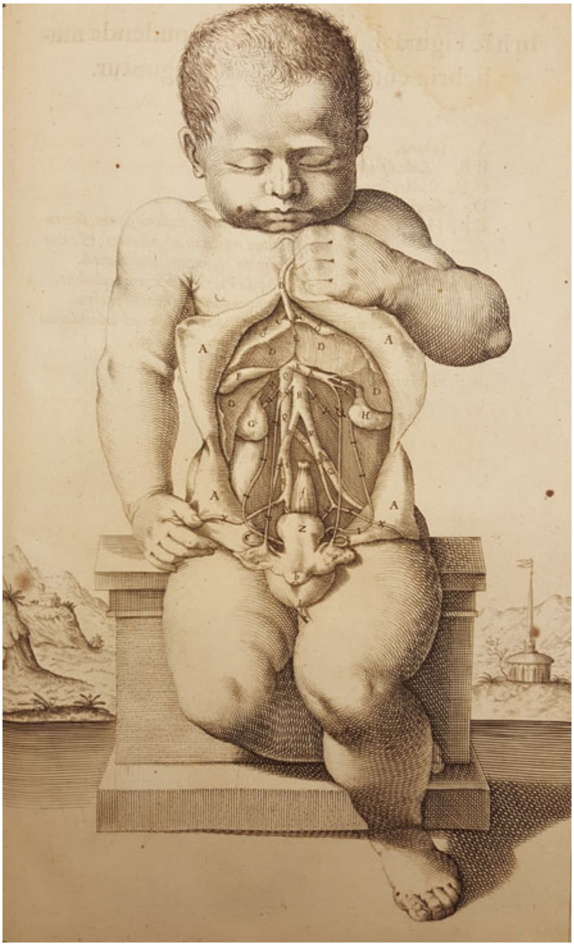

Casseri, Giulio 1552–1616 De formato foetu tabulae (Adriani Spigelii Bruxellensis… Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden… Amsterdami: apud Iohannem Blaeu, 1645)

Chapter 1
Scientific Evidence and Proof. Towards a Personalized Justice

Cuno Jakob Tarfusser

Abstract The concept of “Personalization” developed in medicine allows attention to be focused on the individual with respect to the diagnosed disease; this change has made a multidisciplinary synergy possible that characterizes scientific research. The same concept is, however, difficult to apply to Justice, where the subject of personalization is not clear and unambiguous as in the medical field, even though the possibility of adapting the general and abstract laws to concrete facts under investigation does remain.In spite of the aforementioned antithetical nature of the “Personalization—Justice” binomial, there are points of union between the latter and medicine. The first of these is represented by the individual, the focus of both disciplines; the second is the scientific evidence that allows Justice to acquire ever higher levels of reliability and validity. These levels, however, can only be reached if, as an indispensable condition, a mutual knowledge, derived from an understandable communication and a common formation, enabling the two disciplines to define themselves as complementary, is generated.

I am not a scientist. I am not an academic. I am a lawyer and as a lawyer a practitioner or, as I usually qualify myself, a “worker at law” as opposed to the academics which I define as artists. As such I have spent my whole professional life —17 years as investigative Public Prosecutor, 8 years of as head of a Public Prosecution office here in Italy and now more than 7 years as Judge at the International Criminal Court—on crime scenes and in jails, in courts and in courtrooms, and I will try to acquaint you with my personal experience, with what I see, live and think on a daily basis when it comes to scientific evidence. And I do this very humbly, trying only to open for you a window into, and give you a better understanding of, the world, the way of thinking as well as the point of view of a

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lawyer and provide some suggestions on how to improve the not always easy relation between Forensic Science and Justice.

At the outset I will make one amendment to the title of the topic of this report: a question mark. Indeed, I will approach the argument by placing a question mark at the end of the title, which then reads: “Scientific evidence and Proof. Towards a Personalised Justice?” This is because without a question mark the title seems to imply a certainty: the certainty that the two concepts “Personalised Medicine” and “Personalised Justice” are indeed overlapping—something which has to be investigated–, and the certainty that similar to the developments in the field of medicine, the field of justice is also in a phase of developing its personalisation.

Is it or is it not? Is it even possible or is it not? In the positive, are the two kinds of personalization comparable? Can Justice at all be personalized and, if yes, how and under which conditions and inside which boundaries? Finally, is it not that Justice is somehow already “personalized”?

It is against this background that I will take two steps back by analysing if, how and where Medicine and Justice have contact points in the context of a potential personalisation. I will deal with the meaning of the concept of “Personalization of Justice” as opposed to the concept of “Personalized Medicine”, and I will try to come to a conclusion by responding to the question of whether justice is moving towards personalization, how this is possibly linked to scientific evidence, and what can be done to enhance the interface between scientific evidence and justice.

While studying for this topic I learned that Personalized Medicine implies—and I will try to say it in my own words—placing the focus of healthcare, holistically intended, on the person vis-à-vis the illness as opposed to the illness vis-à-vis the person.

I understand that the ideal situation would be that the person, any person, goes to the tailor (doctor) where he/she is measured in detail (medically screened) so that during the whole life of the person, for any event (illness) he or she risks facing or is actually facing, the tailor tailors the appropriate dress (prevention, therapy) on the basis of the measures taken, as opposed to what is happening today–and I use the same exemplification—with the person going to a store (hospital) where, advised by the salesperson (doctor) on the basis of the description of the event (symptoms), he or she receives an outfit (medical care) tailored to the event, with the result that all persons going to the same event are dressed the same way.

I understand also that this inversion of parameters from a relation health care/illness versus person/patient towards a relation person/patient versus health care/illness, which is undoubtedly an extremely important and welcome development, has become possible through multidisciplinary synergies in scientific and technological research. In particular in the field of biomedicine, genetics, genomics and neuroscience which now allow Medicine to know, understand, prevent and individualize.

Having learnt this much about the concept of “Personalised Medicine”, I will now turn to the field of Justice and attempt to outline whether a development of Justice towards a personalization similar to the one which is ongoing in the field of Medicine is at all possible, is even envisaged, or is perhaps already in place.

I will now state, immediately anticipating my conclusion, that there is no doubt that the paradigm of Personalized Medicine as set out cannot be applied to the field of Justice.

This is so for many reasons.

By way of principle, Justice and Personalization are two antithetical concepts. Justice is symbolized as a blind goddess holding a sword and scales. All three symbols strongly militate against any personalisation: the goddess is unable to see who is judged, the scales are balanced and with the sword she is able to strike hard.

Furthermore, is it not true that "everybody is equal before the law" in all countries based on the rule of law?

If we now try to compare medicine and justice, we can easily acknowledge that the two fields of human knowledge are ontologically distant from each other:

- Medicine is a science, Justice is not
- Medicine is universal, Justice is systemic (common and civil law)
- Medicine is transnational, Justice is the expression of State sovereignty
- Medicine is progress, Justice is conservation
- Medicine is (implies) illness, Justice is (implies) conflict
- Medicine has a Nobel price, Justice does not.

Furthermore, while in the field of medicine all actors involved (doctors, patients, health administration, relatives of the patient, etc.) pull the same strings and therefore the subject of personalization is clearly identified, justice is ontologically a field of controversy, of mutual dispute, of thesis and antithesis where a third party has to find the synthesis (be it in criminal or in civil cases).

Who would be the subject of personalisation in the judicial system? Who would be the doctor in a legal dispute (the judge? the prosecutor? the defence lawyer?) and who the patient (the accused? the victim? the plaintiff? the opponent?). Personalization for whose benefit, considering all parties involved in the conflict? What happens in the case of multiple accused or victims? What happens in the event that a legal person, as opposed to a physical person, is involved in the conflict?

And furthermore, how can the 4 or 5 "Ps", namely, prediction, prevention, personalization, participation, and psycho-cognition be applied to Justice?

All rhetorical questions, in light of what I have just stated, unless we want to work on a new, a modern Cesare Lombroso approach. A sort of a Lombroso 2.0 approach, which would be highly debatable!

After having tried to highlight the dividing elements between Medicine and Justice from the perspective of Personalization, I will now turn to the unifying elements, to the ones which tie together Medicine and Justice when it comes to personalisation.

The first and fundamental element I am referring to is that at the core of both, Medicine and Justice, are people, individuals, their lives, and their destinies.

And this brings me back to one of the questions I raised at the beginning: does Justice not already have its own “personalisation” in place?

Of course it has. Personalised Justice—admittedly not known under such heading—is normal practice in the field of Justice; I would even go so far as to say that it is inherent to Justice, although in different terms to those applying to Medicine.

Just think of an actual case under investigation and, even more, an actual case brought before a judge. In such a situation, a complex system considering the persons involved in relation to a fact in dispute or to be investigated—something which undoubtedly can be considered as personalization—is in place. And while in the field of Medicine the personalization follows, and is dependent upon, scientific progress and is then very much left to the doctor/patient relationship, in the field of justice the personalization is—would you believe it?—disciplined by law.

In general terms, I think of the fundamental rights of any accused person to have a fair and expeditious trial, to remain silent, to a technical defence, etc. More specifically, I think of the numerous factors and circumstances that characterise concrete proceedings and which the parties in their submissions and observations and the judge in his/her decisions have to take into account in order to adapt the cold and abstract legal provisions to the concrete facts and responsibilities under scrutiny.

To give you an example, I am referring to the so-called aggravating and mitigating circumstances, which can be objective (related to the crime) or subjective (related to the conduct of the suspect/accused);

I am referring further to the environmental and social circumstances in which the accused person has grown up or lived, or those in which the facts have occurred and which the Judge has to take into consideration;

I am referring also to the behaviour after the criminalised conduct in terms of regret and damage restoring;

And finally I am referring to the possibility to graduate the punishment in terms of typology and the length of the penalty.

By giving you these examples, I do not want at all to subliminally argue that Justice is fine with personalization and even more progressive than Medicine, which should get in line. I would not dare!

What I rather want to say is that the medical concept of “personalization” cannot be applied and reapplied paradigmatically to the field of Justice, and as a logical consequence the response to the initial question of whether we are moving towards a Personalized Justice cannot but be denied.

What seems to me to be misleading is to link the concept of scientific evidence in relation to Justice with the concept of “personalization” instead of with the concept of “certainty”.

I would therefore even further re-formulate the title of my speech as follows: “Scientific Evidence and Proof. Towards a more Certain Justice!” and now not with a question mark, but an exclamation mark!

And this is the second unifying element between Medicine and Justice: the scientific evidence.

It is undisputable and undisputed that Legal Medicine—though I would enlarge the spectrum and speak more generally about Forensic Science and Technology—is becoming more and more important, is influencing Justice and its decision-making processes towards more certainty, towards more just decisions.

More and more judicial decisions will rely and be based on evidence which has nothing to do with what we have been used to for decades and I am referring here to the so-called "traditional evidence", in particular the testimonies of persons, be it eye witnesses or police personnel—too unreliable, too controversial, too weak, too easy to be influenced. This particularly applies when it comes to proceedings based on *indicia* as opposed to proceedings with clear and uncontroversial evidence, and even more when it comes to proceedings in the field of organized crime or of international crimes, i.e. crimes against humanity, war crimes, genocide and crime of aggression.

I am thinking in particular of two fields, crucial in judicial proceedings, where scientific evidence can and will, if not supersede, certainly overtake the traditional evidence in importance:

(i) the proof of the crime, the conduct, the fact on the one side, and
(ii) the assessment of criminal liability of the suspect and the accused on the other side.

I will not discuss here the different types of findings which science and technology have developed in the last decades and from which judicial proceedings have benefitted in terms of certainty: DNA, fingerprint screening, biometrics and trace evidence in general are only some of them and are also concepts well known to investigators, lawyers and judges.

I only want to briefly refer to an example of more recent scientific development which will soon, I am sure, supersede the classical psychiatric expertise in the field of assessing criminal liability, namely the recourse to the neurosciences, which will help judges in achieving a more in-depth "personalised" —and therefore certain— Justice when it comes to culpability. No doubt, it is a very delicate field of medical investigation applied to justice, because it goes to the very heart of the question of knowledge and intent, of mental capacity, and therefore it must be handled with particular care.

Up until now, parties and judges have used psychiatric expertise in order to decide upon the liability of an accused person. I have always considered forensic psychiatry as a highly unreliable and contradictory medical science. And I think that such classic psychiatric expertise is now generally considered to be a "weak" science, which is no longer able to provide the necessary certainties to judges. There has been no case I have investigated and prosecuted where, if the liability of the accused was challenged, I was not confronted with at least three different psychiatric experts: one saying that the person had the intent and knowledge to engage in the conduct and was aware of its consequences, another saying exactly the opposite, and a third, usually the expert appointed by the judge, who concluded on the limited or partial intent and knowledge of the person.

On the horizon we can now see a change, a change in tendency.

As far as I know, the U.S. has already initiated at the end of the last millennium the use of the neurosciences in court. In Italy, I know of four decisions, the first in 2009, two in 2011 and one in 2013, which have approached with great care this new field of forensic knowledge. It would be too long and out of context to enter into the details of these decisions. What I can say very briefly is that in all four cases, the judges wanted to ascertain the mental element through test, through morphological and morpho-metrical brain analysis, through genetic analysis, and they relied in their decisions, in addition to all other evidentiary elements, on the outcome of such neuro-scientific expertise.

Maybe it is also worth noting what immediately caught my eye: out of these four decisions, three were taken by first instance single judges. This appears to be a clear indicator that novel sciences are more readily resorted to in trials before "novel" judges, young people who have another, a better, or maybe more open-minded and less conservative understanding of and approach to the latest developments in science.

But, as previously stated, it is beyond the scope of the present to enter into the discussion of the various types of scientific evidence. Instead I will outline how, in more general terms, science can aid justice to achieve more certainty, on what our needs are when it comes to scientific evidence. On how justice can fully use the enormous potential of scientific evidence and in so doing reduce the uncertainty of its decisions.

No doubt that reliability is the key word in this respect: the reliability of the science and reliability of the scientist, the expert. It is also clear that the science must be understood by the non-scientist involved in the proceedings, be it the Prosecutor, the Defence lawyers or the Judges.

But what is reliability and what does it mean for forensic science and forensic scientists to be reliable? This is even more relevant, since reliability is determined by the Judge in the decision.

But is it or is it not that an expert or expertise can be found reliable by a Judge more on the basis of the way in which the expert understandably and clearly expresses him or herself in writing and is eloquent in the oral hearing while questioned by the parties and the Judges than on the basis of his qualification and expertise? In other words, Judges could be more impressed by the person and his/her personality rather than by the professional qualities of the expert.

On the other hand, an internationally recognized expert in his/her field of forensic science could be judged as not sufficiently reliable simply because he/she is "too much of a scientist", so to speak, in expressing him or herself orally and in writing in a way which is not understood or not easily understandable by lawyers.

If this is true, and we can say without any hypocrisy that it is, what is the pre-condition for giving to the word reliability a reliable meaning?

The answer is that the pre-condition is knowledge, is mutual knowledge.

I am convinced that this knowledge can be achieved only through understandable communication and through common training.

I have always had the impression that the judicial system (starting from the investigation up to the trial phase) on the one side and the world of science (forensic science, in particular) on the other, look at each other with a certain sense of suspicion, of reciprocal scorn mixed with envy, instead of looking at each other as complementary.

I submit that there is no reason for the forensic sciences to exist without the judiciary. In turn, the judiciary, without a continuously evolving and improving forensic science, would be barbarous and inhuman as it was, even only decades ago.

As far as mutual knowledge is concerned, we all (lawyers and forensic scientists alike) have nothing more than a general and global knowledge of each other's fields. And while a scientist with difficulties bears the formalities of the law, the lawyer ontologically distrusts a field he does not really understand and which on top of this appears to consider itself superior, which to a lawyer is intimately unacceptable.

It is for this reason that I truly believe it is high time that, instead of lawyers (possibly even further divided into groups of judges, prosecutors, defence lawyers) on the one hand and forensic scientists on the other talking at cross purposes, we talk together and discuss the problems that link both branches.

These problems—and I speak from my own point of view as "worker at law", be it judge or public prosecutor—are very often extremely basic, starting with the question: is there anyone who could be of assistance to me on a particular subject?

Currently, a "worker at law", after discussing with colleagues and friends, who discovers that a certain problem arising in the judicial process can be addressed from a scientific point of view, then has to look for an expert on that particular subject. This search, too, is currently done by contacting colleagues on a mailing list who may have been in analogous situations before, by asking for reference contacts of the expert they used, of course only after having been assured by the colleague of the qualities of such an expert.

How it is possible, in the third millennium, in a world which is hyper-technological and always connected, that in the field of Justice and Forensic Science people still have to rely on "word of mouth" instead of on a system or platform which is accessible as needed and which would contribute to keeping the legal world up to date on the scientific and technological developments in the field of forensic science as well as on the experts who may be contacted and appointed?

In order for such a system or platform to work most effectively, these two worlds which have a perfect awareness of their need for one another and of the need for cooperating should also strive, in my view, to find a mutually intelligible language. In particular, science needs make itself understandable to those who make decisions within the justice system in the name of the people, because it is science which in this context aids justice and it is the latter which in all its forms and stages, from the investigators to the Judge, from the prosecutor to the defence lawyer, evaluates the results of scientific analysis and places it together with all other reliable evidentiary elements. In other words, we have to find a common way, first and foremost in terms of language, to understandably communicate with each other.

This also implies that, in the fields of justice and science, any training done with a view to making the most effective use of forensic science for purposes of achieving a more certain justice should necessarily be continuous and common.

- Actors within the judicial system must know how to act and what to pay attention to, bearing in mind any potential scientific proof that may be requested at a later stage. I myself have always been convinced that from the very first moment of any investigation, the investigator has to proceed being well aware of the scenario that will most likely occur in the context of the investigation unfolding, and in this way also has to take into account if and what kind of scientific investigation or analysis will become necessary in the future. Therefore, the investigator has to know how to behave at the crime scene, which traces can be scientifically analysed, and how to collect and preserve such traces in order to avoid contamination, etc.

  This training of actors in the judicial system also needs to be more broadly oriented to include lawyers who need to have a better understanding and knowledge of the most likely scenario in which an investigation will unfold, including of the continuously advancing technological and scientific findings which can be of interest to justice (the lawyer has to understand, not simply rely upon, scientific findings and analyses).

- At the same time, forensic scientists must be trained in the very strict procedures they have to follow, in what is expected of them in terms of their written analysis, in the modalities in which they may be required to be confronted in court with the results of their consultancy, but also on judicial and deontological ethics.

  Forensic scientists need to understand that—although it is increasingly important, often even determinant—forensic science is only one element among (many others) which, after having been discussed by the parties in the courtroom and having been freely assessed as to its reliability, admissibility, relevance, probative value, etc. by the Judge, contributes to establishing the conviction of the Judge and leads him/her to a final conclusion for which only the Judge is responsible.

If, as I am convinced, mutual knowledge through common training and understandable communication is critical for a better mutual understanding of Forensic Science and Justice with a view to reaching an always higher degree of effective reliability and in so doing creating a position in which it is possible to render judicial decisions which are always, not more personalized, but always more certain, and to think and to debate very concretely the possibility of:

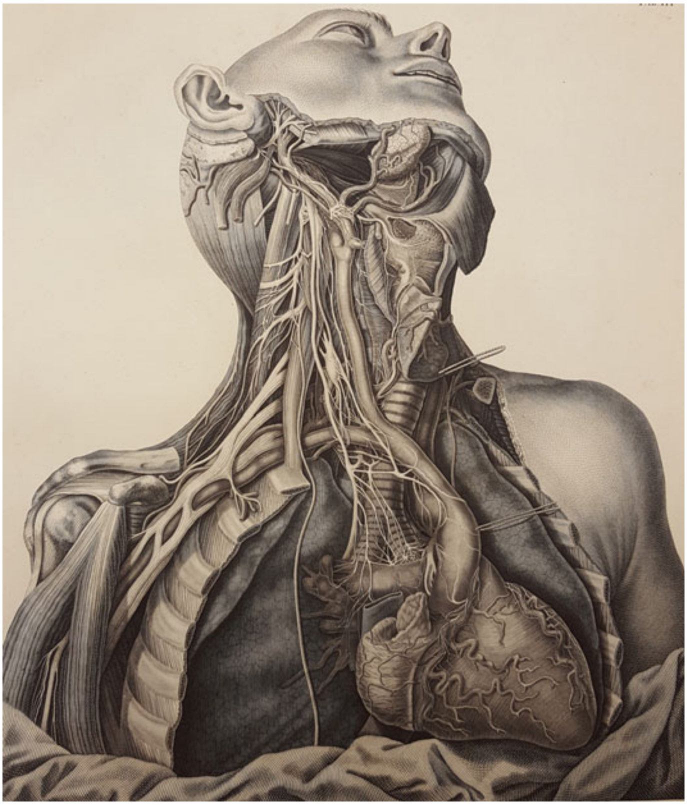
(i) drafting a sort of manual of the forensic (medical) sciences; I am thinking about a sort of *vademecum* written by forensic scientists for lawyers in which it is explained what the sciences are about, what they can achieve, and how they need to be taken into account in the context of evolving investigations;
(ii) to establish a sort of international "bar council"/board of experts in forensic sciences, divided in the different branches of the Forensic Sciences, and regulated in its admission by way of evaluation and continuous performance control, maybe through appraisals by those who have benefitted from the expert.

All this, the manual and the list of experts, as living documents, should be accessible on-line and continuously updated, amended and implemented by the users.

A first step to finding a better mutual language and understanding for Justice and Science which may eventually contribute to a more "Certain Justice" could be seen in the fact that I, a lawyer, was given the privilege and the honour of expounding my reflections in this interdisciplinary and international volume of information on the Bio-Medicolegal Sciences.

Part I
P4 Personalized Medicine Scientific-Technological Innovation and Epistemological Perspectives

Casseri, Giulio 1552–1616 Tabulae anatomicae 78 cum supplemento 20 tabularum Danielis Bucretii (Adriani Spigelii Bruxellensis ... Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden ... Amsterdami: apud Iohannem Blaeu, 1645)



Scarpa, Antonio Antonio 1752–1832. Tabulae nevrologicae ad illustrandam historiam anatomicam cardiacorum nervorum, noni nervorum cerebri, glossopharyngaei, et pharyngaei ex octavo cerebri. Auctore Antonio Scarpa… Ticini: Apud Balthassarem Comini, 1794

Chapter 2
Metabolomics and Molecular Imaging in the Post-genomic Era

Linda Illig and Thomas Illig

Abstract Metabolomics and Molecular Imaging are important tools in targeted medicine for better understanding disease pathoetiology and etiopathogenesis, as well as for improved diagnostics and therapy. Advances in analytical biochemistry have recently made it possible to obtain global snapshots of metabolism. In particular, the combination of different molecular omics techniques shows major differentiations in the metabolic make-up of the human population. Metabolites may determine the risk for a certain medical phenotype, the response to a given drug treatment, and the reaction to a nutritional intervention or environmental challenge. Molecular imaging (MI) is based on the idea that diagnostic tracers are concentrated in specific areas because of their interaction with molecular species that are distinctly present in a diseased state. Current molecular imaging techniques include positron emission tomography (PET), magnetic resonance imaging (MRI), ultrasonography (US), and computed tomography (CT). MI is non-invasive, allows serial investigations and can monitor the therapeutic efficacy of drugs during the entire course of treatment.

2.1 Introduction

Metabolomics, the science of the "systematic study of the unique [bio]chemical fingerprints that specific cellular processes leave behind", is an auspicious upcoming field in medical research [1]. The prospect that biological fluids reflect the health of an individual by a "metabolic profile" has been seized consistently in medical history. Whereas in former times, for instance, the analysis of urine was

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used to detect high glucose concentrations or high albumin secretion and hence diabetes or other metabolic disorders, today the options are much more manifold. Cells of distinct tissues, plasma, serum or whole blood are available as well as urine, stool, or saliva. Due to well-developed physical quantification methods, such as different forms of mass spectrometry (MS) like direct flow injection (FI), gas chromatography (GC)-MS, liquid chromatography (LC)-MS and nuclear magnetic resonance (NMR) spectrometry, it has become possible to measure numerous metabolites, including lipids, carbohydrates, amino acids, peptides or even xenobiotics, cofactors and vitamins in the biological material [2]. Nowadays, the techniques are customarily utilized for testing in relation to common as well as on rare diseases, such as diabetes, chronic kidney disease, phenylketonuria and medium chain acyl-CoA dehydrogenase deficiency, to name but a few [3–5]. Generating metabolic patterns from diverse singular metabolites in order to enable medicine to detect the prevalence of different kinds of diseases and even to prognosticate their incidence in an applicable time frame are definitely realistic future dreams, which now are starting to become true. Last year, real-time metabolome profiling was demonstrated for the first time by biologists at the ETH Zurich, using a new method in bacteria [6]. This achievement opens an enormous range of scientific investigations, following medical as well as prophylactic therapy.

In contrast to genetic studies, metabolomics studies have shown to be much more challenging due to different metabolite concentrations and concentrations in different tissues or other biological material. In addition, varying environmental influences, drug intake or dietary differences are able to overwhelm studies with disruptive factors. Therefore, sample quality and equality is now one of the main, if not the major challenge in metabolomic studies.

Due to high diversity of all the different metabolites in human bodies the analysis of the whole metabolome is a major challenge. Lipids, carbohydrates, amino acids, peptides etc., all belong to this group. Therefore, chromatographic methods have mostly combined with mass spectrometry in metabolomic studies. Nevertheless different concepts have been developed to investigate the roles of metabolites in human beings.

Many endeavours have been made in research to approach molecular imaging (MI). MI is based on the idea that diagnostic tracers are concentrated in specific areas because of their interaction with molecular species that are distinctly present in a diseased state. MI is noninvasive and allows serial investigations, and can therefore potentially monitor the therapeutic efficacy of drugs during the entire course of treatment. Current MI techniques, besides MALDI-MS, include positron emission tomography (PET), magnetic resonance imaging (MRI), ultrasonography (US), near infrared imaging (NIR) and single photon emission computed tomography (SPECT). A lot of networks and organizations have been founded to enhance the facilities of MI. The U.S based Society of Nuclear Medicine and Molecular Imaging (SNMMI) holds the Center for Molecular Imaging Innovation and Translation (CMIIT), which is an organizational component within the SNMMI dedicated to all aspects of molecular imaging in the detection and management of disease. The center is currently facilitating initiatives to bring molecular imaging discoveries from the laboratory to the patient and to advance "personalized" medicine [7]. In Europe, the European Society for Molecular Imaging (ESMI) represents an academic community developing and validating imaging technologies and multimodality imaging biomarkers in the life sciences and the use of innovative imaging methods to support basic and clinical research (http://www.e-smi.eu).

2.2 Metabolomics. Practises and Requirements for Quality Control

Whereas genetic disease studies have proved to be utmost effective and useful in defining rare diseases, complex diseases like diabetes, myocardial infarction or neurological or atopic diseases could not be explained fully by genetic factors. Gene variants showed weak to moderate effects [8–10]. Therefore, presuming that environmental factors play a critical role in the development of certain complex phenotypes, it is important not only to consider genetic variants whilst searching for explanations. Advances in analytical biochemistry have recently made it possible to obtain global snapshots of metabolism. In particular, the combination of different molecular-omics techniques (which implies the analysis of the totality of something) shows major differentiations in the metabolic make-up of the human population. Metabolites may determine the risk for a certain medical phenotype, the response to a given drug treatment, the reaction to a nutritional intervention, or environmental challenge. Understanding the role of genetic predispositions and their interaction with environmental factors in complex chronic diseases can be very well examined by metabolomics.

For instance, Illig et al. have combined concentrations of 163 serum metabolite traits with genetic polymorphisms, and thus could show a direct connection between eight genes encoding for enzymes or solute carriers (*FADS1, ELOVL2, ACADS, ACADM, ACADL, SPTLC3, ETFDH and SLC16A9*) whose functions match the metabolic traits. The impact of the loci on the concentrations explained between 6 and 36% [10]. The variants identified in that study could afterwards be examined in GWAS with clinical parameters. For several loci, associations with clinically relevant parameters have even been reported previously. *FADS,* for example, as a supposed risk locus for perturbed blood lipid parameters [11] has been found to be associated with levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL) and total cholesterol [12, 13]. Many biomarkers, including metabolite levels, show correlations with disease, but those correlations do not automatically mean causality. If metabolite levels show a high significance to genetic variants, association tests are done in Mendelian randomization experiments. The metabolite should show both an association to a disease and an association to a gene or genetic region that encodes for instance for an enzyme that is involved in the metabolite´s metabolism. Those enzymes then emerge as potential drug targets [14].

Another metabolic study that has been combined with genome wide association studies (GWAS) has shown gender differences in metabolic and genetic biomarkers: Mittelstrass et al. could prove that glycine profiles of males and females are significantly different and, furthermore, that specific genetic variants in the *CPS1*-locus (carbamoyl-phosphatase synthetase 1) show sexual dimorphism [15].

2.3 Advances in Pharmaceutical Research

One major problem in therapy is the advanced stage of damage in the body caused by the side effects of a long period without treatment. Often those secondary complications lead to irreversible transformations. Therefore, early markers are extremely important. Side-effects of drug therapy can also lead to harmful affections. Furthermore, it has been proven that distinct groups of people tolerate drug therapies differently [16]. Metabolomic studies are aiming to detect biomarkers that indicate the patient´s compatibility towards a certain drug in order to prevent harmful or even lethal destructive processes in the body caused by drugs. The approach has major impact for a better efficiency in drug discovery, for instance by providing a more careful selection of animals in preclinical studies, for a better stratification of patients in clinical drug trials and for personalized therapies [17]. Currently, Xu et al. examined the effects of metformin, a typical first-line drug in the treatment of type 2 diabetes (T2D), intake on human metabolism. Their results indicate an activation of AMP-activated protein kinase (AMPK), therefore a down-regulation of *FADS1* and *FADS2* expression that consequently leads to reduced levels of acyl-alkyl phosphatidylcholines (PCs) and low density lipoprotein-cholesterol (LDL-C). This suggests that the intake of metformin has potentially beneficial effects on cardiovascular diseases [4].

A metabolomics approach has also been used to identify candidate biomarkers of pre-diabetes. Patients with impaired glucose tolerance (IGT) showed significantly lower levels in the metabolites glycine and lysophosphatidylcholine (LPC) (18:2), whereas their acetylcarnitine concentrations were higher. As impaired glucose tolerance is also known as a pre-diabetic state, a combination of those levels different from the standard indicates a possible development of diabetes in the near future [3].

Another illustrative example is the work of He et al., who were able to picture a metabolomic plasma signature for schizophrenic patients. Five metabolites differed significantly from neuroleptics-free participants and therefore are strongly suggested as candidate biomarkers for schizophrenia [18]. Here personalized medicine might be used to predict a person's risk for schizophrenia and to try to prevent an outbreak of the disease with the help of drugs and lifestyle changes.

Very recently, we published a population-based metabolomics study that represents a pharmacogenetic footprint of ACE-inhibition: Angiotensin-I-converting enzyme (ACE)-inhibitors function as anti-hypertensive in applied medicine. We observed differences in the concentrations of several dipeptides, and of ratios of di-to oligopeptides between ACE-inhibitor users and non-users that were shown to have genotype effects. Major homozygote carriers showed high changes in the metabolite status, smaller differences were shown by heterozygote carriers, and minor homozygotes had almost no changes in the peptide levels. Furthermore, the genotype dependent dipeptides, which include aspartylphenylalanine and phenylalanylserine, were significantly associated to blood pressure, thus qualifying them as markers for ACE-activity [19].

2.4 Quality Requirements

Ideally, metabolomics traces the totality of all metabolites at a specific time in a biologic material like blood, urine, tissues etc. In contrast to pure genetic studies, metabolite profiles vary from matrix to matrix. For gaining correct answers, the kind of source used for metabolite harvesting has decisively to be taken into account. Yu et al. pointed out significant differences between metabolite profiles measured in human plasma or serum. About 25 percent of metabolites had to be excluded from the study due to low measurement stabilities. In serum more different metabolites could be verified, whereas in plasma the stability of the metabolites proved to be significantly higher [20].

The metabolic status of a person varies rather rapidly. Human serum metabolites, for instance, have been shown to be age dependent [21]. Environmental influences as well as diet are crucial for the momentary composition. So the metabolic state changes in the moment a person leaves his house and comes into contact with sunlight, or it is dependent on the date and time of day the matrix is taken from the patient, to name but a fraction of the difficulties the study designer is confronted with. Only limited numbers of samples are available, though. The majority of studies take advantage of bio-banked samples. Most laboratory errors occur in the pre-analytical implementation. Thus standard operating procedures (SOPs) for this phase and quality markers are strongly demanded. Anton et al. pointed out the concentration changes under different laboratory conditions, such as temperature variations from room temperature to dry or wet ice. They also measured concentration loss or gains due to different handling delays under a constant temperature. In particular, observed high-level increases in lysophosphatidylcholines and decreases in phosphatidylcholines predetermine these substances as quality markers for appropriate samples. Also −80 °C storage directly after centrifugation and a rapid separation of cells from plasma proved to be necessary in order to maintain optimal quality. Last but not least, it should be standard to test sample quality from run to run [22]. The goal is to detect molecular markers that can show quality of used biomaterials, since this can often not be proven, especially for old biomaterials without any documentation.

2.5 Further Potential Molecular Imaging Techniques

Many of the studies hitherto specified are based on molecular imaging through mass spectrometry. However, several studies have been aiming to developing other strategies as further kinds of techniques are imaginable for noninvasive molecular imaging. Biomarker detection using magnetic resonance imaging (MRI) is in need of contrast agents with a high specificity. Peptides, antibodies or small ligands have been used for targeting. Preclinical molecular MRI of inflammatory processes and its interactions with the human brain is a central research topic in several neurological disorders focusing on a set of molecules expressed by endothelial cells, such as adhesion molecules like VCAM-1, ICAM-1, P-selectin or E-selectin, which emerge as therapeutic targets and biomarkers for neurological diseases [23].

^{1}H NMR spectrometry, for instance, was used to compare the metabolic profiles of cerebrospinal fluid samples from drug-naive patients with matched controls [24]. Near infrared imaging (NIR) uses near-infrared wavelengths (from about 700 to 2500 nm) in a spectroscopic method. Several studies have demonstrated the practicability of that method: NIR fluorescence imaging was for example used for noninvasive trafficking of scaffold degradation [25]. Currently, Xu et al. were able to fulfill real-time imaging and tracking of ultrastable organic dye nanoparticles in living cells with the help of NIR imaging [26]. Several further molecular imaging techniques are in development for use in molecular research, such as single photon emission computed tomography (SPECT), positron emission tomography (PET) or ultrasonography (US).

Once those methods have been brought together effectively with metabolomics, this will open up new horizons in the field of metabolic research. Individual phenotyping will then be done easily for a huge amount of parameters. Holistic treatments will become possible. Therapies based on metabolite status will replace therapies resulting from snap-shots of single parameters like measurement of blood pressure or blood sugar, giving a much broader overview of the patient´s state of health. It is even imaginable that a metabolic snap-shot will be done each time as a preventive medical check-up.

2.6 Conclusions and Outlook

In targeted medicine Metabolomics and Molecular Imaging are important tools for better understanding disease pathoetiology and etiopathogenesis, as well as for improved diagnostics and therapy.

The pioneer of -omics-technologies has been genomics, which could be conducted as GWAS in huge population studies with thousands of participants [27, 28]. Those single studies were further merged to meta-analyses, which contained ten or even hundreds of thousands of samples [29, 30]. Genomic approaches like whole exome or genome sequencing proved to be very effective in determining genetic causes for rare diseases [31]. Unfortunately, they have so far not led to the eagerly awaited breakthrough for new forms of therapies of complex diseases. Although GWAS revealed associations of hundreds of disease risk-loci, the effects of common genotypes only proved to be from weak to moderate [9, 32]. Therefore, and building on the previous work, scientists also shift nowadays to so-called post-genomic methods like transcriptomics, proteomics and metabolomics. The explored patterns are nearer to the outcomes and therefore promise to show stronger effects on the phenotypes, entailing a better understanding of complex diseases, their diagnostics and their treatment. They can also be used in combination with GWAS in order to enable genetic forecasts. Strengthening of associations could be achieved by looking at ratios between metabolite pairs and the reconstruction of metabolic pathways by Gaussian graphical modeling [33]. Non-invasive in vivo imaging is emerging as an important tool for basic and preclinical metabolic research. Individualized medicine is hardly imaginable and feasible without post-genomic methods. There is immense variation in the individual response to drugs and yet that variation is not usually recorded. Drugs are approved on their average performance. Metabolomic's vision of the future is a stratified medicine that separates patients into different groups, aiming at a precision medicine enabling medical treatments to be tailored to the individual patient group based on their predicted response or risk of disease.

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Chapter 3
The Origin of Personalized Medicine and the Systems Biology Revolution

Marco Carraro, Silvio C.E. Tosatto and Rosario Rizzuto

Abstract The complete sequencing of the human genome has opened up many avenues of research. Among these, the notion of personalized medicine is becoming increasingly common. In this chapter, we review the major implications of the genomic era for improving the diagnosis and treatment of diseases. Medicine has witnessed several paradigm shifts in the course of the last two centuries, and personalized medicine is bound to be seen in the same way. Sequencing technology has evolved by orders of magnitude, coming into the range of $1000 for a complete human genome. Diseases are increasingly diagnosed with the help of genomics data. Combination with other high-throughput omics data further provides novel opportunities to improve treatment in the light of systems biology. However, much work still needs to be done to provide adequate analysis for personalized medicine to fulfill its potential. Regulatory challenges also lie in wait in order to guarantee that the right conclusions are drawn from the novel data. Despite the current limitations, personalized medicine is revolutionizing clinical practice.

3.1 Introduction

The completed sequencing of the human genome in 2003 was a scientific watershed with great potential to improve medicine. The resulting technological advance has opened the possibility to sequencing individual genomes in a short amount of time and at a reasonable price. The promise of identifying genetic variants that influence our lifestyles and make us susceptible to diseases is now becoming reality. A new

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era for healthcare is beginning, the era of personalized treatments. This was anticipated at the end of the nineteenth century by Sir William Osler, a Canadian Physician, who said that “If it were not for the great variability among individuals, medicine might as well be a science and not an art” [1].

Until the last decade, the prevalent idea was that susceptibility to diseases could be described as a normal distribution, considering the incidence of a specific phenotype in the general population. Influenced by this idea, for many years, pharmaceutical research focused its attention on the discovery of drugs that could be effective on the general population. Increasing cases of individuals with reduced or toxic effects reveal that personal genetic variations in the normal phenotypical distribution have to be considered carefully.

Two pioneers of the genetic era were among the first to have their genome sequenced: James Watson (Nobel Prize for discovering the DNA structure) and Craig Venter (lead scientist of Celera genomics at the time of Human Genome Project). This created great expectations among the general public, especially with the publication of their genetic code. Venter published his entire genome sequence, revealing the presence of polymorphisms that make him potentially susceptible to antisocial behavior, alcoholism, obesity, stroke and Alzheimer’s disease [2]. Interestingly, Watson decided not to publish a short part of his genome containing the APOE gene, which is linked to Alzheimer’s disease onset [3], stating, inspiringly, that “Since we can’t do much about Alzheimer’s disease, I didn’t want to know if I was at risk” [4]. The hard reality is that the great advances in diagnosis often have no reflection on our ability to treat genetic diseases.

The advent of personalized medicine promises to achieve a shift in future healthcare not only with a predictive, but mainly a proactive approach, to medicine, where emphasis should be placed more on disease prevention than treatment. A change of paradigm in research is also needed to achieve this goal. Different disciplines can no longer be considered as separate and patient data obtained by different high-throughput techniques has to be necessarily integrated in a conceptual data cloud. That which over a dozen years ago could only be considered fiction will only become reality in this way. The time is ripe for the next revolution in healthcare: personalized medicine.

3.2 Paradigm Shifts in Medicine

In his famous essay on the nature of scientific revolutions, Thomas Kuhn theorized that scientific research proceeds through long, stable periods of normal science intermittently punctuated by events in which new paradigms for science emerge [5]. To overcome “normal science”, often decades of new evidence are required. The history of medicine is rich in examples of “normal science” becoming obsolete, and sometimes newer theories ignored because they did not fit the old paradigm.

Table 3.1 Paradigm shifts in medicine as chosen in the British Medical Journal poll [6]

Rank	Paradigm shift	When	Who	Votes (%)
1.	Sanitation			15.8
	Link between cholera and sewage-polluted water	1854	John Snow	
	Infrastructure for sewage disposal and tap water	1848	Edwin Chadwick	
	Hands disinfection for physicians	1847	Ignaz Semmelweis	
2.	Antibiotics			15
	Discovery of drug against syphilis (Salvarsan)	1909	Paul Ehrlich	
	Discovery of penicillin	1928	Alexander Fleming	
3.	Anesthesia			14
	First surgery under anesthesia	1846	William Morton Horace Wells	
4.	Vaccines			12
	Smallpox vaccine	1796	Edward Jenner	
5.	DNA structure			9
	Publication of the DNA structure	1953	Francis Crick, James Dewey Watson, Rosalind Franklin	

A consensus about medical revolutions is very hard to define and could also be considered rather unfair. However, readers of the British Medical Journal have been called to vote on a shortlist of 15 milestones in medicine [6]. More than 11,000 participants (as summarized in Table 3.1) ranked a relatively unexpected revolution at the top: sanitation. Pioneers in the sanitary revolution were John Snow, who first linked epidemic cholera with sewage-polluted water, and Edwin Chadwick, who came up with the idea of infrastructure for sewage disposal and to bring tap water into houses. Interestingly, we have to recognize how this is still a theme, as sanitation remains a serious problem in much of the developing world, with more than 1 million estimated deaths per year due to diarrheal disease. Another contribution to the medical field is the introduction of sanitization. The history of sanitization is indissolubly liked with the fight against child-bed fever. In the first part of the nineteenth century, in Europe and America, thousands of young women died due to this infectious disease, also known as puerperal fever. Women were generally affected within the first days after giving birth, while the disease progressed rapidly and caused acute symptoms such as severe abdominal pain, fever, and debility often leading to death. Two main actors in the fight against puerperal fever were Oliver Wendell Holmes and Ignaz Philipp Semmelweis. Both were inspired by hearing about the death of colleagues a few days after performing postmortem exams on a woman who had died of puerperal fever. Semmelweis speculated that he and his physicians were carrying "cadaverous particles" from the autopsy room to the mothers they examined in the maternity hospital. From that moment on, Semmelweis began to insist that anyone who attended autopsies washed their hands with calcium chloride before entering the maternity unit. As a consequence, the maternal mortality rate fell dramatically [7].

In the British Medical Journal survey, sanitation was followed closely by the discovery of antibiotics. The beginning of the "antibiotic era" is associated with Paul Ehrlich and Alexander Fleming. Ehrlich found a drug against syphilis, an endemic disease that was almost incurable at that time after a systematic screening. Amazingly, the mechanism of action of this compound is still mostly unknown, and its chemical structure has been solved only recently. Something even more serendipitous happened in 1928, with the discovery of penicillin by Fleming. Interestingly, he was also among the first who warned about the potential resistance of bacteria to antibiotics [8]. Third on the podium of revolutions in medicine is anesthesia. William Morton and Horace Wells could be considered the fathers of modern anesthesia. When in 1846 the first patients underwent painless surgery, John Collins Warren said "Gentlemen, this is no humbug" [9]. At that time, just the idea of a painless surgery seemed a kind of miracle. Until the discovery of anesthesia, surgeries were rather quick and often brutal. Patients were completely conscious and many paintings representing surgery before anesthesia illustrate something that looks like torture. With its use, anesthesia allowed safer, longer, and even more invasive surgical procedures.

Runners-up in the medical revolutions poll are the introduction of vaccines and the discovery of the structure of DNA. The question is: what will be the next big revolution that will improve, once again, medical practice?

3.3 Omic Sciences and their Interaction

New technologies and knowledge developed in the context of the Human Genome Project opened the field to the so-called omics revolution during the beginning of the twenty-first century [10]. The technological effort needed to sequence the human genome lead to the definition of new protocols and technologies, suitable for the production and analysis of an enormous quantity of scientific data. These technologies, generating a previously unbelievable amount of data thanks to the high processivity of the new approaches, defined new "high-throughput" standards of performance. In fact, high-throughput technologies were essential in order to achieve the ambitious aim of the Human Genome Project. Without the ability to rapidly and accurately measure thousands of pieces of data in a short time period, there would be no way to sequence an entire genome. See Table 3.2 for a comparison of technology before and after the Human Genome Project.

Like the new approaches developed in the context of the Human Genome Project, all disciplines focused on the development of new techniques and on their

Table 3.2 Quantitative advances since the human genome project (HGP)

	HGP begins	HGP ends	10 years later
Cost to generate a human genome sequence	$1 billion	$10–50 million	$3–5 thousand
Time to generate a human genome sequence	6–8 years	3–4 months	1–2 days
Vertebrate genome sequences	0	3	112
Prokaryotic genome sequences	0	167	8760
Human single nucleotide polymorphisms	4.4 thousand	3.4 million	53.6 million
Genes with known phenotype/disease causing mutations	53	1474	2972
Drugs with pharmacogenomics information on label	4	46	104

Data provided by the National Human Genome Research Institute (*URL* https://www.genome.gov/)

data analysis have been call "omics", from "-ome", a term derived from the word genome. Genomics has been the first "omic" discipline to be defined in the context of the Human Genome Project. Genomics focuses on the sequencing and analysis of genomes and exomes. For example, single nucleotide polymorphism genotyping (SNP genotyping) measures individual genotypes for several hundred thousand SNPs in the genome. Approaches like Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) were not anticipated. Other assays to sequence and analyze a small amount of the genome have been proposed to focus only on positions that could be causal of disease onset. After more than 15 years since the conclusion of the Human Genome Project, genotyping technologies are now accurate and affordable, but analysis at DNA level sometimes presents limitations. DNA sequence variations tend to be very common, generating a lot of noisy signals that can be hard to decipher. In addition, other epigenetic modifications and environmental factors may modify gene expression in a non-predictable way. Even so, SNP genotyping is currently considered among the most useful techniques to predict disease risk.

Use of high-throughput technologies is not limited to genome analysis, but at least three other omics disciplines can be identified: Transcriptomics, Proteomics and Metabolomics. Transcriptomics is the simultaneous measurement of gene expression levels in a cell or tissue by oligonucleotide arrays in which hundreds of thousands of probes capture RNA molecules. Proteomics, instead, focuses directly on protein levels in a tissue mainly obtained by mass spectrometry. The size of each peptide is defined after protein extraction and digestion. Proteins can be identified by comparing the size of the peptides extracted from the tissue with a database containing the digest of all known proteins. Last but not least, metabolomics is the high-throughput measure of metabolites present in a cell or a tissue. In general, each discipline offers a different perspective on the molecular mechanisms underlying disease initiation and progression.

The omics revolution also opens up new challenges, as laboratories usually do not have sufficient computational resources and storage to process this large amount of data. Since storage and analysis costs are not falling as fast as data generation, this represents a new bottleneck for advancing the field. New kind of scientists and technical infrastructures are needed. One way to address these challenges is the training of an ever-increasing number of bioinformaticians. Cloud computing is a promising technology to fill the gap between data generation and storage, such as the Embassy cloud, which is part of the European ELIXIR bioinformatics infrastructure [10].

3.4 Personalized Medicine. Evolution and Perspectives

New achievements of high throughput sequencing did not stop with the end of the Human Genome Project. Year after year, this technology continues to evolve, chasing the goal of the $1000 genome. Pushed by public research and private companies, this new challenge has led to a rapid decrease of DNA sequencing costs. Over the last 15 years, the sequencing cost for a human genome dropped from $100,000,000 (estimated cost of the Human Genome Project) to less than $10,000 for a genome. During the last few years, several companies claim to have reached the goal of $1000 dollars. Illumina HiSeqX Ten in particular seems to have found this “holy grail”.

At present, state-of-the-art technology allows the genotyping of any sample of interest at an affordable price in a short period of time, sometimes less than a couple of weeks. WGS and WES have become affordable tools for understanding the genetic basis of human phenotypes and diseases [11]. Analysis of genetic variants in an individual genome has permitted the examination of the genetic basis of disease with an unprecedented level of detail. The huge amount of data generated for both healthy and diseased individuals has not only helped in the definition of the molecular basis of genetic diseases, but is also transforming future healthcare. The combining of genomic data with medical patient records will soon permit the prediction and prevention of disease onset, enabling new pro-active therapeutic strategies.

In this context, the National Cancer Institute (USA) in 2011 coined the term “personalized medicine” for healthcare, considering information about the individual genome, proteins and the environment for diagnosis and treatment of diseases [12]. This will transform the perspective of future healthcare from disease diagnosis and treatment to personalized health monitoring and preventive medicine. The first examples of personalized healthcare are based on the analysis of patient genomic markers to define whether a person is likely to respond to a given pharmacological therapy, adjusting dosage to optimize drug efficacy and safety. One of the most famous attempts of personalized treatment based on the analysis of genetic variants is warfarin, the most commonly used anticoagulant worldwide. Warfarin targets the vitamin K epoxide reductase complex subunit 1 (VKORC1) enzyme. Inhibition of VKORC1 by warfarin leads to the production of coagulation factors with reduced activity. Several VKORC1 mutations have been identified and most are common variants affecting VKORC1 expression influencing warfarin dosage within the normal range. Rare mutations have been associated with warfarin resistance, requiring an increase in drug dosage. Without knowing the personal characteristics of patients and their genetic background, it could take months of trial-and-error testing to find the right drug dose. The problem could be more widespread than expected. Mutations can also affect enzymes involved in the process of drug metabolization, such as the Cytochrome P450 family members. To give an example, for Cytochrome P450 2D6 (CYP2D6), one of the best studied drug-metabolizing enzymes, about 10% of the general population has a slow-acting form, while another 7% have a super-fast-acting form [12]. Some subjects may therefore process drugs too rapidly (ultra-metabolizers), rendering them ineffective, or too slowly (poor metabolizers), causing an increase in blood concentration and potentially leading to toxic effects. At the moment, several studies are trying to optimize drug dosage after SNP genotyping and the first on-line tools become available to identify and suggest if patients need a more specific drug dosage based on their genetic background. A growing number of drugs are expected to have companion diagnostics, as about 10% of marketed medications will propose or recommend genetic testing for treatment optimization in the future.

Another field of personalized medicine that has greatly advanced is precision disease diagnosis (Fig. 3.1). The decrease in WGS cost permits causal gene

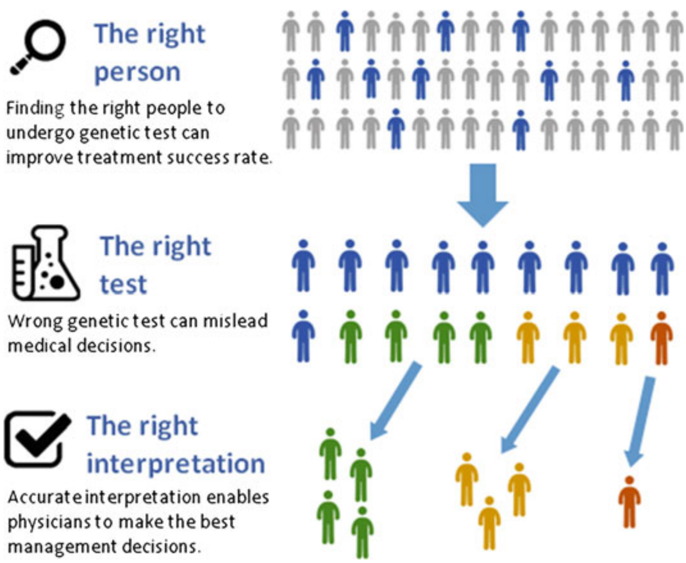

Fig. 3.1 Three main steps of precision disease diagnosis

identification for diseases and complications at a personalized level. For example, Bainbridge et al. sequenced the complete genomes of a twin pair and identified compound heterozygous mutations in the SPR gene responsible for the dopa (3,4-dihydroxyphenylalanine)-responsive dystonia in both twins [11]. Precise identification of the specific causal variants open up the possibility to improve child health by supplementing L-dopa therapy with 5-hydroxytryptophan, the serotonin precursor whose synthesis depends on SPR. Precise diagnosis in cancer research has markedly benefited from WGS/WES. Hundreds of cancer genomes have been sequenced, allowing previously unimaginable collaborative efforts that have led to the creation of fundamental resources, such as the Cancer Genome Atlas. In addition to bulk cancer sequencing, single-cell cancer exomes have also been examined. When compared to normal tissues, somatic mutations for specific cancer genomes, as well as molecular markers for cancer subtyping, could be identified. Børresen-Dale propose to classify breast carcinomas on the basis of different gene expression patterns, to link tumor characteristics with clinical outcome. For patients that had received the same therapy, estrogen receptor positive tumors could be divided into at least two groups, each with its specific gene expression profile and different prognosis [13]. These data could provide potential targets for personalized cancer treatment in the future. WGS could also help to identify spontaneous mutations in the 'normal' genome of cancer patients that may lead to carcinogenesis. Sequencing has already been applied to patients with suspected increased cancer susceptibility, such as those with multiple primary tumors. For example, a germline de novo p53 deletion was identified in a patient who developed 3 different cancer types in 5 years [11].

So far, we have described how disease diagnosis and treatment are changing in the "omics" age, but something is still missing. The personalized medicine revolution seems to be dramatically deeper than could be expected. By knowing the biological background of the patient, preventive risk assessment will become possible, defining individuals as "not-yet patients". Not-yet patients are individuals with a genetic background predisposing for a specific disease, who do not present any symptoms (yet). This situation could be very dramatic in the case of patients at risk of a lethal or disabling disease that may never develop. Recently, actress Angelina Jolie brought the field of personalized medicine to public attention, as she was found positive for a BRCA gene mutation in genetic testing. BRCA1 and BRCA2 tumor suppressors ensure the stability of genetic material by helping in DNA damage repair. When either is mutated, DNA damage may not be properly repaired and cells are more likely to develop additional genetic alterations that can lead to cancer. Specific BRCA1 and BRCA2 variants increase the risk of several cancer types [14, 15]. In particular, BRCA gene mutations account for about 5–25% of hereditary breast cancers and around 15% of ovarian cancers [16]. Positive to BRCA mutations and with a strong familiarity for cancer, Angelina Jolie decided to have a double mastectomy and later to surgically remove her ovaries and fallopian tubes, to prevent a pathology that she may never have developed. An additional chapter could be dedicated to incidental or secondary findings. These are unexpected results, not related with the clinical condition for which sequencing has been performed. New specific regulations are needed to manage these situations, ranging from the uncommon "misattributed paternity" to findings of critical medical value like possible predisposition to degenerative diseases [17].

3.5 Systems Biology. A (further) Starting Point

Genomic information may not always be sufficient to predict a person's health, environmental factors can contribute to disease development or even trigger disease onset in susceptible individuals. For complex and multifactorial diseases, many authors consider WES/WGS to be of limited value for predicting disease outcome. To give an example, Baranzini et al. failed to find evident genomic or transcriptomic differences in monozygotic twin pairs discordant in multiple sclerosis [11]. Despite a strong genetic component having been postulated for this disorder, it is likely that other factors contribute to disease onset. Access to large omics data could provide new insights into the treatment of human diseases. Transcriptomic, proteomic and metabolomic information could be considered a more precise index of human health than the genomic sequence alone. Combining genomic information with a scheduled monitoring of these omics parameters should serve to obtain real-time information of an individual's condition.

The integration of different omics data has led to a new discipline called systems biology, aiming to model complex biological interactions integrating information in a holistic manner. In contrast to treating a mixture of factors as single entities, systems biology relies on experimental and computational approaches to provide mechanistic insights [10]. In systems biology, data are often elements integrated into networks. Just think about information coded in our genome and environmental signals. In systems biology, these two information types have to be considered together, integrated into the individual organism to produce their phenotype—normal or diseased. These two information types and the phenotypes they produce are considered part of biological networks that capture, integrate and transmit the information to molecular machines. A fundamental postulate in systems biology is that disease arises from networks perturbed by genetic changes and/or environmental signals. The resulting altered molecular machinery encoded by the perturbed network leads to the disease pathophysiology [18]. Integrated omics data analysis could monitor molecular profiles and detect subtle changes that may indicate network perturbation. For example, Snyder and colleagues studied the omics profile of healthy volunteers monitored for 14 months with a so-called integrative Personal Omics Profile (iPOP) analysis [11]. The individual's genome was sequenced at high accuracy with WGS/WES and genetic predispositions for diseases and drug responses were identified. The physiological state changes occurring during two viral infections and onset of type 2 Diabetes were monitored with information from the transcriptome, proteome and metabolome. The generated integrative profile could observe both trend changes, associated with more gradual changes, and spikes of particularly enriched genes and pathways, especially at the beginning of each physiological event. This integrative analysis provided a much more comprehensive view of the biological pathways changing during disease onset. Importantly, thanks to the previous genome sequencing and active monitoring, diabetes onset was detected in its early stage and could be effectively controlled by proactive interventions, such as diet change and physical exercise. It is clear that "genomic medicine" is not sufficient to describe the new horizons offered by systems biology. Genomic medicine is one-dimensional in nature, considering only nucleic acid information. In contrast, this new medicine is holistic, using all types of biological information from DNA and RNA to proteins, metabolites, interactions, cells, organs, and external environmental signals, integrating them in predictive models for health and disease. A new term for future systems biology-based medicine has been coined: systems medicine.

The field of systems medicine is not limited to the analysis of "classical" omics data of individuals. Profiling other omics data such as gut microbiome and microRNA profiles may also be important for health monitoring. The gut microbiome, including all symbiotic microorganisms in the human gastrointestinal tract, is considered an "extended genome", which may play an important role in immune host response and drug metabolism. To give an example, it can contribute to the interpersonal variation of simvastatin degradation, a commonly used drug for cholesterol control. MicroRNAs are another important profile for personalized health monitoring. This layer of posttranscriptional regulation could be important in monitoring various tumor initiation drivers and extracellular microRNA may serve as biomarkers for various diseases [11].

3.6 Conclusions

Enormous volumes of data have been generated in the context of high-throughput omics sciences over the years, as never before during the history of modern science. For the first time this data opens the possibility of personalized reports about individual genetic background and metabolites profiles. A new kind of medicine is approaching, where personalized treatments start to be possible. However, the speed at which personalized medicine is translated into the clinic appears to be slower than expected. This discrepancy seems to be a clear symptom that cultural, technical and methodological bottlenecks remain be solved [19]. According to a poll from 2012, American physicians recommended personal genetic tests for only 4% of their patients [19]. High-throughput omics science has led to progress in the medical sciences, but the rate of progression is far from uniform. Oncology seems to be the field that is adopting this genomic-era medicine most rapidly, with many cancer centers developing personalized medicine initiatives [19]. At the moment, most personalized medicine tools could be considered to be largely proof-of-principle. It seems evident that in this time of enthusiasm, and a little confusion, there is a pressing need to clarify the appropriate level of confidence that these methods should achieve to be translated into the clinic, and which classes of approaches are most suitable for each disease class.

The Critical Assessment of Genome Interpretation (CAGI) was created in 2010 to address this problem. CAGI is a community experiment to assess the performance of computational methods predicting the impact of genetic variations, currently at its fourth edition in 2016. The organizers provide unpublished genomic data for which the associated phenotypes have been experimentally determined and groups from all over the word have a few months to submit predictions. Evaluation of the submissions is performed by independent assessors and discussed in a final meeting after a couple of months, usually in San Francisco. The main goal of CAGI is to accelerate the development of software to predict the phenotypic impact of variations both at the molecular and organism level. This community experiment aims to identify critical points in genome interpretation and propose strategies to solve them. It is also an opportunity to connect researchers from different fields on the interpretation of human genetic variants. Several challenges were proposed in every edition and can be divided into two main groups: gene-oriented and phenotype-oriented predictions. Gene-oriented predictions aim to identify relations between mutations of a specific protein and an observed phenotype. Phenotype-oriented predictions aim to identify variants that could be causal of complex diseases. Exomes or genomes are released to predictors, who have to classify individuals into healthy and affected, and often also have to identify the disease-causing variants.

Apart from scientific and technical limitations, other concerns are slowing down translation of personalized medicine into the clinic. Very few studies have considered the economic aspects of personalized genetics. There is a general consensus that costs in omics studies will be more related to data interpretation than data generation, shifting the challenge from “the $1000 genome” to “the $100,000 analysis” [20]. Only a minority of the few personalized tests that included economic information were cost-saving, suggesting that a promise of better health is not necessarily associated with lower expenditure [10]. In this context, it is clear that costs associated with personalized medicine still remain ambiguous. However, not only economic concerns are emerging with the market release of the first genetic tests. The need for new rules regulating the commercialization of these tests has emerged with the marketing of genetic tests by 23andMe. The company sold “spit kits” for $99, which provide an analysis of the genetic code with both information about ancestry and health risk. In 2013, the American Food and Drug Administration asked the company to immediately discontinue providing health-related information due to concerns about test accuracy. Since then, 23andMe only releases information to customers about their ancestry. New ethical challenges also have to be addressed, as precision medicine will pave the way for a new commercialization of health, especially in the context of preventive medication. This will open the market to new kinds of prevention drugs that may have to be taken for life, even before having contracted a disease. An interesting debate is also whether these precision treatments should be merely pharmaceutical or mainly lifestyle based.

Despite various clinical and technological concerns, we still believe that personalized health monitoring and preventive medicine will greatly improve the health of the general public. As often happens in science, it will probably take another few years before any tangible results are brought before the public, but it is only a question of time. The revolution has already begun.

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Chapter 4
Epistemological and Ethical Implications of the Personalized Justice

Giuseppe Zaccaria

Abstract The history of the recognition of human rights, which still aspires to the pursuit of a "personalized justice", has seen three major periods: the cultural revolution of the Enlightenment and the abstract universalistic dimension of the rights of every human being; the recognition of the rule of law and human rights anchored in the existential context; and the legitimation of the rights to differences and to the identity of the individual. The process of evolution towards a personalized Justice is still an obstacle in the concept of equality, a fundamental pillar of Italian constitutional law. Going beyond traditional law can be achieved with a historic reversal of the criminal law, from a punitive system to a restorative-retributive one that assesses on a case by case basis, even through mediation, taking into account differences and peculiarities. Restorative justice, despite not having the ability to become a universal paradigm for conflict resolution, is evolving towards a personalized justice.

At first sight, and without considering the matter in any particular depth, the expression "personalized justice" might appear to be an oxymoron, the combining of two contradictory terms. Indeed, in western juridical culture, the law is presented to citizens as a guarantee of generality and equality of treatment. The ancient motto, clearly visible in courts of law, that "the law is equal for all", perfectly conveys the idea, linked to the earliest conceptions of justice, regarding the need for a rule that is equal and valid for all so as to avoid arbitrary conclusions. Equal cases—it has always been maintained—must be adjudicated in an equal manner, without, that is, any diverse treatment.

Today, nevertheless, it is apparent to everybody how, in the context of an increasingly pluralistic (pluralism of ethics and values, juridical pluralism) and depersonalised society caused by the processes of globalization, differences seek a

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specific recognition, which is not possible simply on the basis of the general and abstract nature of the traditional rule. In our time, given that the most perceptible social value is personal or group identity, the sacrifice of particularity and concreteness in the individual case seems intolerable. *Today* everybody asks for and expects a "particular" justice from the law, that is, according to the Hannah Arendt's concept, "the right to have rights". Increasingly invoked is a justice that, while not undermining the value of equality, adheres more to the concreteness and specificity of people and specific cases and is able to "defend the human being in his most individual and singular reality" [1].

As we mentioned before, one could argue that an oxymoron hides in the expression "personalized justice": nonetheless, if we engage in a somewhat more considered and deeper reflection, it is not difficult to observe that many of our initial perplexities recede, or at least adapt. The long history of the recognition of rights—"a great invention of our civilisation" [2]—although recounted briefly here, tells us that personalized justice is nothing other than the conclusion of a process and parabola that have seen broad and profound transformations. The idea of human rights is a grand and original moral idea that has been historically translated throughout the centuries. The long history of rights moves towards their progressive contextualization.

Everything began in the IV century A.D, when Aristotle, addressing the practical sciences, or rather the sciences that have as their object not knowledge but action, in book V of the *Nicomachean Ethics*, dedicated entirely to the analysis of justice, shows that he is well aware of the extreme complexity of the concept of equality: equality is not only *arithmetical*, but also *geometrical*, capable of being traced back, that is, to symmetry and parity of treatment. Here, in giving to each his due, is the underlying essence of the logic of justice, which finds in equality its fundamental medium, based precisely on the absolutely analogous recognition of due responsibilities. *The equal is just*: this is the affirmation of Aristotle that has remained a point of reference in the perennial doctrine of justice and from which it is necessary to take inspiration so as to correctly formulate the issue of personalized justice.

The recognition of rights constitutes an age-old promise, at the origin of the juridical civilisation to which we belong. If the primitive populations of ancient Greece and Rome were not conversant with the language of rights, it was eight centuries ago, in 1215, in the *Magna Charta Libertatum*, that a significant and decisive step forward was made in the delineation of the sphere of justice: for the first time the importance of the requirement of reciprocity in order to specify the characteristics of justice was recognised. John Lackland, relinquishing the exercise of arbitrary power over the bodies of individuals, laid the foundations for a universal recognition of rights that in the coming centuries will be ascribed an ever greater importance.

The narration of this extremely long process, which has still not reached its conclusion, will have to be reconstructed not in an analytical manner, but in condensed form, unable to give an intricate and complex account of a historical and cultural evolution, but only a trend line, within which can be distinguished three diverse epochal structures, three diverse historical and conceptual approaches, but also anthropological, with which rights are related to differences.

The first phase, dating back to the historical moment in which human rights were present in the form of *natural rights*, can be aligned with the beginning of the modern age, between the XVII and XVIII century. It is the phase in which the *universalistic* dimension of rights, which must apply everywhere, in every corner of the world, is underlined, *non quia christiani sed quia homines* [3]. By the mere fact of belonging to humanity, every human being possesses *natural rights* independently of differences of sex, race, language, religion, culture, political opinions or social conditions. This means that rights are recognised regardless of differences, considered as inessential for defining man and identifying the fundamental rights; but also, that rights must be equal for every individual, without any kind of distinction. The universality of human rights is fastened to the principle of equality as a fundamental ethical postulate.

If before this period the expression "human rights" did not even exist within the lexicon, it is during the cultural revolution of the enlightenment that the decisive and exemplifying moment of the modern language of human rights occurs. "Mais si l'homme"—wrote the Marquis Marie-Jean-Antoine Nicolas Condorcet in his *Esquisse d'un tableau historique des progress de l'esprit humaine*, 1795—"n'y est pas tout ce qu'il doit etre, la dignité de sa nature n'y est point avilie; quelques-uns de ce droits son tau moins reconnus; on ne peut plus dire qu'il soit esclave, mais seulement qu'il ne sait pas encore etre vraiment libre". In short, next to the discovery of the existence of natural rights, which itself already poses an (imperative) ethical truth of a universal character, the necessity to guarantee their practice and application starts to become manifest.

The second phase of the history of the relationship between rights and differences marks the beginning of a new understanding: differences can no longer be placed between brackets, but, on the contrary, must be considered as important aspects of the human being. Individuals become aware of the circumstance of being born and living in a context of social relationships in which they can discover their identity on the basis of differences. Identities aspire to recognition, claiming rights.

National and International institutions commit themselves to the recognition of these rights and oblige themselves with regard to their holders. The strategic role that rights perform is that of limiting sovereignty, protecting the space of the individual from potential abuses of power, and directing the legislator in predisposing measures protective of civil goods. There is a radical upheaval of perspective compared to the past in the state-citizen relationship, which is increasingly considered from the viewpoint of the rights of citizens rather than from that of the sovereign. From the first Declarations (the Declaration of Independence of Virginia, 1776, to the Declaration of the Rights of Man and of the Citizen, 1789, and the Universal Declaration of Human Rights, 1948) we arrive at the definite formation of the Rule of Law and the Constitutional State, as well as to the international recognition that the holder of human rights is the "human person". There is, in brief, a gradual extension of the recognition and protection of rights to a sphere above individual States.

The evolutionary process of rights is accompanied by their transformation: Norberto Bobbio has spoken of the passage “from the consideration of abstract man to that of man in the various phases of his life and its various states.” Compared to the abstract universalism of a self unmoored from existential contexts, a human life is considered in its various modes of being: as a child, a minor, a woman, an elderly citizen, a disabled person, a sick person, and so on. It should be pointed out how this process of specifying holders of rights, which corresponds to greater difficulties of protection and to a related willingness for better and more appropriate protection of the diverse ways of life of people, constitutes an important intermediate step, but one that has not completely left behind the previous universalism. In other words, these states of life, linked to criteria such as age, sex or gender, the particular physical conditions or the particular biological demands, unite vast groups of people, beyond cultural differences, but also belong to the entire human family. All of the citizens are able to understand such demands. The rights of minors, women, the elderly, and so forth, are designated on the basis of a general interpretation of the particular states of life. Furthermore, a multiplication of the anthropologies posited by rights takes place, the expression of the human world in a plurality of diverse forms of life, but in the background the idea remains that it is not permissible to introduce a difference of value between people who all possess equal dignity, in spite of differences.

And we come to the third phase of our narration, that in which we find ourselves today. It is the phase in which one speaks of rights to differences, of the request to protect one’s own self-perception and identity, defending them from cultural homologation and social assimilation.

If previous phases of the evolution of human rights were based on *cosmopolitanism*, today the aspect of *interculturality* in the determination of rights seems firmly to prevail [4]. Differences are mostly expressed as cultural experiences, reflecting a certain mode of experience of the world and these diverse modes risk being tendentially incommensurable, difficult to communicate and often incompatible. The processes of globalization, however, also emphasize the aspects of cultural deterritorialisation, being those specific forms of life protected outside of their original environments; and as a consequence the particular claims of individuals and groups must contend with the historical-cultural accumulation of the associative life. Fragmentation and diversity are produced within once compact cultural units: it becomes impossible to consider society as a unified whole and as defined once and for all: different *ethics* develop, in which the value of personal autonomy becomes prevalent, the free will of every individual to give to their own existence the form that they feel to be most fitting. The ethical disagreement on rights has become a structural phenomenon [5]: we are faced with an unprecedented connection between the abstraction of rights and the reality of needs [6].

Naturally, in such a framework the issue then becomes the finding of a common humanity, and before that a common language, in the articulation and plurality of the forms of life. The “canonical” principle of the territorial validity of rights must be combined with its personal and cultural character, and this last can find its basis in constitutional guarantees such as equal protection, freedom of association and religion, the right to defence and to a fair trial.

Up until now we have attempted to succinctly reconstruct a history of rights from the point of view of the progressive emergence of differences between men: on the long path of the affirmation of rights that has made the language of rights the "lingua franca" of global public discourse [7], it was initially intended that differences be placed between brackets, but in the final phase of their evolution this was no longer possible, even if it does not seem possible to be able to speak in a proper and full sense of a "personalized justice". What impedes the full completion of the prospect of "personalized justice" and constitutes a constantly unstable point of equilibrium is precisely one of the pillars of the idea of justice, namely, the concept of equality. To treat equal cases in an equal way is the very principle of the equality of all before the law.

We will now endeavor to find some confirmation of our thesis with reference to some practical forms of the ongoing attempt to greater personalize justice in contemporary juridical experience and correspondingly developed in theoretical studies.

I shall point out immediately that I do not principally intend to refer to the institutional efforts and those of contemporary criminal practice to personalize the sentence, reduce and individualize the punishment and mitigate its severity, expand the range of sanctions with greater recourse to conditional freedom, or even suspend execution of the sentence. It is enough to remember that, even though at the origins of modern conceptions of punishment the entire sphere of criminal law had to be rigorously pre-determined in tribute to the cardinal principle *nullum crimen nulla poena sine lege* of the Enlightenment, and thus also the sanctions that the court was called upon to apply mechanically, already in an authoritarian criminal code aimed at the toughening of penalties, namely, the Italian penal code of 1930, article 132 provided that "within the limits set by law the court shall apply the punishment *discretionally*; it must indicate the reasons justifying the use of that discretionary power". A great deal of progress has therefore been made, but the fact remains that traditional punitive justice—think of procedural forms, for example—is structurally inadequate to meet the real needs of people involved in the punitive context.

I would rather refer to horizons that have not yet been entirely explored, to attempts, which have multiplied in recent decades, to introduce a diverse type of justice, differing from the traditional paradigm.

All modern statutory law implies that we can prosecute and punish only the infringement defined by law. When we define, as the cardinal principle of liberal societies, the fact that one can only punish those who have been identified as being punishable by law (*the principle of legality of offenses and punishments*) what is most important to underline is the very idea of legality: in other words, society punishes according to legality when crimes are not defined arbitrarily and penalties are measured against any excess. In modern societies the right to punish is distinctly separated from revenge because, unlike the latter, it focuses on the offense defined by law.

This is precisely the difficulty encountered when trying to imagine other paradigms in the exercise of criminal law: the three essential cornerstones of reference are, on the one hand, the perpetrator and the victim, and, on the other, a third, which in traditional criminal conceptions of modernity is represented by the law. From Kant to Beccaria positive law is described as the most convincing expression of the ideal model of criminal law. The retributive model refers the punishment to the single infringement of the law, privileging, that is, the role of the third party compared with those of the victim and the perpetrator. This supposition, highlighting the limits that the *retributive* model comes up against, gave rise to the will to radically rethink, in terms of its basic assumptions, the general structure of contemporary criminal justice, recovering the forgotten dimension of interpersonal relationships. We can therefore say that one must always understand the term *personalized justice* as a justice attentive to interpersonal relationships, not as something completely tailored to the individual needs of the person, as happens, instead, in the case of *personalized medicine.* It concerns, more precisely, a restoration of *community justice* with respect to the *state justice* model. In this sense, the ideas that the crime is first and foremost an offense, that it should be repaired rather than punished, that reparation consists in a satisfaction and that this last must pass through a negotiation, constitute the resumption, obviously in a completely different context, of a medieval experience of justice, which refers to the *community* nature of negotiated justice (Sbriccoli).

International legislation (the United Nations, Council of Europe, European Union) (Mazzucato) has for some years now provided, among the prime objectives of democratic criminal law systems, the reparation of relationships damaged by crime.

In the alternative model of *Restorative Justice*, which arises from the diverse experimental models that emerged in order to overcome the marginalization or even the exclusion of concrete relations produced by the contemporary criminal justice system, the guiding concept is no longer that of retribution, still dominant in our criminal systems, but that of the reconstruction of the organic social bond that holds a human community together. In placing the emphasis on the wrong done to the victim ("to put right the wrongs" [8]), taking charge of their sufferings and exigencies in order to repair them, but at the same time involving the victim in the procedure that leads to the imposition and enforcement of the penalty, there is the risk of "concealing" the position of the institutionalised third party, and with this its guarantee and commonality, which could be harmed by a failure to acknowledge its role. In other words, the restoration of the rule, where the concept of reparation aims to reconstitute a relationship of dialogue between those involved in the specific conflict, could work with regard to the relationship between the wrongdoer and the injured party, but not in relation to the more general issue of the restoration of criminal legality that follows on from the unacceptable disorder produced by the crime. The actual and personal damage that the offense caused is not easily reducible to the terms of a mere "conflict between private individuals" [9]. Above all, an issue emerges: as the basis of dispute resolution can negotiated or consensual justice replace the role played by the authority, understood as guaranteeing the avoidance of a repetition of the offense manifested in the crime? Or, in other words: if the right to punish is the right to defend, even by use of force, the implicit ethical order in the life of a community, who is the guarantor, in the *Restorative Justice* model, that will protect the bedrock of common values around which a human group is constituted and in which it recognizes itself? As the abrasion of the rule has a public valance, so the response to such an abrasion must necessarily maintain a public character, so as to provide precisely those limits and those guarantees that most legal systems affix to the penalty.

To safeguard the possibility of a comparison between the solution of a particular case and that of analogous cases, it is necessary that both the general parameter of conduct set by the rule as well as the solution to the abrasion of said rule are also shareable by third parties, and that the response to the conflict maintains a fundamental "proportionality test" in relation to the abrasion. We must not forget, however, that "classic" criminal law, despite all of its shortcomings, is a guarantee of a patrimony of principles, providing guarantees, which must not be lost.

Ultimately, the Restorative Justice model, even with all of its shortcomings and indeterminacies, in wanting to overcome the interpersonal conflict originating from the crime, has in any case the advantage of restoring the right as the guardian of an intersubjectivity that has been violated and that must be reconstructed, proposing a dynamic order based not on imposition but on sharing. It suggests practically distinguishing and evaluating each case on its merits, situation by situation, taking into account differences and peculiarities, with particular attention to the expectations of the people involved. Both the wrongdoer as much as the victim are considered in the concreteness of their existential and social relationships. On the other hand, such a model has the limitation of fracturing the comparability of single situations, thereby obscuring the general valency of the rule, since reparation is not strictly related to a measure of general character. The perspective on which to work will probably be that of *complementarity* between the model of retributive justice and that proposed by the new reparative paradigm, the approach of which is able to genetically mutate the fundamental idea of substantial criminal law on a number of points. Therefore, at least in the short term, it is not possible to *completely* eliminate every punitive element of the sentence, by maintaining that all types of crime are ontologically reparable. But the fact remains that the reparative idea directs us towards a new understanding, a new epistemological basis of the same general meaning of the penalty, no longer built solely on the basis of retributive logic and on the identificatory fact of the offense, but more attentive to personal dimensions. In a word, the model of restorative justice opens up a new pathway and today represents the hope for an epistemological transformation of the penalty (Donini).

This matter would be worth enlarging upon, extending it in a more comprehensive manner to mediation in other configurations (that of civil and commercial, in particular), as an informal method of *conflict resolution*, equipped with procedures overseen by a third party equidistant from the principal parties. Just to give an example, it is enough to note here that in the civil sphere of *alternative dispute resolution* (ADR), an extra-judicial solution of disputes, the recognition of the humanity and dignity of the other is not necessarily required, while it is, however, specific to the *restorative approach* in the criminal context.

The term mediation signifies the arbitration of opposing claims, which refer to those persons in dispute [10]. It is necessary to move from this definition in order to take into account the fact that mediation does not view the conflict as a *pathological* social event, but as a *physiological* phenomenon, and that mutual expectations may lead to an irredeemable conflict or, alternatively, to the establishment of communication between the persons involved. The role of a fair and impartial third party is nevertheless decisive, and it is this role that I would like, in conclusion, to draw attention to. Said succinctly, the task of the third party is to establish dialogic methods of conflict management. The power of the third mediator, however, is inseparable from the force of the law and its awareness of the legal framework within which it operates; its impartiality and independence from the parties are themselves indistinguishable from the exchange of arguments. If transferred from the level of violence to that of language and discourse [11], the conflict consists in a dispute between different theses and several different subjective positions regarding an issue. What the culture of mediation reminds us is that in such a dispute the possibility of dialogue, communication and respect for the dignity of “enemy” must be guaranteed. In this sense some procedural conditions—although reduced—must be ensured, if the mediation is to be an acceptable method for the parties. And among these there must be the awareness that mediation is a form of protection of rights through the achievement of an equitable agreement. If the proceedings were to be biased in favor of one party, or completely unregulated, reaching a fair agreement between them would be impossible. The informality of the proceedings cannot mean inequality and arbitrariness.

To conclude, although certainly without underestimating the ever increasing diffusion and innovative capacity, I believe that reparative justice and mediation lack the capacity to become a universal paradigm of conflict resolution: not everything is capable of being mediated or repaired, while mediation and reparation cannot presuppose juridical precepts and sanctions; nevertheless, between reparative and retributive justice, between ADRs and ordinary justice, there can and should be not opposition but interaction, interaction that in the law must be given between coercion and dialogical opposition.

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Part II
Innovation, Unitariness and Evidence. Bio-Medicolegal and Juridical Sciences

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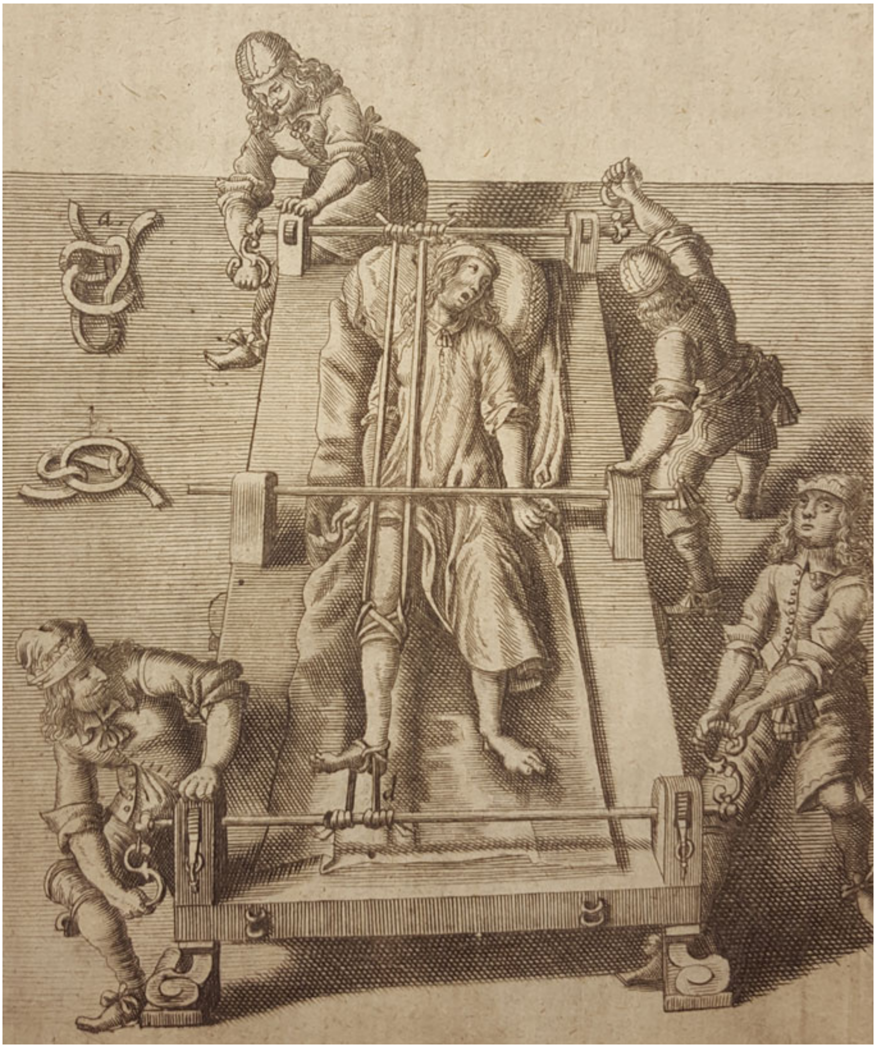

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Chapter 5
Bio-Medicolegal Disciplines and Scientific Evidence

Santo Davide Ferrara

Abstract The chapter focuses on the systematization process that is transversely involving the bio-medicolegal sciences for the ante- and post-mortem ascertainment on living and dead persons, and traces a synthetic historical profile and the state of the art of some of the disciplines most directly involved in modern forensic ascertainment, from genetics to toxicology, radiology, anthropology, entomology, to medical law and ethics. The evolution in the evaluation of the causal value and causal link, to the present "criterion of scientific probability" and "counterfactual reasoning", the progressive *fragmentation* of bio-medicolegal knowledge, due to the proliferation of ultra-specialised sub-disciplines and branches, the *loss of unitariness*, the importance of methodological and criteriological rigor and the achievement of quality and accuracy in order to increase the value of scientific evidence, are specifically traced.

5.1 Introduction

Through the observation, documentation, assessment, and scientific interpretation of findings deriving from clinical and post mortem ascertainment, the bio-medicolegal sciences apply bio-medical knowledge and methodology to the resolution of legal questions and problems concerning both individuals and society, providing evidence to criminal, civil and administrative law.

The chapter focuses on the systematization process that is transversely involving the bio-medicolegal sciences for the ante- and post-mortem ascertainment on living and dead persons.

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5.2 Ante-mortem Ascertainment

The systematization process of bio-medicolegal sciences is clearly evident in the development of *clinical forensic medicine*, in the fields of criminal, civil, administrative and insurance law, related to acts of violence and personal injuries resulting from malicious acts, negligence, accident or from medical malpractice, and related to the assessment of damages.

5.2.1 Clinical Forensic Medicine in Criminal Law

Overlapping with many other specialised areas of medicine, clinical-legal and forensic medicine includes the examination and report on victims of physical or sexual assault, road traffic and industrial accidents, elder, spousal and child abuse, neglect and starvation, torture, self-infliction, criminal abortion or poisoning, and alcohol and/or drug intoxication.

Between the end of the 20th and the beginning of the 21st centuries several guidelines and documents have been published in the fields of forensic medical examination of living persons [1] and in particular in the examination of victims of different kinds of violence, such as, for example, those on torture and other cruel treatment [2–4], child abuse [5] and sexual violence [6]. Nowadays, the forensic medical examination of victims of violence, aimed at collecting evidence to be used in court in criminal cases, is a routine activity in numerous countries. According to R. Dettmeyer "*the examination and expert appraisal of victims of violence takes place against a backdrop of both national and international legal requirements, foremost the Universal Declaration of Human Rights (UN, December 10, 1948)*".

The ascertainment requires a prompt physical examination that has to meet minimum standards, such as, for example, the description and photo-documentation of each injury, taking into consideration that some standards may be different based on the subjects and on what kinds of violence are suspected of having been perpetrated (child abuse, sexual violence, etc.). It is extremely important that the physical examination is conducted according to recognized medical standards, developed with the aim of obtaining evidence to use in criminal proceedings. Nevertheless, quality requirements for the examination and documentation of injuries in victims of violence are often not included in medical education and training. The examination of these people necessarily includes the ascertainment, documentation, and interpretation of injuries within the framework of orderly criminal proceedings aimed at punishing perpetrators, and in this context, the independence of the expert is of particular importance, increasingly more so in cases of violence perpetrated in police or other official custody. In order to be allowed to perform an objective and neutral appraisal without fear of repression, the medical expert in criminal proceedings must not be in a condition susceptible to corruption.

Despite the over-mentioned standardization process, the implementation of guidelines for the assessment of victims of violence has not been sufficient up until now, considering the importance of the issue, and there is still much progress to be made.

5.2.2 Medical Malpractice

Medical malpractice lawsuits were very uncommon prior to the 1950s, while in the second half of the 20th century the epidemic of denunciations and litigation for cases of presumed "malpractice or bad healthcare" has increased in a dramatic manner worldwide, particularly in Europe.

In cases of suspected medical malpractice, the ascertainment of injury and evaluation of treatment are complex and often difficult issues. Moreover, the clinical point of view may differ from medico-legal opinion, medical environments vary from state to state, and medical malpractice legislation is usually handled and regulated at national level. Despite these criticisms, the methods of ascertainment and criteria of evaluation of a medical malpractice case should not differ substantially in the developed countries and should be based on internationally shared scientific principles. Legal Medicine and the international medicolegal community have commenced the praiseworthy and difficult endeavour concerning the handling of Medical Malpractice. Indeed, in order to provide effective guidance, the International Academy of Legal Medicine developed and published the first international guidelines [7] and a monograph depicting the European state of art on medical malpractice and liability [8]. These documents, in spite of their importance, are only the first step in the complex harmonization process of the legislative–juridical, operational and institutional practices of medical liability cases, but they do provide a universal tool for the medicolegal community.

5.2.3 Personal Injury and Damage Assessment Under Civil/Tort Law

The other broad field of development in the medico-legal ascertainment of the living is personal injury and damage assessment under civil/tort law.

The medical expert's ascertainment and evaluation of the claimant's permanent impairment and/or disability is essential to the determination of compensation and its quantum in many jurisdictions, including, for example, workers' compensation, motor vehicle accidents and medical malpractice.

With the aim of avoiding inequality in damage compensation and in order to provide guidance for the harmonization of the damage evaluation, many national and international "baremes" have been developed. Currently, the methods proposed in the "AMA Guides to the Evaluation of Permanent Impairment" are the most widely used for impairment rating. The said work is the result of the revision and expansion of the first edition, dating back to 1971, which was developed from a series of articles published between 1958 and 1970 [9]. It provides a methodology for the evaluation of permanent impairment involving the various body systems (i.e. limbs, spine, pelvis, mental and behavioural disorders, pain-related impairment, etc.).

A preliminary effort to share terminology and vocabulary has been made so as to unify the methods of ascertainment. In particular, the usage and meaning of terms such as "impairment", "disability" and "handicap" were clearly defined by the International Classification of Impairments, Disabilities, and Handicaps (ICIDH), published by the World Health Organization (WHO) in 1980 [10]. Unfortunately, the Guides and other similar instruments developed in other countries, often conflate the concepts of impairment, disability and handicap. For these and other reasons, the international IALM Working Group on Personal Injury and Damage was recently created, aimed at developing guidelines on the assessment of physical and psychical damage, providing recommendations on methods of ascertainment and criteria of evaluation under civil/tort law [11, 12], whiplash associated disorders [13] and psychic and existential damage [14]. In fact, in order to guarantee objectivity, reproducibility and rigour in the collection of scientific evidence, to be used as credible evidence at civil court and accepted as scientific proof, the methodology to be used to verify the reality and degree of the injury/damage must be based on scientific accuracy. Personal injury ascertainment and evaluation remain very complex issues from clinical, medico-legal and juridical points of view and the legislative frameworks remain heterogeneous. However, as in the field of medical malpractice, these international consensus documents, focusing on the ascertainment of both physical and psychical injuries and damage, standardize the very early stages of the compensation procedure.

5.2.4 Malingering

In the assessment of the living, whatever the aim, the problem that the medico-legal examiner has to face is the patient's "fabrication" of symptoms of mental or physical disorders for a variety of "secondary gain" motives, commonly denoted with the term *malingering*.

Although a number of strategies and tools are available, the detection of malingering remains one of the most significant challenges for medico-legal examiners. Subjective impressions, in fact, still have a negative influence on the conclusion of many cases, leading to a high misclassification rate. In this perspective, techniques based on reaction-times (RTs) recently introduced (for example, the *RT-based version of the Concealed Information Test* or the *autobiographical Implicit Association Test*) detect longer latencies and the higher rate of error associated with the additional cognitive processes involved in lying, based on the assumption that a more cognitively demanding task would be more time-consuming. However, the main limit of these techniques is that they only study the latency in the response, so the liar can falsify the evidence by checking this unique parameter. Therefore, the study of new methods for malingering detection must be continued and deepened.

5.3 Pathology and Post-mortem Ascertainment

Forensic pathology, which still remains at the centre of the post-mortem ascertainment, is often considered, among all of the bio-medicolegal and forensic sciences, as the one most subject to interpretation by the forensic examiner, because of the lack of pathognomonic findings and the necessity, in each case, for an integrated evaluation of all data. However, thanks to the standardization of procedures and the progressive development of the microscopic examination (i.e. techniques of microscopy, new staining, new markers in immunohistochemistry, etc.) and the advantages of other disciplines (i.e. toxicology, radiology, genetics, etc.), the value of the scientific evidence provided by the pathologist has undoubtedly increased in recent years.

At the end of the 20th century, in light of the necessity to implement uniform methods of investigation shared by the bio-medicolegal scientific community for improved forensic autopsies [15], several books, monographs, articles and documents were published [16–19]. In February, 1999, the Recommendation R(99)3 of the Council of Europe concerning the Harmonization of Medico Legal Autopsy Rules was published [20]. Subsequently, in 2015, the European Council of Legal Medicine, in accordance with the EU Recommendation, published an accreditation/certification procedure restricted to post-mortem investigations for forensic pathology services in Europe [21]. Moreover, specific procedures were described for peculiar types of death, with a description of alternative methods of dissection, types of autopsy samples and types of complementary analysis and examinations to be carried out (histology, toxicology, genetics, microbiology, entomology [22]). The 21st century has so far been witness to the publication of further guidelines or best practices [23], elaborated by Working Groups and Consensus Conferences under the patronage of International Scientific Societies (i.e. IALM, NAME, AAFS, IAFS, nMAFS, ECLM, AFIP, AECVP, ABP, RCP), on the subject of methods of ascertainment, diagnosis and the classification of natural deaths (i.e. sudden cardiac death [24], sudden infant death syndrome [25], pulmonary thromboembolism [26], sepsis [27] stillbirths [28–30]), violent deaths (i.e. drowning [31], anaphylaxis [32], firearm deaths, suicide cases [33], infant victims of abuse [34, 35], death in prison [36]) or drug related deaths (i.e. cocaine [37], heroine [38], ecstasy [39], doping [40, 41]).

Moreover, a uniform method of investigation has been proposed for forensic bio-archaeology monitors of mass grave exhumations [42] or in cases of mass fatality incidents (i.e. environmental, medical, vehicle, industrial or terrorist) [43–45] for personal victim identification and/or collection of evidence for prosecution.

Microscopic and histological examinations have also experienced a great evolution from the second half of the 20th century, when the first publications specifically concerning forensic histopathology appeared. In 1977 Janssen published the first book of forensic histology [46, 47] and other texts were published by Cecchi and Marchetti (1999, updated version in 2009) [48], Lau (2008), Dettmeyer (2011) [49], Cummings (2011), Jose Blanco Pampin (2012) and Murty (2014). Currently, histopathological ascertainment is essential in order to identify and date natural disease patterns (i.e. ischemia, thrombosis, infection, inflammation) [50], those resulting from violence (haemorrhage, asphyxia, fractures), as well as those related to acute or chronic exposure to xenobiotics (drugs of abuse, doping [51], drugs [52]), with the final aim of reconstructing accidental [53–55] or criminal [56] dynamics, even in cases of medical malpractice [57, 58]. Furthermore, the evolution of microscopy has opened up new fields of research, as evidenced by the examples listed below.

– *Scanner electron microscopy* (SEM) permitted the analysis of cadaveric tissues (the heart and lung), and the detection of the shape, dimension, and composition of metal particles on wounds (i.e. gunshot residue or electric and thermal injuries).
– *Transmission electron microscopy* (TEM) was able to demonstrate the intensity of hemorrhages and of edema in asphyxia deaths [59], as well as silicon in lung silicon embolism.
– *Laser scanning confocal microscopy*, permitting the simultaneous observation of more fluorochromes, the change of fluorescence in a time interval of live cells and the multidimensional analysis (2D, 3D and 4D) of the images, has been applied, for example, in assessing the cell's activation stage, and for the detection of metals in tissues [60].
– *Environmental scanning electron microscopy*, an upgrade from the standard SEM, has permitted the analysis of materials or tissues without specimen preparation [61] in order to characterize, for example, saw or stab marks on bones [62, 63] or detect diatoms in cases of drowning [64], and can be used in forensic cases of intoxications [65] or gunshot-related deaths for the estimation of firing distance [66, 67].
– In situ *hybridization* (ISH) is used in the search for viral DNA, in particular for the study of the cases of diagnosis of SIDS [68], and in the identification of gender [69].
– *Immunohistochemistry* (IHC) [70] is mainly focused on the analysis of the vitality and chronology of skin lesions, the chronology of cerebral hypoxia and myocardial ischemia, as well as the diagnosis and duration of asphyxiation [71–73]. Recently, *double-color immunofluorescence* analysis combined with *confocal microscopy* is used for the identification of the type of cells and of the specific proteins expressed, thus providing information on the stage of cellular

activation helpful for assessing the chronology of lesions and the cause of death (i.e. asphyxia death) [74, 75].

Useful markers for the determination of wound age are closely related with the process of wound healing, such as collagen, fibronectin, adhesion molecules, etc. [76, 77], as well as numerous others delineated in Chap. 39 of the present monograph.

5.4 Bio-Medicolegal Sciences

The synthetic historical profiles and the state of the art of some of the sciences most directly involved in modern ante- and post-mortem ascertainment, from genetics to toxicology, radiology, anthropology, entomology, up to medical law and ethics, are outlined below.

5.4.1 Genetics and Genomics

The evolution of forensic genetics has been driven by the analysis of human genetic variation, which began over a century ago with Karl Landsteiner's discovery of the human ABO blood group variants (termed polymorphisms).

After its initial description in 1985 [78], forensic DNA analysis revolutionized forensic science. The full potential of DNA analysis became evident in the very first case in which it was used when, in 1986, Sir Alec Jeffreys applied the novel technology to identify a man who had raped and killed two girls [79], but three major milestones of technological development led to a wide acceptance of DNA analysis in forensic science: polymerase chain reaction (PCR) [80] was introduced into forensic science, along with the use of short tandem repeat (STR) loci and a fluorescent dye detection technology [81]. A further important development was the move from gel electrophoresis to capillary electrophoresis. By the start of the 2000s, STR typing using laser-induced fluorescence detection of dye-labelled PCR products, which are subsequently visualized and analyzed by capillary electrophoresis (CE) size-based separation, had evolved as the method of choice in forensic laboratories around the world [82].

Along with the technical development of STR typing strategies, nomenclature and interpretation guidelines were developed [83, 84]. Soon, the biostatistics interpretation of DNA profiles became a major focus of research, introducing the Bayes theorem and likelihood ratio approaches into forensic science [85]. After the first government national database (NDNAD), set up in England and Wales in April 1995, over the past two decades NDNADs have grown constantly and now form an important tool for police investigations. After the ratification of the Prüm Convention, a novel set of markers were introduced, called the European Standard

Set (ESS) of loci [86], and as a result of this new multiplex reactions are now available from all major suppliers. In the same period there was also a great amount of research into additional markers, including the analysis of mitochondrial DNA [87] and Y-chromosomal polymorphisms [88], with the introduction of the Y-chromosomal haplotype reference database (YHRD) in 2002 [89] and the EMPOP database in 2007 [90]. Further increase in the sensitivity of DNA detection was achieved by the introduction of miniSTR markers [91] and single nucleotide polymorphisms (SNPs) [92].

The standardization process of forensic genetics addresses both technical and procedural standards. Technical standards encompass the genetic systems to be used (including type, nomenclature and methodology), the statistical methods for evaluating the evidence and the communication of the final report. Procedural standards include matters of operation, such as laboratory accreditation and performance, accreditation and licensing of personnel, record keeping and proficiency testing. Molecular genetic analyses now play an increasingly important role in the medico-legal context. Paternity testing cases, criminal casework (i.e. biological stains, contact samples, hairs), identification of human remains, criminal DNA databases, and a variety of applications of non-human DNA typing are the kinds of expertise usually requested by the judges regarding forensic experts. The identification of unknown human remains by means of DNA analysis is required regularly, mainly in cases where other identification strategies have failed or are unavailable.

In the last few years forensic genetics has also been applied in other fields of legal medicine, namely in forensic pathology, through the genetic diagnosis of sudden cardiac death and also toxicogenomics. Genetics is perhaps the forensic science that provides the most accurate scientific data, and therefore the highest level of evidence, but uncertainty always exists and must be measured with the standard of probability. Likelihood ratios are nowadays used for weighing the value of the evidence and for communicating this value to the courtroom. The Bayesian approach to inference provides a coherent framework for interpretation.

5.4.2 Toxicology

Forensic toxicology developed as an autonomous branch of sciences from the first half of the 20th century. The second half of the 20th century was dominated by rapid technological evolution; with the advent of gas chromatography (GC) and dedicated detectors (i.e. flame ionization detector—FID or nitrogen-phosphorous detector—NPD), hyphenated techniques involving a separation step before detection (i.e. GC-FID or GC-NPD), became the golden standard for forensic toxicology. In parallel, clean-up techniques were needed to treat post-mortem samples, and the traditional liquid/liquid extraction (LLE) or solid liquid extraction (SLE) were accompanied by solid phase extraction (SPE) with a variety of phase modifiers. With the advent of modern, benchtop GC coupled to mass spectrometry (MS), GC-MS became the gold standard for analytical forensic toxicology, due to its unparalleled specificity for qualitative analysis, offering, at the same time, the possibility of general unknown screening by the use of mass spectra library.

In the 1960s and 1970s hair had been used for evaluating exposure to toxic heavy metals, and, ten years later radioimmunoassays were developed and validated for drug analysis in keratinized matrices, rapidly followed by GC-MS methods [93]. Atomic absorption for metals was subsequently substituted by inductively coupled (ICP) mass spectrometry (MS), applicable to virtually all elements. However, large, polar or thermolabile compounds of forensic interest still required the use of liquid chromatography, with UV or diode array or refractive index detection. Liquid chromatography coupled to mass spectrometry (LC-MS) entered forensic toxicology at the end of the century, in some cases rapidly substituting GC-MS for a large variety of compounds. The newest generation of high resolution mass spectrometry (HRMS), with user-friendly instrumentation and software, became the most powerful tool for a general unknown screening. Currently, high resolution-mass spectrometry (HRMS) with time-of-flight mass spectrometry (TOF-MS) or the Orbitrap are used for a comprehensive general unknown screening. However, an analytical strategy must be applied in order to cover the widest range of substances [94]. The first step is generally quantification of ethanol and other volatiles using head-space (HS) GC coupled to a FID or to MS. Immunoassays or enzymatic assays are generally available for urine screening, but they can be adapted to other fluids. On blood, organs/tissues and keratine matrices a systematic toxicological analysis (STA) should include extraction, a GC-MS screening for acidic, neutral and basic compounds (both derivatized and non-derivatized), and a LC-MS screening for common drugs of abuse and psychoactive substances; specific class screening (e.g. anticholinergic drugs, pesticides, steroids, beta-blockers) may be indicated by the circumstances [95]. GC-MS in single ion monitoring, GC-MS^n, LC-MS^n or LC-HRMS^n are the techniques that can be applied for the qualitative and quantitative determination of targeted compounds in the confirmation analysis. Only when a validated MS method is not yet available (e.g. with proteins of forensic interest such as CDT or erythropoietin) can other detection techniques be used. A separation method prior to detection is always necessary.

Although it is difficult to reach the levels of evidence provided by genetics, forensic toxicology is certainly one of the disciplines that reach the highest level of probability in its results. The elaboration and implementation of guidelines and operative protocols, which can be considered as an indirect expression of evidence-based medicine in bio-medicolegal sciences, provide support for this assertion.

5.4.3 Radiology and Imaging

A distinction between applications in the living and in the post-mortem can be made in each forensic sub-discipline, but it is in forensic radiology that this partition reaches its highest level, establishing two completely different areas.

The main radiological techniques, both in the living and in the dead, are currently applied according to the availability, costs and purpose, taking into consideration the radiological exposure for living persons.

5.4.3.1 Ante-mortem Ascertainment

In the forensic ascertainment of living persons, radiological imaging techniques without biological hazard are preferred with respect, for example, to those that imply radiation exposure. Therefore, information offered by X-rays and CT mainly depend on the availability of data previously obtained following clinical indications, while techniques using ultrasound or magnetic force, such as MRI, may find wider application.

Radiology in living persons, besides its important role in the identification and objectification of lesions, is widely used for dating injuries and for age assessment. In the second half of the past century, the need to study precise and accurate methods of *age estimation* has been one of the main problems, with increasing interest in western countries, due to the different systems of sanctions and guarantees in existence, according to the age of the examined subject in the various legal systems. Furthermore, there are an increasing number of individuals without identity documents, who have immigrated illegally, or those with suspected irregular birth certificates. In age estimation of the living the same problem arises as in the other bio-medicolegal sciences, namely the request made by the legal system for definite and certain answers, difficult to satisfy in this field of bio-medicolegal science, which only provides probabilities.

As demonstrated in several applications, the accuracy of a method or a formula for calculating the probability that an individual is an adult changes according to the population studied, and many ethnicities and groups have never been studied, so the problem is far from being solved. Furthermore, the multifactorial approach has further contributed to the appearance of new problems that require a correct statistical approach in order to be effective. Furthermore, the development of new techniques has also created new ethical issues.

Another area of primary interest in clinical forensic medicine is *age determination of hard and soft tissue injuries*. Different kinds of injuries, in fact, have to be assessed in order to understand whether they have been inflicted by a third party and, if so, in which manner. In cases of inflicted injury, the determination of the time of occurrence plays an important role as it can lead to the inclusion or exclusion of possible offenders as well as to the differentiation of multiple events, with obvious repercussions in cases of maltreatment of vulnerable persons, torture and abuse. It is also useful in the reconstruction of violent events, particularly in including or excluding potential suspects, and may be of importance in cases of insurance litigation. Fracture dating is based on radiographic methods that determine callus status and thereby rely on the expert's experience. As a novel approach, a study investigated the applicability of magnetic resonance imaging (MRI) for bone fracture dating, concluding that quantitative assessment of T1 and T2 behavior over time in the fractured region enables an objective age determination of a fracture [96]. The duration of the healing process of internal or externally visible subcutaneous hemorrhages and/or hematomas was also studied with different radiological techniques, in order to estimate the age of the injuries.

Reflectance spectroscopy was investigated, resulting in a model capable of predicting the age of a hematoma with an accuracy of approximately 1 day [97]. Magnetic resonance imaging (MRI) has proven its use in dating intracranial hemorrhage [98] and, more recently, in the estimation of a soft tissue hematoma's age [99]. In spite of these results, the current standard regarding age estimation of hematomas is an external inspection and visual evaluation of the color of the bruise, even if prior studies have shown that the external aspect of subcutaneous bleedings is not reliable [100].

5.4.3.2 Post-mortem Ascertainment

Post-mortem Forensic Radiology has gained increasing importance in the examination of the cadaver, and today, due to the rapid development of radiological techniques, modern cross-sectional imaging methods are used. Many techniques have been investigated in detail during the last decade and research projects have emerged in different countries, of which the most famous is the Virtopsy project, founded in 2000 [101, 102].

In general, the different techniques can be divided into basic methods, which are non-invasive imaging techniques that can be used alone (conventional X-rays, CT, MRI), and complementary methods, minimally-invasive and combined with basic methods (imaging-guided sampling and PMA). Ultrasound has also been applied post-mortem, but mostly in combination with other techniques [103], as outlined below.

– *Conventional x-ray* is the oldest technique of forensic imaging and it has almost been eclipsed by the CT in modern imaging.
– *Post-mortem computed tomography* (PMCT), at the present time, is the most widespread technique in post-mortem forensic imaging. The high special resolution, rapid examination times, easy handling of the CT-unit and the possibility to detect any foreign material, such as projectiles and surgical material, make this method an excellent screening tool and adjunct to the conventional autopsy. The technique is also applicable to the investigation of victims of mass catastrophes, since it allows a rapid inspection of lesions and the detection of specific elements, such as medical implants, that may aid the identification of the victim [43]. Additionally, by investigating the virtual skeleton it is possible to estimate age and gender, providing other clues in relation to the identity of the victim [104]. Furthermore, 3D images present such information in a clear and accessible way to judges or other non-medical experts [105]. The main weakness of the method is the low contrast for organ visualization and thus a limited sensitivity for the detection of organ findings, which is why the technique is

suitable for examining cases of traumatic death, such as gunshot cases and polytrauma, but is only of relatively small help in investigating cases of natural death [106].

- *Post-mortem magnetic resonance* (PMMR), conversely, is useful for visualizing the soft tissue, especially organs. Although this technique has the potential to overcome the limitations of PCMT, it is only rarely used in forensic imaging as it is a complex technology requiring specific training, while the maintenance costs of MRI-units are high. The high soft-tissue resolution makes MRI a perfect tool for detecting natural causes of death [107, 108] and for examining traumatic soft-tissue injuries [109]. The absence of any radiation allows the examination of living patients and therefore renders it an important tool in clinical forensic imaging, especially for examining victims of survived strangulation [110].
- *Imaging-guided biopsies* or liquid aspiration permit the obtainment of different matrices, such as organ-tissue or body fluids from the inside of the body, in a minimally-invasive way [111]. They also permit the radiological exam (mostly PMCT) to be combined with histological, toxicological, or micro-biological investigations.
- *Post-mortem angiography* (PMA) is one of the most promising methods in Forensic Radiology. Depending on the method used, a selective angiograph of coronary arteries [112, 113] or a whole-body PMA can be performed [114, 115]. The injection of the contrast agent in combination with PMCT also leads to an increase of contrast in the soft tissue, allowing the detection of organ findings not visible in unenhanced PMCT. Today, the most widespread technique of MPMCTA is Multi-phase PMCT-angiography (MPMCTA) [116], a whole-body angiography that includes the performance of a native CT-scan and consecutive CT-acquisitions after contrast agent (Angiofil®) injection with a perfusion device (Virtangio®), in three phases (i.e. arterial phase, venous phase and dynamic phase of MPMCT). The diagnosis is done by comparing images of the different phases and the native CT [117].

Nowadays, the indication for post-mortem imaging strongly depends on the applied technique. For the detection of foreign bodies, PMCT or conventional X-ray techniques are the most suitable and permit rapid detection, even in putrefied, carbonized or otherwise highly damaged bodies. They then permit the guidance of the pathologist in sampling the foreign bodies, if necessary (e.g. projectiles), and the finding of indications, which may lead to the identification of a given individual (e.g. the detection of a prosthesis). PMCT is the method of choice for investigating cases of traumatic death, as the bone system may be investigated, which can provide a great deal of information concerning the biomechanical origins of fractures, thereby contributing to the forensic reconstruction of a case.

The easy and rapid creation of *3D-images* makes it especially effective in court and during meetings with non-medical professionals (e.g. prosecutors and police investigators). In cases of fatal hemorrhage, such as in sharp and gunshot trauma, and especially in cases of suspected medical error, PMCT should be extended by adding whole-body PMCTA, as this technique permits the discovery of the source of bleeding and the visualization of the trajectories of knives, needles and projectiles. In order to investigate the cause of a natural death, specifically in cases of sudden cardiac death, PMCT is not the method of choice, since visualization of the organ's parenchyma and the vessels lumen is not feasible. The methods of choice are PMCTA, which permits a detailed investigation of stenosis or other lesions of the coronary arteries, and PMMR, which shows the myocardium and the possible lesions within it. As each technique has its particular advantages and limitations, knowledge is essential so as to enable the correct choice of method for solving a medico-legal case.

As previously mentioned, the quality of evidence provided by the post-mortem ascertainment has been increased by the interdisciplinary work of the pathologist, with experts in other forensic sub-disciplines. In this context, forensic radiology is certainly one of the most promising and expanding specialties. An example of this increase in the value of evidence is given by MPMCTA. With this approach, in fact, the sensitivity of PMCT for detecting pathological findings in medico-legal cases can be increased from 65 to $\sim$81%, rendering the performance of the radiological exam similar to that of conventional autopsy [118]. This technique has been shown to have enormous advantages, even compared to conventional autopsy, such as in cases of sudden cardiac death, where it allows the detailed investigation of coronary arteries [119–121]. It is also used in cases of fatal hemorrhage where it permits the finding of even the smallest sources of bleeding [122], stabbing and gunshot trauma [116, 123] and especially in cases of suspected medical error [124]. It is proven to be particularly useful for investigating cases of in-hospital death and may therefore be an interesting tool for quality-control in hospitals and after medical intervention [125].

5.4.4 Anthropology

The first half of 20th century saw the emergence of the early pioneers of forensic anthropology, but it was not until the 1940s that forensic anthropology gained recognition as a legitimate discipline, growing into a separate field within the forensic sciences during the decades that followed.

Anthropology assumes a primary importance in those cases where there is no suspicion concerning identity, thanks to its ability to provide a biological profile. Furthermore, and not of secondary importance, the anthropologist provides those identify factors, which are offered by the uniqueness of bones.

Anthropology relating to humanitarian action has recently emerged, revealing a broader sense of the need for special techniques, methodologies and research in order to face the specific types of problems encountered in these applications.

Skeletal trauma remains one of the most challenging issues, for which the assessment of a traumatic mechanism and pattern has been significant, also in defining the weapon used to produce a lesion or date a bone callus.

The *age of bone fractures* and distinguishing between peri-mortem and post-mortem trauma are major issues, in relation to which taphonomic and environmental conditions, time elapsed since death, type of bone tissue, post-mortem changes, etc., must be evaluated taking into consideration that the term "perimortem lesion" means a lesion occurring shortly before or shortly after death and that any injury directly associated with the manner of death or the "immediate" handling of the remains is considered a perimortem injury. Besides the radiological methods, the tools used for analysis are still macroscopic and stereoscopic observation, decalcified and non-decalcified thin section microscopy, and scanning electron microscopy with edx. The search for traces of hemorrhaging, markers of inflammation and additional indicators, such as biological markers, could be useful.

For the *identification* of decomposed, burned, mutilated or otherwise unrecognizable remains, to the investigation and documentation of genocide and mass graves, the first objective is establishing a biological profile, using conventional osteological methods to estimate sex, height, age at death and ancestry of the body or of its remains, and, in some cases, a CT scan on virtual bones. If the biological profile suggests a match with a missing person, further methods, such as radiographic comparison, skull-photo superimposition, dental status, or forensic DNA analysis could confirm the decedent's personal identity. The difference between identification and recognition is crucial and the primary and secondary methods based on a comparison between ante and post mortem files include dactiloscopy, DNA and odontograms.

Dactiloscopy, especially in mass disasters, plays a major role in the identification of the victims, particularly in the initial stages of the process, as well as in the analysis of clothes and personal belongings. *DNA* matching is the best way to identify body parts. The constant advances in genetics have entailed the achievement of better results from degraded and fragmented evidence, but always with the premise that ante mortem data must be available for comparison. *Odontograms* are of paramount importance for identification in cases of suspicious identity, or in close mass disasters.

5.4.5 Entomology

Forensic Entomology plays a role in determining the post-mortem interval (PMI). Indeed, the forensic pathologist can estimate the amount of time that has passed since the death of the victim with several scientific parameters. However, a defect of such parameters is that they inevitably reach a point of stabilization in the first days. After a few days have passed, forensic entomology, by studying stages of decomposition, patterns of arthropod succession or by estimating the age of the immature stages of the arthropods present at the scene, is considered the most accurate method for the PMI estimation. The microfauna that colonises the body can, in fact, provide useful additional information besides that already obtained during the routine and customary forensic ascertainment. The estimation of an immature insect's age that has fed on a dead body permits the estimation of the minimum Post Mortem Interval (mPMI). Furthermore, entomotoxicology, which is the disciplinary field that deals with the qualitative detection of toxic and narcotic substances in scavenger insects found on or next to the corpse, also studies how these substances interfere with the normal growth of insects, providing more precision to the mPMI estimation. Thanks to the possibility of identifying substances on insects, this sub-discipline is also useful in those cases where it is not possible to perform toxicological analysis of blood, urine and tissues samples, such as in cases of skeletonization or advanced decomposition.

Despite the developments of recent years, this discipline requires further exploration, and researchers should take various factors into consideration and compile a global database. Indeed, it is important to maintain databases of insects of forensic importance from different geographical locations, with variation in all the factors that affect entomology, such as temperature, altitude, habitat, sun-shade, indoor–outdoor, and so forth.

5.4.6 Odontology

Forensic Odontology, also known as forensic dentistry, is the application of dental knowledge to criminal and civil cases in the interests of justice. Comprising the entire relationship between dentistry and law, it deals primarily with the proper handling and examination of dental evidence and the proper evaluation and presentation of dental findings [126]. The first time that a dentist was used as an expert witness dates back to 1814 [127]. A few years later he assumed the role of expert witness in US courts [128]. In the 20th century forensic odontology grew into a separated field, playing a major role in solving cold cases and providing strong evidence in court. Thanks to the extensive interactions with law enforcers, the judiciary and other forensic experts in various forums, such as hands-on workshops, conferences and continual education programs, odontology has gained greater importance among the forensic sciences, today playing a major role, particularly in the identification process.

The aims of forensic odontology are mainly related to the identification both of the dead, as regularly applied, and of the living, such as in age estimation, or through bite-mark analysis, that is, in the individuation of an assailant who has used his or her teeth as a weapon [129]. The role of forensic odontology in personal identification has been recognized almost worldwide, thanks to human teeth characteristics. Human dentition, besides having the advantage of longevity and resistance to high temperatures, is one of the unique characteristics of human morphology, and its uniqueness is enhanced by dental treatments. Its important role in the identification process is also demonstrated by the use and effectiveness of forensic odontology in mass disasters. To give an example, 80% of the more than 92% of non-Thai victims who were identified after the Tsunami of December 2004, in south East Asia, were identified using forensic odontology [130]. On the other hand, bite-mark analysis plays an important role in the criminal justice system for the identification of the assailant.

Under civil/tort law, conversely, forensic dentistry is aimed at the detection and the evaluation of injuries, disease or impairments to the jaws, oral tissues and teeth, and for further study of such topics it is necessary to consult the specialist material.

5.4.7 Medical Law and Ethics

Medical Law is the branch of law that focuses on the responsibilities of medical professionals and the rights of the patient, while Medical Ethics is concerned with the set of moral principles that apply values and judgments to the practice of medicine. While identifying their roots in ancient times, the issues addressed by these disciplines are always of current interest, mainly because of the unending progress of science, technology and biomedicine.

The development of codes of medical ethics began in the 19th century, but the rules of professional ethics are receiving greater attention both at the national and international level, thanks to their role in standardizing some aspects of medical conduct that may have crucial consequences for the patient's fundamental rights and for medical practice itself. Currently, these codes, which include sets of rules of professional behavior for physicians, are devised by authoritative medical associations recognized by physicians and usually by the State as well, although in different ways based on their legal stature. The growing influence of ethics in contemporary medicine can be seen, in fact, in the increasing use of Institutional Review Boards to evaluate experiments on human subjects, in the establishment of hospital ethics committees and in the expansion of the role of clinician ethicists. The study of the controversial ethical issues that arise from the new possibilities brought about by advances in the bio-medical sciences is the task of bioethics, which addresses all the ethical questions that emerge from the relationships between the life sciences, bio-medicine, biotechnology, politics, philosophy, and law. It is such a complex discipline that bioethicists often disagree among themselves over its precise limits. Nowadays, bioethics has managed to address many issues, such as, for example, surrogacy, boundaries of life, access to care, allocation of scarce healthcare resources, morality of medical treatments, and the right to refuse medical care, etc.

Further challenges concern the development and application of biotechnologies that will affect future evolution. In particular, genomics and other 'omics'-research and the development of new technologies hold great promise in relation to potential new discoveries in healthcare, but they are constantly accompanied by the rise of new ethical issues. Innumerable instances can be cited, such as the treatment of patients in a locked-in state, stopping venoarterial extracorporeal membrane oxygenation (VA-ECMO) [131], etc. An emblematic example is that of single nucleotide polymorphisms, which can calculate the individual's susceptibility to particular diseases and the varied responses to pharmacological interventions, and are one of the best illustrations of personalized medicine (as well as an exciting new therapeutic science in the genomic era). However, it is not easy to interpret all the information provided by this method in a clinically meaningful manner and within the ambit of ethical and legal considerations.

5.5 Causality

Causality is central to every legal case, despite the variety of its philosophical and legal conceptions, and the theme of causation has long been a subject of debate. It is unanimously accepted that the mere statistical correlation between antecedent and subsequent is not enough to provide a causal explanation or identify a causal link between a process and another process or a state, which is the effect. A great effort has been made to come to an agreement on what the "quid pluris" is that substantiates the existence of a causal link. The most famous modern attempt to standardize causality dates back to 1948, with the so-called deductive-nomological model of Gustav Hempel and Paul Oppenheim, which reconnected the explanation of an event to subsumption under universal laws, with a deductive structure hinged on the accurate prediction or postdiction of the phenomenon to be explained. Due to the existence of a few universal laws, Hempel later admitted the possibility of using statistical laws, on the sole condition that they were carriers of high probability, thereby introducing an inductive-statistical model. Despite criticism of these models, statistics continues to play a vital role in the scientific explanation of the causal value, although not conclusive. Currently, causal value and causal link are evaluated by means of a "criterion of scientific probability", such as universal law, statistical law or criterion of rational credibility: universal laws, by means of deduction; statistical laws, by means of inference; in the absence of such laws, according to a criterion of rational credibility. To identify the degree of probability of causal value in both the criminal and tort contexts, a further check of the causal link is made by applying counterfactual reasoning and any additional criteria; providing a conclusion in terms of near-certainty, probability or the exclusion of the causal value–causal link. Whenever possible the percentage of probability should be estimated.

Further reading on the state of the art of the issue of "causality" can be found in the extensive works edited by the present writer [8–10].

5.6 Unitariness

In the last decades, bio-medicolegal knowledge has seen a progressive *fragmentation*, due to the proliferation of ultra-specialised bio-medicolegal sub-disciplines and branches, such as *forensic* pathology, genetics, toxicology, psychopathology, criminology and anthropology. Some branches have reached such a high level of ultra-specialization that they have become autonomous with respect to their original discipline (i.e. Forensic Psychiatry, Criminology, Genetic, Toxicology, etc.) and this fragmentation is also witnessed by the increase in bio-medicolegal publications outside specifically forensic journals [132]. The process of disciplinary fragmentation, although it has resulted in a greater identity of each of the sub-disciplines, has negatively affected and continues to affect bio-medicolegal knowledge and has been responsible for the *loss of unitariness* and the subsequent impoverishment of unitarian knowledge achieved by the bio-medicolegal sciences [133, 134]. It is equally foreseeable that the original unitariness is more likely to be restored by means of interdisciplinary research and trans-disciplinary innovations, in a dynamic analytic process of Hegelian and Vichian evolution of history, in general, and of science in particular, as will be reiterated in Chap. 39 of this monograph.

5.7 Evidence

Achieving quality and accuracy is fundamental in order to increase the value of scientific evidence and, therefore, reaching the final goal of legal medicine, which is "to discover the truth and ensure justice".

The law has always sought so-called incontrovertible truths, and science has been one of the main interlocutors in trying to achieve this objective. However, the expectations generated by the world of science have been disappointed, as shown by the decisions of the highest courts of many national judicial systems, following the influential decision of the US Supreme Court in Daubert vs. Merrel Dow Pharmaceuticals (509 U.S. 579, 1993). In light of the assumptions derived from this sentence, the valorisation of scientific evidence provided by the expert as evidence in the context of the trial has been revised. The "general acceptance" of the published literature subjected to peer-review and the expert's reputation are fundamental but not exhaustive repositories of evidence in court, particularly in cases where there is an open debate within the scientific community. The justified criticism of the courts can be dispelled only after the scientific community attains a renewed awareness concerning its limits and consequently rejects the notion of Promethean infallibility. On the same footing in the present era as the observance of methodological and criteriological rigor, essential for any scientific endeavour, is the almost unanimous consensus concerning the allocation of highest evidence to the "experimental sciences" based on the "laboratory bench" and the reference of lesser evidence, gradually decreasing down to the scientific disciplines based on clinical findings, of a subjective and prevailingly interpretive imprint.

The approach of the following chapters will be demonstrative of how and how much "radiomic" integration, including omics and imaging, will entail a significant acceleration of the ongoing process to improve the level of quality and quantity of evidence in the higher perspective of "personalized justice".

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Chapter 6
From Scientific Evidence to Juridical Proof

Gabrio Forti

Abstract The valorization of scientific evidence provided by the expert as evidence in the trial framework has been revised, as well as the role of the judge, in light of the assumptions derived from the Daubert Judgment. The criterion of "general acceptance" of evidence obtained from the published literature in journals subjected to peer-review and the expert's reputation can be helpful, particularly in cases where there is an open debate within the scientific community. It is the judge, however, who must take on the role of gatekeeper of the validity of evidence, to consent to its probatory admissibility, through a preliminary comprehension of the methods of science. The evaluation of reliability and admissibility of evidence is also reflected in the areas of medical malpractice and professional liability. Standards of care may be derived from an analysis not only of the guidelines, but also of other scientific sources to be subjected to the usual proof of admissibility, as well as new research paths that take account of personalized medicine and the application of guidelines to the individual.

6.1 Introduction

The use of scientific evidence as scientific proof in trial is a long-standing and delicate issue. The recourse to expert testimony has increased dramatically in recent years, but scientific uncertainty as well. Lawyers and Judges turn to science on a variety of legal subjects: from intention and causation to insanity and standards of care. However, technical and scientific topics in modern complex societies are quite

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difficult to govern, as shown in the debate on the evaluation of risk [1] or recently in the discussion on the use of the Bayesian statistical methods in courts in weighing evidence [2].

Over the years, the Italian Supreme Court, like many other courts worldwide, has assigned judges the task of "gatekeepers", assessing the scientific validity of the proffered scientific evidence. In the leading *Cozzini* case,[1] a judgment basically sticking to the holding of *Daubert*[2] (further developed in *Kumho*),[3] it required them to establish relevance and reliability of scientific theories for use in trial.

However, as recently stated, «*Daubert* remains an enigma, with courts and commentators continuing to disagree over what it means for a trial court to guard the gate» [3]. The decision has «left many procedural and substantive questions unanswered» [4], while the great challenge for lawyers and judges remains, «in this age of science», to «build legal foundations that are sound in science as well as in law» [5].

It is «both unrealistic and highly unlikely that judges will ever have the expertise to be able to evaluate the wide range of scientific subjects that come to court» [3]. The judge is at risk of suffering a condition of «cognitive inferiority» towards the expert he has summoned in trials [6]. After twenty-plus years of experience following *Daubert*, we do not have much confidence «that judges have risen to the challenge» and thus we can certainly agree that «something more is needed», including a way that makes allowance for the fact that judges *do not need* such a deep scientific expertise [3].

Such "more" does not seem simply to lie in an opening to mechanical or quantitative criteria like an overdue confidence in peer reviewing. As pointedly stated with regard to "trials by mathematics", while there has been a rapidly growing interest in the conjunction of mathematics and the trial process, «surely the time has come for someone to suggest that the union would be more dangerous than fruitful» [7].[4] If "good grounds" are needed in order to admit scientific insights in trials, such grounds must be found or devised within the scope of legal aims.

Perhaps some or even most of the problems arising in the relationship between law and science could be better framed and met, keeping in mind that science and law are divided «fundamentally in their objectives», owing to the need to take into account this difference: «the objective of the law being justice, that of science truth [9]. If justice certainly «seeks truth» and «it requires that clear decisions be made in a reasonable and limited period of time», this latter objective stems from the need for law to provide human relationships with a reasonable certainty and thus to give to all citizens the ability to rely on a kind of regularity in the behavior of other

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[1]Cass. pen., sez. IV, 13 dicembre 2010,. n. 43786, *Dir.pen.proc.* 2011, 1341, ann. by P. Tonini.

[2]*Daubert v Merrell Dow Pharmaceuticals*, 509 US 579 (1993).

[3]See *Kumho Tire Co v Carmichael*, 526 US 137, 119 S. (1999).

[4]See also Tillers 2011 [8], who recommends that an agreement be reached between mathematicians, logic and legal professionals upon the purposes that any given mathematical or formal analysis of inconclusive argument about uncertain factual hypotheses can serve.

people. The great challenge lies in the achievement of a good balance of these two objectives, provided that «despite all these differences», both science and the law «seek, in structured debate and using empirical evidence, to arrive at rational conclusions that transcend the prejudices and self-interest of individuals» [9]. As aptly stated, «a court proceeding, such as a trial, is not simply a search for dispassionate truth. The law must be fair. In our country, it must always seek to protect basic human liberties» [5] and we cannot forget that the history of science is «a graveyard of mistakes» [10].

More precisely, we could say that law and especially criminal law must stick to science in order to comply with the principle of legality and fundamental human rights, that require that law be clear, ascertainable and non-retrospective, and that judges decide disputes by applying legal rules that have been declared beforehand. This means, among other things, that a kind of procedural way must be devised, in order to achieve, if not absolute scientific soundness, at least a level of reliability of the summoned expertise, which corresponds to the need to find a solution to the case within a reasonable time span and for judges to perform decently their gatekeeping function, complying with the need that criminal liability requirements be proved beyond any reasonable doubt [11, 12].

On the other hand, in the Italian judicature, the *Franzese*[5] decision, while quite groundbreaking for its discussion of subtle epistemological issues in criminal trials, also highlighted the distinction, in the realm of causation, between frequentist and logic probability, to the effect that, as aptly stated, the law has imposed its own vision on scientific causality [6]. If the *Franzese* solution about causation assessment in the criminal trial seems debatable, it does, however, show the necessity for law to hold its ground somewhat in front of science, selecting data and scientific standards according to normative criteria tuned to the aims and values at stake in the relevant field of legal experience, being well aware of the watershed dividing the requirements for criminal and civil liability, respectively.

Thus the *Daubert* opinion is one-dimensional in that it relies on only one principle, validity, to set forth the standard for admitting scientific evidence, namely, in evidentiary terms, trustworthiness or accuracy. It seems to have neglected one second major foundational principle that joins trustworthiness (according to the provisions of the U.S. Federal Rules of Evidence): necessity. Actually admissibility of evidence «requires judges to take into account the state of the science in light of the nature of the legal issues involved». Scientific statements contain error rates that qualify their value, but there are different costs associated with making mistakes in different legal contexts [13], thus demanding greater accuracy in matters where the judicial decision may deeply impinge upon the rights of the defendant, being foremost the divide between criminal and civil proceedings, as well as, within criminal cases, between different kind of punishment provided for wrongdoing.

One sensitive ground where these topics are discussed is the issue pertaining to the scope and limits of criminal medical liability in case of malpractice. Apparently

[5]Cass. sez. un. 12 luglio 2002, Franzese, *Foro italiano*, 2002, II, 601 ann. by Di Giovine.

aimed at achieving more reliability in judgments on medical malpractice in Italy, art. 3 of d.l. 13 September 2012, n. 158 has stated that compliance with guidelines and best practices validated by the scientific community plays a decisive role in the evaluation of criminal negligence of clinicians. The decisive role of guidelines has been more recently confirmed, with far-reaching effects on the overall features of criminal liability, by the law 8 March 2017, n. 24. Such approach has raised concerns in the judiciary and among legal scholars, especially on account of the often doubtful reliability of clinical guidelines, as well as of cases when departing from them would benefit the patient's health, according to so-called personalized medicine. Actually, clinical guidelines do not entirely overlap with standards of care relevant to the assessment of negligence and have different aims [14, 15]. Sheer compliance with such guidelines may give rise in itself to an insidious kind of defensive medicine, one of the main problems in current health care systems [16]. On the other hand, also the evaluation of published scientific literature could sometimes be problematic considering, for example, the lack of access to raw data, the existence of questionable research practice and scientific frauds. The problem is well known in the scientific community as "replicability crisis".

Doctrine and jurisprudence have made a special effort to define the penal treatment both of clinicians who decide to ignore guidelines and of those respecting these recommendations, even in specific situations when they consider it to be in the patient's interests to depart from it, in accordance with s.c. personalized medicine.

Moving from a background of legal doctrine and judicial decisions in the field of criminal law, the chapter aims at highlighting the difficulties encountered by courts in actually sorting through scientific knowledge when, as is often the case, science itself is divided on the topic at stake and, especially, when confronting "new" scientific evidence, i.e. novel unpublished findings and practice. Moreover, it will discuss the conditions that allow judges to use guidelines in criminal trials as scientific proof and sketch some new paths of research, deemed conducive to a better understanding and treatment of professional liability, even making some allowance for clinical "epistemic injustice" and the privilege enjoyed by practitioners.

6.2 Looking for Evidence in Modern Science

Any discussion on scientific evidence and scientific proof in trials cannot help focusing on the evaluation about the validity of scientific expert opinion. One mainstay for such an assessment is usually considered the groundbreaking decision of the United States Supreme Court in *Daubert v. Merrell Dow Pharmaceuticals, Inc.,*[6] which established that the rule in *Frye v. United States,*[7] over "general acceptance", could not be deemed as «a necessary precondition to the admissibility of scientific evidence».

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[6] 509 U.S. 579, 113 S.Ct. 2786 (1993).

[7] 293 F. 1013 (D.C. Cir. 1923).

Going beyond *Frye*, *Daubert* has burdened judges with the gatekeeper's task of deciding about "evidential reliability" on the basis not of the sheer conclusions offered, but of the methods used to reach those conclusions, in particular according to the following four criteria: 1. The theoretical underpinnings of the methods must yield testable predictions by means of which the theory could be falsified 2. The methods should preferably be published in a peer-reviewed journal. 3. There should be a known rate of error that can be used in evaluating the results. 4. The methods should be generally accepted within the relevant scientific community [17].

Daubert has thus assigned to the trial judge «the task of ensuring that an expert's testimony both rests on a reliable foundation and is relevant to the task at hand» and, drawing on U.S. Federal Rule 702 over the requirement of reliability, has stated «that an expert's testimony pertains to "scientific … knowledge", since the adjective "scientific" implies a grounding in science's methods and procedures, while the word "knowledge" connotes a body of known facts or of ideas inferred from such facts or accepted as true on good grounds».[8]

As stated by Justice Breyer's in *Kumho Tire,*[9] a primary purpose of the «*Daubert* gatekeeping requirement … is to make certain that an expert, whether basing testimony upon professional studies or personal experience, employs in the courtroom the same level of intellectual rigor that characterizes the practice of an expert in the relevant field» [3].

Daubert has thus prompted an approach to scientific evidence that requires judges to have the basic skills needed to read and understand scientific methods and to integrate scientific knowledge in their decisions. As similarly remarked by Chief Justice Rehnquist's in *Joiner,*[10] judges (and thus lawyers) need some understanding of the methods of science to effectively screen proffered scientific evidence [3, 18] although «a judge is not a scientist, and a courtroom is not a scientific laboratory» [5], nor should he/she be an expert in any one area, much less in all areas, of science [13].

While making the relationship of courts with science more active and critical, heightening the standard of their scientific culture [19], *Daubert* is, however, bound to raise at least two concerns for practitioners: not only how wide a judge's knowledge must be, but also «where the scientific sea begins and ends» [13].

Indeed, one of the problems for judges is that real science is *not* easily distinguished from pseudoscience, and thus what philosophers call "the problem of demarcation" is far from being solved: «there is no simple, mechanical criterion for distinguishing real science from something that is not», although «the Supreme Court, in the *Daubert* decision, has made a respectable stab at showing how to do it» [9].

In Italy, the Supreme Court in the well-known *Cozzini* judgement,[11] while noting that it is not for the supreme court (rather than lower courts) to establish

[8]509 U.S. 579 (1993), at 590.

[9]*Kumho Tire Co. V. Carmichael* (97-1709) 526 U.S. 137 (1999) at 152.

[10]*General Electric Co. v. Joiner* 522 U.S. 136 (1997).

[11]Cass. pen., Sez. IV, 13 December 2010,. n. 43786, in *Dir.pen.proc.,* 2011, p. 1341, annotated by P. Tonini, especially point 16. See also: Cass. pen. Sez. IV, 29 January 2013,, n. 16237, rv. 255105, Cantore.

whether a given scientific rule is reliable or not, has claimed for itself the task of checking the rational route of the investigation in fields which, albeit not entirely new, reveal some dark sides, marked by many contradictory studies and involving a broad international debate. Thus, the decision has admitted that the scientific nature of the evidence can be recognized by the courts themselves even when there is «an unresolved conflict» among different theses, which should require from judges the direct verification of the reliability of studies supporting them, on account of «the factual basis on which they are conducted, the breadth and rigor, the objectivity of the research, the degree of factual support of the thesis, the critical discussion that has accompanied the development of the study» as well as «the explanatory potential of the theoretical elaboration together with the level of consensus on that thesis in the scientific community».

As remarked by some scholars, while displaying similarities with the statements of *Daubert*, the *Cozzini* judgment seems to depict and require an intermediate kind of expert: not only able to report on the state of the scientific debate, but also to evaluate it, thanks to his (possibly higher) knowledge, on the basis of his/her scientific views, without neglecting, in the analysis of the case, also scientific theses that the expert could personally not share. Thus, «the expert's credibility while being a key credential, does not legitimate to limit himself to the "ipse dixit"» [20].

The *Cozzini* standard, actually not much followed by the posterior Italian judicature,[12] cannot replace the consensus criterion, having to depend on a knowledge which is already recognized as reliable by a significant part of the scientific community, as no parameters seem to ensure the quality of science, apart from the prevalence of the views expressed by scientists [20]. However, this judgment, no less than *Daubert*, should work as a stimulus for judges to seek better training, beyond their legal education, e.g. in the field of basic statistics and research methodology [13].

6.3 Role of the Scientific Peer-Review System in Forming Evidence

Dealing with the role of the scientific peer-review system in forming evidence, *Daubert* stated that «the fact of publication (or lack thereof) in a peer reviewed journal thus will be a relevant, though not dispositive, consideration in assessing the scientific validity of a particular technique or methodology on which an opinion is premised».[13]

[12] See Cass. pen., sez. IV, 24 maggio 2012, n. 33311, in *De Jure*; Cass.pen., sez. IV, 22 marzo 2012, n. 24997, rv. 25330; 33; Cass. pen., sez. IV, 21 novembre 2014, n. 11128, in *De Jure*.

[13] 509 U.S. 579 (1993), at 594.

However, while "general acceptance" publication in a peer-reviewed journal and reputation of the scientist-expert may help judges in their difficult task, «ultimately, they [the judges] must come to appreciate the complexity of scientific inquiry. They must come to understand those questions scientists can answer and those they cannot» [19]. Judges should thus try to practice at least three levels of scientific thinking, which are often confused, but must be considered in order to assess the validity of scientific expert opinion: «the theory or principle that provides authority for the conclusions that are drawn from the data»; «the general technique or procedure that produces the data»; and «the specific practices used to obtain the data» [13, 19, 21].

Recently, Faigman, on account of the troubles lawyers and judges could meet in order to acquire the understanding of the methods of science that *Daubert* requires, has proposed «to return to the sensibilities of the *Frye* test in order to accomplish the objectives of *Daubert*». Thus, «lawyers and judges should embrace the insight of *Frye* of asking scientists about the expertise offered in court so that *Daubert*'s validity assessment can be carried out» and to this effect a recourse to «the venerable institution of scientific peer review» has been suggested [3].

In spite of such recent support for the use of peer review in trials, great caution is required, as the double-blind peer review system does not ensure the reliability of scientific judgment, being conditioned by the specific expertise and possibly by the academic rank of the referee, and ill-suited to studies that use research methods that are not new or outside of the mainstream [20]. In fact, peer review is deemed «inherently conservative», as it «tends to reinforce dominant views in the field, and may be unavailable or unduly restrictive regarding certain fields of study» [3].

One of the «salutary benefits» for judges of taking peer reviews into account is the possibility they offer to look into the mainstream views of the respective scientific field, allowing them to understand whether the disagreements arising in court «reflect real debates in the field, rather than being the products of the litigants' choosing outlier experts» [3]. The judge's basic knowledge of the peer reviewing data relevant to the case should thus allow him to evaluate and possibly discard an expert opinion in case this has neglected or at least not adequately discussed the results of peer reviewed science [4].[14]

The good use of peer reviewing would in any case require that its conservative tone be balanced by a procedural setting benefitting of «the natural polarizing effect of the adversarial process», as such «especially well designed to frustrate the reception of the middle ground of scientific opinion» [3]. «The *Daubert* standard operates in a procedural setting, not a vacuum». In *Daubert*, the Supreme Court noted that «[v]igorous cross-examination, presentation of contrary evidence, and careful instruction on the burden of proof are the traditional and appropriate means of attacking shaky but admissible evidence».[15] Adversarial testing «presupposes

[14] 463 F.3d 710 (7th Cir. 2006).

[15] 509 U.S. 579 (1993), at 596 (citing Rock v. Arkansas, 483 U.S. 44, 61 (1987).

advance notice of the content of the expert's testimony and access to comparable expertise to evaluate that testimony» [22].

If science «is an arena in which ideas do battle, with observations and data the tools of combat», the institution of peer review is especially useful «to separate valid science from nonsense», as well as «to ensure that the current paradigm has been respected», while it is of little help in choosing between competing valid ideas, and in catching cheating or fraud, as scientists usually believe that their fellows are rigorously honest in the reporting of scientific results, which makes it easy for a purposefully dishonest scientist to fool a referee [9].

Moreover, peer reviewing seems to be of little help in providing and evaluating what has been called "diagnostic evidence", which must be distinguished from "framework evidence", also in order to decide whether the evidence is admissible, namely according to standards like *Relevance, Qualifications, Validity, Added Value (Helpfulness), Prejudice* [17].

6.4 From Evidence to Proof

The predicament of lawyers and judges confronting the *mare magnum* of scientific debate cannot be ignored. We must generally agree with the statement that «law should lag science, not lead it», that «the courtroom is not the place for scientific speculation» and «the expertise offered in the courtroom should reflect mainstream opinion, and not the polarized views of a small group of outliers» [3].[16] Actually the judge is and must be a consumer not a producer of scientific knowledge [23].

However, "consumer" does not mean passive receptor [24]. Law is law and should thus filter out from the huge proffered science what is suitable to its aims and complies with its own criteria.

Law should find a balance in the level of understanding of the rigors of scientific research it should take on, based upon the kind of judgment it must make and the values as well as rights involved in the consequences thereof.

We could hardly deny that «all applied science is probabilistic and infused with uncertainty» and «this uncertainty creates doubt» [3]. Thus, if the overall social acceptance and legitimacy of the law lies in its ability to convey a reasonable safety in human relations and in the solutions of even difficult issues [14, 15], then not only conventional rules of evidence, but also conventional rules of judgment, should help judges (and lawyers) to cross the quagmire of scientific uncertainty. The huge debate on the relationship between science and the law should thus

[16]Faigman quotes from *Rider v. Sandoz Pharmaceuticals Corp.*, 295 F.3d 1194 (11th Cir. 2002) ("Given time, information, and resources, courts may only admit the state of science as it is. Courts are cautioned not to admit speculation, conjecture, or inference that cannot be supported by sound scientific principles. 'The courtroom is not the place for scientific guesswork, even of the inspired sort. Law lags science; it does not lead it.'") [*quoting Rosen v. Ciba-Geigy Corp.*, 78 F.3d 316, 319 (7th Cir. 1996)].

encompass a larger perspective than only the task of solving the riddle of intricacy and innumeracy inherent in scientific knowledge.

The word "law" is differently meant in science and law itself [9]. This difference in meaning must be considered, especially when we are confronted with attempts to draw on scientific (and technical) laws, in order to define what are *legal* laws. Actually, the relevance in trials of a scientific/technical law depends upon the evaluation thereof on the part of (legal) law itself; an evaluation which takes account of the balance among various interests and aims [25].

One first caveat would suggest that the balance between the two levels of knowledge required in trials and from expert testimony—framework knowledge and diagnostic knowledge—be tipped towards the latter (i.e. heightening the requirements for proof) the more deep and lasting the judicial decision impinges on constitutional human rights of the defendant. A remark which bears heavily in the field of causation, allowing to keep general and individual causation a long way apart, only the latter being appropriate for criminal liability and the "beyond reasonable doubt" requirement it must fulfill [25].

But not only criminal law trials in general would be a case in point. Within this same field, the standards of scientific proof could vary according to the "invasiveness" of the kind of criminal penalty the judge could inflict. It is enough to provide a reminder of what, in this respect, has been stated by the European Court of Human Rights from the *Engel* case onwards, dealing with the effects of considering, under Article 6 of the European Convention, that a procedure involves "a criminal charge".[17] If not requiring the judge to become an amateur scientist, in these cases he/she should at least comply as strictly as possible with his/her role of gatekeeper charged with the task of screening scientific evidence for its empirical soundness.

As exemplified in the *Franzese* decision, the judicial evaluation should avoid the pitfall (reported as often yawning in toxic tort cases) of examining and rejecting separately each study on which the plaintiff's expert relied and rather look jointly at all the relevant scientific studies produced in trials as expert-based opinions. As it has been aptly reminded, «scientists, including epidemiologists, typically state that they would look at the totality of the evidence before reaching a conclusion» [26].

I would add another criterion, which has been somewhat outlined while dealing with the role of the precautionary principle in criminal law [27]. We must be aware that in many criminal cases, when the defendant is a professional, an entrepreneur or a corporate manager, his/her knowledge of the technical and scientific data and theories relevant to the case is generally far superior to the expertise possessed by prosecutors, judges, complainants and often even expert witnesses. Such "epistemic privilege" is enjoyed also by the practitioners and institutions of contemporary healthcare services [28].

[17]ECHR, Case of Engel and Others V. The Netherlands *(Application no. 5100/71; 5101/71; 5102/71; 5354/72; 5370/72)*, Judgement, 8 June 1976.

In the combination of criteria that judges must take into account in order to assess the adequacy of scientific evidence, the unwillingness of the expert-defendants to share their own knowledge should be considered somewhat relevant for reducing the standard of scientific evidence required for proof in trials even to the point of contenting oneself with a higher amount of evidence mainly based on "framework" knowledge, namely, of evidence that describes general scientific propositions, rather than on a diagnostic one every time very good grounds exist for assuming that such a defendant possessed a superior knowledge (useful for both the empirical framework and its application to the given case), that could have been submitted to the court. This would somewhat remedy the «distributive unfairness in respect of epistemic goods» [29], especially seen from the quite mobile perspective of social systems and institutions [30], which more often than not has brought about or at least aggravated "victimizations", which trials should not completely disregard.

The undeniable dangers associated with making a mistake in the legal context in which science is presented should be accepted only through an accurate balance between the level of certainty the relevant scientific data the party enjoying an epistemic privilege can provide (also related to the level of such privilege) and, as said above, the consequences inherent in the legal decision to be adopted.

Such scientific and technical disclosure of sorts expected from all parties enjoying an epistemic privilege, albeit needing a further procedural scrutiny and legal fine-tuning, does not seem at odds with the legal protection against self-incrimination (as derived in the United States Constitution from the Fifth Amendment) or with the right to silence (somewhat already amended in England and Wales by the Criminal Justice and Public Order Act 1994).

Apart from hindrances that this approach might have to confront in different legal systems, it seems well suited to a "narrative" and interactive way of dealing with complex issues, and seems a reasonable compromise in order to cope with «the basic problem endemic in the integration of law and science», namely that «the law's search for truth is time-bound, whereas science strives for truths unencumbered by limitations periods and crowded dockets» and that «the law desires truth, but realistically settles for justice and fairness», and «fairness in particular mandates that the evidentiary rules for scientific evidence include a necessity component». A "basic problem", which has suggested the recourse to a "necessity principle" in the field of expert testimony rules, allowing «more flexible and subtler judgments regarding the state of scientific research» [13].

Thus "justice" and "fairness" should be taken into account only to implement a fair rebalancing of epistemic injustice through procedural tools [27] which could find their proper place in those case-management techniques (such as pretrial conferences, pretrial hearings where potential experts are subject to examination by the court, and by specially trained law clerks or scientific special masters), which have been deemed useful to narrow the scientific issues in dispute [5], and that could be framed in a wider context inspired by what has been called a "responsive regulation" [31].

6.5 Standards of Proof of Medical Malpractice According to *Daubert* and the Dubious Weight of Clinical Practice Guidelines in Assessing Standard of Care

Medical malpractice, or medical negligence, occurs when a health care provider, with an action taken or by the failure to take a medically appropriate action, violates the governing standard of care in the treatment of a patient, causing the patient to suffer an injury. The evaluation of the correctness of the conduct is carried out by comparing what the practitioner has done or failed to do, with an ideal standard of care that can be drawn from referenced scientific sources and especially from what has been validated among the practitioners in the relevant medical profession.

In assessing liability for medical malpractice, the real weight carried by clinical practice guidelines (CPG), namely those «systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances»,[18] is currently a moot, albeit extremely relevant, question.

The one-way-street approach of the Maine law[19] [32] is well known, namely, the allowance of clinical guidelines as a "shield" (exculpatory use) for practitioners, according to which «a physician could point to his or her compliance with an approved guideline as proof that they gave good care, but a patient could not use the physician's non-compliance with a guideline as evidence that they did not» [33]. However, such lop-sided allowance of scientific evidence has been deemed a violation of the equal protection and due process clauses of the Fourteenth Amendment [33].

In Italy the passing of d.l. n. 158/2012 and, more recently, of l. n. 24/2017, which have somewhat linked the evaluation of medical negligence to the compliance with the «best practices and guidelines accredited by the scientific community» or, respectively, with «guidelines defined and published according to law», has posed the ticklish question (among others) of when such "guidelines and best practices" may be deemed as having bearing on the case, especially considering that, according to a long established judicature, the practitioner should deviate also from the most consolidated medical guidelines whenever suggested for the particular case by the "rules of the art" of the medical profession [20].

In the U.S.A. the Obama administration is nowadays supporting the idea of guidelines and a regulatory safe harbor, through the improvement of guideline uniformity in view of a federal law that excludes the responsibility of the doctor who has complied with them.[20] A program prompted, among other reasons, by the

[18]Committee to Advise the Public Health Service on Clinical Practice Guidelines, Institute of Medicine, Clinical Practice Guidelines: Directions for a New Program 8 (Marilyn J. Field & Kathleen N. Lohr, eds. 1994).

[19]*Me.Rev.Stat.Ann.* tit. 24, §§ 2971–2978 (repealed 1999).

[20]Patient Protection and Affordable Care Act, Pub. L. 111–148, 124 Stat. 119 (2010) (to be codified as amended in scattered sections of the Internal Revenue Code and 42 U.S.C.).

worry about the “warped system” of so-called “defensive medicine”, namely the excessive care which doctors provide in an attempt to maximize reimbursements.

Defensive medicine is certainly abetted by the loose judicial criteria used to assess the due standard of care, and thus by the «selective perspective» and the frequent hindsight bias of the judicature, «which neglects how the most appropriate course of action may involve a treatment that likely leads to a patient’s recovery but also involves a small chance of exacerbating the patient’s condition», also due to the «lack of the relevant evidence on the comparative benefits of the treatment, especially with new treatments» [34].

However, mechanisms devised to introduce guidelines more prominently into the legal process and help courts decide which guidelines should be regarded as authoritative, also through «a national guidelines certification program» [35], as it has happened with the latest Italian law, hardly seem a solution. The current reluctance on the part of courts to rely too much on guidelines has good grounds, stemming from «political and legal issues that can arise with the development of guidelines» and the awareness on how «political sensitivities, conflicts of interest, and potential lawsuits often silence otherwise innovative and potentially useful guidelines [36]. As stated by authoritative medical legal doctrine, «failure to follow a guideline is not prima facie evidence of negligence» as compliance by doctors with the required standard of care may be inferred by a detailed analysis of available scientific sources extending beyond the mere scope of guidelines [37].

Moreover, the “path to personalized medicine”, namely to «the tailoring of medical treatment to the individual characteristics, needs and preferences of a patient during all stages of care, including prevention, diagnosis, treatment and follow-up» [38], contradicts the restriction on physicians «to one procedure or series of procedures for a specific condition», as «no two patients are exactly alike and no two conditions are exactly alike». Indeed, practice guidelines apply to the general case, but could not apply, at least without some extrapolation, to a particular individual, especially «when multiple diseases exist, as they frequently do in the elderly, or when treatment entails competing risks» as exemplified in the recommendations of anticoagulation for patients with atrial fibrillation (an abnormal heart rhythm disturbance) to prevent blood clots, which can, however, also lead to life-threatening bleeding [36]. As stated, «clinicians and policymakers should understand the evidence but individualize decision-making to the specific patient or situation»[21] [39, 40].

Moreover, while no better method is probably conceivable for clarifying the standard of care than use of CPGs before courts [41], medical malpractice issues are particularly exposed to the risk of unduly muddling framework knowledge and diagnostic knowledge, only the latter being relevant in most cases. As the judicature

[21]U.S. Preventive Services Task Force, *Screening for Carotid Artery Stenosis: U.S. PreventiveServices Task Force Recommendation Statement,* 147 Annals Internal Med. 854–59 (2007) as quoted by Wong et al. 2011, 727.

is prone to consider guidelines in a conservative and backward-looking way [33, 34], the risk of such muddling is quite real.

Thus, any legislative endorsement of "state" guidelines, as recently passed in the Italian legislation, poses serious problems, also due to their lagging behind the progress of scientific research: «even if a guideline reflects current best evidence when written, medical advances could soon render such a guideline obsolete» [34, 41]. Moreover, the reliability of such "governmental" guidelines would be marred by their not infrequent laxness, as agencies, besides often lacking the resources to set the regulations efficiently and update them, «are vulnerable to interest-group capture, perverse political preferences by the government, and self-aggrandizing administrators» [34]. Personal conflicts of interest corrupt the guideline issuance process [42]: «by favoring one test over another, or one therapy over another, guidelines often create commercial winners and losers, who cannot be disinterested in the result and who therefore must be separated from the process» [43].

Another delicate issue relates to the role of expert witnesses in assessing the required standard of care of clinicians and thus medical malpractice, as they tend to perpetuate the prevalence of custom, as «generally, experts do not testify as to what the expert himself would have done, or to whether what the defendant did was reasonable, but rather an expert testifies to what other physicians ordinarily do» [41], thus discouraging, just like the "general acceptability" standard, the development of a real evidence based medicine. Moreover, it is likely that what an expert believes about a community standard is what the expert personally believes *should be* the standard of care: «in practice, questions to an expert about a community standard are really more like questions about a personal belief»; the problem arises from the complexity of human biology, diseases, and medicine, which «simply exceeds the capabilities of human subjective reasoning» and thus reveals its inadequacy in dealing with medical liability [44].

As a result, we are often confronted with a «battle of experts debating what is the standard of care for the particular procedure under consideration», problematic under many respects, including lack of objectivity and overconfidence in habits [45]. A battle which, more often than not, is a «battle of the guidelines» [41].

As «the "safe harbors" concept rests either on an illusion or on a deception», what the law should try to achieve is to provide physicians with parameters that give them «the flexibility to utilize their own skills within an acceptable range of options» [42]. As stated, «medicine is, after all, often too variable or too subtle to be captured in concrete standards» [46]. A remark which perhaps mainly explains «the natural resistance of professionals to accept standardization of practice» and to «give up the latitude that has traditionally been accorded them to exercise their clinical judgment as they see fit» [33].

6.6 Conclusions

Guidelines are relevant in defining malpractice and medical standards of care, but in conjunction with other sources, such as consensus documents (national and international), operational procedures (local, national and international) and evidence-based publications (national and international literature) [37]. As with any other form of evidence, they should also be subjected to the usual eligibility test according to the criteria established by the judicature.

Although this could make it difficult to apply the *Frye*, or even the *Daubert* standard to medicine, some noteworthy attempts have been made to comply with *Daubert* in the use of clinical guidelines in trial [47].

A first step has been to establish their admissibility as evidence, based on whether they are «relevant to the conduct in question» and «whether they are reliable sources for delineating sound or proper medical practice» [47]. Afterwards, judges should establish «whether the standard articulated in the proffered guideline has risen to the level of a nationally accepted standard, and whether a respectable alternative school of thought exists. Moreover, if a guideline is offered as an affirmative defense, the judge «must decide whether the defendant followed it appropriately in making treatment or coverage decisions, prescribing medical products, or rendering medical care» [47].

Relevance of guidelines in the case should be evaluated also considering whether the primary objective of the guideline is «consistent with the physician's screening, diagnosis, or treatment objectives» and if the «recommendations contained in the guideline must be applicable to the plaintiff». Moreover, relevance could be indicated by the guideline accounting for significant new developments in medical research and technology, on the basis of two important dates: «(1) the date of publication of the most recent evidence employed; and (2) the date the final recommendations contained in the guideline were made. Out-of-date guidelines may be irrelevant per se in light of new medical developments» [47].

Compliance of guidelines with *Daubert* standard should also require that they «can be (and has been) tested», that seems to imply that they are evidence based, as only these methods «includes structured and comprehensive reviews of scientific literature, synthesis and evaluation of the literature, and expert panel review» [47]. Evidence-based medicine has been defined as « the conscientious, explicit and judicious use of current best evidence in making decisions about the care of the individual patient. It means integrating individual clinical expertise with the best available external clinical evidence from systematic research».[22] It encompasses the use of mathematical estimates of the risks of benefit and harm, derived from high-quality research on population samples and it «stresses a structured critical examination of medical research literature: relatively speaking, it deemphasizes average practice as an adequate standard and personal heuristics» [36, 48, 49].

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[22]Evidence-Based Medicine Working Group, *Evidence-Based Medicine. A New Approach to Teaching the Practice of Medicine,* 268 JAMA 2420–25 (1992).

This is just what certainly can and should be improved in the domain of guidelines, one of their major weakness being just the lack of scientific evidence supporting them [42]. Their role could be enhanced once the process of construction and selection thereof could be further advanced, as attempted in the definition by IOM (Institute of Medicine) of their trustworthiness. Being «recommendations intended to optimize patient care», they should be «informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options».

Thus to be trustworthy, guidelines should: «(a) be based on a systematic review of the existing evidence; (b) be developed by a knowledgeable, multidisciplinary panel of experts and representatives from key affected groups; (c) consider important patient subgroups and patient preferences, as appropriate; (d) be based on an explicit and transparent process that minimizes distortions, biases, and conflicts of interest; (e) provide a clear explanation of the logical relationships between alternative care options and health outcomes, and provide ratings of both the quality of evidence and the strength of recommendations; (f) be reconsidered and revised as appropriate when important new evidence warrants modifications of recommendations».[23]

As to the second *Daubert* standard, namely that guidelines have been subject to *peer review*, *Daubert* itself states that «[p]ublication is not a *sine qua non* of admissibility; it does not necessarily correlate with reliability».[24] Actually there are legitimate guidelines, by definition, escaping external peer review, like actuarially derived guidelines reflecting scientifically tested principles, but not subjected to the peer review process [47].

As to the stated need for courts to ordinarily consider «the known or potential rate of error»,[25] this sounds particularly relevant for guidelines, whose credibility will require the «drafters' candor about the uncertainties regarding the harms or benefits of the suggested interventions, and whether the recommendations provide flexibility in accommodating those uncertainties inherent to even thoroughly conducted clinical trials» [47]; thus, in such instances, guidelines considered as allowing a wider flexibility will be needed to establish whether «the judgment of a reasonably prudent practitioner under similar circumstances» has been exercised: «reliable guidelines acknowledge the use of opinions, as well as the existence of differing views, to allow room for "legitimate disagreement"» [47].

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[23]Inst. Of Med., Clinical Practice Guidelines We Can Trust 25–26 (Nat'l Acad. Press 2011), *available at* http://www.nap.edu/catalog.php?recordid=13058.

[24]Daubert *V.* Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993) at 593.

[25]Daubert *V.* Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993) at 594: «Additionally, in the case of a particular scientific technique, the court ordinarily should consider the known or potential rate of error, see, *e.g.*, *United States* v. *Smith*,869 F. 2d 348, 353-354 (CA7 1989)».

As to the requirement of the "general acceptance" finally deemed by *Daubert* as having a bearing on the inquiry,[26] once applied to clinical guidelines and medical malpractice replicates the general troublesome issues arising where two or more "schools of thought" and thus different guidelines compete, without gaining universal or also general acceptance. As stated, while the aim for the medical communities should be to achieve the creation of scientifically sound, evidence-based guidelines for clinical decision-making» [47], in the evaluation of malpractice, when the alternatives are deemed medically legitimate by well-respected members of the medical profession, «physicians are allowed to defend their treatment choices in situations where hospitals or small physician practices have limited resources and cannot necessarily utilize the most expensive equipment or technology» [47].

Confronting the many loopholes and uncertainties arising in the relationship between law and science in general and in dealing with medical malpractice cases in particular, new paths can and should be devised, including a pretrial role assigned to that innovative way of alternative dispute resolution which is restorative justice [16]. A way often conducive in itself to a far better comprehension and selection of relevant scientific issues than that allowed by traditional procedures, also through a rebalancing of the epistemic injustice and the epistemic privilege enjoyed by practitioners and health care structures that possess a superior *diagnostic* knowledge of scientific data to be applied to the individual case. And, especially, thanks to the ability of restorative justice to promote and sustain a dialogue among all of the parties involved, which could somewhat defuse the "battle of experts", whose outcome is often only a pyrrhic victory, at least for the sake of truth and justice.

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[26]«A "reliability assessment does not require, although it does permit, explicit identification of a relevant scientific community and an express determination of a particular degree of acceptance within that community" …Widespread acceptance can be an important factor in ruling particular evidence admissible, and "a known technique which has been able to attract only minimal support within the community"».

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Part III
Innovation, Unitariness and Evidence. Forensic Pathology and Anthropology

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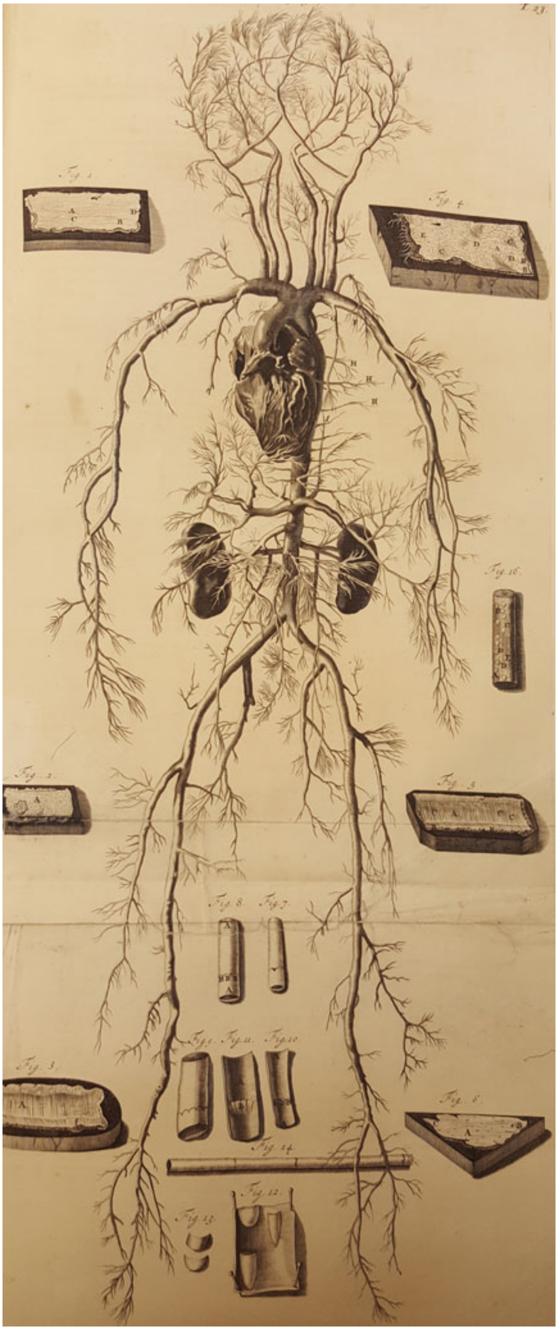

Bidloo, Govard, Anatomia humani corporis centum & quinque tabulis, per artificiosiss. G. de Lairesse ad vivum delineatis, demonstrata, veterum recentiorumque inventis explicata plurimisque, hactenus non detectis, illustrata. Amstelodami: Sumptibus viduae Joannis à Someren, haeredum Joannis à Dyk, Henrici & viduae Theodori Boom, 1685

Chapter 7
Forensic Pathology Historical Roots and Modern Evolution

Stefan Pollak

Abstract When defining the position of a scientific discipline, it is important to remember its roots, to analyze its current state and to meet new challenges resulting from social evolution. The roots of modern forensic medicine and especially forensic pathology date back to the second half of the 19th century. Since then, circumstances of life, the manifestations of crime and thus the issues of forensic pathology have changed considerably. Some incidents such as neonaticides have become rare nowadays. This involves the risk that basic knowledge regarding formerly all-important topics may slide into obscurity. On the other hand, some new pathomorphologic conditions have to be assessed in forensic autopsies, such as sequelae of high-tech medical treatment and changes due to the prolonged survival of severely injured persons. Other problems result from multimorbid patients in a society having a disproportionate number of old people who often suffer from dementia and even succumb to minor trauma. Considering the role of forensic pathology in the future, it will be crucial to continually strengthen the scientific basis through high-quality research work.

7.1 Introduction

Forensic pathology deals with the medicolegal investigation of unnatural, suspicious and unclear deaths. Though by no means the only kind, in Continental Europe this field of activity constitutes an essential topic of forensic medicine, which has been defined as “the application of medical knowledge in the administration of justice” [1]. Apart from daily routine casework carried out for legal and police authorities, forensic (legal) medicine and its sub-discipline forensic pathology are integral parts of medical faculties and therefore intensely engaged in research and teaching.

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Contrary to clinical and anatomical pathology, which are focused on the diagnosis and detailed classification of diseases as well as the determination of (natural) causes of death, *forensic* pathology uses the diagnosis as a starting point to reconstruct what happened. The main purpose of forensic autopsies is not so much to find out the cause of death as such, but to clarify legally relevant circumstances and contexts [2]. Forensic pathology aims at solving suspicious fatalities by doing a complete autopsy and additional histological examinations [3–5]. Therefore, the medicolegal examination of the dead body (and its clothing) is the central issue of forensic pathology. This includes the inspection of the scene together with the assessment of relevant traces and any injurious objects.

To overcome future challenges one has to be aware of the past. With regard to forensic pathology, the historical roots of external and internal body investigations go back to ancient China [4]. In Europe, the earliest medicolegal dissection took place at the University of Bologna in the 13th century. In 1612, the first encyclopedic text on legal medicine ("Questiones medicolegales") was written by Paolo Zacchia [6]. At Basel (Switzerland), Felix Platter (1536–1614) performed a great number of autopsies and elucidated the relation between a disturbed anatomical structure and the pathological function of the respective organ. In his monograph "Observationum, in hominis affectibus …" Platter presents a professional and scientific approach specific to legal medicine [7].

Clinical autopsies became more common only in the 19th century, in the era of pioneering pathologists such as Carl von Rokitansky and Rudolf Virchow. At that time, an independent development of forensic medicine was hindered by some representatives of pathological anatomy who were of the opinion that their own teaching would sufficiently cover the forensic aspects as well [8]. When Eduard Hofmann was appointed professor of forensic medicine in Vienna (1875), he founded a school of evidence-based somatic forensic medicine outlined in his classical textbook first published in 1878 [9].

For several decades, forensic medicine and pathology have been subject to progressive specialization in separate fields such as forensic imaging, anthropology, odontology, entomology, biomechanics, ballistics, and so on. This tendency implies the danger of a fragmentation into separate entities lacking an integral comprehension of the discipline and a common medicolegal view. At least with regard to forensic autopsies, a supranational recommendation on the harmonization of autopsy rules has been adopted by the Council of Europe [10].

The following statements do not claim to portray the present state and its potential evolution in an objective manner. On the contrary, the author admits to express his personal opinion based on a professional experience of more than four decades.

7.2 Forensic Pathology: Status Quo and Future Perspectives

7.2.1 Changing Tasks in the Course of Time

The issues of forensic pathology are subject to time-related circumstances. Over the years, only some fundamentals remain valid (such as the principles of blunt and sharp trauma). Suicidal intoxications may serve as an example of a constantly varying phenomenology, which reflects the societal changes and the shifting availability of certain toxic agents. This has already been pointed out in the context of carbon monoxide and other poisons [11]: town gas, which played a major role as a source of poisoning after the Second World War, has long been replaced by natural gas, which does not contain carbon monoxide. Up to the 90s of the last century, non-accidental carbon monoxide intoxications were mostly brought about by conducting exhaust fumes into the interior of a car, but nowadays this method has lost its former importance due to the common use of catalytic converters in automobiles [12]. At present, an increasing number of suicidal carbon monoxide poisonings is due to charcoal fires in closed spaces [13].

A similar change can also be observed with other poisons formerly used for committing suicide: arsenic poisoning verified by autopsy has drastically declined, just as have intoxications by caustic and topically irritating agents such as acids, lyes, certain metal salts, phenol and its derivatives, which were frequently seen before the Second World War. In the 1950s and 1960s, E 605 (parathione), an insecticide belonging to the substance class of phosphorous acid esters, was so commonly used for committing suicide or poison murders that there really was an "E 605 fashion". Even the spectrum of drug intoxications has changed in the more recent past (decrease of barbiturate intoxications, increased use of tricyclic antidepressants, neuroleptics and parenterally administered insulin preparations as a means to commit suicide). Nowadays, suggestions for "alternative" suicide methods are often obtained from the Internet, such as suffocation by breathing a helium enriched atmosphere [14]. Recently, suicidal ingestion of toxic plants such as Taxus baccata has also been inspired by respective instructions on the internet ([15], Fig. 7.1).

Compared with the past, fatalities due to illicit drugs underwent significant changes in terms of consumption habits, the external findings on the body and the age distribution [16]. In Central Europe there has been a change in the last decades, especially concerning the current predominance of mixed intoxications and the rather inconspicuous appearance of drug victims, who frequently belong to advanced age groups. Due to the more common use of insulin syringes with very thin needles, the injection sites are often difficult to detect. The nutritional status of drug addicts does no longer differ from the normal physique. In the majority of drug victims no evident neglect or lack of personal hygiene is found. As there is a general fashion trend towards tattooing, the mere presence of tattoos cannot be

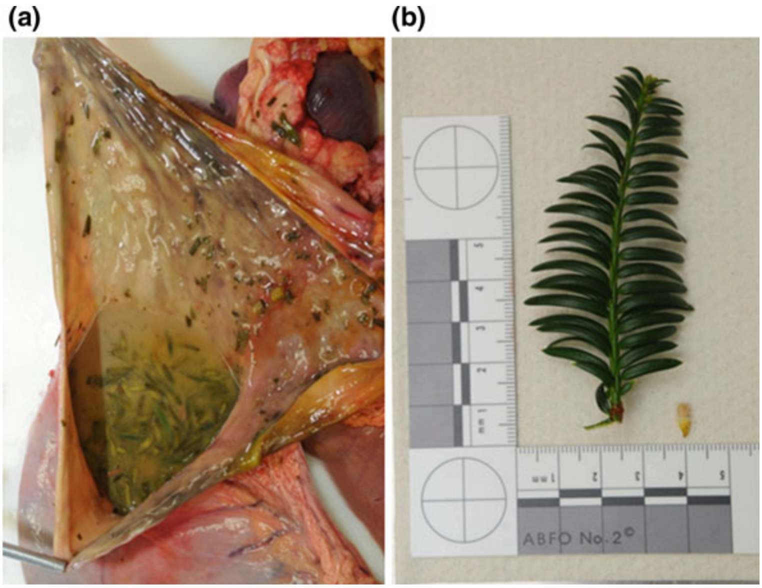

Fig. 7.1 Suicide by poisoning (46-year-old female victim). **a** Stomach containing partly chewed up yew leaves. **b** Taxus baccata (European yew) shoot together with a separated male cone

regarded as an indicator of narcotic addiction except where the image motifs are characteristic for drug abuse ([17], Fig. 7.2).

Some topics that were of utmost forensic importance until several decades ago have become rare nowadays. This applies, for instance, to death from illegal abortion. Today's generation of medicolegal experts has hardly any experience of their own in this field. Up to the 1970s, the autopsy material comprised all of the fatal complications resulting from improper abortion methods, such as venous air embolism, intrauterine infection followed by sepsis, haemorrhage and peritonitis from uterine perforation, as well as intoxication after use of drugs and chemicals to induce miscarriage.

It is apparent that the decrease in the number of deaths associated with abortion is due to the fact that legal termination of pregnancy is now permitted under certain conditions. Easing the restrictive grounds for legal abortion also implicated a sharp fall of neonaticides, so that young medical examiners are no longer familiar with the demanding autopsy techniques indicated in such cases. The same is true for the correct interpretation of the morphological findings regarding neonatal conditions (caput succedaneum, umbilical cord, vernix), maturity of the child, criteria of a separate existence (born alive?), viability, natural and violent causes of perinatal death, birth trauma and injuries from assisted self-delivery.

Another instance to illustrate the time-dependant changes in forensic pathology is death from vehicular accidents. In most developed countries such as in Germany, road traffic fatality rates have declined considerably since the early 70s of the last century. Apart from the decreased frequency, the injury pattern of the victims has

Fig. 7.2 Left forearm of a 51-year-old male drug addict who died from mixed intoxication (heroin, cocaine, ethanol). The colored amateur tattoo shows a cannabis leaf

also changed considerably. This is true both for vehicle occupants and pedestrians. Due to the higher safety standards of modern cars including seat belts, airbags and many other precautionary measures (e.g. rigid passenger compartment, telescopic steering column, windscreen made of laminated safety glass), striking skin injuries from direct impact of structural parts are less common nowadays. Nevertheless, even passengers whose integument remains intact may show internal injuries causing a fatal outcome. Belts and airbags naturally limit the collision-induced movement of occupants and therefore reduce the injury potential. However, beyond the limits of biomechanical tolerance the inner organs and blood vessels may sustain lethal injuries from crucial deformation and/or deceleration.

In car-to-pedestrian collisions, characteristic patterned injuries depicting structural elements of the car front (e.g. radiator grill, front-light edging, direction indicator, bumper bar, windscreen wiper, radiator mascot) have become rare. Both in vehicle occupants and pedestrians, an increasing number of victims belong to the senior age group being at risk even in cases of minor trauma. Due to preexistent multimorbidity, elderly persons are prone to complications so that death may result from secondary trauma sequelae. In cases of prolonged survival, intercurrent infection, pneumonia, multiorgan failure and pulmonary thromboembolism are often fatal complications of an originally non-lethal injury [18].

7.2.2 *Forensic Pathology in Relation to Medical Criminalistics [2]*

The reconstruction of suspicious fatalities has always been a domain of forensic pathology, which is therefore a natural partner of criminalistics. The purpose of medicolegal death investigation goes beyond purely medical findings to questions of evidence (estimation of the time of death, differentiation between self-inflicted injuries and lesions caused by another person, as well as the analysis of wound findings with regard to the object used, etc.)

Medicolegal research draws an essential part of its ideas and questions from practical forensic work. Moreover, the findings and data collected for expert opinions constitute the basis for an empirical approach to research. Without the study material of medicolegal routine work, no new findings could be obtained in many forensically relevant areas. On the other hand, the close relation to university medicine ensures a high scientific standard and independence. Without proper research activities, forensic pathology and medical criminalistics would rapidly fall behind general scientific progress.

From the multitude of research subjects, only a few can be dealt with in this context. The collection of epidemiological data, which is possible only on the basis of medicolegal practice, is of importance for society and legal policy in general. The results of such systematic investigations also help to reveal health risks. For example, a medicolegal multicenter study on HIV prevalence in drug victims demonstrated that intravenous drug consumption is one of the main causes of HIV infections in Germany. In the course of the observation period (1985–1994), the prevalence level declined from 29% to about 7% [19]. The medicolegal monitoring thus impressively demonstrates the success of educational campaigns and the importance of prophylactic measures to prevent an infection by sharing unsterile needles. Epidemiologic research on sudden infant death was and still is also essentially conducted by the institutes of legal medicine [20], as in most of these cases both the manner and cause of death are unclear, so that infanticide cannot be ruled out from the very beginning.

An important aspect of medicolegal publications is to direct the attention of criminalists to diagnostic problems. Serial killings in hospitals and geriatric care may serve as an example. Knowing the relevant methods, which do not necessarily leave any external injuries on the often multimorbid patients, is an important prerequisite to become suspicious and to induce the performance of an autopsy [21].

An attempt to answer the old question of how many homicides go unrecognized was made in a joint study of 23 institutes of legal medicine [22]. Among 13,000 autopsies of the study material, 92 "discoveries by chance" were reported where a natural death was indicated on the death certificate. These cases comprised 49 fatal accidents, 10 homicides and 19 deaths from medical complications. On the basis of this multicenter study, it was estimated that there are at least 1200 homicide cases per year which do not appear in Germany's official statistics, as they are classified as natural deaths.

Most methods used in clinical and basic research are applicable to forensic pathology on a rather limited scale. For medical criminalistics the comparative phenomenology of exactly defined study groups is essential. The evaluation of current case series provides an authentic picture of the criminalistic phenomena in homicides and bodily harm. The development and statistical confirmation of morphological criteria help to characterize typical patterns of findings. Although case reports play an important role, forensic research must go beyond the description of unusual individual observations. Many questions cannot be answered by experience alone, but require experimental research. Experiments performed on appropriate test models to clarify the biomechanical basis of injury formation are such an example.

A special challenge is the systematic analysis of morphological findings for the purpose of achieving a better understanding and more reliable interpretation (e.g. with regard to the underlying trauma and causative mechanism, the specificity of changes, the probability of occurrence, and the influence of contributing factors). This may be illustrated by an example from the field of forensic wound ballistics: simulants, such as gelatin and transparent glycerin soaps, turned out to be appropriate target media, since their behaviour is similar to that of human organs and soft tissue from a physical-ballistic point of view. So there is a high level of comparability between damage to the simulants and injuries to the human body. The application of composite models offers manifold possibilities of an interdisciplinary approach to forensic wound ballistics bringing together medicolegal experts, physicists, weapon engineers, chemists, radiologists and microbiologists [23].

7.2.3 From Casuistry to Hypothesis-Based Studies [24]

Just as in other academic disciplines, permanent research efforts are also necessary in the field of forensic pathology. The profile of requirements undergoes constant change, because of the general progress in medicine and the natural sciences. Most new topics arise from concrete challenges faced in the daily routine. As a consequence, problematic findings encountered in forensic casework should be published in order to avoid the uncritical application of conventional textbook opinions. Exact observation and analysis are still indispensable prerequisites for scientific work oriented to the demands of forensic practice.

In forensic pathology and medical criminalistics, systematic research is often based on the careful documentation of concrete cases. There are multiple reasons why casuistry plays a special role in this area of work, as follows [25].

- Usually, crime sequelae and individual case constellations cannot be simulated in experiments.
- Case reports describe new/rare forms of offences and injury patterns.
- Reconstructive problem solutions are often based on interdisciplinary collaboration, the results of which are mainly published in the form of case reports.

- Such publications may describe the application of novel investigation methods.
- Reports on adverse treatment events can draw attention to potential risks and complications.
- Preventive measures can only be taken after risks have been identified.

Quite often, concrete observations not yet fully understood lead to hypothesis-based studies, which can either be experimental or epidemiological and usually apply "abductive reasoning" (a concept developed by Charles Sanders Peirce). The term refers to the process of arriving at an explanatory hypothesis. It is the only bona fide knowledge-expanding method of inference—using still speculative findings for which there is still no reliable proof. After formulating the hypothesis by abduction ("new idea"), predictions are made from the formulated hypothesis by deductive inference and, in a third step, facts are sought that verify the assumptions (induction). Thus, individual cases confirming the hypothesis can verify the theory. Experiments on test models may provide valuable information, for instance in the field of biomechanics. Systematic analysis of morphological changes and their reproducibility under standardized conditions constitute a reliable foundation for evidence-based interpretation.

Medicolegal knowledge is always impermanent and will be valid only as long as it is not disproved by reality. Statements considered right today may require modification or even be recognized as wrong tomorrow. Whether something is "right" or "wrong" always has to stand the test of reality, as reflected by our daily case observations. For medicolegal practice this means that casuistry is an indispensable means of (self)-criticism. It induces us to question traditional doctrines and to correct them, if necessary.

Case libraries, such as those presented in atlases of forensic medicine, help to train the diagnostic eye ("You only see what you already know!"). By describing frequently seen patterns of findings, archetypical behaviours in committing offences or suicides are identified [17]. Even mass disasters with a large number of deaths can be the starting point of important discoveries and the use of new methods: after the disastrous fire in the Vienna Ring Theatre on 8 Dec 1881, Eduard Hofmann and Eduard Zillner were the first to detect that the inhalation of fire fumes results in the formation of carbon monoxide haemoglobin. On that occasion, odontological data were used for the first time to identify charred bodies.

Every systematic investigation of trauma to mechanical and pathophysiological problems must start with a critical analysis of the phenomena. In 1947, Walther Schwarzacher pointed out "…that all scientific work in the … medical field has to begin with the observation of the research object and that studies … in patients or corpses are the 'raw material' which permits the discovery of general principles … and a better insight …". What one has to learn above all is to see things in as naïvely a way as possible and nevertheless remember all of the former pictures in one's memory as though on the respective page of an atlas [26].

Although forensic pathology shows many features of an empirical science, traditional opinions must always be open to doubt. Eduard Hofmann, one of the most important forensic pathologists, expressed this idea already in 1881 in the preface to the 2nd edition of his famous textbook: "By handing … my book over to the inclined reader, I would like to add that I have tried to base the teachings presented not only on my own … experience and that of others, but to verify them by experiments, where appropriate; this method … supplies the most pleasing results in forensic problems and should thus be used more than it was in the past" [27].

7.2.4 Problems and Challenges [24]

In September 1905, the 1st Congress of the German Society of Forensic Medicine was held in Meran. In his opening address, Fritz Strassmann strongly advocated an intensification of medicolegal research and stated: "There is nothing that will contribute more to the reputation of forensic medicine than an increase of our scientific achievements." According to Art. 1 of the statutes, the aim of the association was to offer a platform for joint scientific work. One hundred and eleven years later, the academic presence of forensic medicine as an independent discipline of the medical faculties is still jeopardized in some countries, even in Europe. The research output, especially that of forensic pathology, may be regarded as insufficient, when journal impact factors and the volume of external funding are taken as a basis.

Fortunately, in most European countries forensic medicine is taught at the universities, which ensures its independence and scientific character. Humboldt's concept of the unity of teaching and research—supplemented by casework—guarantees practical and yet state-of-the-art training, application-oriented research and evidence-based expert opinions. Consequently, every effort has to be made to maintain the university status of forensic medicine and its firm integration into the curriculum of the medical faculties, also in the future. Neglecting research work would endanger the scientific level of expert opinions as well as the quality of pre- and post-graduate training. In university institutes, all of these three tasks mutually benefit from each other.

In recent years, the output of bio-medicolegal research in Europe has been analyzed by a working group from Padova [28, 29]. The authors ascertained a potentially irreversible fragmentation process within forensic medicine, causing knowledge to crumble into a multitude of sub-disciplines and specialties. Among these branches, forensic pathology accounts for a large share in the number of publications (22.1%) and the total impact factor (20.3%). Upon closer examination, the following topics of forensic pathology were the most frequent ones dealt with in scientific journals: injury mechanism (17%), histopathology (15%), radiology and sudden death (9% each). A bibliometric analysis revealed that an increasing number of articles are published in English.

When categorizing the papers of the corresponding author by nationality, Germany ranks first with respect to the total impact factor in forensic pathology, toxicology, genetics, anthropology and criminalistics. It is presumed that this leading role is at least partially due to the German medicolegal system, which focuses on synergy between professional and academic scientific work, since forensic practice is prevalently the domain of the universities.

Nowadays, a medicolegal expert working in the field of forensic pathology has to master a wide and challenging range of knowledge including "classical" topics such as thanatology, traumatology and sudden natural death as well as modern technologies (e.g. forensic imaging and molecular pathology) complementing the traditional spectrum of methods. On the other hand, the importance of personal experience cannot be overestimated in such a diversified area as forensic pathology. Seen from this angle, the decline of forensic autopsies in most European countries is worrying. If the annual number of autopsies performed by a prospective medicolegal specialist is less than one hundred, the candidate will hardly ever acquire a sufficient background experience.

To correctly interpret morphological findings it is extremely important to train the "diagnostic eye". Visual memory mostly sets the course for the analysis of an individual case, even if additional examinations using technical equipment are necessary to furnish proof [17]. External and internal signs of lethal traumatization may be scanty and discernible only for the experienced eye, for instance in some cases of strangulation and electrocution (Figs. 7.3 and 7.4).

Finally, a seemingly marginal problem shall be addressed, namely the official—i.e. national—language used in court. English has meanwhile become the lingua

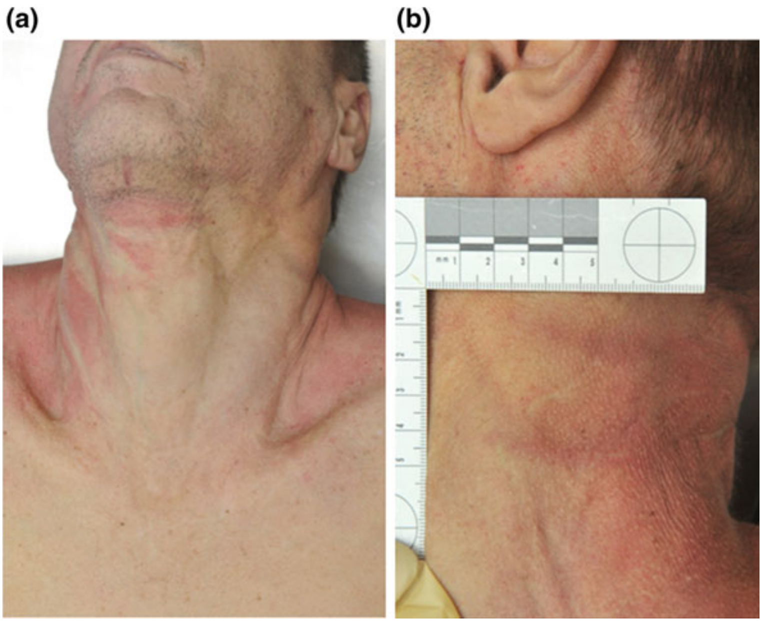

Fig. 7.3 Autoerotic death of a 49-year-old male (accidental hanging). The noose was made of a wide leather belt. Note the inconspicuous hanging mark (**a**) and the petechial hemorrhages below the earlobe (**b**)

Fig. 7.4 Left hand of a 62-year-old man who died from electrocution when handling a defective electrically-operated pressure washer (residential voltage). The fingertips reveal atypical, *greyish-white* electric burns with slightly elevated margins

franca in scientific medicine, including forensic pathology. This implies a loss of familiarity with the native tongue when describing medical issues such as morphological findings. The resulting language deterioration leads to less precision in the autopsy report and the conclusions drawn from it.

7.3 Conclusions

Pekka Saukko and Bernard Knight quoted Giovanni Battista Morgagni (1682–1771), celebrated as the father of modern anatomical pathology, at the beginning of their standard textbook "Forensic Pathology": "those who have dissected or inspected many bodies have at least learned to doubt, while those who are ignorant of anatomy and do not take the trouble to attend to it, are in no doubt at all." In the preface to the first edition, this thought is taken up again: "all too often, dogmatic opinions are derived from an unsound factual base, learned from lectures or textbooks that repeat previous dogma with little sense of critical evaluation. In some parts of the world forensic pathology is learned by rote from teachers who studied themselves in the same fashion…" [30].

Today, performing an autopsy means more than the ascertainment and recording of traumatic or pathological characteristics. Interpretation should not reproduce stereotyped associations, but consider functional disorders and pathophysiological effects that go along with certain morphological alterations. This includes post-mortem chemistry: biochemical analyses of body fluids may provide valuable information on antemortem carbohydrate metabolism, especially in diabetes mellitus. Other markers help to substantiate the diagnosis of alcoholic ketoacidosis, hypothermia, sepsis, anaphylaxis and hormonal disturbances [31]. Besides, it has to be emphasized once more that an autopsy is not complete without histology. Both sampling and microscopy should preferably be in the same hands and carried out by the same medicolegal expert [5]. As far as available, pre-autoptic use of modern imaging techniques is recommended, especially in victims with complex trauma patterns [32].

Apart from the primary body damage caused directly by a trauma, also (late) complications often characterize both the clinical course and the autoptic findings. Victims who have undergone multi-day intensive care in the premortal period typically show the sequelae of progressive organ failure accompanied by generalized inflammatory reaction and interstitial edema. In the presence of severe pre-existing diseases and only minor trauma it can be difficult to categorize the manner of death as unnatural. This problem is particularly relevant in vulnerable persons such as geriatric and care-dependent patients. Forensic pathology reflects the shift in the demographic structure of Western societies.

According to Bernard Knight [30], prospective forensic pathologists should be strongly advised to think twice, question and disagree. To scrutinize allegedly established knowledge is a first step in the right direction—towards scientific activity aiming at a sound factual base.

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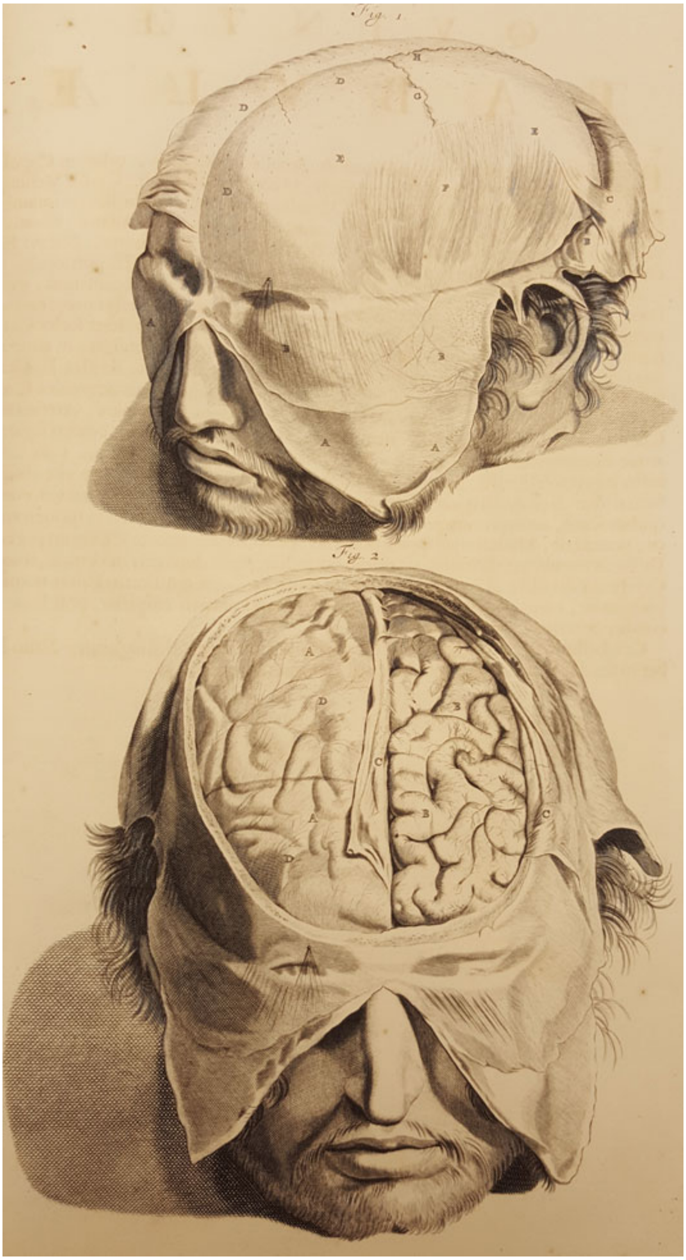

Bidloo, Govard, Anatomia humani corporis centum & quinque tabulis, per artificiosiss. G. de Lairesse ad vivum delineatis, demonstrata, veterum recentiorumque inventis explicata plurimisque, hactenus non detectis, illustrata. Amstelodami: Sumptibus viduae Joannis à Someren, haeredum Joannis à Dyk, Henrici & viduae Theodori Boom, 1685

Chapter 8
Forensic Pathology and Malpractice in Cardiology and Cardiac Surgery

Massimo Montisci

Abstract Medical liability has become a fact of life in the physician's modern practice and each malpractice claim gives rise to a scientific challenge. Under this scenario, the role of Legal Medicine is essential to prevent erroneous interpretations of scientific evidence and the medico legal expert should always be involved in cases of alleged medical liability on cadavers. Focusing on malpractice claims in the field of cardiology, the considerable complexity of the management of cardiac pathologies implies the importance of a multi-disciplinary approach, together with the application of a shared ascertainment methodology. In particular, it is essential for the medico-legal expert to collaborate with specialists, such as cardio-pathologists, cardiologists or cardio-surgeons in cases of alleged medical liability in the cardiologic field and to follow the guidelines which have been produced to assist the expert dealing with deaths reflecting cardiac disease, in order to prevent criticism of case analysis in medico-legal environments.

8.1 Introduction

From the latter half of the twentieth century, medicine has become a victim of its own success and medical liability has become a fact of life in every physician's modern practice.

Nowadays the phenomenon of malpractice has the dimensions and the severity of a pandemic, whose transversal invasiveness does not spare nations, structures, politico-institutional regimes, social classes, professional contexts, or cultural and ideological orientations [1].

Medical errors are considered to be the third cause of death in the United States and therefore they require greater attention. In particular, the literature estimated preventable adverse events using a weighted analysis and described an incidence

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range of 210.000–400.000 deaths a year associated with medical errors among hospital patients [2].

The main consequences of this phenomenon are new economic pressures, loss of physician autonomy and increasingly "defensive medicine".

Despite the inevitability of human error (and in particular in the context of healthcare), the problem should be better analysed in order to design safer systems.

Strategies to reduce death from medical care should include three steps:

- making errors more visible when they occur so their effects can be intercepted;
- having remedies at hand to rescue patients;
- making errors less frequent by following principles that take human limitations into account.

Currently, deaths caused by errors are unmeasured and discussions about prevention occur in limited and confidential forums, such as a hospital's internal root cause analysis committee or a department's morbidity and mortality conference.

These forums review only a fraction of detected adverse events and the lessons learnt are not disseminated beyond the institution or department. Therefore, in order to achieve a more reliable healthcare system, it's important to share data about medial errors both nationally and internationally and to create a culture of learning from mistakes.

Each malpractice claim gives rise to a scientific challenge requiring specific expertise in the analysis and evaluation of the clinical case in question, also due to the hyper specialization in every field of medicine.

In particular, focusing on malpractice claims in the field of cardiology, the considerable complexity of the management of cardiac pathologies implies the importance of a multi- and trans-disciplinary approach.

Indeed, cardiac patients present multiple clinical features, such as the concomitant presence of metabolic, vascular and neurological pathologies, which render these cases particularly complex and risky and it is essential for the medico-legal expert to collaborate with the cardio-pathologist and with the cardiologist or cardio-surgeon in the evaluation of complex liability cases.

Therefore, under this scenario, the role of Legal Medicine is specific and essential to prevent erroneous interpretations of scientific evidence and hasty scientific verdicts.

8.2 Malpractice Claims in the Field of Cardiology and Cardiac Surgery

Medical liability is a significant burden, especially for cardiologists and cardiothoracic surgeons, as described in a recent study, which has shown that average medical liability defense costs are higher in cardiology than in other specialties [3].

The annual percentage of cardiologists and cardiothoracic surgeons facing medical professional liability (MPL) claims reported in that study was respectively

8.6 and 18.9%, compared with 6.6% among general internists and 11.6% among gastroenterologists. In all other specialties, 7.4% of physicians experienced a claim in a year on average (Table 8.1).

In order to improve the quality of care and risk management and to reduce the number of claims and lawsuits, the knowledge of the clinical characteristics and outcomes of lawsuits against cardiologists and cardiothoracic surgeons should be implemented [3–5].

Concerning clinical conditions involved in claims against cardiologists, the aforementioned study highlighted that 69.4% of the included patients prompted a claim for cardiovascular conditions, among which the most common was the acute coronary syndrome (ACS-44.2%), while claims related to management of heart failure or arrhythmia were infrequent (3.2 and 5.5% of all claims, respectively) (Tables 8.2 and 8.3).

Under this scenario, given the considerable complexity of cardio-pathological cases and the essential role of Legal Medicine to prevent erroneous interpretations of scientific evidence, it is fundamental for the medico-legal expert to follow internationally approved medico-legal guidelines governing the ascertainment and evaluation process.

In the past, the multiplicity of regulatory frameworks and operative systems has inevitably caused heterogeneity and fragmentation in the evaluation of medical liability. However, in 2013, the EALM Working Group on Medical Malpractice [6, 7] published International Guidelines in the International Journal of Legal Medicine, to be followed in the assessment of medical liability cases. In 2015, the aforementioned guidelines were adopted by the International Academy of Legal Medicine, to be applied on a global level.

Table 8.1 Annual percentage of cardiologist with MPL claims compared other physician specialties (modified from Mangalmurti et al., Am Heart J 2014, 167: 690–6)

	Cardiologists	Internists	Gastroenterologists	Cardiothoracic surgery	All other physicians
No. of physicians	777	9,880	639	437	29,183
No. of physician years	4155	53,026	3,981	3,187	169,389
Any claim in year (%)	8.6	6.6	11.6	18.9	7.4
P		(P < 0.001)	(P < 0.001)	(P < 0.001)	(P = 0.016)
Claim with indemnity payment in year (%)	1.0	1.2	1.3	3.8	1.7
P		(P = 0.173	(P = 0.097)	(P < 0.001)	(P = 0.003)
Claim with payment $\geq$ $1 million in year (%)	0.1	0.1	0.0	0.2	0.1
P		(P = 0.702)	(P = 0.092)	(P = 0.170)	(P = 0.987)

Table 8.2 Characteristics in MPL claims against cardiologists (modified from Mangalmurti et al., Am Heart J 2014, 167: 690–696)

Characteristics	No. of claims	Percent
Total no. of claims	530	100.0
Indemnity payment		
No	458	86.4
Yes	72	13.6
Severity		
Nonfatal	226	42.6
Fatal	304	57.4
Cardiology procedure involved		
No	321	60.6
Yes	204	38.5
Unknown	5	0.9
Surgery performed		
No	350	66.0
Yes	143	27.0
Unknown	37	7.0
Inpatient status		
Inpatient	379	71.5
Outpatient	149	28.1
Unknown	2	0.4
Patient sex		
Female	202	38.1
Male	320	60.4
Unknown	8	1.5

In such guidelines there is a specific Section focused on the *methods of ascertainment on dead persons,* which must be known and applied by the specialist in Legal Medicine and/or in Forensic Pathology operating as an expert or consultant in cases of alleged medical liability in the cardiologic field regarding death of patients.

8.2.1 Cardiology and Cardiac Surgery Malpractice Claims: Methods of Ascertainment

The method of ascertainment includes the examination of clinical and documentary data, the execution of the autopsy and possible further analyses.

In general, during the evaluation of the clinical and healthcare documentation the need to involve medical specialists in the ascertainment phase could emerge, in order to ensure better definition of the case in question. This is especially recommended in cases of medical liability lawsuits in the cardio-pathological field, given the considerable complexity of these cases.

Therefore, according to the specificities of each case, the medico-legal expert should require the collaboration of multiple specialists, such as cardiologists,

Table 8.3 Clinical conditions involved in MPL claims against cardiologists (modified from Mangalmurti et al., Am Heart J 2014, 167: 690–696)

Conditions	No. of claims	Percent
Total	530	100
ACS	234	44.2
CHF	17	3.2
Arrhythmia	29	5.5
Other cardiovascular	136	25.7
Perioperative	30	5.7
Cardiac medications	26	4.9
Resuscitation	18	3.4
Failure to diagnose aortic dissection	13	2.5
Valvular abnormalities	11	2.1
Procedural complication	7	1.3
Misinterpretation of electrocardiogram or echocardiogram	7	1.3
Failure to diagnose pulmonary embolism	5	0.9
Congenital or pediatric	7	1.3
Miscellaneous	12	2.3
Noncardiovascular	66	12.5
Failure to diagnose cancer	13	2.5
Fall or mechanical injury in hospital or clinic	12	2.3
Infection	10	1.9
Trauma	6	1.1
Gastrointestinal	7	1.3
Mental health	3	0.6
Pulmonary	4	0.8
Miscellaneous	11	2.1
Unknown	48	9.1

cardio-pathologists, cardio-surgeons, clinical geneticists and genetic counselors [8] in order to determinate the precise cause of death. This involvement should preferably take place before the autopsy, as the specialist may profitably contribute to the choice of pre-autopsy examinations, ascertainment and possible integrative examinations.

Moreover, the autopsy is a moment of prime and essential importance in medico-legal ascertainment for medical liability on corpses, as indicated in Recommendation no. R (99) 3 of the Committee of Ministers to Member States on the Harmonisation of Medico-Legal Autopsy Rules, which deals in great detail with the question of autopsy procedures [9].

The investigation, description, documentation and sampling during a medico-legal autopsy should primarily follow medical and scientific principles and simultaneously consider the judicial requirements and procedures.

Given the considerable variation in the way in which pathologists approached cases concerning sudden cardiac death, the Association for European

Cardiovascular Pathology published the Guidelines for autopsy investigation of sudden cardiac death in 2007 [10]. In these guidelines the role of the autopsy in sudden death is precisely defined, and consists in establishing or considering:

- whether the death is attributable to a cardiac disease or to other causes of sudden death;
- the nature of the cardiac disease, and whether the mechanism was arrhythmic or mechanical;
- whether the cardiac condition causing sudden death may be inherited, requiring screening and counselling of the next of kin;
- the possibility of toxic or illicit drug abuse and other unnatural deaths.

Therefore, pathologists are responsible for determining the precise cause of sudden death and the aforementioned guidelines represent the minimum standard required for the assessment of sudden cardiac death, including the protocols for heart examination, for histological sampling and for toxicology and molecular investigations.

Moreover, in 2015, the Royal College of Pathologists [11] has published further guidelines, primarily addressed to consultants performing medico-legal autopsies, which are designed to be a focused bench-top guide with step-by-step examination suggestions. These guidelines have been created to address the needs of the autopsy pathologist dealing with deaths reflecting cardiac disease and to indicate a technical approach and investigation that should prevent criticism of case analysis in medico-legal environments.

Improvements in standards of practice will allow meaningful comparisons between different communities and regions and, most importantly, permit future trends in the patterns of disease causing sudden death to be monitored.

8.2.2 Malpractice Claims in the Field of Cardiology and Cardiac Surgery: Criteria of Evaluation

Following the ascertainment steps, the medico-legal expert, together with the collaborators (i.e. cardiologists, cardio-pathologists, cardio-surgeons, clinical geneticists and genetic counselors) [8], should conduct a comparative evaluation of all data collected and identify the pathological features which have led to the death of the patient. Subsequently, they should collegially reconstruct the physio-pathological pathways composing the actual chain of events, through the link between the initial, the intermediate and the final pathological features.

Moreover, the *ideal medical conduct* which should have been followed by the physician during diagnosis, prognosis and treatment must be reconstructed. The reconstruction of the ideal medical conduct is carried out by analysing a specific scientific sources hierarchy, consisting of the national and international guidelines, followed by consensus documents, operational procedures, evidence based publications and other literature data. Concerning evidence based publications and other literature data (i.e. treatises and articles published in peer reviewed journals preferably with impact factor), it is important to point out that the expert should take into account the so-called *pyramid of evidences* [12]. The scientific sources considered for the evaluation of the case must predate or be contemporary with the facts and accredited by scientific associations or institutions.

Guidelines[1] and *protocols*[2] have particular importance and they are on the top of the scientific sources hierarchy [13].

In the field of cardiology and cardio-pathology there are many useful guidelines and protocols in which diagnosis, prognosis and therapy of various problems (like acute myocardial infarction, acute coronary syndromes) are described in detail [14–16]. However, the strict observance of such guidelines and protocols is not sufficient, because the cardiologist must take into account all clinical signs and all instrumental evidence, which could indicate the necessity to deviate from standard conduct.

Therefore, it is important to keep in mind that adherence to guidelines does not necessarily constitute a full defense against a claim of negligence, and on the other hand, that the decision to deviate from guidelines does not necessarily imply negligence, because some clinical situations may imply the need for a deviation from standard guidelines. The ultimate decision about the patient must be made by the physician [14].

Once the "ideal conduct" has been reconstructed, the medico-legal expert must conduct a comparative analysis between ideal conduct and true conduct.

The evaluation of the correctness of the various diagnostic, prognostic and therapeutic phases may lead to the identification of *errors*[3] and/or *non-observances of rules of conduct*,[4] which must be classified as described (errors in consent, diagnosis, prognosis, surveillance, therapy) [6].

In the absence of errors and/or non-observances, the negative event is classified as a *no fault medical accident* which implies no medical responsibility.

This concept is related to the importance of differentiation between error and complication. In fact, the concept of ***error*** derives from a deviation by the medical

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[1]*Guidelines* = statements systematically developed to assist practitioner's decision based on the best available evidence at the time the document was prepared.

[2]*Protocols* = rigid and predefined patterns of diagnostic and therapeutic operational behaviors.

[3]A medical *error* is defined as a violation of a rule shared by the national and/or international medical community. It can be classified into three types.

- *Real error* = a material error of omission or commission, due to violation of universal and/or epidemiological scientific laws, or of consolidated rules of experience and competence.
- *Pseudo-error* = an apparent error due to a general absence of scientific knowledge on a specific issue at the time of the event. It can also be related to an unpredictable and inevitable event.
- *Conscious error* = an error made by the healthcare professional in full conscience.

[4]*Non-observances of required rules of professional medical conduct* are defined as the non-observance of the rules of scientific medicine as taught in degree courses and in specialization schools, which are permanently updated through the scientific literature, congresses and training courses. These rules are mainly orientative in nature and must be applied to each individual case, according to the diagnostic and therapeutic features of the clinical picture.

doctor from the “ideal conduct” described by the scientific community by means of guidelines, protocols and medical literature. By contrast, when the negative outcome for the patient derives from the absence of scientific knowledge in the specific field, or from an unpredictable and inevitable event, or from an intrinsic therapeutic risk, such a negative event must be classified as a ***complication***. In this case, the healthcare professional cannot be held responsible for the event.

In case of error it is important to verify whether there is a *cause for justification* of the error, for example, the special technical difficulty of a surgical operation.

Finally, the causal link between error and event must be identified, by means of the criterion of scientific probability described by universal scientific laws, statistical laws or rational credibility.

In particular, the degree of probability of the causal link must be identified and counterfactual reasoning must be applied.

The conclusions must be expressed in terms of near certainty, probability or exclusion of the causal value-link.

8.2.3 Cardiology and Cardiac Surgery Malpractice Claims. Proposal of Practical Cases

Herein it is proposing a series of 4 cases in which the prosecutor asked the medico-legal expert to identify the cause of death, to evaluate errors and/or non-observance of required rules of professional medical conduct and, in case of error identification, to establish the existence of the causal value of the error and the relationship of an actual causal link between error and event.

Case 1
A baby girl born at 38 weeks of gestation was subjected to echocardiography after less than a month from birth, which revealed left ventricular hypokinesis without dilated ventricle. In the following months many echocar-diographies were performed, without evidence of significant alterations.

At the age of 9 months she was hospitalized for heart failure and after three days she died during transport via helicopter rescue to a hospital where ECMO (Extra Corporeal Membrane Oxygenation) was available.

The prosecutor ordered the autopsy and the appointed medico-legal expert, in accordance with the aforementioned guidelines, requesting the collaboration of an expert cardio-pathologist who dissected the heart in line with standard text guidelines [10]. Moreover, the heart-lung block was conserved and histological samplings of all organs, together with heart and spleen sampling for molecular studies, were performed.

The necroscopic ascertainment revealed the presence of cardiomegaly with biventricular marked dilatation and endocardial thickening (Fig. 8.1) together with a framework of congestive heart failure with pulmonary oedema, hydrothorax and hydropericardium. The histologic examination showed significant endocardial

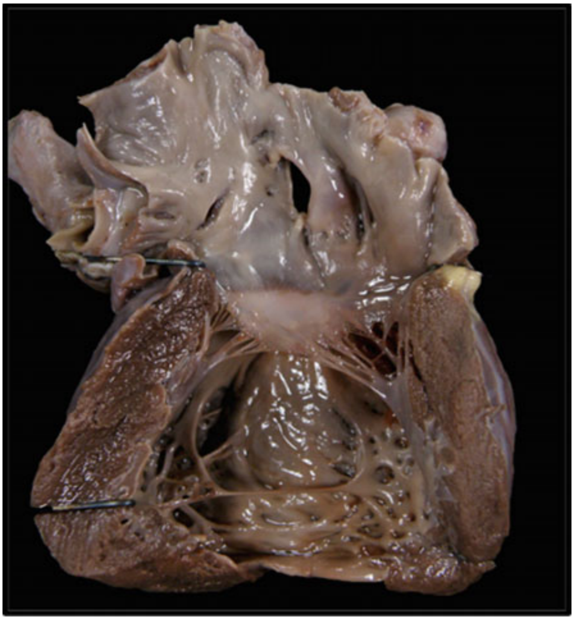


Fig. 8.1 Cardiomegaly with biventricular marked dilatation and endocardial thickening

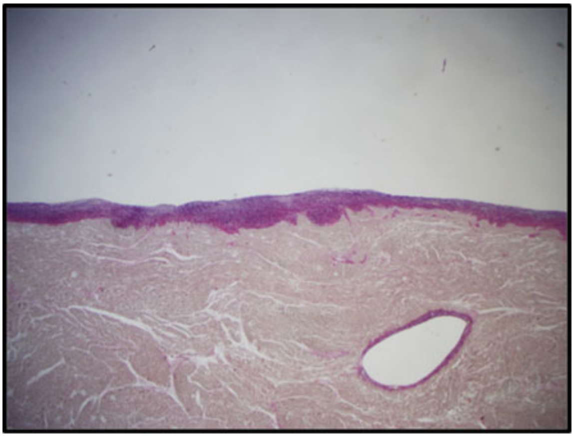


Fig. 8.2 Endocardial fibroelastosis (Weigert-Van Gieson 10X)

fibroelastosis (Figs. 8.2 and 8.3) and molecular studies detected the Cytomegalovirus genome (Figs. 8.4 and 8.5).

The process of analysis and comparative evaluation between *ideal* versus *true* conduct lead to the identification of a *prognostic error*, since a cardiac transplantation in a baby with a history of intrauterine CMV infection and a suspected diagnosis of endocardial fibroelastosis was not planned.

However, the evaluation of the causal link between error and death and the application of counterfactual reasoning excluded the causal link in terms of near certainty. In fact, even in the case in which the baby had been inserted in the

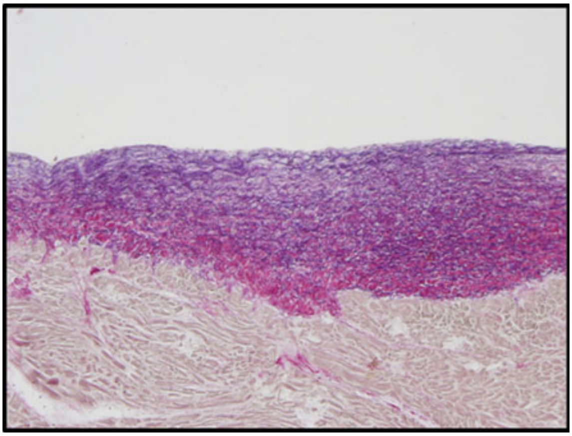


Fig. 8.3 Endocardial fibroelastosis (Weigert-Van Gieson 50X)

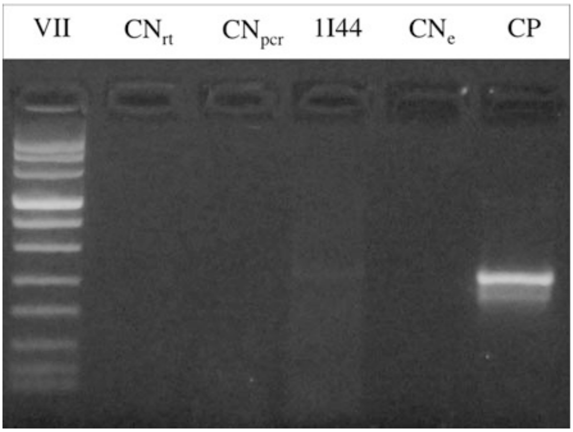


Fig. 8.4 Cytomegalovirus genome detected through molecular studies

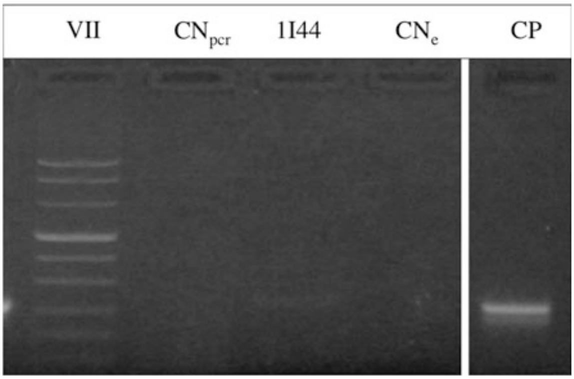


Fig. 8.5 Cytomegalovirus genome detected through molecular studies

transplant list after a correct diagnosis of endocardial fibroelastosis, death would have occurred anyway, since endocardial fibroelastosis is a serious heart disease with poor prognosis and the waiting time for transplant patients already on the list was not less than 108 days.

Case 2

A 65-year-old obese woman suffering from psoriatic arthritis, hypertension and impairment of peripheral circulation was admitted to the hospital for acute low back pain. During hospitalization the patient suffered recurrent episodes of vomiting, hypertensive peaks, persistent fever, hemoglobin drop, episodes of respiratory failure, bilateral pleural effusion, abnormal repolarization and a coronary catheterization was indicated. A few hours after the coronary catheterization the patient suffered a hypertensive peak followed by hypotension, tachycardia and after the appearance of pulseless electrical activity she died.

The prosecutor ordered the autopsy and the appointed medico-legal expert, in accordance with the aforementioned guidelines, required the collaboration of an expert cardio-pathologist who dissected the heart and the vessels of the right iliac-femoral axis in line with standard text guidelines [10] after formalin fixation. The necroscopic ascertainment revealed a massive retroperitoneal bleeding infiltration extended from the right psoas muscle to the right iliac fossa and pelvic cavity with a massive infiltration of perivescical fat departing from ipsilateral femoral vein, together with a transmural tear in correspondence with the external right iliac vein bifurcation and a transmural tear in the superficial femoral artery at the point of the right external iliac vein bifurcation (Figs. 8.6 and 8.7).

The cause of death was identified as haemorrhagic shock resulting from retroperitoneal hematoma due to a tear of the left femoral vascular axis in recent cardiac catheterization with right femoral access.

The process of analysis and comparative evaluation between *ideal* versus *true* conduct did not lead to the identification of possible error and/or non-observance of required rules of conduct. In fact, given the presence of acute pulmonary oedema and the increase in the troponin level, an acute coronary syndrome was suspected and the coronary catheterization was correctly indicated. During the catheterization a tear of the left femoral vein was accidentally produced and the blood made its way through the thin retroperitoneal space of the posterior abdominal wall.

Despite the correct technical execution of the exam and the adequate post-surgery haemostasis, the literature reports an incidence of retroperitoneal haemorrhage of about 0.5%. Therefore, given the correctness of the various diagnostic, prognostic and therapeutic phases, the negative event was classified as a *no fault medical accident*, which implies no medical responsibility. In fact, when the negative outcome for the patient derives from an unpredictable and inevitable event, or from an intrinsic therapeutic risk, such a negative event must be classified as a *complication* and the healthcare professional cannot be held responsible for the event.

Case 3

A 63-year-old obese man suffering from obstructive coronary artery disease and atherosclerosis was admitted to the hospital, because of low back pain persisting for

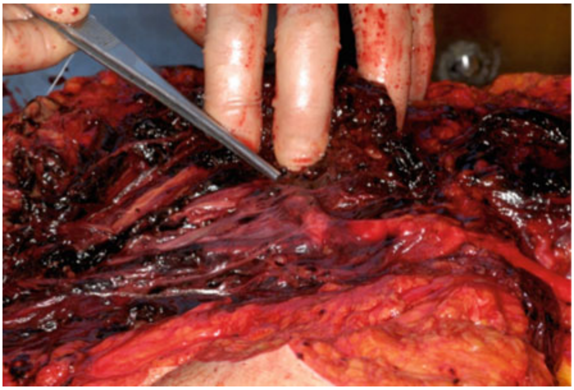


Fig. 8.6 Massive retroperitoneal bleeding infiltration extended from the right psoas muscle to the right iliac fossa and pelvic cavity with a massive infiltration of perivescical fat departing from ipsilateral femoral vein

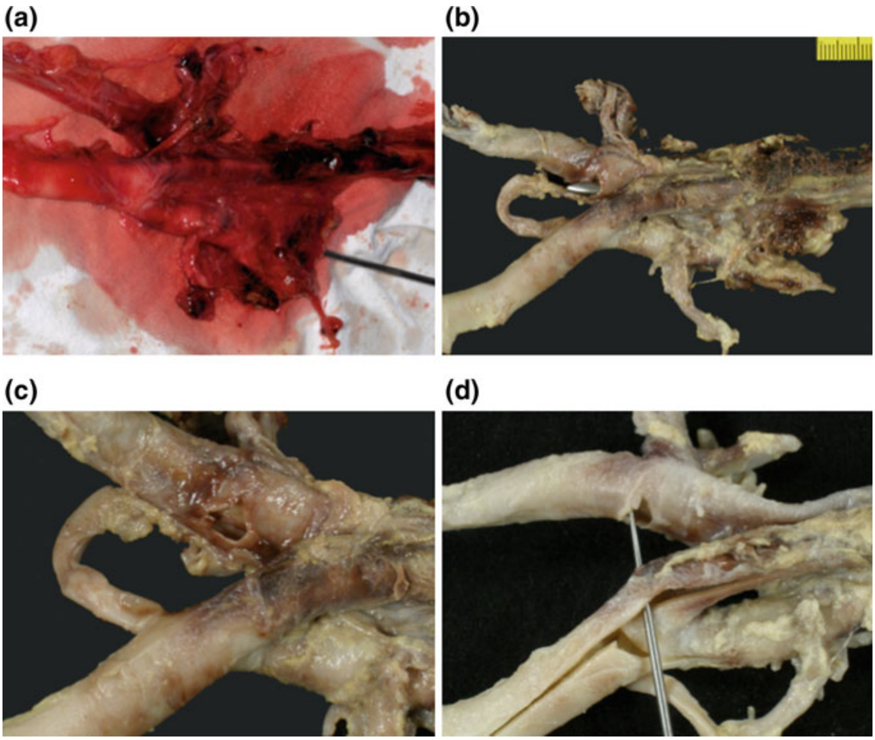


Fig. 8.7 Transmural tear in correspondence of both the external right iliac vein bifurcation and the superficial femoral artery at the point of the right external iliac vein bifurcation, before (**a**) and after formalin fixation (**b**–**d**)

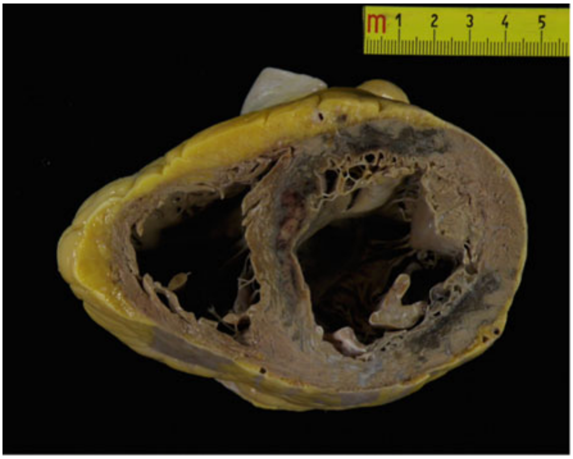


Fig. 8.8 Myocardium necrosis, sclerosis and transmural hemorrhagic infarction

15 days and chest pain with breathing difficulties during the afternoons. During hospitalization the patient suffered acute and subacute myocardial infarction and subsequently died of cardiogenic shock.

The prosecutor ordered the autopsy and the appointed medico-legal expert, in accordance with the aforementioned guidelines, required the collaboration of an expert cardio-pathologist. The heart was fixed in formalin 10% and then dissected by the cardio-pathologist in line with standard text guidelines [10]. The observation of a transverse section of the heart revealed the presence of necrosis involving more than 50% of the left ventricle myocardium, posterior and septal ventricular myocardial sclerosis and anterior-septal and posterior transmural hemorrhagic infarction of the left ventricle with papillary muscle involvement (Fig. 8.8).

The histologic examination of the heart samples highlighted the presence of obstructive plaque of the right coronary artery (Fig. 8.9a) and coagulative necrosis of the myocardium (Fig. 8.9b) with neutrophil infiltration (Fig. 8.9c).

The process of analysis and comparative evaluation between *ideal* versus *true* conduct led to the identification of *therapeutic/prognostic error* consisting in substantial delayed revascularization and in the transfer of a hemodynamically unstable patient with severely impaired left ventricular function to a non-equipped center.

However, after the application of counterfactual reasoning, the causal link between the errors and death was excluded, since the clinical situation was so strongly compromised at admission and the probability of a successful revascularization so low that it was not possible to conclude, in terms of near certainty, that an early revascularization would have saved the patient.

Case 4

A 30-year-old pregnant woman (37°+1 weeks of gestation) was admitted to the emergency room after a lipothymic episode with loss of consciousness, dyspnea

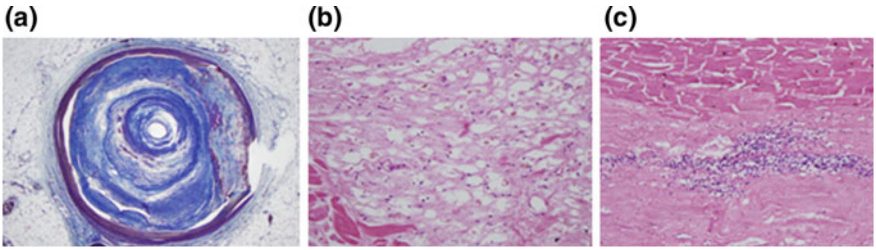
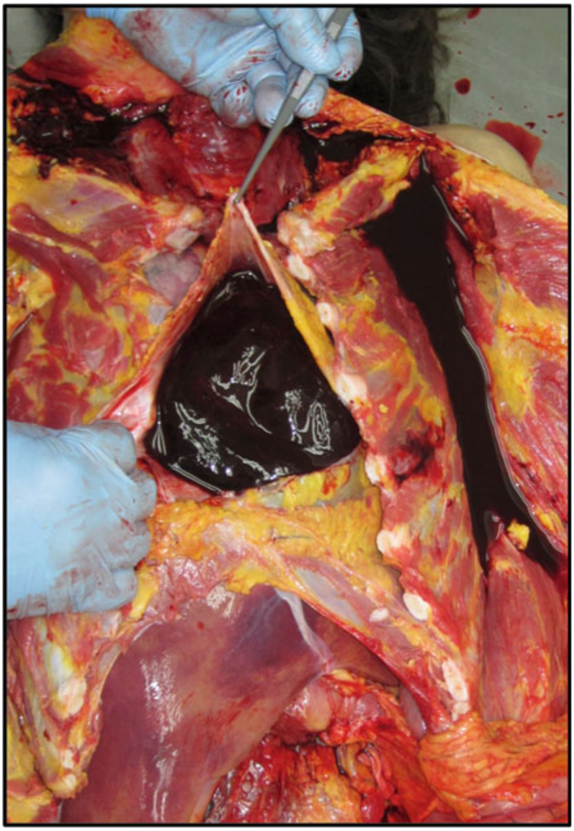


Fig. 8.9 Obstructive plaque of the right coronary artery (AZAN 15,6X) (**a**); Coagulative necrosis of the myocardium (EE 160X) (**b**); Coagulative necrosis of the myocardium with neutrophil infiltration (EE 80X) (**c**)

Fig. 8.10 Cardiac tamponade



and dorsal pain during inspiration, and after a negative clinical examination she was discharged. The following day she was admitted again to the emergency room for dorsal pain during inspiration and was discharged again after a negative clinical examination.

Fig. 8.11 Intimal transverse tear

On the third day she was rescued at home and the emergency medical services identified the presence of Pulseless Electrical Activity. The patient was therefore admitted again to the hospital and transferred to the operating room for an emergency cesarean. After the cesarean the patient died. The prosecutor ordered the autopsy and the appointed medico-legal expert, in accordance with the aforementioned guidelines, requested the collaboration of an expert cardio-pathologist. During autopsy a cardiac tamponade was identified (Fig. 8.10). The heart and the aorta were fixed in formalin 10% and then dissected by the cardio-pathologist in line with standard text guidelines [10]. The presence of both ascending aorta and aortic arch dissection with an intimal transverse tear of about 3 cm, located about 5 mm above the aortic valve plane, was identified (Fig. 8.11).

The cause of death was identified as obstructive shock related to cardiac tamponade from type A aortic dissection with aspects of cystic degeneration of the tunica media in a pregnant woman.

The process of analysis and comparative evaluation between *ideal* versus *true* conduct led to the identification of a *diagnostic error*, since the origin of the lipothymia and of the dorsal pain weren't investigated.

However, the evaluation of the causal link between error and death and the application of counterfactual reasoning excluded the causal link in terms of near certainty. In fact, it was not possible to state that the death would not have occurred if the diagnosis had been made correctly.

8.3 Conclusion

Medical liability has become a fact of life in the physician's modern practice and each malpractice claim gives rise to a scientific challenge.

In this scenario, the role of the forensic pathologist and/or of the cardio-pathologist is divided into two phases and internationally shared guidelines related to both of these phases must be known and followed.

In the first phase, during the medico-legal autopsy, the pathologist should:

- establish if death is related to cardiac disease or another process;
- establish the nature of the cardiac disease;
- consider whether the cardiac disease is related to systemic disease;
- consider whether any cardiac disease is likely to be inherited;
- consider whether the cardiac disease is related to illicit activities;
- consider whether the cardiac disease could have been treated.

In the second one, with regard to the evaluation of medical liability, the role of the pathologist is:

- to identify pathological features;
- to identify damage;
- to reconstruct the physio-pathological pathways and the *ideal medical conduct*;
- to reconstruct and verify the *true medical conduct*;
- to identify and classify *errors* and *non-observances*;
- to evaluate *ex-ante* the error and to identify possible causes of justification;
- to evaluate the causal value and causal link between error and damage/event;
- to identify the degree of probability of causal value and causal link.

Moreover, standardized data collection and reporting processes are needed to build up an accurate national picture of the problem. In fact, measuring the consequences of medical care on patient outcomes is an important prerequisite for creating a culture of learning from our mistakes.

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Chapter 9
New Molecular and Innovations in Forensic Pathology

Toshikazu Kondo, Yuko Ishida, Akihiko Kimura and Mizuho Nosaka

Abstract In forensic pathology, the novel molecular biomarkers as well as innovative devices and techniques are always explored for the correct diagnosis of the cause of death, postmortem intervals, wound ages, and so on. In wound age determination, application techniques, and target cells and molecules are main elements. Bone marrow-derived cells such as fibrocytes and endothelial progenitor cells can contribute to skin wound healing, thus implying that those cells would be candidate cells for wound age determination. Analyses of aquaporins that are water channels in mammals would be helpful for the differential diagnosis of saltwater and freshwater drowning. Circadian rhythm is well controlled by the time-dependent expression of "clock genes", indicating that analyses of the biological clock would be powerful methods for the estimation of the time of death. Postmortem computed tomography (PMCT) has rapidly and widely spread, and assists forensic autopsy. Actually, it is unnecessary to mention that the advancement of molecular and imaging innovations is able to contribute to the progress of forensic pathology.

9.1 Introduction

Forensic medicine is one of the general medicines that is composed of the following scientific parts: forensic pathology, toxicology, and genetics (serology). The history of clinical medicine is correlated with the advances of basic researches, because no advances in basic research lead to lack of development of clinical medicine. Likewise, forensic medicine is applied medical science, but not basic science. Thus, it is always necessary that novel and basic knowledge is applied to forensic practice

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as much as possible. In 1985, Jeffreys et al. reported that DNA fingerprinting would become one of the most powerful tools [1]. Since then, genetic polymorphism-based personal identification and individualization have been progressively promoted in forensic practices. In forensic toxicology, the development of the analytical apparatuses such as GC-MS and LC-MS/MS strongly contributed to the detection of drugs and poisons in minute biological samples.

In forensic pathology, conventional macroscopic observations and histopathological analyses are still the main tools. However, forensic pathologists are always exploring the novel biomarkers and innovative techniques for the correct diagnosis of the cause of death, postmortem intervals, wound ages, and so on. From the 1980s to 1990s, lots of small glycoprotein molecules, so-called "cytokines", were cloned in immunology and biochemistry. At that time, numerous clinical and basic scientists tried to explore the relationship between diseases and cytokines. At the present, the pathophysiological roles of several cytokines have been cleared, and molecular target biomedicines have been developed. Thus, for these 20 years, from the viewpoints of forensic pathology, we have focused on cytokines that play important roles in cell-cell communication in the immune, endocrine, central nervous systems, and so on. In particular, immunohistochemical techniques are utilized for the detection of cytokines in the field of forensic pathology. At present, we have demonstrated that some cytokines are good molecular markers for the estimation of skin wound ages [2, 3]. Moreover, our recent studies implied that cytokines also provided useful information for the estimation of thrombus ages [4–6]. In addition to cytokines, we examined the expression of several types of aquaporins in human organs [7–9]. Analyses of aquaporin expression would be helpful for the differential diagnosis of saltwater and freshwater drowning. In diagnosing the cause of death, Maeda and colleagues carried out the comprehensive analyses of biochemical markers, endocrinological hormones, and cytokines using biological fluid samples such as blood, urine and pericardial effusion obtained from forensic autopsy cases [10, 11]. Their study would contribute to improve the accuracy in diagnosing the cause of death and to estimate the process of death, indicating the importance of forensic molecular biochemistry.

There are still many issues remaining in the field of forensic pathology. For example, the estimation of postmortem intervals is carried out based on the appearance of postmortem phenomena such as postmortem rigidity, hypostasis and rectal temperature, which has made almost no progress in 100 years. There are no reports about molecular-basis estimation of postmortem intervals. The circadian rhythm is controlled by the time-dependent expression of several genes, the so-called "clock genes". Thus, we performed experimental and practical studies by analyzing the expression of clock genes such as Per2 and Bmal1 in several organs, and demonstrated that gene expression analyses of the biological clock could be powerful methods for the estimation of the time of death. This study gives lots of possibility to the estimation of postmortem intervals from the aspects of molecular biology.

The application of medical devices to forensic autopsy is always necessary in order to improve the quality of forensic diagnosis. Previously, we employed a binocular surgical microscope at forensic autopsy in order to examine various types of mechanical skin wounds. Simultaneously, the microscopic images visualized on a monitor were photographed and recorded. This system could detect minute alterations, such as small epidermal tags of abrasions and continuous bridges of connective tissue in lacerations that could hardly be recognized with the naked eye. Alternatively, this system could also contribute to the teaching of new forensic pathologists and medical students. In clinical medicine, the development of imaging techniques such as computed tomography and MRI have greatly contributed to the improvement of image diagnosis. Similarly, it is well known that postmortem radiological examination is useful for the detection of bone fractures and metal objects in the body.

Herewith, we would like to introduce our observations on new molecular and imaging innovations in forensic pathology.

9.2 Wound Age Determination

9.2.1 Historical Background

The skin is one of the important tissues for the prevention of external insults such as microbiological infection and mechanical stresses. Thus, the disruption of skin construction and function can easily cause severe systemic illness, resulting in lethal events. Thus, in forensic practices, forensic pathologists can get various kinds of information from the nature of the skin. For examples, the color of postmortem lividity would give some clues for diagnosing the cause of death. Moreover, it is also important to observe the disruption of skin construction, and to find so-called injuries or wounds. When a wound is found in forensic autopsy, it is always required to determine the type of wound, wound vitality, wound age and relationship with the cause of death. Especially, the determination of wound age is one of the classical but still modern issues in forensic pathology. Historically, Walcher [12] and Orsos [13] theoretically proposed the necessity and importance for the determination of wound vitality or wound age in forensic practice. In the 1960s, Raekallio performed pioneer works on wound age determination through the evaluation of enzymatic activities at skin wound sites [14]. So as to follow, Berg et al. [15, 16] demonstrated the relationship between wound vitality and the levels of serotonin and histamine at the wound edges. Thereafter, immunohistochemical techniques as well as biochemical methods have been applied to wound age determination as the major tool. The principle of wound age determination is closely related with the process of skin wound healing. In mammalians including human beings, skin wound healing is primitive and indispensable biological phenomena. The process of skin wound healing consists of three phases such as inflammation, proliferation, and maturation. These phases consecutively proceed with well-organized interaction between various tissues and cells. In the process of skin wound healing, leukocyte recruitment and the remodeling of extracellular matrices are hallmark events. Thus, several independent groups found useful markers such as collagen, fibronectin, adhesion molecules, and so on [2, 3]. Moreover, cytokines and growth factors play essential roles in each phase of skin wound healing, suggesting that those molecules would be a good candidate for wound age determination.

9.2.2 How to Explore Several Candidate Molecules for Wound Age Determination

In order to explore several candidates for wound age determination, animal experiments using genetically engineered animals can be carried out [17]. Among a group of cytokines, IL-1, IL-6, IFN-γ, and TNF-α are the major inflammatory cytokines that are crucially involved in the development of inflammatory diseases such as rheumatoid arthritis, ulcerative colitis, pneumonia, and so on. In line with this, our groups have proceeded with basic and practical projects for wound age determination and investigated skin wound healing in several kinds of knock-out mice. The local production of IL-6 would be essential for skin-wound healing process as evidenced by the fact that the genetic disruption of IL-6 impaired skin wound healing [18]. Although it is well known that there are several redundancies in the biological activities between IL-1 and TNF-α, these tow cytokines have differential roles in skin wound healing. The enhancement of IL-1 activity through the absence of the IL-1 receptor antagonist (IL-1ra) retarded skin wound healing with a concomitant of the exaggerated inflammatory responses after injury [19]. On the contrary, the lack of TNF-signaling promoted skin wound healing with the acceleration of angiogenesis and collagen production [20]. During skin-wound healing, the signal pathway of TGF-β, a potent fibrogenic cytokine, is indispensable for collagen production, and it is regulated through other cytokine signal pathways such as interferon-γ (IFN-γ) and NF-κB [21]. Moreover, the biological significance of chemokines were investigated in skin-wound healing. In particular, the loss of CX3C chemokine receptor 1(CX3CR1) inhibited macrophage recruitment and myofibroblast function in skin-wound healing [22].

9.2.3 Cytokines and Chemokines

Our experimental study using mice suggested that proinflammatory cytokines such as IL-1, IL-6 and TNF-α could become powerful indicators for wound age determination [23]. Thus, several practical studies have been carried out using human skin wounds with known different ages, because practical application is the goal for every study in forensic medicine. As set out in Table 9.1, our group showed that the detection of IL-1α-positive infiltrating cells such as neutrophils and macrophages would give significant information to determine wound age ranging from 4 h to

Table 9.1 Summary for our previous studies

Markers	Positive ratio	Wound age
IL-1α	>30%	4 h–1 days
COX-2	>50%	8 h–2 days
IL-8	>50%	1–4 days
MCP-1	>30%	1–7 days
MIP-1α	>40%	1–9 days
MMP-9	>30	3–14 days
MMP-2	>20	7–12 days
VEGF	>50%	7–14 days
Ubiquitin	>30%	7–14 days
ORP-150	>40%	7–21 days

1 day [24]. In line with our observations, Grellner's group indicated that inflammatory cytokines such as IL-1, IL-6, and TNF-α would be useful for the determination of wound ages in the early phase after injury [25].

Cell migration is well regulated under the guidance of chemotactic factors. Cytokines possessing chemotactic activities is called chemokine. Chemokines have four main subfamilies such as CXC, CC, CX3C and XC family based on the difference of amino acid sequences at N terminal. All of these proteins interact with G protein-linked transmembrane receptors called chemokine receptors, which are selectively found on the surfaces of their target cells. Matsushima and colleagues firstly identified IL-8/CXCL8 with chemotactic activity against neutrophils and other cells [26]. Recent studies demonstrated that several kinds of chemokines played important roles not only in leukocyte recruitment, but also in reepithelialization, tissue remodeling, and angiogenesis [26]. We focused on IL-8/CXCL8, monocyte chemoattractant protein (MCP)-1/CCL2 and macrophage inflammatory protein (MIP)-1α/CCL3 predominantly recruit macrophages/monocytes. The gene expression of these chemokines is enhanced in murine skin wounds [18–22], suggesting that they might be candidates for the marker for wound age determination. Indeed, these chemokines could be strongly detected in skin wounds with the postinfliction interval of 1–4 days, thus suggesting that they are available as markers of wound age determination [27] (Table 9.1). More than 30 chemokines and their receptors have so far been found, and investigating other chemokines would develop the research field of wound age determination.

9.2.4 *Bone Marrow Cells and Skin-Wound Healing*

9.2.4.1 Fibrocytes

It is well known that bone marrow seems to be the origin of hematopoietic cells such as erythrocytes, leukocytes and platelets, because stem cells/progenitor cells that can be differentiated into specialized cells are present in bone marrow for the renewal of circulating hematopoietic cells. Moreover, non-hematopoietic stem cells/progenitors cells are also present in the bone marrow. In particular, when organ/tissues are damaged, bone marrow-derived non-hematopoietic stem cells/progenitor cells are recruited at the damaged sites, and directly or indirectly contribute to tissue repair including skin wound healing and fibrosis of kidneys and lungs [28–30]. During the process of skin wound healing, cell population is changed in accordance with post-wounding time. Thus, as the conventional method, it is usual to find neutrophils, macrophages, T lymphocytes and myofibroblasts in human skin wounds. In line with this, the detection of bone marrow-derived non-hematopoietic stem cells/progenitor would give additional significant information for the determination of wound ages.

Fibroblasts, being present in the connective tissue, play important roles in the proliferation phase of skin wound healing through the production of matrix proteins such as collagen and fibronectin. Traditionally, the activation of resident fibroblasts in the connective tissues is considered to be essential for the remodeling of damaged tissues, suggesting that activated fibroblasts are migrated from adjacent tissue. However, Bucala and colleagues expectedly found large numbers of fibroblast-like spindle cells as well as circulating inflammatory cells in the early phase of wound healing using a wound chamber model, suggesting that fibroblast-like spindle cells might be recruited from peripheral circulation. They could determine that the fibroblast-like cells were not conventional fibroblasts. The fibroblast-like spindle cells expressed both CD45 (common leukocyte antigen) and CD34 (a marker for hematopoietic stem cells), indicating that these cells belonged to one type of leukocyte derived from bone marrow. Moreover, these cells also expressed mesenchymal antigens such as collagen I, collagen III, vimentin and fibronectin. Bucala et al. have named these cells “fibrocytes”, one of the bone-marrow-derived stem/progenitor cells [31]. Several lines of accumulating evidence demonstrated that fibrocytes could contribute to tissue fibrosis in kidneys, lungs and skin wound healing through the differentiating to myofibroblasts [28–30].

Ishida et al. [32] focused on the recruitment of fibrocytes at the wound sites during the healing process. In unwounded specimens, $CD45^+$/Col I^+ fibrocytes were not detected. Dual-positive fibrocytes initially appeared in skin wound samples with a postinfliction interval of 4 days, and their number increased in lesions with advances in wound age (Table 9.2; Figs. 9.1a and 9.2). Thus, fibrocytes would be one of the novel marker cells for wound age determination in forensic practices.

Table 9.2 Wound age and bone marrow-derived cells

Bone marrow-derived cells	Initial appearance	Wound age (cell number)
Fibrocytes	4 days	9–14 days (>15)
EPC	2 days	7–12 days (>20)

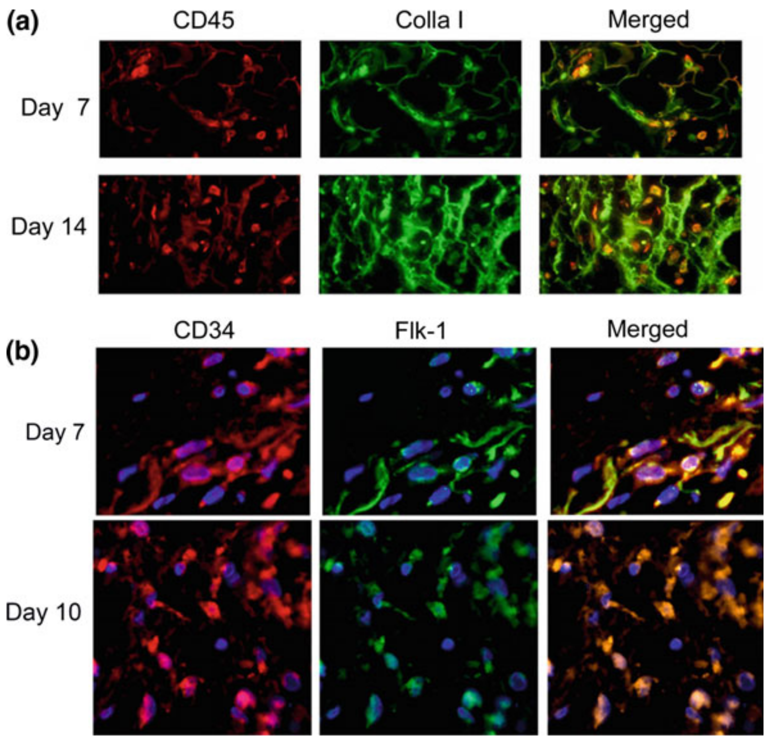

Fig. 9.1 Detection of fibrocytes (**a**) and EPCs (**b**) in human skin wounds. (Cited from Refs. [32, 36] with kind permission of Springer)

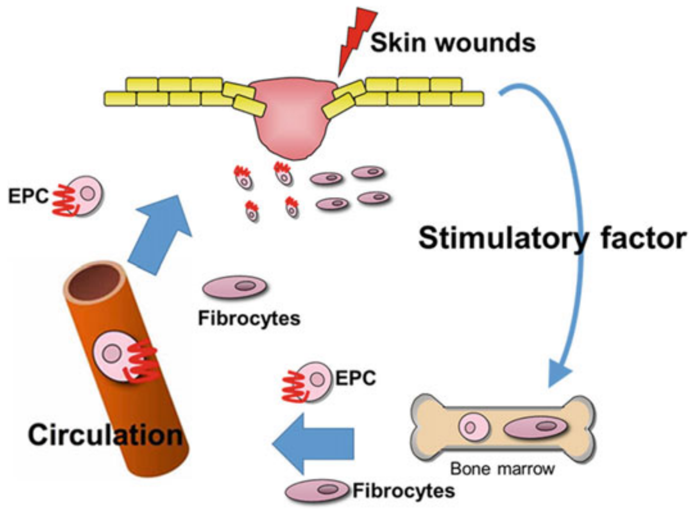

Fig. 9.2 Scheme of recruitment of fibrocytes and EPCs during skin wound healing process

9.2.4.2 Endothelial Progenitor Cells (EPCs)

In skin wound healing and tumor metastasis, neovascularization is indispensable. Several kinds of angiogenic factors such as VEGF and bFGF promote the migration of endothelial cells and the following tube-like formation. In 1997, Asahara and colleagues [33] have identified a unique cell population which could differentiate into mature endothelial cells. These observations implied that endothelial precursor cells seemed to be present in the peripheral blood circulation, and to be recruited to tissues and organs. These cells shared certain antigenic determinants, including Flk-1, Tie-2, c-Kit, Sca-1, CD133, and CD34, thus implying that these were derived from bone marrow. Eventually, these cells are named endothelial progenitor cells (EPCs). Actually, EPCs play important roles in tumor angiogenesis, ischemic vasculogenesis, and vascular homeostasis. EPCs can be recruited and partially incorporated into new vessels in granulation tissues and tumor metastatic lesions, thereby promoting neovascularization [34]. Recently, EPCs sorted from mononuclear cells isolated from the peripheral blood of the patients are one of the tools used in cell therapy against ischemic diseases in clinical practices [34].

From the viewpoint of forensic pathology, it is easy to recognize that EPC would be one of the candidate cells for wound age determination. Our previous study using mice demonstrated that EPC were recruited at wound sites [35]. Moreover, in our practical studies using human skin wound specimens with different ages, $CD34^+$ and Flk-1^+ EPCs were never found in unwounded skin samples. Subsequently, EPCs initially appeared in 2-day-old skin wounds, followed by an increase of EPC numbers in accordance with advances in wound age. Morphometrical analyses revealed that the EPC number of >20 s would indicate a wound age of 7–12 days (Table 9.2; Figs. 9.1b and 9.2). Taken together, our observations indicate the detection of EPCs would be useful for wound age determination [36].

Moreover, it is interesting to explore the mechanism of mobilization and recruitment in EPC, from bone marrow and peripheral blood circulation. There are several chemotactic factors such as chemokines and growth factors for EPCs [35], EPCs expressed several chemokine receptors. In particular, our animal experiment demonstrated that EPC expressed CC chemokine receptor 5 (CCR5), and EPC massively accumulated at the wound sites of the skin. In mice lacking CCR5, EPCs were less recruited during the skin wound healing process, eventually resulting in delayed skin wound healing with attenuated neovascularization. CCR5 has three different ligands such as CCL3, CCL4 and CCL5, meaning that the chemokine systems have redundancy in the ligand-receptor interaction. CCR5 ligands such as CCL3, CCL4, and CCL5 can also use another chemokine receptor, CCR1 [37]. Each chemokine receptor employs multiple signaling pathways such as Jak/Stat, MAPK, and PI3 K, depending on the ligand and cell type involved [38]. Indeed, CCL5, but not CCL3 or CCL4, could promote EPC migration in vitro. These observations suggest that the interaction between CCL5 and CCR5 is specifically involved in EPC homing from the circulation to injured sites. Thus, our experimental study suggests the possibility that the detection of CCL5 and CCR5 might provide valuable information for skin wound age determination.

9.2.5 Autophagy and Wound Vitality

In the wound examination of forensic autopsy, it is always necessary to clarify how a wound (wounds) is (are) related to the death. Thus, when a wound is found, forensic pathologists always have to judge the presence of vital reactions such as hemorrhages in the wound. However, in cases where death has occurred immediately after the injury, it is often difficult to differentiate antemortem wounds from postmortem damage. Despite numerous studies on the vital reactions of skin wounds [39–41], more reliable and applicable methods for the determination of wound vitality are expected.

Autophagy is one of the intracellular catabolic processes for degraded protein with the formation of autophagosomes, which are delivered to the lysosome for degradation and recycling [42, 43]. For the assessment of autophagy, Western blotting analysis is mainly employed with targeting microtubule-associated protein 1 light chain 3 (LC3-I). In the process of autophagy, LC3-I is conjugated with a lipid, which is called LC3-II. LC3-II is one of the membrane components in autophagosomes involving degraded proteins [43]. Concomitantly, the degradation of sequestosome 1 (p62), which plays important roles in transferring ubiquitinated protein to autophagosomes, is associated with the autophagy process [43]. Thus, autophagy promotes the attenuation of p62 [43]. These observations indicate that both LC3 and p62 are indispensable as the marker for autophagy evaluation. Various kinds of stimulation including infection and mechanical stress enhance autophagy in order to maintain homeostasis [44, 45].

Thus, in order to explore novel markers for vitality of acute mechanical wounds, we investigated autophagy in skin wounds of both mice and humans. In our experimental study, mouse skin wounds aged >5 h demonstrated the reduction of LC3-II and reciprocal increase of p62, compared with the uninjured skin tissues (Fig. 9.3a). On the contrary, neither LC3-II nor p62 levels could be detected in postmortem skin wound samples. These observations implied that autophagy might be suppressed at the antemortem wound sites (Fig. 9.3b). It is necessary to confirm whether this hypothesis would be applied to human skin wound samples. In unwounded and wounded human skin tissues from identical individuals, we examined the expression of LC-3 and p62 using Western blotting analyses. In line with results obtained from animal experiments, LC3-II expression obviously decreased in skin wound samples, compared with the unwounded ones (Fig. 9.3c, d). On the contrary, intradermal p62 levels were more elevated in the wounded skin tissues (Fig. 9.3c, e). Thus, analyzing the intradermal expression of LC3 and p62 would contribute to differentiate antemortem skin wounds from postmortem skin damages in forensic practices, indicating that these molecules are good targets for the determination of wound vitality [46].

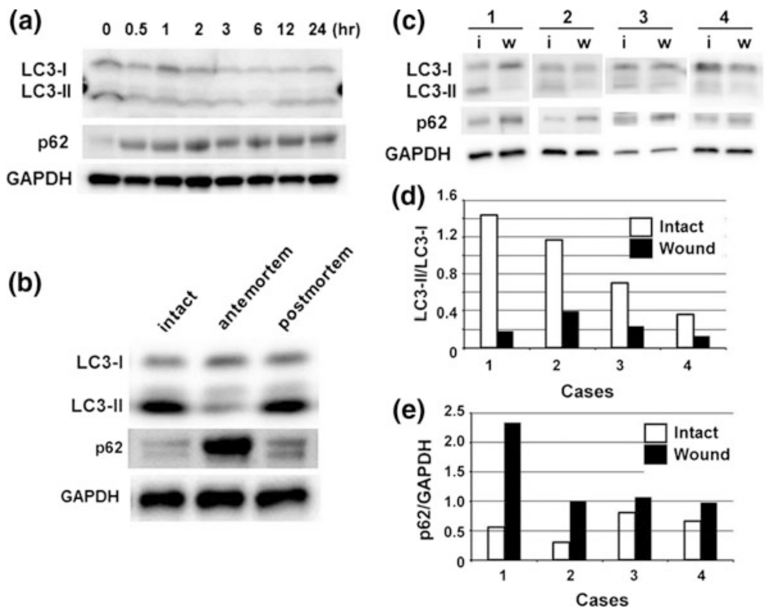

Fig. 9.3 **a** Temporal expression of LC3-I, LC3-II and p62 during murine skin wound healing. **b** Detection of LC3-I, LC3-II and p62 in intact skin, antemortem wounds, and postmortem wounds in mice. **c** Detection of LC3 and p62 in uninjured and wounded skin tissues of humans. LC3-II/LC3-I **d** and p62/GAPDH **e** were densitometrically calculated and presented. All values represent means (at least n = 3). (Cited from Ref. [46] with kind permission of Springer)

9.3 Drowning and Aquaporin

Immersed bodies mean that the cadavers are found in baths, sea, river, lakes and so on. In forensic autopsy cases of immersed bodies, forensic pathologists should pay much attention in order to diagnose the cause of death, because the cause of death in immersed bodies is not always drowning. In drowning cases with short term of postmortem intervals, several characteristic autopsy findings such as froth paste in the air passages, ballooning of the bilateral lungs, and pleural effusion are often noted. However, these autopsy findings do not always indicate drowning. Thus, additional examination is required for the postmortem diagnosis of drowning. At the present, the detection of diatoms from multiple organs such as lungs, livers and kidneys can strongly support the antemortem aspiration of drowning water [47]. As known, there are two drowning cases of saltwater and freshwater. Thus, it is sometimes important to discriminate freshwater drowning (FWD) from saltwater drowning (SWD) [48]. As the conventional macromorphological differences, larger amounts of pleural effusion and heavier lung weights are found in SWD than in FWD [49, 50]. Several lines of accumulating evidence demonstrated the differences of pulmonary ultrastructural morphology between FWD and SWD using animal drowning models [51–53]. In particular, the osmotic ultrastructural alterations such as severe cellular disruption, mitochondrial swelling, and endothelial destruction are more evident in FWD than in SWD. However, SWD but not FWD showed vacuole formation, and discontinuity of alveolar lining cells. Moreover, serum electrolyte concentrations, atrial natriuretic peptide, iron (Fe), or strontium were evaluated for the differential diagnosis between SWD and FWD [54–58].

Under the physiological conditions, water channel proteins play essential roles in the regulation of osmolality in the whole body [59–61]. Aquaporins (AQPs) are a family of small ($\sim$30 kDa/monomer), homologous water-transporting proteins [18–20]. In mammals, AQPs are mainly expressed on epithelial and endothelial cells in which water and ions are exchanged. Moreover, keratinocytes, adipocytes, and astroglias, that are thought not to carry out fluid transport, also expressed several kinds of AQPs. Now, 13 AQPs have been identified in mammals, and several members of AQPs are expressed in organ specific manners. AQP1 and AQP4 are mainly expressed in the brain [62], and presumed to be involved in neuropsychiatric disorders such as Alzheimer's disease, multiple sclerosis, and schizophrenia [63, 64]. AQP1 and AQP4 appear to be part of mechanisms of cerebral volume regulation following ischemia, trauma, tumors, inflammation, and metabolic disturbances [65, 66]. AQP5 is expressed in alveolar, tracheal, and upper bronchial epithelium in order to regulate lung water homeostasis. An in vitro study showed that hypertonic stress up-regulated AQP5 expression in murine lung epithelial cells [66]. In the kidney, seven aquaporin members (AQP1, 2, 3, 4, 6, 7, and 8) are expressed [67], and four members (AQP1, 2, 3, 4) are responsible for renal tubular water transport and body water balance [67]. AQP1 was expressed in the apical and basolateral membranes of the proximal tubule, descending thin limb epithelium, and glomeruli, and AQP2, 3, and 4 in the collecting duct principal cells [67]. Thus, the pathophysiological roles of organ specific AQPs led us to assume that the aspiration of hypertonic/hypotonic water during drowning might affect AQP expression in each organ.

Our animal experiments using mice implied that, in FWD group, intrapulmonary AQP5 expression was significantly suppressed at both gene and protein levels, compared with SWD group. Moreover, postmortem immersion had no influence on intrapulmonary AQP5 expression. Consistently, in human lung samples obtained from forensic autopsies at less than 48 h postmortem, the intrapulmonary gene expression of AQP5 in FWD was significantly attenuated, compared with SWD [7] (Fig. 9.4a). In the brain, both AQP1 and AQP4 were expressed on GFAP-positive astrocytes asrtrocytes, and AQP1-positive reaction was also observed in the endothelial cells of the brain. Morphometrically, the magnitude of intracerebral AQP1 expression was of a similar level between FWD and SWD. On the contrary, the number of AQP4-positive astrocytes was significantly larger in FWD cases than in SWD [8] (Fig. 9.4b). In the kidneys of FWD and SWD, the expressions of AQP1, 2, and 4 were immunohistochemically examined. AQP1 expression was found in both proximal tubules and glomeruli, and AQP4 was observed in the collecting ducts. However, no significant differences in AQP1 and AQP4 expressions were found between FWD and SWD, implying that neither AQP1 nor AQP4 would be available markers for discrimination between SWD and FWD. On the other hand, AQP2 was expressed in the apical plasma membrane of the collecting

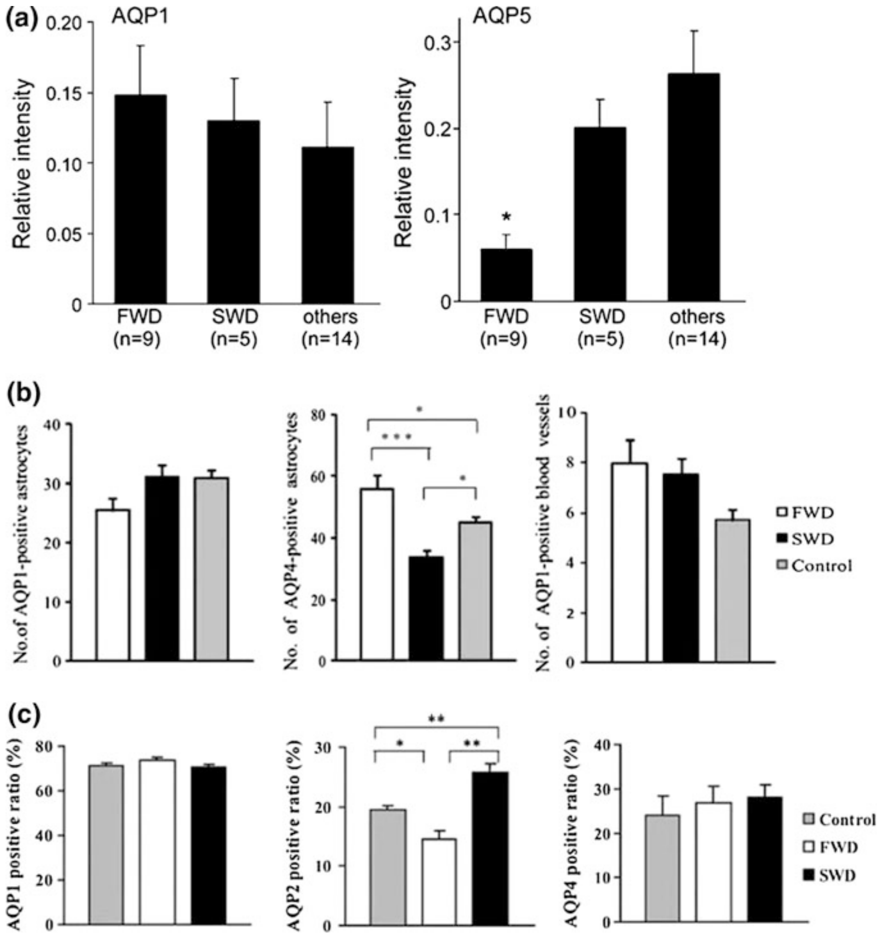

Fig. 9.4 The expression of AQPs in FWD and SWD. **a** Lungs, **b** Brains, and **c** Kidneys. $*p < 0.05$, $**p < 0.01$ $***p < 0.005$. (Cited from Refs. [7–9] with kind permission of Springer)

duct principal cells of FWD and SWD. Its expression was significantly enhanced in SWD group, compared with FWD one [9] (Fig. 9.4c). Collectively, immunohistochemical detection of AQPs in the lungs, brain and kidney can be considered a valuable tool in order to differentiate between FWD and SWD.

9.4 The Clock Gene and the Estimation of Death Time

In forensic practice, it is important to estimate the time of death and postmortem intervals (PMI). However, estimating the time of death and PMI is often difficult for forensic pathologists. Conventionally, PMI was estimated based on postmortem changes, such as rigor mortis, the cooling of rectal temperature, hypostasis, potassium concentration in vitreous humor, development and growth of insects in the corpse, and the degree of putrefaction of the body. However, these postmortem changes are strongly influenced by endogenous and environmental factors. Examples of concurrence methods include reading the time on a wristwatch stopped by a traffic accident or determining the extent of digestion of the last known meal, which may contribute to estimating the time of death.

Accumulated evidence indicates that biological clock systems work in most cells in most tissues (peripheral clock) in addition to the core clock in the hypothalamic suprachiasmatic nucleus (SCN), and several genes have been identified as associated with the biological clock [68–70]. In organisms from cyanobacteria to humans, the circadian rhythm has been found in biological, physiological, and behavioral processes. As the molecular mechanisms, it is well known that the circadian rhythm is regulated by the expression of several kinds of genes, so-called "clock genes" that are conserved from drosophila to mammals [71]. There is a feedback loop with Bmal1–Clock and Period–Cryptochrome (Per–Cry) complexes in the circadian clock system of mammals. Briefly, Bmal1–Clock complex induced the expression of Per and Cry genes as one of the transcriptional activators, and Per-Cry complex reciprocally suppresses the transcription activity of Bmal1–Clock complex, eventually resulting in the circadian biological clock [72, 73]. Thus, if the biological clock could be read in corpses, it might be useful for estimating the time of death in forensic practice.

In experimental studies using mice, there was a clear and rigid oscillation of mBmal1 and mPer2 gene expression in fresh kidney, liver, and heart, implying that the well-controlled breeding condition (light/dark cycle) demonstrated a rigid circadian rhythm (Fig. 9.5a). Since mBmal1 and mPer2 gene expression oscillates in the anti-phase, the mPer2/mBmal1 ratio seemed to be a suitable parameter for reading the biological clock (Fig. 9.5b).

In the next series, we examined the expression of clock genes using human liver, kidney, and heart obtained from forensic autopsy cases with less than 72 h of postmortem interval. In those human organs, a circadian oscillation of hPer2, hBmal1and hRev-Erbα could be found (Fig. 9.6). The ratios of hPer2/hBmal and those of hRev-Erbα/hBmal1 were considered to be more suitable for estimating the time of death (Fig. 9.7). Taken together, these findings indicate that gene expression analyses of the biological clock could be powerful methods for the estimation of the time of death [74].

Postmortem imaging is one of the most powerful tools in order to roughly grasp internal findings before autopsy. In particular, it is well known that postmortem radiological examination is useful for the detection of bone fractures and metal objects in the body. Recently, postmortem computed tomography (PMCT) has been widely spread in forensic autopsy. For example, in shotgun wound cases where numerous bullets exist in the body, it is too difficult to find them during forensic autopsy. PMCT makes forensic pathologists confirm the localization of bullets in the body, and intracerebral hemorrhages and rib fractures are easy to find in PMCT. Moreover, PMCT angiography has also been developed for the detection of

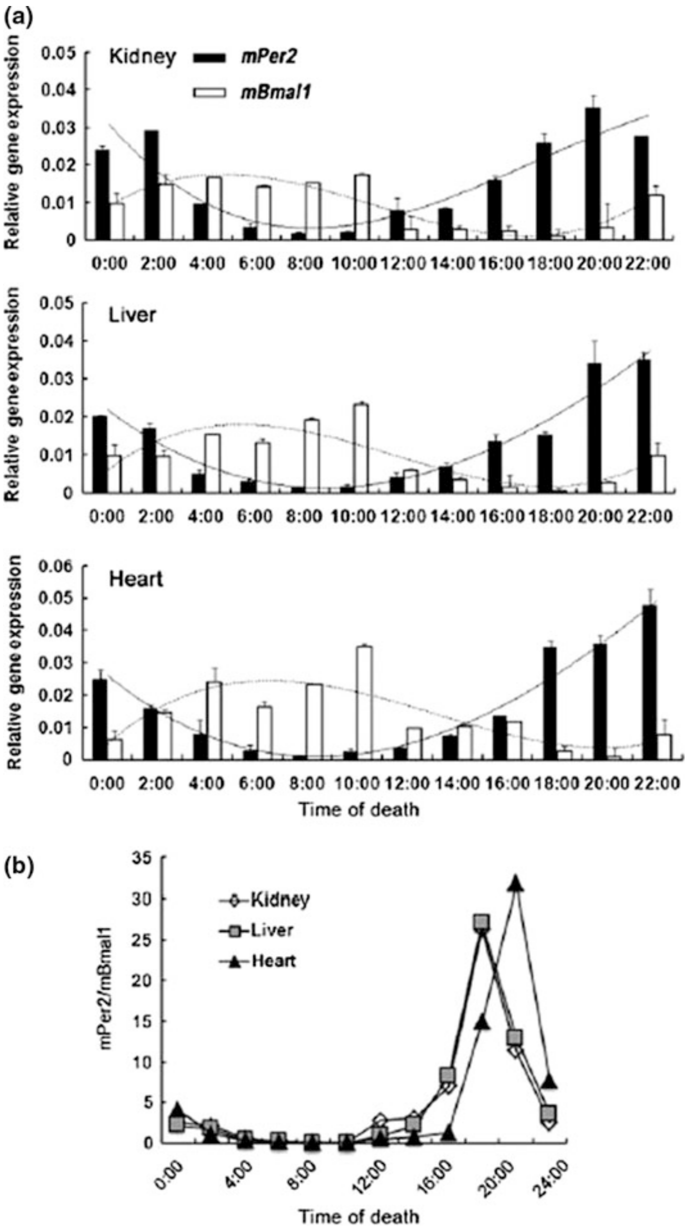

Fig. 9.5 **a** Circadian expression profiles of *mPer2* and *mBmal1* in murine kidney, liver, and heart. **b** Circadian oscillation profiles of mPer2/mBmal1 ratio in mouse tissues. (Cited from Ref. [74] with kind permission of Springer)

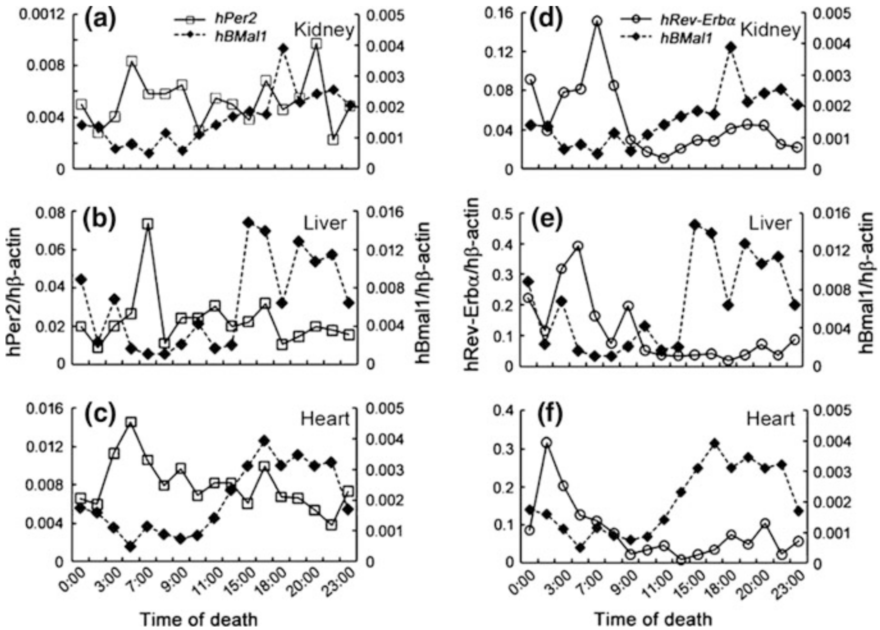

Fig. 9.6 Circadian gene expression profile for hPer2 (**a**–**c**), hRev-Erbα (**d**–**f**), and hBmal1 (**a**–**f**) in autopsy samples. (Cited from Ref. [74] with kind permission of Springer)

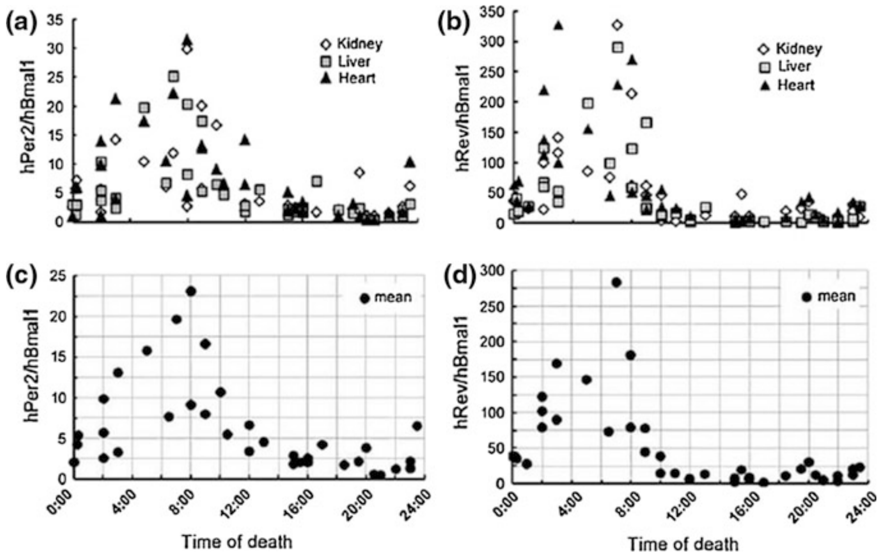

Fig. 9.7 Circadian oscillation profiles of hPer2/hBmal1 (**a**) and hRev/hBmal1 (**b**) in the kidney, liver, and heart from autopsy cases. (Cited from Ref. [74] with kind permission of Springer)

vascular ruptures and injuries. However, the employment of only PMCT is still insufficient for completely diagnosing the cause of death, because postmortem changes are sometimes misunderstood as significant findings such as gas embolization and subarachnoid hemorrhage, thus meaning that PMCT is just an alternative method. In the future, PMCT has other possibilities for forensic practices. Actually, we experienced cases that PMCT combined with computed data processing provided useful information for ballistic analysis.

9.5 Conclusion

In modern society, the importance of forensic medicine is increasing. In order to fulfill social requirements, modern knowledge and techniques, in addition to conventional methods, have to be applied to forensic medicine. In particular, forensic pathology is one of the major elements based on the macroscopic and microscopic observation of cadavers. Thus, it is needless to say that the advancement of molecular and imaging innovations is able to contribute to the progress of forensic pathology.

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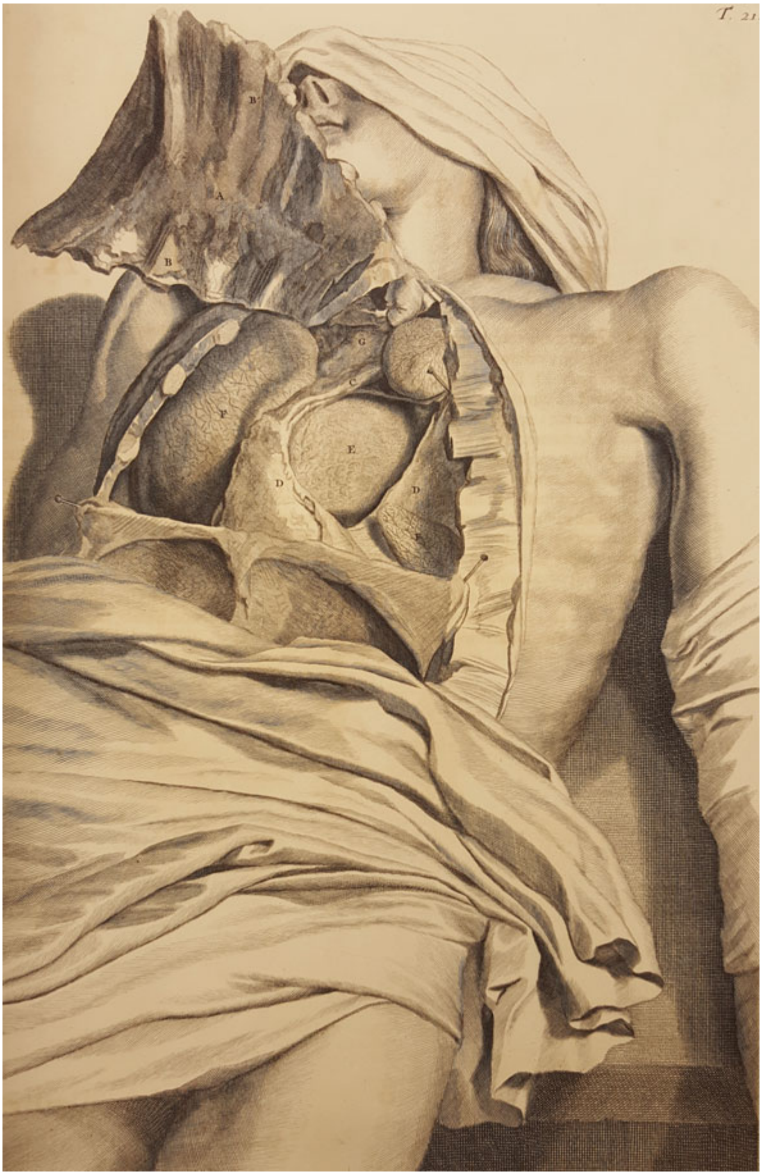

Bidloo, Govard, Anatomia humani corporis centum & quinque tabulis, per artificiosiss. G. de Lairesse ad vivum delineatis, demonstrata, veterum recentiorumque inventis explicata plurimisque, hactenus non detectis, illustrata Amstelodami: Sumptibus viduae Joannis à Someren, haeredum Joannis à Dyk, Henrici & viduae Theodori Boom, 1685

Chapter 10
Current Practice of Forensic Anthropology on Dead Bodies

Marcel A. Verhoff and Frank Ramsthaler

Abstract A badly decomposed cadaver is found. Preliminary investigations confirm the human specificity and suggest that the remains are contemporary and represent one unknown individual. Because the remains are so badly decomposed, it is impossible to recognize identifying characteristics such as facial features, external sex organs, or tattoos. Furthermore, fingerprints can no longer be obtained. In such cases, the first forensic anthropological objective is to identify the decedent by establishing a biological profile. Conventional osteological methods are used to estimate the decedent's sex, age at death, body height, and ancestry. Where needed, a CT scan may be performed and the examinations conducted on virtual bones. Bony scars and dentition status can also provide further identifying clues. If the biological profile suggests a match with a missing person, further methods such as radiographic comparison, skull-photo superimposition, dental status, or forensic DNA analysis are used to confirm the decedent's personal identity.

10.1 Introduction

The outset of every forensic anthropological analysis is marked by the discovery of a badly decomposed or skeletonized human cadaver, or parts thereof. Due to putrefaction, autolysis, or animal scavenging, the decedent can no longer be described well enough to be recognized by persons who might have knowledge of the individual's identity. A typical example would be the discovery of a decomposed corpse in a forest clearing or a skeleton during construction work.

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Although unidentified corpses may also be discovered in domestic environments [1] or after mass disasters [2], these settings differ in that there is usually some guiding information with which to narrow down the individual's possible identity, such as passenger or guest lists. These corpses frequently do not have to be demographically characterized, and the identification procedure can commence at the second stage, which attempts to positively identify the decedent. In mass disasters, particularly if they are large scale, logistical challenges may demand modification of the identification procedure, e.g., organization or suitable data processing methods [2].

A preliminary question when skeletonized remains are found is whether they are human or those of some other mammal [3]. Dog owners occasionally bury their departed "darling" at one of its favorite spots, perhaps, in a forest clearing, along with its blanket and favorite toys. Then, because digging with a pick and shovel is quite onerous, the cadaver is frequently not buried deep enough, and scavengers dig it up. When people, walking in the woods, stumble across such sites, they often mistake the scattered bones for those of a child's. This faulty conclusion is further compounded by the presence of toys and the smallness of the bones. The distinguishing criterion here would be that children's bones (like those of other immature mammals) have epiphyseal growth plates, because they are still growing. These growth plates can, however, rarely be demonstrated in children's skeletons because the unfused epiphyses tend to fall off the diaphysis during decomposition and are frequently lost. The visible bone ends thus represent the diaphyseal face of the growth plate instead of the articular surface. Consequently, children's bones may sometimes not be recognized as human: to the inexperienced examiner, the peculiar shape of these "reduced" bones may suggest a nonhuman origin (Fig. 10.1).

Once the discovered remains have been established as human, a further preliminary question is whether they represent a single or multiple individuals. The discovery of duplicate bones (e.g., more than one right humerus) is an indication

Fig. 10.1 *Upper image*: Right femur of an adult stag. *Lower image* Right femur of an approximately 4-year-old child. It is easy to recognize that the stag femur is nonhuman. Because the epiphyseal caps are missing, the child's femur could be mistaken for a nonhuman bone [39]

that the commingled remains represent more than one individual. Even when only one bone of each type is present, striking differences in the overall appearance of a single bone in contrast to the others (e.g., significantly more delicate) may also suggest commingled remains. Other explanations for differences in the appearance of single bones, such as pathological changes, should also be considered, e.g., in an individual with polio, the bones in an affected leg could be more delicate than the ones on the healthy side.

Once the preliminary analysis has established that the remains appear to be those of a single individual, the actual identification procedure begins. The process has two stages: The first is to determine the individual's demographic profile; the second, to positively identify the individual. Determination of the demographic profile means gathering as many individualizing characteristics as possible from the remains so that the decedent can be compared to individuals on missing person lists, or be recognized from the description by someone who knew them in life. In English-speaking countries, the demographic profile is often called the "biological profile" [4]. This profile encompasses the following main characteristics: sex, age at death, body height, and ancestry, but may include further helpful characteristics such as healed bony injuries or diseases that affected the individual's appearance (e.g., scars, deformities), or led to noticeable physical disability (e.g., limping, stiff arm, etc.). Although the postmortem interval (PMI) is not, strictly speaking, part of the biological profile, it is, nevertheless, an important identification criterion, as it helps pinpoint the time of death. Further, the PMI helps establish whether or not a case is forensically relevant. It should, therefore, be estimated as early as possible in the identification procedure.

A popular identification method for badly decomposed or skeletonized bodies that ought to be mentioned in the context of individualizing characteristics is facial (soft tissue) reconstruction. This method is a kind of "last resort" when all of the other demographic characteristics have failed to produce a match with a missing person. In this technique, layers of a modeling mass are sculpted over the skull in scientifically established thicknesses to simulate the soft-tissue layers. In the best-case scenario, the reconstructed face, rendered more realistic by hair and clothes, approximates the unidentified person's face. A picture of the reproduced face is then publicized on TV or in local newspapers, in the hope that someone will recognize the decedent from it.

If a potential match between the biological profile of an unidentified decedent and a missing person is found, the match needs to be confirmed in the second stage of the identification procedure. Methods such as forensic DNA analysis, forensic odontology (dental comparison), radiographic comparison, and skull-photo superimposition are used to positively identify almost completely skeletonized human remains. If enough skin is preserved, a comparison of fingerprints or tattoos may also be feasible. The choice of methods used in the attempt to establish the decedent's personal identity is determined largely by the kind of information or material available for comparison, both on the side of the missing-person (antemortem material) and that of the decedent (postmortem material). The postmortem dental status can usually still be easily determined from skeletonized or badly decomposed remains for comparisons with antemortem records. Since most people in Germany regularly visit a dentist, antemortem dental records are usually available for most missing persons here. This highlights the enormous significance of forensic odontology in countries with high standards of dental health care.

Caution must, however, always be exercised when comparing antemortem and postmortem material. If for example, a razor or toothbrush that purportedly belonged to a missing person is handed to the police so that an antemortem DNA profile can be obtained, a measure of uncertainty remains whether the objects really belonged to that person and were used exclusively by them. The authenticity of antemortem material for comparisons is thus as critical an issue as its availability is.

A special feature of methods using DNA-profiles and fingerprints is that national and international databanks (which store personal data for existing DNA-profiles) are available for comparisons. Although these databanks only store personal information for people implicated in criminal cases, numerous positive matches have been found from the DNA profiles or fingerprints of unidentified corpses in this way. Presumably because homicide and body disposal frequently occurs in the setting of organized crime, these databanks seem to contain a certain "preselection" of matching profiles.

Although forensic odontology is also helpful in the first identification stage, it is mainly used for positive identification. A postmortem dental report can be distributed among dentists in the region where the decedent's remains were found or be published in dentistry journals, in the hope that a dentist may recognize a former patient from his or her highly characteristic dental status.

All of the steps to gain individualizing demographic characteristics can also be performed for commingled remains, provided they represent only a few individuals. When the remains of more than three or four unidentified individuals are found commingled, the likelihood that they were the victims of a "simple crime" diminishes. A historical context is possible, especially if the remains are skeletonized, because, in Central Europe, at least, mass burials have not been practiced since the Second World War. Fortunately, the biological profiles from a large number of individuals from commingled remains can, when seen together, reveal import clues about the origin of the collective and its historical background. Distinctive shared features of the skulls may suggest relatedness or a common ethnicity of the individuals. An illustrative example would be the case in which, in January 2008, numerous skeletons were uncovered during construction work on the University of Kassel's campus grounds. Sixty skeletons could be examined on-site by medicolegal experts (Fig. 10.2). Strikingly, they all shared the following demographic characteristics: young adult; male (with the exception of three questionable cases); Caucasoid; old injuries only, no recent injuries; no dental repairs. Already the absence of dental repairs in such a large group of individuals suggested a historical burial site, and the fact that–with the exception of the three uncertain cases—all of the individuals were young males suggested a military context. Since the lack of recent injuries made it unlikely that the men had died in action, the conclusion was that they were likely the victims of an epidemic. Further

Fig. 10.2 View of the grave field in Kassel (January 2008) showing approximately 60 partially excavated skeletons. It is striking that often 2 or more skeletons were placed extremely close together. On the whole, the burials appear orderly, unlike those from a mass grave. The space between the skeletons is too narrow for them to have been buried in coffins. The burials are in keeping with the interment of victims during an epidemic, with several deaths occurring every day: The victims are placed in conventional graves, but there is neither the time nor the material to make coffins

examination of the site and historical research later confirmed that the victims were soldiers who had died during a typhoid epidemic in 1814 [5].

10.2 Time Since Death

The estimation of the postmortem interval (PMI) from human remains basically performs two functions: first, to determine whether the PMI is still forensically relevant (FRPMI); and, if so, to pinpoint the time of death and likely time of the crime.

For the investigating authorities, non-contemporary bones are of little interest. This raises the question of when to consider a bone "historical," and when forensically relevant. In the international literature, the cut-off line for forensic relevance is usually considered to be a span of 50 years because it is unlikely that details of a crime older than that can still be reconstructed. Out of practical considerations, such as cost and effort, old crimes are only investigated if there is still a good chance of recovering sound evidence, or the case is of major public interest. Thus, a FRPMI of 50 years still seems appropriate.

Characteristics traits have been established to distinguish contemporary bones from those with a PMI of over 50 years [6]. This distinction may be somewhat difficult for exposed remains, e.g., the climatic conditions in Central Europe, in mid-summer, are such that an exposed cadaver can be largely skeletonized within six weeks (Fig. 10.3).

If live insects are still found on partially skeletonized remains, forensic entomology can be the most promising method of accurately estimating the PMI [7]. Forensic entomology is based on the knowledge of the necrophageous insects that colonize dead bodies, and how their juvenile stages develop in dependence to the ambient temperature. This knowledge is used to calculate the age of immature insects present on the body. Based on the assumption that colonization occurs immediately after death, the age of the oldest immature stages found on the corpse corresponds to the minimum PMI. When the first wave of colonization has finished its metamorphosis and hatched, this method loses its accuracy.

Currently, isotope techniques offer the only approach to absolute dating that is independent of ambient factors and thus allows exact determination of the PMI.

Fig. 10.3 These fully skeletonized human remains were found next to the tracks of a railway line, in 2004, in summer. The biological profile matched that of a man who had been missing for six weeks. Because of the advanced stage of decomposition, the match seemed extremely doubtful at first. However, DNA analysis provided positive identification. Apparently, under certain conditions, exposed corpses can be completely skeletonized within six weeks also in Germany

Radiocarbon dating (carbon-14 dating), a tool often used in archeological contexts, is, however, of little use in determining the FRPMI. Due to inherent limitations, e.g., half-life of 5730 years, measuring inaccuracies, and geographic differences in C-14 concentrations, the method cannot be used to accurately distinguish between a PMI of 20, 30, or 60 years. However, due to nuclear bomb testing, the atmospheric concentration of C-14 has risen (“bomb carbon”), and radiocarbon dating can now be used to distinguish contemporary from non-contemporary bones. “Bomb carbon” in bones implies that an individual lived during or after the nuclear weapon era [8]. In determining the PMI, factors like age at death and element turnover also need to be considered. The most promising approach to estimating element turnover is the analysis of C-14 concentrations in tooth enamel. Once a tooth has developed, this C-14 signature theoretically remains preserved throughout life. Coupled with knowledge of when specific teeth erupt, the C-14 signature can be used to estimate the PMI. Although this method, which Spalding first described, currently appears to be the best radiometric approach, it was tested in a rather small study and needs to be further evaluated [9].

Other nucleotides have been tested as PMI markers, but have not proved useful in practice. Most notably, the radiostrontium method (Sr90) had to be abandoned despite the promisingly short half-life of the isotope, as man-made Sr70 can apparently also mask the genuine isotope signatures in historical bones, probably via secondary diagenetic exchange of alkaline elements (e.g., through precipitation or dampness). The method is thus unsuited as a tool to identify contemporary samples.

As the preceding discussion shows, there are numerous constellations in which the PMI cannot be narrowed down, or a FRPMI be conclusively excluded, with the currently available methods. Such cases should always initially be considered forensically relevant.

Because the PMI can often be estimated from associated finds, archeological recovery methods can be of use. Associated finds may, however, also be misleading: In a case from 2004, a female skeleton was recovered along with old, handmade buttons that were dated to the early 20th century by a historian. Through coincidence, the decedent was later identified as a nun who had disappeared in the late 1990s. However, she had liked to wear traditional garments.

Nonetheless, associated finds can provide important information for the estimation of the PMI. If, for instance, a plastic bag from a particular chain store is found in a design that was only used for two years, it can help determine the time since death.

10.3 Demographic Characteristics—Biological Profile

All forensic methods used to determine the demographic characteristics of a decedent from skeletal remains have limitations in terms of error or inaccuracy. Theoretically, they allow a decedent to be described, for example, as a 43-year-old, 183 cm tall Caucasoid male. Any medicolegal examiner who delivered such an exact description to the investigating authorities would be likely to impress at first. This would be akin to the awe that visitors to a museum feel for the amount of detail scientists can wring out of a few bones, when they read the label to a showcased skeleton. In a forensic context, however, the level of precision described above could mean that the investigating authorities might exclude a 42- or 44-year-old missing man as a possible match with the decedent, or one who was reported to be 182 or 184 cm tall. If, on the other hand, the demographic characteristics of a decedent are described too broadly, e.g., possibly male, maybe also female; aged between 21 and 93 years; height somewhere between 160 and 203 cm, the profile would be of little value to the investigating authorities. The trick is therefore to describe the demographic characteristics close enough to usefully narrow down the circle of potential matches with missing persons, without narrowing it down so far that a factual match might be excluded. An employable profile might read like this: "Man, middle-aged (approx. 35–50 years-old), Caucasoid, between 179 and 186 cm tall."

All of the traits used to establish a decedent's biological profile are population dependent. Thus all charts, formulas, and indices only apply to the populations for which they were evaluated. For the same reason, the current methods have to be constantly re-evaluated, as populations may change over time or through migration.

Because computed tomography (CT) now achieves high resolutions, virtual, 3D, bones that can be examined, measured, and evaluated [10, 11] can be reconstructed from clinical CT-datasets. A drawback is that these are inevitably incomplete, and bone sections are frequently cut off. Due to technical problems, postmortem CT also frequently fails to provide full-length scans of long bones. This is likely the reason why most digital forensic osteological studies were performed on skulls or cranial bones [10, 12].

10.3.1 Sex

The morphological attribution of sex is accomplished by anthroposcopic and anthropometric assessment of sexually dimorphic characteristics [13]. The pelvis and skull exhibit the greatest number of these dimorphisms. Bone size and roughness of muscle insertion sites are considered sexually dimorphic attributes, with male skeletons described as larger, heavier, and more rugged than female skeletons.

The chances of correctly attributing sex are highest when both the undamaged pelvis and skull of the same individual are available for assessment. Useful dimorphic features of the pelvis include the size of the subpubic angle (female: blunt; male: sharp), and the shape of the pelvic inlet (female: oval; male: heart-shaped) and obturator foramen (female: triangular; male: ovoid). The dimorphic features of the skull include the Glabella (female: unpronounced; male: very pronounced); the Mastoid process (female: very small; male: very voluminous); and the contour of the nuchal area (female: flat-smooth, males: very rugged, pronounced muscle attachment ridges). The degree to which each feature is expressed is graded on a scale as hyper-feminine, feminine, indeterminate, masculine, or hyper-masculine. The category most often scored for all assessed features determines which sex is attributed: female, indeterminate, or male [10, 14].

Morphometric methods were developed to counter concerns that anthroposcopic methods are unscientific and subjective [15]. Discriminant function analysis has become a popular morphometric method with which to attribute sex [16–18]. Discriminant functions that can be used to attribute sex from human skeletal remains are developed from the measurements of various sexually dimorphic bone lengths and distances taken from random samples. Another way of objectifying the assessment of traits is to quantify established anthroposcopic characteristics.

Both with morphometric and morphological methods, the expression of dimorphic features may be found to overlap and preclude the attribution of sex in forensic contexts. This situation is often encountered with children's skeletons because many sexual characteristics are not fully developed before puberty. Nevertheless, with the established methods, sex can be correctly attributed in about 85–90% of cases—despite challenges like secular change, ancestry, or incomplete skeletons.

In the past few years, numerous studies have attempted to develop alternative methods for the attribution of sex, for instances, from cranial bones, or bones other than the skull and pelvis, to allow sex attribution even under challenging case constellations. Unfortunately, none of these are currently reliable enough for forensic casework.

10.3.2 Body Height

The calculation of body height is based on the circumstance that long limb bone length is linearly proportional to height. This fact was used to derive linear regression formulas for the calculation of body height from length measurements of whole or partial long bones. Various authors have emphasized that not only the acceleration of secular change needs to be considered when choosing regression formulas, but also the fact that they are sex and population specific and can only be applied to the populations or skeletal collections for which they were derived [19]. Because the calculated height is an estimate, the result should either be expressed as a value, with standard error, or as a confidence interval.

Sometimes the decedent appears not to have been reported missing, and may, therefore, not have been native to the region in which the remains were found. This situation creates a dilemma, since the formulas for height calculation are extremely population-specific.

Forensic anthropologists should be familiar with various regression formulas specific for their region so that they can, if necessary, use different formulas to calculate height from all available long bones and estimate the decedent's height by comparing results.

10.3.3 Age at Death

Numerous empirical methods are available with which to estimate the decedent's age at death. Influencing factors such as individual aging, way of life, and regional life conditions also deserve consideration. Noninvasive (macroscopy, dentition status, overall appearance, or X-ray) and invasive (chemical or histological analysis of tooth or cortical bone samples) techniques may be used to determine the age of adults and subadults.

Many osteological approaches to estimating age at death make use of the fact that some bone features change during life and can be used to estimate age. The majority of these approaches are anthroposcopic or semi-quantitative in nature, e.g., classification of epiphyseal union; dental abrasion; abrasion or age-dependent rarefaction of intraosseous bone structures; changes at the pubic symphyses [20] or rib ends [21]; or degenerative alterations. Additionally, there are also less common approaches such as histological or chemical methods, e.g., asparagine racemization. Further, degenerative skeletal changes or antemortem loss of teeth can also provide clues to age, but are strongly influenced by life circumstances [22].

The age of subadults is best estimated by assessment of epiphyseal ossification and dentition status (age-dependent development and eruption of specific teeth, both deciduous and permanent).

The utility of assessing the closure of cranial sutures is still being hotly debated [23]. Other, unusual, approaches to estimating age, such as tooth root translucency [24, 25] are also being explored.

It is important to remember that the available methods only allow an estimate of biological age, not of chronological age. The older the decedent, the more difficult it becomes to accurately estimate age because many useful characteristics become obliterated over the course of a lifetime. Currently, age is, thus, best estimated by combination of the available methods.

10.3.4 Ancestry

In light of the rising migration trends all over the world, forensic experts are increasingly being confronted with the task of having to attribute ancestry or provenance to skeletal remains because this information can decisively aid the criminal investigation and help identify the decedent.

There is wide-spread agreement that the external physical attributes and derived categories associated with the term "race" do not adequately describe the diversity of physical traits in human groups, or their biological variability. This insight, does not, however, by inversion, mean that the morphological, often genetically determined, differences between different populations are random characteristics that preclude attribution of individuals to one of the large, geographically defined, population groups, i.e., Caucasoid; Mongolid; Negrid, or that it is fundamentally impossible to attribute ancestry by assessing skeletal remains, e.g., the skull [26]. Because the well-defined biological concept of "race" cannot satisfactorily be applied to distinguish human populations on the basis of external or skeletal characteristics, use of the term "ancestry" was proposed [27, 28] to describe the phenotypical differences that help forensic investigators narrow down the search parameters for an identity match. The only sociological implications these differences have are those that people ascribe to them. The ancestry model with only three major groups is imperfect, but helps sort human groups to specific populations through differences in skeletal size and shape, in particular of the skull. Howells' studies on population differences showed that individuals could be successfully classified to different populations alone by metric differences of the skull [27, 29].

Ousley and Jantz developed FORDISC®, an interactive discriminant functions program, which uses up to 25 different metric traits to tentatively classify an individual to a population by means of multivariate analysis and Mahalanobis distances [30].

Apart from the difficulty that the statistical algorithms with which FORDISC® operates are problematic to apply to other populations because they were derived from US data, a further weakness is that though they may show good separation between two populations, e.g., Caucasoid versus Negroid, they do not reveal possible collateral relationships to other populations, e.g. Mongoloid. Consequently, other classifications may result when the search is differently defined (e.g.: 3-fold: Negroid vs. Mongoloid vs. Caucasoid) [31].

The analysis of stable isotopes can offer important clues when ancestry is defined as provenance. Apart from determining the decedent's tentative ancestry, this method can also provide information pertinent to the forensic investigation, such as whether the person had a history of migration [32].

10.4 Positive Identification

10.4.1 Forensic DNA-Analysis

Forensic DNA analysis is a reliable tool with which to positively identify a decedent from skeletal remains. The most promising sites for DNA samples are teeth or cortical long bone, for which special extraction and purification procedures have been developed [33]. Due to problems such DNA degradation over time, or deterioration through environmental agents or heat, the success of the analysis is, however, hard to predict; further, DNA binds tightly to the inorganic hydroxyapatite in bone and may resist extraction.

If the STR regions (STR, "short tandem repeats") [34] can be amplified, the sequences from the decedent can be compared to those in a DNA database.

Mitochondrial D-loop sequencing [35, 36] is another option. Although mitochondrial DNA analysis is more likely to succeed than STR-analysis, it is only useful for direct comparisons with DNA from a missing person. This limits its utility as an identity search parameter.

10.4.2 Dental Status

Dental records [37] inventory the presence or absence of teeth and record dental repairs. In determining the dental status, the condition of each of the five surfaces of the tooth crown is individually assessed, e.g., original versus repaired. All repairs are described, e.g., amalgam filling, gold filling, open faced crowns. In odontological comparisons, the decedent's dental remains are carefully examined and compared with the antemortem dental records of a potential match. Dental repairs in the decedent that are not noted in the antemortem dental records do not necessarily disprove a match because the decedent may, at a later date, have visited another dentist; however, if the antemortem records mention tooth repairs for which no correlates are found in the decent, identity can basically be ruled out. The dental remains are, however, carefully re-examined to see if a ceramic inlay or crown may have been overlooked. A further possibility is that the antemortem record contained an erroneous entry. It is difficult to define how many correlates between the ante- and postmortem dental status suffice to positively identify an individual—in the absence of exclusion criteria and solely by odontological comparison. Because dental repairs may range from the commonplace to the rare and highly individual, this decision should be left to odontologists. While a single characteristic correlate between the ante- and postmortem dental status may sometimes suffice for positive identification, in another, 20 correlates (for example, composite occlusal fillings) may still not be decisive.

10.4.3 Radiographic Comparison

For most adults, antemortem radiograms will have been acquired for clinical reasons at some point and are available for comparison with postmortem radiograms taken with the same technique. Cranial or dental radiograms are most promising for this method (Figs. 10.4 and 10.5).

10.4.4 Skull-Photo Superimposition

In contemporary society, the availability of portrait photographs has burgeoned. Suitable, authenticated antemortem photographs are thus frequently available for

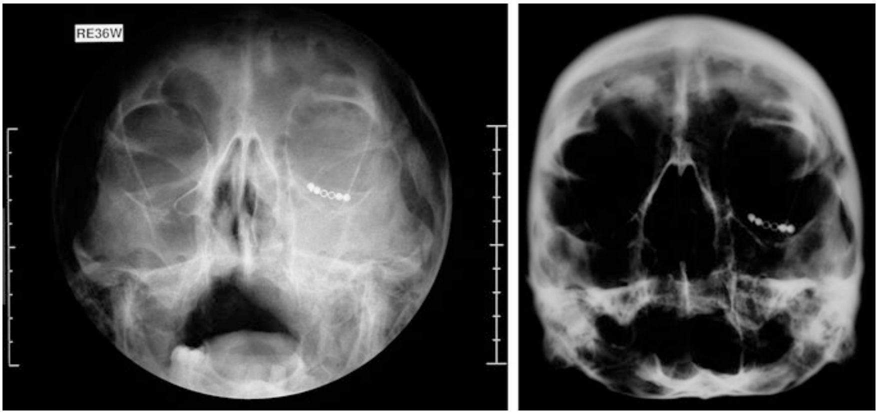

Fig. 10.4 Positive identification through radiographic comparison. The *left image* shows a postoperative control radiogram that was acquired after maxillofacial surgery. A fracture of the left maxillary sinus was repaired with a six-hole plate. The *right image* shows the postmortem skull X-ray from an unidentified corpse. The postmortem X-ray was taken in the same technique as the reference radiogram to avoid beam divergence. The agreement between both X-rays for the position of the metal plate is conspicuous. A comparison of the frontal sinuses shows high congruence between their highly individual structures in both X-rays. Under the condition that ante-and postmortem X-rays are taken with the same technique, congruence of the structures of the frontal sinuses can provide almost as much proof of identity as matching fingerprints

comparisons. Skull-photo superimpositions are used to see if an antemortem photograph of a potentially matching missing person "fits" the skull of the unidentified decedent. This not only entails photographing the skull in the same photographic plane, and at the same distance, as the face in the antemortem photograph, the soft tissue thicknesses over anthropological landmarks on the skull also need to be taken into account. In practice, this problem is solved by placing spacers over these points to simulate the established soft-tissue thickness for the estimated age span for the decedent [38]. The prepared skull is then photographed in the same perspective as the antemortem portrait. This is frequently done using a digital superimposition technique that allows correct orientation and comparison of the skull and the photograph. The proportions of the face and skull and expected contours of the soft tissue layers are assessed for concordance with those in the portrait (Fig. 10.6). A mismatch between the superimposed skull and face is readily apparent, even in simpler superimpositions that do not use soft tissue markers. If there is concordance, the question is whether the agreement is convincing enough to allow positive identification. Unfortunately, there is no easy answer to this question. The more antemortem portraits, in different perspectives, are available that each show multiple points of concordance with the superimposed skull, the more likely it is that the decedent and the person on the photograph are the same.

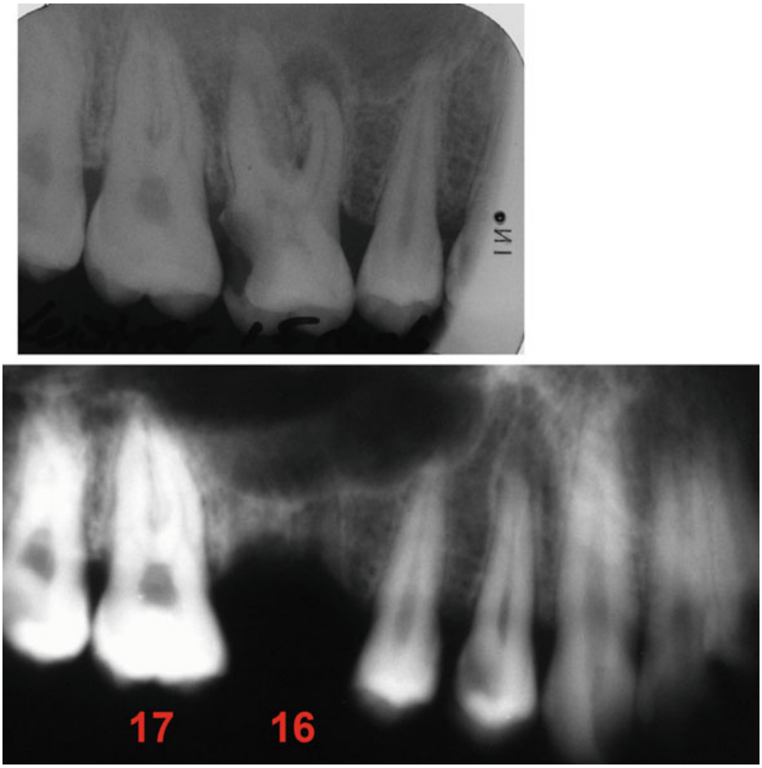

Fig. 10.5 Positive identification through radiographic comparison of a dental spot film. The *upper image* shows a spot film that was acquired for tooth #16, which the dentist later extracted. Tooth #16 was also missing in the unidentified decedent. The *lower image* shows the postmortem radiogram of the decedent's jaw that was acquired using the same technique as for the antemortem film. Congruence of the remaining teeth and jaw contours, in particular those of the maxillary sinuses, confirmed a positive identification [40]

10.5 Conclusions

The procedure of identifying an unknown decedent is complex. Forensic osteological methods prove particularly helpful when badly decomposed or fully skeletonized remains are found. If the corpse is not yet skeletonized, a full-body CT scan is recommended. Due to increases in resolution in conventional CT, more and more osteological details can be assessed from virtual bones. Over the last ten years, "digital forensic osteology" has been used to help re-evaluate conventional forensic-osteology parameters for modern populations and evaluate new ones.

When human remains of possible forensic relevance are found, conventional forensic osteology methods are used to answer the preliminary questions of whether the bones are human or not, whether the PMI is, indeed, still forensically relevant, and whether the remains represent a single or multiple individuals.

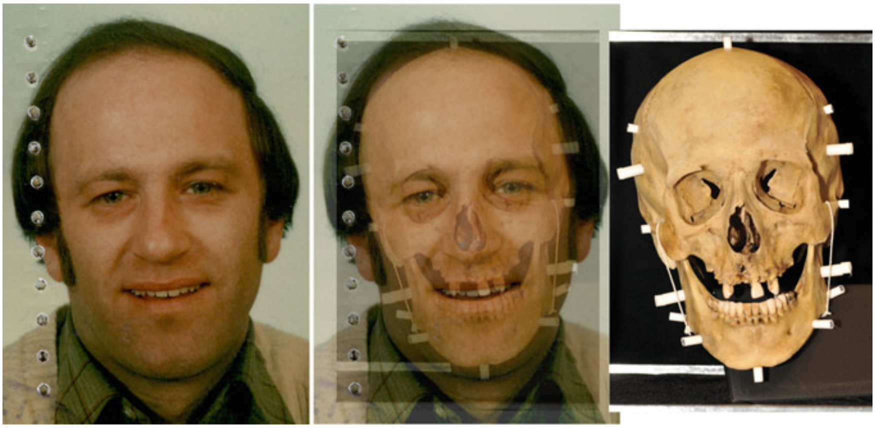

Fig. 10.6 Positive identification through skull-photo superimposition. The *left image* shows the skull from an unidentified corpse. Soft-tissue markers were applied only where they were needed for the comparison of facial contours with the reference passport photo (*right image*). Using the Live-View software feature, an image of the passport photo was projected over the skull, which was fastened to a craniophor. The skull was then positioned to match the perspective and size of the projected face, and photographed [38]. In the last step, the picture of the skull and the passport picture were superimposed using Photoshop® (*Center image*) [1]

Once it has been established that the remains represent a single, unidentified human and a FRPMI cannot be excluded, a two stage identification procedure commences.

In the first stage, as much information as possible is gathered from the decedent. Apart from the biological profile (age, sex, body height and ancestry), clues, such as, old or fresh, injuries and associated findings may be important. The crucial issue at this point is to supply the investigating officials with a set of information that allows them to narrow down their search parameters for possible matches from missing person files, while avoiding the danger of excluding a potential match.

The efficacy of the forensic anthropological methods used to determine demographic characteristics is not only affected by the condition and completeness of the remains, but also by the correct attribution of ancestry and the examiner's expertise. The more methods, formulas, and reference values for various populations there are to resort to, the easier it is to build plausibility checks into the investigation. In particular in estimating the age at death, it is important to be able to reconcile apparently contradictory findings (e.g., unfused cranial sutures, but strongly abraded teeth) by comparing the results with those from further methods, such as the assessment of additional skeletal age markers, e.g., symphy seal surfaces, tooth root translucency; or technically complex methods like asparagine racemization. It remains to be seen if molecular biological methods will, in future, be of additional utility for the estimation of age. Age-related analyses such as 5k mitochondrial

DNA base-pair deletion or teleomere length provide tools that may, at least, be useful for plausibility checks. Currently, the usefulness of methylation patterns is being studied; however, it is not yet clear how stable these remain after death. Ultimately, every effort to develop new methods to estimate age at death—or to validate old ones—is positive.

In view of the rising migration and genetic blending of entire populations, the concept of "ancestry" will eventually have to be reconsidered. The approach of roughly classifying people to one of the three population groups is too simplistic. On the other hand, the information that a skull found, say, close to a small village in Bavaria, exhibits strong Negroid features can considerably aid the investigation.

Due to the progressive "blurring" of morphological distinctions between ancestral groups, determination of the geographic provenance of individuals will gain importance in future. Stable isotope profiles can provide this essential information; however, reliable databanks still need to be compiled.

The goal of the first identification stage is to find a missing person whose demographic characteristics can be compared to those of the unidentified decedent. The success of this stage depends on many circumstances. For instance, some decedents may have been unknown or unregistered in the country in which their remains were later found. In particular transit countries like Germany, Austria, and France lend themselves to body disposal in the context of organized crime. Facial reconstructions can be used as a last resort; however, if the victim was unknown, the method fails.

Forensic anthropology offers important approaches for the second stage of the identification procedure. The question which methods are best suited for the purpose is largely determined by the type of ante- and postmortem materials that are available for comparison. A crucial aspect in the use of antemortem materials is whether their authenticity can be confirmed. Forensic DNA analysis has become so advanced that STR profiles can be won from most skeletal remains. The greatest limitation of this method is the frequent difficulty of obtaining a reference profile. If the decedent's STR profile is not on file in a national databank, a STR profile has to be determined from antemortem reference material from a purportedly matching missing person. The problem with personal objects (e.g. toothbrushes, razors, etc.) is that there is no guarantee that the objects were actually and exclusively used by that person. When personal material is unavailable, the STR profile from blood relatives can be used for comparisons. However, relatives cannot always be found. Moreover, significant problems can crop up in the interpretation of the results and severely limit the value of the comparison.

The comparison of ante- and postmortem dental records is an expedient tool with which to establish positive identification if the antemortem dental status was reliably recorded by a dentist. These records are often only available in countries with high dental health care standards—and only if the decedent regularly went to the dentist. Further, the method is only useful if tooth repairs were performed. Also, due to dental prophylaxis, growing numbers of people will, in future, have a full set of untreated teeth.

For this reason it is important to maintain and improve the available, established forensic anthropological methods for personal identification, such as radiographic comparison and skull-photo superimposition. In future, radiographic comparisons will benefit from the steadily improving resolution in computed tomography, while skull-photo superimpositions are likely to gain importance in light of the growing availability of portrait photographs, for example, through Facebook and the general “selfie” culture.

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Chapter 11
Post Mortem Anthropology and Trauma Analysis

Cristina Cattaneo, Annalisa Cappella and Eugenia Cunha

Abstract Skeletal trauma remains one of the most challenging issues in forensic anthropology. Whether one is looking at skeletal remains or even well preserved cadavers, forensic anthropology and osteology may be the key disciplines by which to define the weapon used to produce a lesion or date a bone callus. If we omit radiology, which is a forensic discipline *per se*, the tools with which novel research is being performed are still macroscopic and stereoscopic observation, decalcified and non-decalcified thin section microscopy, and scanning electron microscopy with edx. The following paragraphs represent a brief summary of these events and the *status quo*, with particular focus on timing.

11.1 Reading Skeletal Trauma

The methods and techniques employed for studying skeletal trauma can vary greatly: although research can count on many innovative and technological possibilities for improving the interpretation of skeletal lesions, the most common anthropological analyses carried out are still essentially based on macroscopic morphological observation. Luckily, research in the last decades has also focused on many other types of analysis that should be conducted in addition to the usual macroscopic examination of fracture/lesion morphology such as microscopy,

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imaging and chemistry. All of these techniques can add fundamental clues to the interpretation of lesions for the purpose of determining both timing and mechanisms which may be significant for the circumstances of death or pertinent to additional forensic questions [1–5].

11.2 Type of Trauma

Trauma analysis usually involves *ab initio* the interpretation of injury type, which with badly preserved skeletal remains may not always be banal. To simplify, there are three main categories of traumatic injury of anthropological interest: blunt force, sharp force and gunshot trauma. Blunt force trauma is the result of an impact between a body part and a flat resistant surface, which can be as large as a board or as small as the head of a hammer. It results in fractures that depart from the impact site and whose morphology and pattern can sometimes reveal very useful information concerning the type of surface that impacted the bone. Bone undergoes plastic deformation and then finally fracture, which can result in delamination, impact site impression (especially if the impacting surface is small) and the creation of radial and/or concentric fractures (in cases where the impacting surface is large), especially on the cranium. Sharp force trauma is defined as the injury inflicted by blades or pointed tools/weapons such as knives or axes. In this case, depending on the type of tool and the manner of injury production, signs/marks left in the bone tissue can be represented by kerfs, chops or hacks, clefts, gouges, punctures or straight-line cut incisions/alterations. The interpretation and analysis of all the aforementioned marks can offer precious information.

Finally, gunshot trauma is produced by the collision of a projectile that travels at a high velocity and usually causes penetrating trauma. Gunshot injury is characterized by an entry wound, and usually a trajectory and/or exit wound. These typical lesions generally allow one to differentiate gunshot trauma from blunt force injury, while the differentiation between the entrance lesion and the exit wound is based on the bevelling features, the shape and dimensions and the potential presence of chipping. Nevertheless, sometimes the proper identification of these lesions may be very complex.

Whatever the case, the assessment of a traumatic mechanism is dependent on the identification of the fracture pattern: in many cases the anatomical reconstruction of fractured/injured bones should be conducted prior to establishing the trauma mechanism, taking precautions not to lose key residues. More and more osteological trauma examination is indeed focusing on special microscopy [6, 7] and consequent chemical analysis [8] and/or radiology [9], not only for the detection of morphological features useful for trauma interpretation, but also for the collection of extrinsic elements and residues that have nothing to do with the bone, but with the tool involved. Furthermore, the presence of gunshot, sharp force and blunt force tool residues could function as markers of ‘peri-mortality’ if found in a dubious bone lesion. Chemical compounds or residues found near a fracture/lesion or within the lesion can prove the criminal nature of that lesion [7, 9–13]: this is where high power magnification of an instrument, such as the Scanning Electron Microscope with Energy Dispersive X-ray spectroscopy (SEM-EDX) or very sensitive radiological analyses (such as, for instance, cone beam tomography or micro CT's), can be exceptionally useful for searching for metal or other particles [11, 13, 14]. Such findings give important evidence in skeletal trauma analysis conducted on decomposed/and skeletonized cadavers; as a matter of fact their presence has been proven to persist for a long time near/within bone lesions after death and decomposition [7, 10, 11, 13–16] as well as to survive extreme environmental conditions such as burning [9, 11–13]. As always, contamination from the environment needs to be taken into consideration and should be excluded before drawing hasty conclusions [7, 10].

11.3 The Crucial Issue of Timing of Bone Trauma

If defining the type of trauma can at times be difficult, the true challenge for the osteologist is defining the age of antemortem bone trauma and distinguishing between peri mortem and post mortem fractures.

Timing trauma means defining the moment of production of a traumatic lesion with respect to the time of death: the lesions defined as "*antemortem*" have all occurred before death, and the lesions which occurred at or near the time of death (when bone tissue characteristics and properties are similar to those in the living tissue) are defined as "*perimortem*", while all the lesions and alterations produced long after death (once the bone tissue has become dry) are defined as "*post-mortem*".

11.3.1 Antemortem Trauma: The Bone Healing Process and Aging Bone Fractures

The possibility to define when an antemortem traumatic event occurred could reveal precious information, not only for the identification process of skeletal remains, but also in cases of maltreatment, abuse and torture that a person was subjected to during his or her life. Once a lesion has been designated as "*antemortem*", because of the evidence of bone remodelling, no further temporal information is usually determined in a detailed way in case of dry bones; nevertheless, a well-understood series of events normally ensues and some can be recognizable in skeletonised remains through gross macroscopic or microscopic appearance. In fact, bone is a living tissue that is usually exposed to mechanical variables, which challenge its structural integrity. During life, when such integrity is disrupted (as in cases of fractures) the bone tissue (being a living tissue) can repair itself, no matter what the cause of fracture or the type of damage. In this perspective, new osseous tissue can be formed where there is damage or disruption thanks to the regeneration power of bone that is strongly activated during the healing process [17]. Bone healing is a natural process that is only partially understood: it has been studied at a clinical, radiographic, molecular and histological level. It is a very complex process in which many cells and events are involved and in which the consequent result is the reconstitution of the injured tissue and the recovery of the original form, functions and physiology. Histologically, the healing process consists of a coordination of multi-steps where the joint participation of different cells is involved: platelets, inflammatory cells, angioblasts, chondroblasts, endothelial cells, fibroblasts and osteoblasts. All these cells participate actively by synthesizing and realising bioactive constituents of bone tissue components (growth factors, collagen and so forth). Among these types of cells, osteoblasts are considered to be the cells responsible for the synthesis and mineralization of the bone matrix [17–19]. At the same time, resorption activity is performed by osteoclasts, which eliminate the necrotic and dead tissue around the injury site.

In histological terms, fracture healing can be divided into two different healing steps: direct fracture healing defined as “primary” and indirect fracture healing defined as “secondary”. Unfortunately, in the majority of cases the healing events have been studied using experimental fracture healing in animal models (mostly rats) [20] or by using clinical radiological follow-up analyses in human patients [18, 19, 21].

The healing process for most of the authors [17–20, 22–25] consists of six identifiable stages:

1. an initial stage of haematoma formation and inflammation,
2. subsequent angiogenesis and formation of cartilage,
3. cartilage calcification,
4. cartilage removal,
5. bone formation,
6. bone remodelling.

According to Hendrix [22], the healing process is only arbitrarily set into stages, and the literature does indeed show little consistency in terms of stages of bone healing.

There is an agreement on the events which occur in a bone fracture, before the healing and remodelling process: blood pools into the fractured area due to the disruption of vessels where it coagulates and forms a hematoma and a blood clot [25, 26]; osteoblasts proliferate and form new bone as a consequence of the disruption of osteogenic tissue lining the bone (endosteum and periosteum) and finally necrotic and dead bone derived from the separation of bone cells from the vascular system (osteocytes have not longer a proper nutritional connection) is resorbed. This latter event does not necessarily take place immediately after fracturing, but can be included in the subsequent healing process [25]. After fracturing occurs, some osteoprogenitor cells differentiate into fibroblasts and other supporting cells, which form the first organization at the fracture site: a soft fibrous granulation tissue. This tissue will be formed at either end of the fracture segments and extend into the blood clot. It is formed by the activity of a group of cells: osteocytes at this step are not included, because no new bone has yet been synthesized. Instead, osteoblasts start to produce new bone, osteoclasts resorb some necrotic tissue, progenitor cells continue to generate new osteoblasts, macrophages eat away the hematoma, and fibroblasts extend their activity in producing intercellular material. In addition, new blood vessels come into the fracture line from the surrounding soft tissue [22, 27]. Then, a formation of a primary callus across the fracture represents the next stage; when it becomes complete it will give great stability, even if it is composed by woven immature bone. The primary callus, in fact, is a bone tissue not yet calcified and still slightly soft. At this stage it is possible to recognize different parts of the callus based on its position and origin: the intermediate or sealing calluses, which lie at the two fracture ends; the endosteal callus, which is the part of the callus that units the open marrow spaces; and the periosteal callus, which is the most visible, because it bridges the two end fragments around the outside of the fracture.

There are no structural distinctions between these three types of calluses (which are distinguished only as a concept of the process), but the only difference is linked to their origin. The intermediate callus is created on the granulation tissue by osteoblasts that deposit the osteoid into the fibrous connective tissue, where they become ossified, and where their orientation follows the fibrous matrix one. The endosteum callus is formed in the fracture line from osteoblasts derived from the endosteum. The periosteal callus is produced by osteoblasts on the periosteum and it is particular given its often bulging shape; this latter is visible macroscopically in skeletal remains (or bone samples) and more identifiable than the other two on radiographic images [17, 22, 27]. Once a primary bone callus is created, the remodelling stage will take place; the replacement of woven bone with a more organized lamellar bone operated by basic multicellular unit (BMU) will remodel the primary bone into a callus referred to as a "secondary", more stable callus. The BMU are units that remodel the tissues in all stages of the process after fracturing: calcified cartilage is first resorbed and then substituted by woven bone then woven bone is removed and replaced with new lamellar bone. The cells produced in BMU are osteoclasts that remove a packet of pre-existing tissue and then osteoblasts that replace the removal of osteoclasts with a packet of newly bone. The remodelling stage by BMU always follows precise activity in a specific sequence: activation, resorption and formation, which require 3–4 months to build a "secondary" callus from its beginning [27]. The BMU are to all effects the ones responsible for the modelling stage, which means the conversion of calcified cartilage to woven bone and of woven bone to lamellar bone; the resultant mature callus will be sooner subjected to a remodelling stage that permits final healing.

These phases are in general those that represent histologically the healing process and can be seen microscopically, although anthropological literature does not seem to be aware of this completely.

The process of bone healing has also been investigated in the field of radiology for clinical studies (mostly in the orthopaedic field) of the *in vivo* follow-up of the mechanical stability of fracture repair and mineralization as an objective evaluation of the course of a successful healing. Even in radiological assessment, as in the histological analysis, the healing stages are generally classified arbitrarily. As reported in detail by Hufnagle [23], some researchers [22, 28] have presented, in agreement, the best description and outlines of what stages of bone healing are observable in radiographs: up to six stages of bone healing have been described based mostly on the features of the fracture line, fracture gap, and inner structure of the newborn callus, using criteria such as the presence of bridging or obscuration of the fracture line (lucent, sclerotic, or invisible), fracture margin (that can be sharp, blurred, or invisible), visible bridging activity (starting, partial, or complete), and the deposition of new tissue inside the callus and its mineralization.

If, on one hand, the healing process is well understood in forensic pathology, especially concerning soft tissue lesions, the timing of a specific response directly on dry and skeletal remains is still unexplored. Thus, defining the post traumatic interval, namely the time elapsed since the fracture occurred, becomes a difficult task. Bone tissue response also follows a strict time-dependent sequence that can vary depending on multiple variables, such as type of lesion, location, bone type involved, age and health status [27, 29–31]. In literature some works [32–34] report the minimal period required for initial osseous evidence of healing responses on skeletal remains, especially based on the gross appearance. Other important studies focused on histological fracture dating of fresh and dry bone tissue [33–35], reporting detailed healing phases and a timetable (Table 11.1) for natural fracture healing based on documented forensic cases and an extensive pathological literature on fracture healing [34].

Recent research [35, 36] has focused on the evaluation of what features can be still detectable and adequate in the assessment of traumatic lesions on dry bone material by using histology and radiology; while the results have shown that it is still possible to find many features useful in the estimation of the post-traumatic time interval of fractures and amputations, they were based only on archaeological material/callus or fractures of unknown origin, with the intent to prove the possibility of using such features in dating fractures.

This is where research currently stands and where it is heading in this specific field. Table 11.1 represents the *status quo* for dating dry bone calluses and must be taken as the starting point for future immunohistological and biomolecular research, which is still lacking.

11.3.2 Differentiating Peri Mortem from Post Mortem Trauma

Distinguishing between postmortem and perimortem lesions in skeletal remains is another major problem in the forensic anthropological field. There are many factors influencing the success in the identification of a fracture as perimortem or post-mortem, which must be taken into account if a forensic evaluation of trauma is required: the different taphonomic and environmental conditions, the time elapsed

Table 11.1 Timetable of features of the healing process reported by Maat et al. [34] and revised by De Boer et al. [35]

Category of lesion	Healing features	Time interval
Common	Frayed bone lamellae at the lesion margins[a]	Before 48 h
Common	Absorption of the cortical bone adjacent to the lesion[b]	After 4–7 days
Common	First Howship's lacunae at the lesion margins[a]	After 4–7 days
Common	Smoothening of the lesion margin[a,b]	After 4–7 days
Common	Start of endosteal and periosteal osteogenesis separable from cortex[a,b]	After 7 days
Common	Periosteal osteogenesis at distance from the fracture site	After 7 days
Common	Clearly visible endosteal callus formation[a,b]	After 10–12 days
Common	Aggregation of spiculae into woven bone[a,b]	After 12–20 days
Common	Primary bone tissue deposition[a]	After 12–20 days
Common	Osteoporosis of the cortex[a,b]	After 12 days
Common	Margin of the lesion appears more sclerotic[b]	After 12–20 days
Common	Start of the primary woven bone transition into secondary lamellar bone[a]	After 14 days
Common	Cortical 'cutting and closing cones' orientated towards the lesion[a]	After 14–21 days
Common	Fields of calcified cartilage at sites of callus formation	After 14 days
Common	Clearly visible periosteal callus[a,b]	After 15 days
Common	Endosteal callus becomes indistinguishable from the cancellous bone in the marrow cavity[a,b]	After 17 days
Common	Periosteal callus becomes firmly attached (inseparable) to the cortex[a,b]	After 6 weeks
Specific for fractures	First scattered bone tissue spiculae between the lesions ends[a,b]	After 4–7 days
Specific for fractures	Union by bridging of the cortical bone discontinuity[a,b]	After 21–28 days
Specific for fractures	Smoothening of the callus outline[a]	After 2–3 months
Specific for fractures	After inadequate immobilization: Pseudoarthrosis development[a,b]	After 6–9 months
Specific for fractures	After adequate immobilization: quiescent appearance indicating subsided healing[a,b]	After 1–2 years
Specific for amputations	Visibility of cut marks on the amputation surface[b]	Less than 13 days
Specific for amputations	Start of 'capping' of the medullary cavity[b]	After 'not many weeks'
Specific for amputations	Complete capping of the medullary cavity[b]	After 'several months'

[a]Features visible by plain radiographic analysis; [b]Features visible by histological analysis

since death (PMI), the type of bone tissue and the variety of postmortem events which might determine post-mortem changes on the remains.

However, perimortem injuries, postmortem changes and taphonomic alterations (these latter can naturally appear during the decomposition process) may be observable and potentially distinguished [37].

The term “postmortem trauma” refers to a bone lesion occurring after the death of an individual; typical post-mortem lesions are those caused by environmental factors, such as carnivore tooth marks, surface erosions, sun bleaching, weathering etc., and by accidental events produced by weight, human activity, transport and fortuitous trauma [38].

The term “perimortem lesion” in forensic anthropology, means a lesion occurring shortly before or shortly after death; any injury directly associated with the manner of death or the “immediate” handling of the remains is considered a perimortem injury [39]. Nevertheless, according to Wieberg and Wescott [40], the term “perimortem” seems to be ineffective in a forensic context since it refers to a temporal period instead of a physical condition (fresh or dry). Few authors discuss how long perimortem fracture characteristics persist into the post-mortem interval (PMI) or the cause of their changes. It is therefore unclear what exactly constitutes “near death” in terms of skeletal modification.

The morphology of the fracture is the most indicative factor in determining whether a lesion is perimortem or postmortem based on the distinction between fresh or dry bone, depending on the moisture content of the bone tissue [41–46]. A “green fracture”, typical of fresh bone tissue, has characteristics which differ greatly from those exhibited by a fracture typically formed on dried bone: these differences allow us to evaluate when a fracture was produced in relation to the time of death.

The fracture characteristics, in fact, are closely related to the moisture content and the quantity of collagen matrix (which gives flexibility to the bone tissue); at or around the time of death the bone tissue is characterized by the same features observable also in living bone tissue (with the same properties of strength, elasticity and flexibility). Once the bone tissue begins to lose its original components, such as collagen matrix and content of water or humidity (usually because it proceeds in the putrefactive process), its own properties will change radically and invariably. At this time bone tissue becomes gradually “dry”, a type of bone tissue where one can notice a significant loss of flexibility and resistance to subsequent stress [42].

Depending on the bone quality just mentioned, there will inevitably be a different response to the stress applied, which means differences on the fracture features; fractures in bones definable as “fresh” (with a high content of liquids and matrix as well as fat, and collagen) will undoubtedly have a morphological appearance dictated by high moisture content, while, on the contrary, the morphology belonging to fractures that occurred in “dry” bone will exhibit dissimilar characteristics due to loss of moisture. The differences observable in lesions occurring at different times in relation with death may therefore be quite significant, especially if the bone properties in tissues differ so greatly, because the responses to the same insult will appear extremely divergent. However, there will also be a phase in which the properties of tissue will present characteristics intermediate between "green" and "dry" bone tissue, thus causing intermediate responses that will certainly be more difficult to interpret.

What is important in determining changes in tissue properties is not only the passing of time since death, but also all of those cumulative effects resulting from the process of decomposition, taphonomic events and environmental factors that occur over time [39, 40, 45–47].

In this scenario, one must figure out how to deal with a correct trauma analysis on skeletal remains, in which it is fundamental to distinguish between perimortem or postmortem lesions [48].

Several authors have claimed to distinguish easily between peri- and post-mortem fractures on the basis of specific macro-morphological characteristics, which are in their opinion closely correlated with bone quality and properties [38, 45, 48]. They state that the proper assessment of trauma appears totally dependent on the characteristics of the fracture, such as: the fracture morphology, the outline of fracture, the angle and tactile roughness of the fracture margins [38, 40, 45, 46, 48], and, last but not least, the colour of the margins fractured [46, 49]. The features of the fracture are defined similarly by most forensic anthropologists; regardless of their differentiation within the two typologies of fracture (post and perimortem), few studies have focused on the problem of the validity and reliability of such common criteria when they are applied to human skeletal remains. This verification is limited by the lack of human remains where different kinds of fractures are known. In addition, not much is known about potential modification of original lesions with relation to time or as a consequence of environmental factors.

In general, the perimortem nature of an injury is sometimes indicated by the breaking pattern, which is more complex than the breaking pattern of a dry bone; concentric, circular, and spiral fractures are usually typical of green fractures (fractures occurring in living or defleshed bone). Nevertheless, some authors have described such patterns even for dry bones [50] in which typical fracture characteristics include a different production of several small fragments, brittle flaking or shattering, and surface cracking. In fact, Ubelaker and Adams [50] also demonstrated the presence of butterfly or spiral patterns in bones where the fracture occurred 1 year after death. In addition, other fracture-related features of perimortem fractures are bone tear, break-away notch and plastic deformation, with the adherence of small fracture fragments adjacent to the fracture site or impact [45].

The fracture angle and fracture colour are other additional features analysed and considered according to Wieberg [40] as the main characteristics in the determination of post-mortem trauma: dry bones usually show irregular to jagged fracture edges, which are often lighter in colour than the darkened adjacent bone [50]. On dry bone the fractures also exhibit breakage nearly at a right angle to their long axes, with almost flat ends (see the perpendicular, parallel or diagonal breakage). On the contrary, smooth, often bevelled, sharp fracture edges with sporadic sharp projections are associated with perimortem trauma and so are the smooth fracture surface and obtuse or acute fracture angles. In this latter case, these morphological aspects indicate that the injury was inflicted when the bone still had elastic properties, which could be in life or just after death. Furthermore, according to many authors [51, 52] green fractures are characterized by smooth fracture edges, usually of the same colour as the rest of adjacent bone; thus, colour staining can be present due to decomposition fluids, blood, soil, dirty water, leaf stains and organic matter. In this sense, if the colour is homogenous for both fracture margins and the external bone layer, the fractures could be considered as perimortem, since both have been exposed for the same time to staining materials. On the contrary, the difference in colour of post-mortem trauma is connected to the fact that the fracture occurred a long time after death and so the fracture surface has a different colour (lighter) than the adjacent external layer, because it had probably been exposed when the decomposition process had finished.

However, the coloration of fracture edges should be used with caution given its very variable nature, which depends on the depositional environment, as suggested by several authors [40, 46, 53] who have reported an equalization of colour between fracture margins and external cortical bone over time.

The differences between peri and postmortem trauma are briefly summarized in Table 11.2.

However, despite the numerous publications on the subject, the criteria for distinguishing between perimortem and postmortem lesions are based on observations of fractures involving long bones or elements characterized by cortical tissue. When, on the contrary, we consider different types of bone, such as spongy bones, where the cortical tissue is not as present as the main constituent, these parameters no longer work [54, 55].

The main problem however lies in the fact that even when we have a certain perimortem fracture, we do not have the certainty of its vitality. In other words it may be perimortem, although inflicted just after death. This is why anthropology should and is joining forces with pathology in verifying whether signs of haemorrhaging and hence clear signs of vitality of a fracture can be found. Initial studies have proven that there is some hope and again, here also, future investigations must focus on immunohistochemistry and proteomics of these procedures. Indeed, if

Table 11.2 Morphological comparison between fresh and dry bone features according to Johnson [43] and Weiberg [40]

Fracture features	Fresh bone characteristics	Dry bone characteristics
Outline	Radial pattern circling diaphysis	Perpendicular/horizontal fracture surface
Colour surface	Homogeneous colour with external bone	Heterogeneous colour with external bone
Surface	Smooth	Rough
Fracture angle	Obtuse and acute angles	Right angles
Other	Loading point present	Loading point absent
Other	Fracture front never crosscut epiphyseal ends	Fracture front can crosscut epiphyseal ends

anthropology adopted the methods of pathology, it may in fact envisage jumping from the peri-post mortem issue to actually being able to distinguish between a perimortem fracture that is antemortem and one that is post-mortem. This point can be solved only through the search for traces of haemorrhaging and markers of inflammation. The use of additional indicators like biological markers could be fundamental. This is a focal point: if some signs of vitality are found in lesions which seem perimortem, these could be used in the correct diagnosis of the lesion as antemortem, in correlation to a traumatic event that occurred just prior to death [56, 57]. It has been shown in a study which observed the histological fracture margins of apparently dry bone of individuals who had died at known times from the bone fracture (from 10 min to 60 days) that microscopic residues of soft tissue do remain in dry bone and may indicate the presence of an inflammatory reaction and therefore that the fracture was vital. However, further research has to be performed, in parallel with novel proteomic techniques, also in order to investigate the potential of biochemical markers.

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Part IV
Innovation, Unitariness and Evidence. Clinical Legal and Forensic Medicine in Living Person Violence—Criminogenesis and Personal Injury

Casseri, Giulio 1552–1616 Tabulae anatomicae 78 cum supplemento 20 tabularum Danielis Bucretii (Adriani Spigelii Bruxellensis ... Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden ... Amsterdami: apud Iohannem Blaeu, 1645)

Casseri, Giulio 1552–1616, Tabulae anatomicae 78 cum supplemento 20 tabularum Danielis Bucretii (Adriani Spigelii Bruxellensis … Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden … Amsterdami : apud Iohannem Blaeu, 1645)

Chapter 12
Humanitarian Forensic Action. A New Field of Application for Forensic Sciences

Duarte Nuno Vieira

Abstract The chapter, divided into three sections, outlines the emerging role of forensic sciences and legal medicine in the humanitarian action, which includes all the interventions aimed at furnishing aid and assistance designed to protect lives and health, alleviate suffering, protect human dignity during and in the aftermath of man-made crises and natural disasters, as well as prevent and strengthen preparedness for the occurrence of such situations. All of the areas and sub-disciplines of forensic science and medicine can profitably be applied in the humanitarian forensic action, in particular forensic anthropology, odontology, genetics, pathology, and clinical legal and forensic medicine. Forensic expertise in human rights protection serves four main purposes: to help families uncover the fate of their loved ones, to collect and document evidence of inhumane crimes, to use that evidence for convicting the offenders, and ultimately to set up new and more efficient prevention strategies.

12.1 Introduction

Currently, a widely shared and/or universal definition of Humanitarian Action is lacking, due to the fact that the field of analysis (e.g., law, ethics or politics) influences the definition and the principles behind this definition. Putting together the most important international bodies and associations, such as the United Nations (UN) and the World Health Organization (WHO), one could argue that a humanitarian action provides life-saving services and facilitates the return to normalcy for people and communities affected by natural and man-made disasters.

It also seeks to lessen the destructive impact of disasters and complex emergencies. Humanitarian response is guided by the principles of humanity, impar-

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tiality, neutrality and independence, which provide a common framework for organizations involved in the humanitarian action [1].

In this chapter the role of forensic science and medicine in the humanitarian action is presented and discussed. It could seem a new field of application of forensic sciences, although to say the truth it is not really that new, as forensic pathologists and anthropologists have worked in this field since at least 50 years.

The first systematic medical investigation of human rights abuses was used as evidence in an internationally recognized trial in Nuremberg for war crimes involving "medical experimentation" by Nazi doctors [2]. After this contribution there was a long pause in the involvement of medical science in documenting human rights abuses until the mid-1980s, when Dr. Clyde Snow (1984–1985) intervened in Argentina with a team of forensic practitioners investigating the cause of death and injuries sustained by victims of the reigning military junta [3]. Since this first intervention in Argentina, the forensic community has become more conscious and more knowledgeable about his role in recent times, having given it the designation of "humanitarian forensic sciences".

Currently, there is no standard or legal definition of humanitarian forensic action, although it could be synthetized as the range of forensic actions furnishing aid and assistance designed to protect lives and health, alleviate suffering, protect human dignity during and in the aftermath of man-made crises and natural disasters, as well as prevent and strengthen preparedness for the occurrence of such situations [4]. The above definition includes the main aspects that should be comprised in response to motivated humanitarian objectives: to assist people who require such assistance, facilitate the return to normality for affected people and communities, and prevent and strengthen preparedness for the occurrence of potential future situations.

The main part of the chapter addresses the range of forensic activities that seek to protect lives, to alleviate human suffering, to ensure respect for human beings, and to maintain and protect human dignity and human rights. In that sense one must take into consideration the main aspects of any humanitarian actions, namely, humanity, the principles of impartiality, neutrality and independence. Talking about forensic sciences implies the application of scientific knowledge and methodology for the resolution of legal questions and issues regarding individuals and society. As well known, forensic sciences have a big field of applications that include also the humanitarian action, in terms of alleviating suffering, saving lives or protecting health. Reading the documents produced by the United Nations and/or the International Committee of the Red Cross (ICRC) or listening at the multitude of workshops organized by the ICRC, the present and future role of the forensic sciences in the humanitarian action emerges very clearly. Forensic scientists and practitioners can play a key-role in times of armed conflicts, in war crimes, terroristic attacks, natural disasters (such as earthquake, flood, tsunami, etc.) in cases of transport or technological accidents, and in any other kind of mass disaster. All of these are incidents of mass fatality that usually leave behind a large number of victims that deserve the attention of the forensic community, as do their families. These are situations in which the identification of the bodies and their recovery must be a key concern, because the families and the population must have the confidence that everything will be done so that the bodies of their relatives are recovered, properly identified, and are delivered to them as soon as possible. This point is crucial in order for the family to fulfill funeral rituals and to begin the mourning process. In fact, to be informed that a loved one has died is always a personal tragedy, but to remain in the uncertainty of what has happened can cause even greater suffering. In the following section, the main areas where forensic experts apply their specific knowledge, expertise and methodologies is described and discussed.

12.2 The Role of Forensic Sciences and Medicine in Humanitarian Actions

Professor Stephen Cordner has often quoted Sergio Vieira de Mello, a Brazilian United Nations (UN) diplomat who worked for the UN for more than 34 years and believed that missing persons represent one of the most contentious issues when the restoration and maintenance of peace in conflict situations is at stake. In 2003, S.V. de Mello, indeed, said: “my experience has taught me that the disappeared are often the must contentious issue in peace making, the question that makes confidence building all the more difficult” [5].

One of the most important and potentially problematic areas of forensic intervention is represented by the identification of dead bodies. In this kind of humanitarian forensic action it is of upmost importance to differentiate the scenario, that is where, when and why the action took place. Dealing with mass disasters caused by natural phenomena is completely different from acting in situations of terrorism or operating in contexts where violations of human rights have been committed. Such differences create heterogeneous scenarios, requiring different ways of acting and entailing different possibilities of action.

In the occurrence of mass disasters and/or acts of terrorism society usually supports the victims, there is a sense of solidarity and sustenance from the state, as well as funds, and the bodies are generally fresh, with recent ante mortem information.

The scenario is totally different when working on cases of human rights violations, where there is usually no support from the state or, at least, no full support from the state, where investigations can take place years after the events, where in many cases one does not deal with fresh bodies but with skeletonized bodies, and where there is always a lack of funds. In such a context there is also a lack of ante mortem data and DNA analysis can exhibit technical problems (i.e. insufficient biological material for DNA extraction).

There is also another specific and important field where the forensic humanitarian action can take place, that is, those situations of human rights violations with physical and/or psychological torture. Everything began with Clyde Snow in the 1980s [3], when he moved to Argentina with a team of forensic anthropologists to identify Argentinian victims of the military regime; since then forensic medicine has been used on a regular basis in order to discover evidence of inhumane treatments both on the living and the deceased. Colleagues from the National Portuguese Medico Legal Institute have recently worked in Mali carrying out the exhumation of mass graves and identifying victims, thereby obtaining clear proof that there were cases of human rights violations, arbitrary execution and torture prior to killing. Furthermore, there was another high profile case where the Portuguese government decided to send a forensic team to try to recover the bodies of some soldiers who disappeared after the independence of a renown Portuguese colony. The families had been complaining for more than 20 years that they did not know what had happened to their loved ones, to their military familiars, who had remained there, and so a team was sent there to perform the examination of the area and to try to find these bodies, with the result that they were able to recover all of them.

All of the areas and sub-disciplines of forensic science and medicine can be profitably applied in the humanitarian forensic action, in particular forensic anthropology, odontology, genetics, pathology, and the emerging field of clinical legal and forensic medicine.

In fact, in all of the areas of political tension and conflict, where political and religious violence exists, there is always a tendency for an increase in general violence, such as gender violence, child abuse and elder abuse. Legal and forensic medicine can play a key-role in relation to sexual violence, human trafficking, especially in the area of torture and ill treatment in cases of detention, which is something that we find a lot of in countries that are undergoing difficult and violent periods.

To examine victims of violence or torture, to properly recognize and classify these injuries and therefore to ensure the safeguarding of lives, thereby promoting changes and permitting courts to fulfil their role, implies the necessity of a specific know-how and expertise in clinical legal and forensic medicine.

Recently the European Council of Legal Medicine (ECLM) and the European Union of Medical Specialists (UEMS) has approved a denomination and definition, which states that legal and forensic medicine involves the observation, documentation, collection, assessment and scientific interpretation of medical evidence deriving from clinical and/or post-mortem investigation. Thus, beside post-mortem investigations, also clinical forensic examinations under different fields of law (i.e. criminal, civil, work, family and administrative law) have been recognized as a core sub-discipline within forensic and legal medicine. Looking at the content of the residence program in legal and forensic medicine of the main European Countries there is a wide description of what a resident should learn in terms of ascertainment methodology (how to conduct an interview, a clinical examination, how to collect evidence, what kind of complimentary examinations to request, etc.) and criteria of evaluation (i.e. how to interpret clinical data and other evidences).

It is fundamental to know that the doctor-patient relationship changes when you pass from a clinical physician situation to a medical expert situation; collaboration and trust become something more connected with lack of cooperation and some degree of suspicion and you have to know how to surmount these problems and resolve the difficulties that pertain to a clinical forensic examination, namely simulation and dissimulation, or more in general malingering. The clinical interview is crucial. The examiner must be sensitive and empathic, watch out for signs of tiredness or distress, and remain objective. He has to ensure active listening, meticulous communication, courtesy and genuine empathy and honesty in order to obtain the examinee's trust. He must adopt the right posture and know the questions to use, how to pronounce them and which questions to avoid, such as *leading questions* (individuals may answer with what they think the forensic professional wants to hear), *closed questions* (which limit the answer to a choice between the two) or *too many questions at the same time* (the examinee might be confused). Moreover during the interview the ascertaining physician has to observe the body language, the facial expression, the tone of voice, and the gesture of the examinee that can give many clues about the truth of the history and about the consistency of the allegation that are presented.

Another essential step of the ascertainment is represented by the systematic clinical examination, which must follow a "head-to-toe" scheme. Of course the forensic expert has to be prepared to work in conditions that often are not the best ones. For example when examining people in the field of a humanitarian forensic action, violation of human rights, where sometimes the ambient is not the ideal one but the only one possible, then the examiner must be particularly attentive in order to overcome the deficiencies and insufficiencies of the examining conditions. During the clinical objective examination the forensic physician has to systematically ascertain each portion of the body surface through inspection and palpation, to expose only the part observed in each stage of the collection, to examine the clothes very accurately and to collect physical evidence, with a thorough photographic documentation. The forensic expert is specifically trained for the examination and description of injuries, describing the *number*, *location* (anatomical region involved and distance from permanent anatomical marks with a scale), *type* (ecchymosis, hematoma, bruise, laceration, contusion, incised wound, burn, oedema, etc.), *shape* (linear, curvilinear, rounded-shaped, oval, irregular, etc.), *dimensions* (height, width, diameter, depth; use measuring scale), *orientation*, *colour and surface* (scaly, crusty, ulcerating, etc.), *signs of vital reaction and secondary reactions* (discoloration, healing, infections, etc.), *presence of associated foreign material* (soil, sand, wood, etc.). Given this basic description, within the field of clinical forensic medicine, the trained physician must also ascertain and describe impairments and damages, such as reduced mobility, pain, tenderness, and abnormal range of movement. He has to use *international nomina anatomica* and the international classification of functional disability and health (ICF) [6].

In my personal experience, travelling around the world, I have found a lot of colleagues that still perform forensic descriptions using local and national expressions and do not use the international standards. One should know that in clinical forensic medicine it is important to describe the existence but also the absence of any injuries to have a full and reproducible picture. Documentation in diagrams and photos is crucial, using metrical and colorimetric scales in order to allow a second opinion/interpretation to be performed also in a retrospective fashion.

In clinical forensic medicine it is also important to always take into consideration that the situation under observation could have been changed due to the intervention of the emergency team, and that the absence of evidence is not evidence of absence [7]. In many cases the forensic examiner intervenes too late, when many things are corrupted or disappeared. For example, in the field of torture medicine, many victims totally recover from the physical and psychological point of view and when they arrive to the forensic examiner there is no clear evidence of physical and/or psychological torture, but absence of evidence is not evidence of absence.

As discussed in many workshops organized by the International Academy of Legal Medicine (IALM) and during the recent IALM Intersocietal Symposium in Venice (21st–24th June, 2016), clinical forensic medicine is fundamental in the ascertainment and evaluation of migrants and asylum seekers. The aim is to get evidence as whether the case under examination is really a case of torture or persecution or inhumane treatment or not. The new European Directive 2013/32/EU describes a common procedure that should be followed in every European State whenever migrants ask for asylum [8]. A number of substantive changes have been made to the Council Directive 2005/85/EC of 1 December 2005 on minimum standards on procedures for granting and withdrawing refugee status [8]. From a medico-legal point of view it is essential that in every European reality the same ascertainment methodology and criteria of evaluation are adopted and applied to perform a valid differential diagnosis and arrive at a solid conclusion concerning humanitarian protection.

Other areas of forensic action can have a strong humanitarian impact dealing with migrants and asylum seekers, especially forensic genetics. Every day we are seeing migrating families arriving with children that do not belong to those families, which points to the fact that child trafficking is now becoming more and more common. Children are being kidnapped and used in order to try to obtain asylum, so genetics is also very important in this area for trying to see if the family is really a family.

So the role of the forensic sciences is today very well recognized. For example, a Report on the special role that the forensic sciences can play in the investigation and documentation of torture was recognized by the General Assembly of the United Nations in 2007 [9].

Given this special role of the forensic sciences, all of the major international organizations have forensic groups dedicated to the field of humanitarian forensic action and even the International Criminal Court (ICC) decided to create, two years ago, a forensic international advisory board in order to provide advice and expert opinions in all cases with humanitarian implications at trial. Nowadays, all of the major international organizations use forensic medicine and the forensic sciences on a routine basis, whenever they are fulfilling such a role in the field of humanitarian actions.

It is also interesting to note that international scientific forensic associations are paying special attention to such issues; perhaps a special prize should be awarded to the American Academy of Forensic Sciences (AAFS), which created the first Humanitarian and Human Right Resource Center. This center sets up projects, research activities and training with a basis in humanitarian forensics all over the world [10].

12.3 Conclusions

The theoretical and operative roles of the forensic sciences in the present and future humanitarian forensic action can be summarized as follows.

1. Forensic experts can *provide casework*, which means perform forensic ascertainments and evaluations on the field, as already done by the International Committee of the Red Cross (ICRC).
2. Forensic experts and physicians specialized in legal medicine can *promote adequate practices* working and sponsoring this kind of training and capacity building all over the world.
3. Forensic scientists and practitioners can help *developing guidelines and operational standards*, not only of a specific forensic nature. For example, the Minnesota Protocol has just been reviewed in February 2016, and will be soon available on the website of the United Nations [11]. The old manual written in

Vienna in 1989 has been updated with a benchmark for the autopsy procedure all over the world, especially when there are humanitarian and human right issues present. Forensic pathologists have significantly contributed to the revision of this international protocol, which has a strong ethical and humanitarian impact.

4. Specialists in legal medicine and forensic scientists have to stress *capacity building,* where there is still much to be done; capacity building should include not only forensic practitioners, but also all of those who are the recipients and can be directly involved in forensic activities. Capacity building that only takes into consideration forensic practitioners will not be enough, since the participation of prosecutors and judges is essential, as they can learn and come to understand much more effectively what forensic scientists can provide.
5. All of the professionals involved in the humanitarian action must *convert the lessons learned and the experiences obtained into effective prevention mechanisms.* Through the humanitarian forensic actions the forensic community can promote human rights and attempt to make people more sensitive to all of the rights set out in the universal declaration. Everybody knows that human rights are not always a top priority in the majority of Countries. It is essential to insist, persist, resist and never give up, as depicted by the Acción Poética Escobar, an Argentinian cultural and poetical movement, which promotes solidarity. To never give up is of fundamental importance. And I am absolutely sure that in this field of humanitarian action, humanitarian forensic action can really make the difference.

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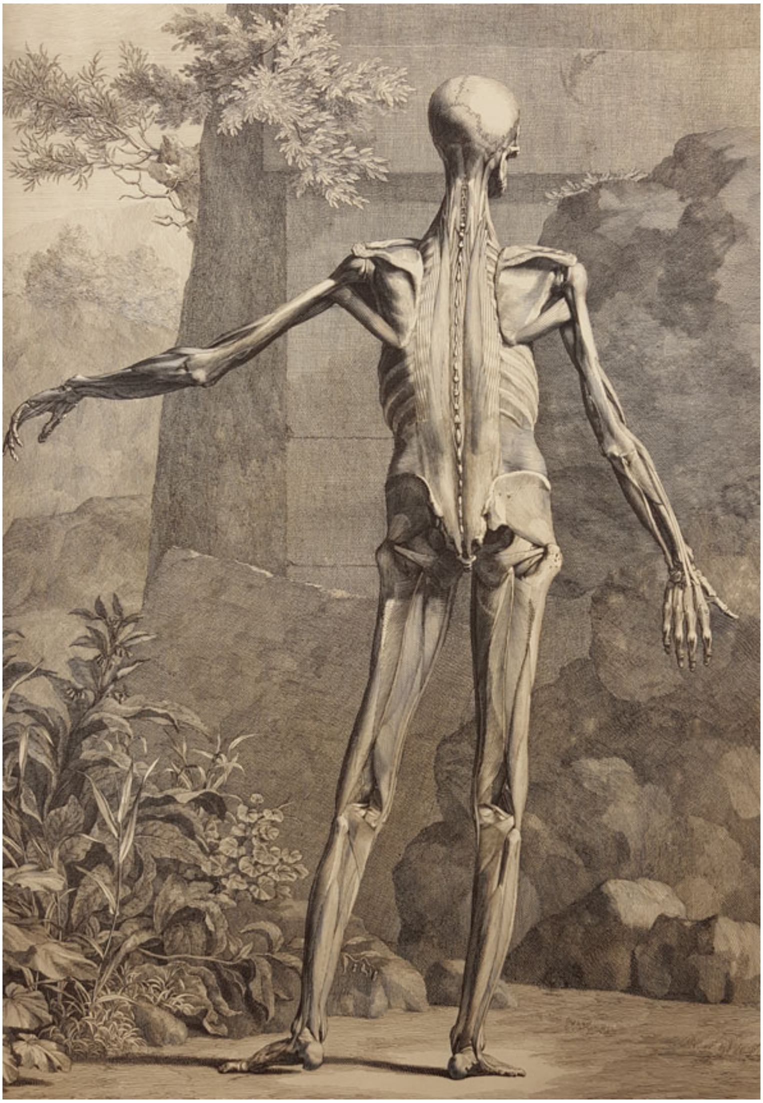

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Chapter 13
Omics and Functional Imaging in Antisocial Behavior

Pietro Pietrini, Giuseppina Rota and Silvia Pellegrini

Abstract Recent advancements in molecular biology have permitted to broad the possibilities already achieved by morphological and functional imaging (PET and fMRI), highlighting not only the brain areas, but also the cerebral circuits and molecules that modulate human violent behavior . Through the omic sciences, epigenetic mechanisms (e.g. Hyper-methylation of MAO-A gene) and genetic variants that are capable of promoting violent and/or aggressive behaviors in subjects exposed to traumatic adverse events have been highlighted. These findings open up important forensic psychiatric perspectives on the use of omic science data to support the conclusions about partial or complete insanity of defendants, igniting a vivid debate about the use of neuroscientific evidence in court.

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13.1 Introduction

"Tom looks and is in robust physical health. His manner and appearance are pleasing. In his face a prospective employer would be likely to see strong indications of character as well as high incentive and ability. He is well informed, alert, and entirely at ease, exhibiting a confidence in himself that the observer is likely to consider amply justified. This does not look like the sort of man who will fail or flounder about in the tasks of life but like someone incompatible with all such thoughts" [1].

The above description of a young psychopath sketched by psychiatrist Hervey M. Cleckley in his famous book "The mask of sanity" draws our attention to one of the most puzzling aspects of psychopathy—its chameleonic and deceitful nature. Since antique times, psychopathy has represented a great menace for society and clinicians have tried to understand its nature and to find remedies for it. Philosophers, also, have been fascinated by psychopathy. Plato, for instance, had already suspected that evil behavior had to be the result of both some biological disposition and the exposure to environmental influence, as no one would willingly choose to be evil: *"No one is willingly evil, but one can become evil for a bad disposition in his body and for a training without a true education; this is hideous for everyone and happens against his will"* (Plato, Timaeus, 86e).

The concept that psychopathic antisocial behavior must be rooted somehow in the individual brain has been proposed also by the great fathers of psychopathology, including the British psychiatrist Henry Maudsley (1835–1918), who wrote: "*As there are persons who cannot distinguish certain colours, having what is called colour blindness, and others who, having no ear for music, cannot distinguish one tune from another, so there are some few who are congenitally deprived of moral sense*".

However, the neural correlates that subtend mental abilities involved in human social interactions, including moral judgment, control of impulses, modulation of behavior, theory of mind, empathy and so on, have remained mostly an authentic mystery until recent times. The body of knowledge gained in the field of neuroscience over the last decades has changed the way we conceptualize mind, behavior, and even human nature. While since the 19th century it has been well known that lesions to the cerebral cortex may lead to impairments in specific cognitive functions and in the ability to modulate behavior, only in recent years, through the development of modern methodologies for investigating brain functions, including positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and magnetic electroencephalography (MEG), it has become possible to investigate the neural circuits implicated not only in cognitive processes like perception, attention, memory and language, but also in more complex and elusive mental functions, including emotions, impulse control, moral judgment and social behavior. In addition, molecular biology and genetics have led to the decoding of the human genome and are now investigating the role that genetic endowment plays in shaping physical traits, but also personality features, as well as the risk of developing body or brain pathologies.

Among the research strands followed by modern pioneers of functional exploration of the brain, of special importance for the law is the study of the neural bases of aggression and antisocial behavior. Recent studies indicate that regions and circuits within the frontal lobes are involved in the regulation of aggressive impulses, moral judgment and behavior in general [2, 3]. The hypothesis that those circuits may be dysfunctional in criminal individuals has found support from the empirical evidence of significant morphological and/or functional differences between the brains of "normal" individuals and that of criminals. Indeed, quantitative measures with high-resolution magnetic resonance imaging showed that psychopathic convicted criminals have over 20% fewer neurons in the ventromedial prefrontal cortex as compared to matched control individuals with no history of abnormal behavior [4, 5]. Interestingly, this difference remained significant after data were co-varied for head trauma, abuse of alcohol or substances and education, indicating that they were not merely the consequence of concomitant factors associated with psychopathological conduct. In addition, specific alleles in genes involved in the metabolism of neurotransmitters can be associated with a significantly higher risk of developing antisocial behaviors and of committing criminal acts [3, 6].

In view of the evidence from neuroscience, the question of the extent to which the individual is free and responsible for his or her actions has found a renewed vigor. The issue reconnects to the millenary debate, within the ethical and philosophical realm, on free will versus determinism—a debate whose echo resonates in the courtroom, as the main foundation of the criminal justice system is that individuals are well equipped with free will that enables them to distinguish good from bad and to decide to act in one way or another.

In the present chapter, we briefly review the utilization of imaging tools and genetic assessment in the study of human behavior and examine findings from our own studies as well as from other labs on the neurobiological correlates of human (anti)-social behavior. We also discuss the potential implications of these findings in the forensic settings.

13.2 The Study of the Neural Bases of Human Behavior

Until the beginning of the 1900s, the study of the neural bases of human behavior relied on clinical observations of neurological disorders or traumatic brain lesions and their correlation with cognitive and/or emotional deficits. The famous case of Phineas Gage, the young worker whose most frontal lobes were injured by a penetrating trauma due to an accidental explosion, is one of the first best described cases of the consequences of frontal impairment on personality and behavior [7].

The first method devised to investigate brain function in a non-invasive manner in intact human beings dates back to the beginning of the last century. In 1929, the German psychiatrist Hans Berger discovered that the spontaneous electric activity generated by brain cells can be measured by applying electrodes to the scalp:

electroencephalography (EEG) was born. EEG records spontaneous electric activity from the whole cortical surface. Specific neuronal activity is associated with physiological arousal, wakefulness-sleep cycle, as well as to distinct emotional and cognitive states. EEG may also be employed to measure discrete brain responses (event-related potentials, ERPs) to sensory or cognitive events. In EEG recording, the spatial localization of the source of electrical activity is relatively poor as compared to other more modern imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). On the other hand, temporal resolution is excellent, as EEG detects neuronal firing itself. Since its discovery, EEG has become an important tool for neurologic and psychiatric diagnosis and intervention and is widely used in clinical settings.

In spite of the fact that the first modern technology for studying the human brain in vivo dates back nearly one hundred years, only in the last few decades there has been a true revolution in the methodologies for dissecting the neural underpinnings of mental function in a non-invasive and unprecedented way. As a matter of fact, the development of more and more sophisticated brain imaging tools, including structural and functional magnetic resonance imaging, has made it possible to deepen the study of the morphological and functional architecture of the human brain.

Altogether, the findings from these studies concur in indicating the crucial role of frontal cortical areas and their links to subcortical structures, including the amygdalae, in the modulation of behavior, action planning and impulse control in physiological conditions and in individuals with abnormal behavior [2, 3].

13.3 Predictive Role of OMIC Sciences in Violence Prediction

Novel interesting findings on the underlying mechanisms that may predispose to aggressiveness and violence in humans originate from studies that investigate the complex interplay between "*nature*" and "*nurture*".

Observational studies on twins and adoptees indicate that violent human behavior is 65% heritable [8], although many environmental factors, including maltreatment, abuse and being neglected during childhood, are known to play a role in expression of aggressive behavior in adults [9].

Following the completion of the whole sequencing of the Human Genome, specific genetic variants have been identified in the serotoninergic and dopaminergic pathways that increase individual vulnerability to impulsive and aggressive behavior, by modulating the impact of negative environmental factors on behavioral traits [6, 10–16]. Among these variants, those with the greatest evidence of an association with aggressive behavior are: a variable number of tandem repeats in the promoter sequence of MonoAminoOxidaseA gene (MAOA-uVNTR); three variants located on the gene that codifies for the serotonin transporter (5-HTTLPR, SCL6A4 rs25531, STin2); a single nucleotide polymorphism (SNP) on the

Catecol-methyltransferase gene (COMT rs4680); and two variants on the Dopamine Receptor D4 gene (DRD4/1-11 and DRD4 rs1800955) [6, 10]. All these allelic variants play a role in regulating the cognitive and emotional processes underlying social behavior, and they can increase the vulnerability to develop violent and antisocial behavior in those individuals who were raised in negative and abusive environments during childhood and adolescence [6, 10]. Of note, combinations of three or more of these polymorphisms, rather than each one of them individually, increase the risk of antisocial behavior in people who have been maltreated and abused as children [6, 17].

Preliminary findings obtained by our own research group indicate that combinations of these risk variants have a greater frequency in individuals with antisocial and criminal behavior than in noncriminal control subjects [18]. This is actually an important issue, as it is well known that genes acting on related phenotypes do enhance their potency—a phenomenon called *epistasis*.

However, if it is true that genetics influences the way we react to the external stimuli, it is also true, on the other hand, that the environment itself exerts some influences on genes by modulating their expression. Specifically, environmental stimuli produce *epigenetic* modifications on our genome—by the means of adding some chemical markers, for example methyl groups to DNA, without changing the genetic sequence—, which regulate gene function. Epigenetics may be considered as the missing link between *nature* and *nurture* [19], as DNA methylation, histone modifications and the expression of specific microRNAs modulate gene expression in neurons and have a role in shaping our behavioral responses to environmental factors [20, 21]. Child abuse, for instance, creates long-lasting changes in methylation of the SLC6A4 gene promoter in lymphoblasts, which are associated with adulthood behavioral outcomes, including antisocial personality disorder [22]. Similarly, MAOA promoter hypermethylation in human blood has been associated with antisocial personality disorder [23].

None of these genetic or epigenetic modifications, however, or their combinations, exert any deterministic effect on behavior, but rather they act as vulnerability factors that make subjects more receptive to external negative stimuli and increase the risk of aggressive behavior. Based on this concept, predicting whether individuals are likely to commit a crime based on their genetic profiles to date is not reliable. On the other hand, the existence of genetic variants that affect the individual susceptibility to a negative environment—especially during childhood—and to violence should not be ignored, as it has several implications for criminology and, potentially, for the management of criminal detention and the rehabilitation for convicted individuals [24, 25].

The most recent findings [26, 27] have led to the hypothesis that these "risk genetic variants" may actually act as "plasticity" alleles, rather than as "vulnerability" alleles. That is, these polymorphisms would enhance the individual susceptibility to the external environment, regardless of its negative or positive features. Thus, the same genetic variants that are associated with an increased risk of antisocial behavior in people with a history of child abuse and maltreatments, on the contrary, would be responsible for an increased inclination toward prosocial behavior in subjects raised in positive and protective environments [28].

This hypothesis definitely excludes a deterministic role of these variants on behavior and carries important implications both in terms of childhood raising interventions and of novel rehabilitation strategies for convicted individuals.

In light of their wider meaning, these findings are gaining more and more interest not only from a neuroscientific point of view, but also from an ethical and legal perspective, giving rise to an active debate among neuroscientists, philosophers and legal scholars.

The next step of research in this field will be the attempt to complete the whole picture by the identification of other genetic variants that, in addition to those already known, may affect our way of behaving in response to the environment. A great contribution will come from the OMIC sciences, like genomics, proteomics and metabolomics, which has already started, by using GWAS (Genome Wide Association Studies) to find a few novel genes that might play a role in shaping human social behavior [29–31].

13.4 Relevance in Forensic Psychiatry

Results from both morphological and functional brain imaging studies and from behavioral genetics have identified specific neurobiological correlates of human behavior. These findings raise important implications that go far beyond the mere field of neuroscientific investigations. Specifically, if behavior is modulated by neural circuits, the question is to what extent an abnormal behavioral control can be due to an anatomical abnormality or to a functional failure within those neural systems. As a matter of fact, studies that combined MRI with genetic investigations indicated that individuals with the MAOA-low alleles have both structural and functional brain differences as compared to MAOA-high allele subjects [32]. Interestingly, both genetic vulnerability alleles and morphological and/or functional alterations in frontal cortical areas have been found in individuals convicted for impulsive crimes [33]. These findings have started to be incorporated in expert witness examinations to support the conclusions about partial or complete insanity, igniting a vivid debate about the use of neuroscientific evidence in court [34, 35]. Furthermore, brain areas involved in behavioral control may undergo depletion, and this phenomenon could play an even more relevant role in more vulnerable individuals. In a series of studies with combined high-density EEG and structural and diffusion weighted MRI, we recently showed that healthy young individuals who perform executive tasks that involve the frontal cortex undergo episodes of 'local sleep' in the same cortical areas after 24 h of sleep deprivation, which are associated with task impairment. An fMRI connectivity analysis indicated that performance impairment may depend, at least in part, on a breakdown in connectivity determined by a "network overload". These results indicate the existence of an association between theta waves during wakefulness (local sleep) and performance errors and may contribute to explain behavioral impairments under conditions of sleep deprivation/restriction, as often occurs prior to impulse crimes. Moreover, sleep deprivation was associated with micro-structural changes in both grey and white matter that were reverted by recovery sleep [36, 37].

13.5 Conclusions

Over the last few decades, developments in cognitive neuroscience methodologies and molecular biology have led to unprecedented tools for investigating the neurobiological correlates of mental functions and dissecting the neural mechanisms of even the most intimate aspects of the human mind [38]. Altogether, these findings from behavioral neuroscience are providing novel bases to the ancient question of whether evil individuals are *bad* or *mad*, that is, malevolent by choice or rather because incapable of being otherwise. This issue in turn will also have important implications for forensic psychiatry and the law itself.

One foundation of the criminal justice system is that individuals are gifted with free will that enables them to distinguish good from bad and to decide to act in one way or another. On such a foundation the retributive jurisprudence, typical of all modern societies, bases culpability and imputability. Insanity is widely recognized as a valid excuse from legal liability. Thus, if control of aggression is associated with specific neural substrates, is it not plausible that whatever alterations of such substrates occur—whether congenital or acquired, morphological or functional—these may lead to abnormal behavior that escapes the individual's control, even in the absence of any evident pathology? Furthermore, does not the possession of a given genetic allele that increases the risk of developing impulsive behavior or loss of control under stressful situations or in response to provocative stimuli, in itself represent a limitation to what we call free will? These are only some of the numerous questions that arise at the crossroads among science, philosophy, ethics and the law and that will represent a challenge for the years to come.

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Chapter 14
Current and Future Evidence in Personal Injury Ascertainment Under Criminal Law

Reinhard Dettmeyer

Abstract The forensic medical examination of victims of violence for the purposes of gathering evidence for use in criminal proceedings requires a prompt physical examination of the victim, a precise description and, where possible, photodocumentation of injuries, as well as their correct medico-legal interpretation according to the incident. Such examinations, documentation, and appraisals are required to meet minimum standards, which may be subject to modification in the case of suspected child abuse and for the purposes of examining victims of sexual violence. It is also essential to ensure that the examiner or expert possesses material autonomy, as well as administrative and organizational independence. An independent expert should not belong to the police force, the public prosecutor's office, or the court in any organizational capacity. Statutory provisions need to exclude the possibility of influencing an expert "through official channels", while at the same time his financial independence should be such that attempts at corruption are at least unlikely. The independence of the expert is of particular importance in cases where individuals have been the victim of violence in official custody, most notably in police custody, in prisons, and following involuntary detention in a psychiatric institution.

14.1 Introduction

The forensic medical examination of victims of violence has been part of daily routine in numerous countries for many years [1, 2]. The examination and expert appraisal of victims of violence take place against a backdrop of both national and international legal requirements. Foremost among these is the "Universal Declaration of Human Rights", proclaimed by the General Assembly of the United Nations on December 10, 1948, articles 3, 5 and 11:

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- ***Article 3:*** *Everyone has a right to life, liberty and security of person.*
- ***Article 5:*** *No one shall be subject to torture or to cruel, inhuman or degrading treatment or punishment.*
- ***Article 11 (1):*** *Everyone charged with a penal offence has the right to be presumed innocent until proved guilty according to law in a public trial at which he has had all guarantees necessary for his defence.*

Manifold efforts have been made since 1948 to improve the protection of fundamental human rights, e.g., by developing additional provisions relating to the collection of evidence and the protection of victims of violence. Reference should be made in this context to the "*Manual on the effective investigation and documentation of torture and other cruel, inhuman or degrading treatment or punishment*", "Istanbul Protocol", issued by the UN Office of the High Commissioner for Human Rights in 2004 [3]. The international professional code of medical ethics requires physicians to not use their medical knowledge contrary to the laws of humanity (The World Medical Association's Declaration of Geneva, 1948, updated by the Declaration of Tokyo in 1975). This precludes an overly strong identification with the respective prevailing state powers in many countries. The physician's responsibility is to his/her patients, the injured victims of violence—any possible loyalty to the state is of lesser importance.

Where this is possible, individuals with violence-related injuries are treated in medical practices or medical centers and hospitals in the course of everyday professional life. The patient's health and, for instance, any necessary surgical interventions, take priority. For this reason, not to mention the sometimes high workload in hospitals, physical examinations of victims of violence solely for the purpose of collecting evidence are often inadequate, if not dispensed with entirely. As a result, the documentation of injuries that is admissible in criminal proceedings is often flawed or lacking altogether.

Quality requirements in terms of the examination and documentation of injuries in victims of violence are frequently not included in medical training and continuing medical education. Institutions, organizations, and the literature occasionally list criteria relating to the care of victims of violence that physicians should adhere to, e.g., European Council of Legal Medicine (ECLM) Guidelines, Examination of living persons. Harmonisation of Forensic and Medico-Legal Examination of Persons [4]. These guidelines make a partial distinction between types of violence.

Violence toward infants and children is considered separately, as are sexual violence, violence in the form of torture or against individuals in official custody, as well as general violence among adults, including domestic violence and violence within families. A conceptual distinction is made between "maltreatment" and "torture". Torture is defined in the UN Convention against Torture, Article 1, as follows:

> For the purposes of this Convention, the term 'torture' means any act by which severe pain and suffering, whether physical or mental, is intentionally inflicted on a person for such purposes as obtaining from him or a third person information or a confession, punishing him for an act he or a third person has committed or is suspected of having committed, or intimidating or

> coercing him or a third person, or for any reason based on discrimination of any kind, when such pain or suffering is inflicted by or at the instigation of or with the consent or acquiescence of a public official or other person acting in an official capacity. It does not include pain or suffering arising only from, inherent or incidental to lawful sanctions.

The present article, however, is not primarily concerned with the appraisal of injury resulting from torture, but rather with everyday violence against individuals and its various forms in different situations.

If a medical examination is insufficient, the associated documentation is poor, and the interpretation of injuries inadequate, the evidence required in criminal proceedings against one or more defendants will ultimately be lacking. In such cases, findings are legally admissible to only a limited extent. Particularly in criminal proceedings, however, the highest standards of proof apply, given that offenses are punishable by law.

The description of standards on the forensic medical physical examination of victims of violence, as well as on the documentation of findings, is subject to variation in the primarily forensic medical literature [5–15]. Other medical professional groups lack, or rarely possess, appropriate experience in the examination of victims of violence. Non-forensic medical experts also lack experience in terms of appearing as an expert witness in court proceedings and providing verbal testimony before court.

If present, normative regulations on ensuring the freedom and independence of (forensic medical) experts in official proceedings vary widely. The de facto organizational integration of forensic medicine in various state institutions and authorities not infrequently raises doubts about the neutrality and independence of the expert, even when, in reality, correct appraisals are made.

The situation looks different when, from the outset and in a manner apparent to all, an expert acts exclusively on the side of one party, e.g., the police or public prosecutor's office. In such cases, independent courts and the law of criminal procedure must ensure that the rights of those victims of violence that are not supported by their own experts, or that are unable to afford an expert, are recognized.

Cases of violence against police officers or other representatives of state authority should be considered as special aspects. Violence is also seen against physicians that help the wounded and injured in times of conflict and war irrespective of the person concerned, thereby becoming targets of violence themselves. Reports of this kind emerge from war zones and regions affected by civil war. It goes without saying that physicians should not be persecuted for practicing their profession. Physicians must be allowed to practice their profession without fear of repression. The same applies to physicians that write expert appraisals on injuries for use in legal proceedings or to inform the public.

14.2 The Forensic Medical Examination of Victims of Violence

Individuals that have sustained injuries must have the right to an adequate physical examination by an independent forensic medical expert for the purposes of documentation and expert appraisal. This applies both to the victims of acts of violence (examination of victims of violence), as well as to those individuals that have been accused of a crime (examination of defendants). The opportunity for a physical examination by an independent expert should also be available in equivocal cases involving an actual or alleged accident.

Ultimately, there may be reasons why a victim of violence has not (yet) informed the police and does not (yet) wish to take legal proceedings, despite the fact that a perpetrator could be punished and the victim could claim damages and compensation. The state must possess the organizational infrastructure, in collaboration with medical institutions, to provide (or create) the possibility for the independent, neutral, and confidential collection of evidence even in such cases; this includes recording traces of injury by means of photodocumentation.

It must always be possible to conduct medical examinations of potential victims of violence of any kind in the presence of an interpreter, where necessary, and without third parties present, unless the victim expressly wishes the presence of a person of trust. It is essential to ensure that the recommendations made by the (forensic) examiner on the basis of the examination are promptly implemented or offered by a specialist physician (gynecologist, urologist, internist, etc.).

14.2.1 The Forensic Medical Physical Examination of Victims of Violence as Evidence of a Criminal Offense

The individuals that most frequently undergo forensic medical physical examination are victims/witnesses of criminal offenses. The injuries identified in this way may originate from widely varying situations with diverse histories. A distinction can be made, for instance, between the following case constellations:

- Violence between adults in a public place or in the workplace;
- Domestic violence between adults;
- Violence against infants, toddlers, children, and adolescents [16];
- Sexual violence involving adult victims;
- Sexual violence against minors (see [17]);
- Violence against individuals in official custody (e.g., police custody, detention centers, barracks, psychiatric units, etc.);
- Violence committed by paramilitary groups (e.g., militias, private or state armed guards);

- Violence against individuals particularly at risk (the mentally and/or physically handicapped [18]);
- Violence against elderly, care-dependent, or defenseless individuals in homes or other institutions;
- Violence committed by physicians, nursing staff, or fellow patients against patients;
- Violence committed by patients or residential care patients against physicians, nursing staff, etc.;
- Violence against enforcement actions undertaken by the state, i.e., primarily against police officers (resistance against state authority);
- Violence in the context of self-harm or self-mutilation.

The description and documentation of injuries need to satisfy minimum criteria, e.g., localization, type, direction, extent, depth, color, and—if possible—the approximate age of the injury. Injuries in the oro-dental region may be worthy of particular attention [19].

The vocabulary used to describe an injury should be suitable in terms of quality and acceptance and should be as unambiguous as possible. A good description should enable other experts to identify and interpret the pattern of injury—possibly even when photodocumentation is lacking. Photodocumentation needs to be technically correct in terms of: image size selection, brightness, sharpness; the scale used on the image for sizing; overview and detailed images; correct numbering and labeling of images.

National legislation can, to varying extents, obligate victims of acts of violence to cooperate in the process of proving a criminal offense. Indeed, victims of violence are generally disposed to cooperate—depending on the national framework—since victims generally have an interest in seeing the perpetrator convicted. However, an individual must have the right to refuse, e.g., in cases where the suspect is a close relative. In the absence of the right to refuse cooperation, a victim is obliged to submit to, e.g., a physical examination and/or possibly blood collection or hair sampling, where appropriate, for the purposes of detecting alcohol or drugs.

Depending on national legislation, the handling of victims of violence can be regulated within the framework of provisions, guidelines, or instructions as follows:

- Considerate handling of victims of violence;
- Where possible, only one round of questioning by the police or preferably a judge;
- Videotaping of questioning, at least in the case of minors;
- Respecting the victim's right to have a person of trust or legal representative present.

Some countries pool victims' rights together in the context of criminal proceedings to include the right to an interpreter and the right to psychosocial support in the event of a trial, while in other countries such an approach is, at the very least, the subject of discussion. A systematic approach to collecting all findings, while at the same time adhering to standards of medical examination, is required most notably in the case of sexual violence [4, 18, 20]. There are separate publications on the handling of child victims of sexual violence and torture [21–26].

Even in cases where no recourse is taken to legal proceedings, legal systems can nonetheless make provision for the compensation of victims if injuries are interpreted by an expert as violence-related and if civil proceedings are taken to claim damages and compensation (“parallel justice”). A number of countries have passed laws to protect victims of domestic violence, the most recent being the People’s Republic of China in March 2016. Laws of this kind enable, for instance, an exclusion order against perpetrators banning them from certain places, or a restraining order specifying that they must maintain spatial distance from, and refrain from contacting, the victim, including by means of electronic media (cell phone, social networks, etc.). However, there is often little concordance between the legal framework on paper and reality.

14.2.2 The Forensic Medical Physical Examination of Accused or Suspected Individuals

In order for an expert to be able to make statements relating to the type and severity of injuries, as well as a possible offense, it is important that not only the victim of violence be physically examined, but also the suspect or perpetrator that has already confessed. It is not uncommon for statements on the sequence of events, as well as the type, and duration of an offense, to differ widely. In such cases, injuries that are present, as well as those that are not present but can be expected, may be relevant to the interpretation and appraisal performed by the independent forensic medical expert.

It is common for the physical examination of defendants or suspects to take place at a time that is not longer in close temporal relation to the offense. However, suspects and defendants should not be refused the option to undergo a physical examination if they so wish. The Committee for the Prevention of Torture and Inhuman or Degrading Treatment (CPT) of the Council of Europe demands that these individuals have the right to be examined by a physician of their own choice and out of hearing and sight of police officers. The results of the examination should be made available to the detainee and his/her lawyer (as already set out in the CPT Report for Germany. Report to the Government of the Federal Republic of Germany carried out by the CPT from 8 to 20 December 1991. CPT/Inf (93) 139, 1993).

The institutional autonomy of a physician also needs to be guaranteed in cases where an apprehended individual’s fitness to be held in custody, undergo questioning, and/or serve a custodial sentence needs to be assessed by a medical expert, or medical measures permissible in legal proceedings are to be taken (e.g., blood sampling; see CPT report).

Some countries have national provisions governing the forensic medical physical examination of suspects for the purposes of classifying injuries. The tendency is that a suspect is obliged to submit to further procedures for the collection of evidence. National legislation and court decisions can and should set limits in this regard.

For this reason, German courts took the decision that, e.g., the following medical procedures for the collection of evidence from suspects are prohibited [27]:

– Angiography;
– Pneumoencephalography;
– Narcoanalysis;
– Phallography;
– Forcible urine sampling by means of urinary bladder catheterization;
– Cerebrospinal fluid sampling;
– Forcible administration of hemetics to recover packages containing drugs.

14.2.3 The Forensic Medical Examination of Victims of Violence Committed by the State

The treatment of individuals that find themselves victims of state violence in police custody, or actual or suspected perpetrators held in official custody, is regulated differently around the world and is, as a general rule, severely under-regulated, if regulated at all.

In some parts of the world, large numbers of people are the victims of state violence (torture, inhumane and degrading violence and treatment) purely on the basis of their religion or ethnicity. In Europe, the European Convention for the Prevention of Torture and Inhuman or Degrading Treatment, which came into force on 1 February 1989 and to which member states of the Council of Europe are signatory, gives the Committee for the Prevention of Torture and Inhuman or Degrading Treatment (CPT) the power to visit places where detainees deprived of their liberty by a public authority are held. The CPT also demands that all individuals held in police custody have the right to be examined by a physician of their own choice. A further demand is that prison physicians be institutionally independent from police and judicial authorities.

However, many countries around the world: either lack a network of legal provisions to protect against violence and torture in official custody; the existing legal provisions are insufficiently differentiated; or the legal provisions are not practised or enforced [28].

The use of physicians in the area of policing is often not regulated in any way on a national level or does not form part of the regulations relating to police custody. There are also, at best, insufficient regulations governing “prison medicine”. Here, regular medical check-ups to monitor the health status of inmates could serve a significant preventive function by deterring potential perpetrators of violence (including, e.g., prison personnel). The extent of violence in police custody and detention centers is largely underestimated in the public consciousness [29–35].

Reports on what at times represents state violence against people on a massive scale are frequently seen in both the national and international press:

Germany 2003: A child kidnapper was threatened with having pain inflicted on him if he failed to reveal the location of the child (inadmissible interrogation technique; German Federal Constitutional Court, decision of 14.12.2014—Case No. 2 BvR 1249/04).
China 2005: Alleged torture of Tibetans; a large number of refugees suffered post-traumatic stress disorder and severe depression [36].
Egypt 2011: Women were sprayed with water, given electric shocks, and forced to submit to virginity tests by a physician (!) in the presence of soldiers (press reports, 03.12.2011).
Germany 2012: Inadmissible interrogation resulting in a confession after at least 38 h of sleep deprivation (prohibited interrogation technique; German Federal Constitutional Court, decision of 21.10.2014, Case No. 5 StR 296/14).
US 2015: According to Human Rights Watch (HRW), mentally ill inmates in US prisons are sedated and abused (HRW, Callous and Cruel: Use of force against inmates with mental disabilities in US jails and prisons. http://hrw.org/node/134861).

Thus, individuals in official custody merit particular attention and protection from violence. Without exception, all injuries inflicted on persons in state custody should—as should naturally also fatalities in official custody—be examined, documented, and appraised by a forensic medical specialist. This applies not only to injuries inflicted by fellow inmates, but also—and especially—to those inflicted by representatives of state authorities (police officers, militiamen, soldiers, etc.).

The dividing line between inflicting isolated injuries and systematic torture is a gray one. The situation becomes all the more complicated when physicians are involved in acts of torture. Situations involving patients forcibly placed in psychiatric care merit special attention. If physical injuries require examination in this context, forensic medical expertise takes the foreground; in principle, however, a psychiatric expert should always be called upon in cases where patients in psychiatric institutions need to be examined as possible victims of violence or also as possible perpetrators of violence.

This is aimed at improving the situation for victims of state violence with regard to evidence. Therefore, the European Court of Justice ruled that a lesser burden of proof applies to detainees in cases of abuse in police custody. In this context, it is sufficient for the victim of violence to convince the court that injuries that were present following release from police custody were not present at the time of arrest (case of Ribitsch v. Austria, Council of Europe: European Court of Human Rights 1995, 504 et seq.).

More differentiated guidelines in terms of examination, appraisal, and treatment also need to be considered in the case of victims of torture, most notably when minors are to be examined [10, 16, 26]. The reader is referred in this regard to guidelines set out by the United Nations [3] and other proposals made by commissions, institutions, and in the literature [5–7, 9, 37, 38].

In countries with accreditation authorities or relevant institutions, the accreditation of clinical forensic medicine, including the examination of victims of violence, can help to set important standards [15].

A recent publication addresses the unsatisfactory situation in terms of the examination, documentation, and appraisal of victims of violence in official custody [36].

The forms of unacceptable acts relate to widely differing situations, including:

– Undue violence in conjunction with the arrest of individuals (blows, kicks, and the use of tear gas, pepper spray, taser guns, and firearms), as well as prohibited restraining measures involving fatalities (positional restraint);
– Inadequate protective measures during the transportation of detainees;
– Prohibited methods during the interrogation of witnesses and accused, including threats of violence to obtain information or a confession;
– Tolerating the use of force by other (also detained) individuals in facilities under state control;
– Targeted mistreatment/injury of witnesses and accused for the purposes of intimidation;
– Systematic torture of detainees (e.g., waterboarding, beatings, electric shocks, etc.);
– Mock executions;
– Denying medical assistance to sick or injured persons;
– The practice of psychological violence alone [39].

However, there is no distinct, established discipline of “prison medicine” (or at best only a rudimentary one), that is endowed with special powers, as becomes clear at the relevant conferences [40].

14.3 The Forensic Appraisal

The forensic appraisal of injuries sustained by an individual must satisfy minimum requirements; a consensus needs to be achieved on these requirements in the form of medical specialist standards within specialist forensic medical societies—a move that has already taken place to a certain extent.

A forensic appraisal of injuries to a person should generally include the name of the commissioning agent, the name of the victim of violence (if known), the type and number of injuries, the number of printed pages comprising the forensic appraisal, and the number of images produced for or included in the appraisal. If sufficient information is available, the forensic appraisal can already allow an assessment of the injuries found, e.g., classify them as injury due to blows or falls, self-defense injuries, abutment injuries, coup and contrecoup injuries, gunshot wounds, stab wounds, incision wounds, thermal injuries, electrical injuries, or suspected signs of torture.

In addition, a forensic medical appraisal can and should indicate the degree of intensity of violence, assuming this is possible according to recognized forensic medical standards.

Thus, for instance, manual or ligature strangulation is described as "massive" if petechiae are detected above the level of compression. One speaks of a potentially life-threatening injury in cases where a victim has suffered a basal skull fracture involving bleeding in the oral cavity, since fatal blood aspiration could have occurred as a result of loss of consciousness due to a blow. However, a forensic medical appraisal should state only facts and provide a medico-legally recognized interpretation of injuries. Formulations on conceivable motives, not to mention the guilt of a suspected perpetrator, should be avoided.

The commissioning agent is the sole recipient of the forensic medical appraisal. In addition, the expert appraiser should always retain an identical copy of the appraisal.

The victim can also receive a copy of the appraisal if requested and with the consent of the commissioning agent. The option to disclose the results of a forensic medical examination and appraisal to other persons, authorities, groups, or the public must be available if the victim gives their express permission or indeed wishes this.

14.4 Organizational and Normative Provisions

Fundamental requirements include guaranteeing the following on a national level, both from an organizational perspective and by means of relevant legal regulations and concrete measures:

- Victims of violence should always have access to a medical or forensic medical examination (clinic forensic medicine);
- Detainees should also have access to a medical examination ("prison medicine");
- The (forensic) medical expert's freedom and autonomy, rather than being impaired, should instead be guaranteed (by means of statutory provisions);
- Furthermore, (international) standards should be met.

The provisions set out by the CPT for the medical care of prison inmates should apply in the same way to the examination of victims of injury:

Health professionals who are to care for detained persons must be able to treat their patients in complete professional independence from the judicial and prison hierarchies that govern the institution [39].

A number of aspects arise in relation to the medical or forensic medical expert's position in legal proceedings.

14.4.1 Organizational and Spatial Division

Forensic medical institutes, or the premises at which forensic medical experts work, should be set up in such as way as to be independent of any conceivable commissioning agents. Ideally, forensic medicine should be established as a university discipline, since this would put into effect additional protective rights arising from the preservation of the freedom to teach and research, depending on national laws.

Accommodating forensic medicine on the same premises as the police or public prosecutor raises concerns about overly close proximity to the criminal prosecution system. Any connection to other governmental or semi-governmental organizations, such as militia or the military, should also be viewed critically.

14.4.2 Statutory and Other Normative Provisions

The freedom and autonomy of experts in general, and more particularly of forensic medical experts in criminal proceedings, need to be regulated by law or ensured by other normative arrangements, e.g., decrees issued by the responsible ministry or internal instructions issued to all representatives of the judicial authorities. This includes the free and impartial choice of a forensic medical expert by police forces, public prosecutors, and courts that are as decentrally organized and autonomous as possible.

The forensic medical expert's independence needs to be additionally guaranteed, for instance, by provisions in the specific codes of criminal procedure.

Such provisions could furnish an independent expert with, e.g., the right to perform an initial and a follow-up forensic medical physical examination on individuals in official custody. The code of criminal procedure should contain provisions stating, in a transparent manner, the (few) exceptions in which a forensic medical expert might be refused. Since technical questions sometimes arise, there should be provisions giving the forensic medical expert the right to be present during the questioning of witnesses and accused. Neither police officers nor defense lawyers that have already attended their client should have the right to be present during the physical examination of the accused/their client to ascertain injuries.

In cases where the forensic medical expert demands that an injured person be examined and treated by other physicians, the law makes provision for that person to be brought to another physician or taken to hospital.

It is essential that an arrangement exists whereby forensic medical findings and appraisals can also be monitored. To this end, legal procedural provisions governing the use of additional experts are required. It should be incumbent upon courts to independently verify expert appraisals made on behalf of one party, as well as "counter expert appraisals," and to clarify open questions. An expert appraiser must have the right, if required for technical reasons, to call upon or propose other experts.

14.4.3 Standards of Personal Integrity Required of the Medical Expert Appraiser in Legal Proceedings

Last but not least, the literature formulates the standards of personal integrity required of an expert appraiser. It mentions criteria that, in the first instance, can be assumed as given, while others are difficult to verify. An expert witness or appraiser in criminal proceedings should fulfill the following requirements.

- Neutrality: No unilateral preference for one view.
- Objectivity: No additions or omissions of objectively established findings.
- Independence: Personal independence from organizations, institutions, or own employer, etc.
- Resilience: To attempts at manipulation.
- Expert qualification: Appraisals should be performed in line with medical scientific standards.
- Capacity for self-criticism: Recognizing the limits of one's own ability.

However, in the interests of legal certainty, it is desirable to have clear provisions making it possible to reject an appraiser. As such, an appraiser's personal integrity may be called into question and result in their rejection by the court if they themselves have been convicted of a relevant crime, for instance, or if serious doubts arise concerning their trustworthiness. Such doubts may be caused by their participation in inadmissible interrogation methods, as well as by statements showing prejudice toward a witness or defendant. Transgressions of competence may also raise doubts concerning an appraiser's objectivity. Refusals to accept an appraiser need to be (officially) substantiated in a transparent manner. The appraiser is given the opportunity to make a statement.

14.5 Conclusion

The fundamental principles of human coexistence, the individual's universal and inalienable rights, as well as the resulting multitude of national and international provisions demand that people be protected from violence and that the victims of violence receive assistance. The indispensable core of any assistance comprises the ascertainment, documentation, and interpretation of injuries within the framework of orderly criminal proceedings aimed at punishing perpetrators.

Injuries may be sustained in the context of criminal offenses in any number of circumstances and situations. All victims of violence, irrespective of their previous history, deserve the protection of the state. Injuries sustained by perpetrators themselves also need to be examined, documented, and clarified with regard to a crime, insofar as this is possible.

It is essential for injuries to be ascertained, documented, and interpreted (in this sequence) by a neutral (forensic medical) expert in the context of a physical examination conducted according to recognized medical standards for the purposes of obtaining evidence for use in criminal proceedings. Information relating to the offense and required to this end must be made available to the extent possible.

However, the implementation of guidelines on the examination, documentation, and appraisal of injuries in victims of violence is hitherto either incomplete or wholly unsatisfactory.

The physician's self-image, in particular, connotes empathy for the victims of violence and compels him to demand that victims' rights be better anchored in law worldwide. It is evident that requirements set out in international provisions are adopted only haltingly in national law, if at all. The discussion on the position of the (forensic medical) expert in terms of ensuring the necessary neutrality and independence is, at best, in its infancy.

All victims of violence, including suspects in official custody, need to be better protected by legal norms. This includes, for instance, the right to an interpreter, as well as the right to a medical examination by a physician of the victim's choice without police officers present.

Special provisions stating the rights and duties of physicians that work with detainees ("prison medicine") are required. Such provisions might include, e.g., the physician's right—or obligation—to report the ascertainment of injuries suspicious for maltreatment, as well as the right to conduct regular, "spot" physical examinations of prison inmates.

Medically documented injuries generally attract attention and largely tend not to be questioned. However, the standards of proof in criminal proceedings need to be particularly high. Therefore, it is essential to ensure a particularly careful physical examination, as well as the documentation and, where necessary, qualified interpretation of injuries in accordance with professional standards. The sole aim here is to reconstruct a probable offense and evaluate the severity of injuries sustained.

It is not the role of the forensic medical expert, either in legal proceedings or other contexts, to make judicial evaluations of whether a perpetrator intended to kill, or even of his/her degree of fault. The appointed court alone is charged with the responsibility of assessing the degree of fault.

The medical expert's position in criminal proceedings needs to be structured, both legally and de facto, in such a way that the expert is not susceptible to corruption. It is essential for him to be able to make an objective and neutral expert appraisal without fear of repression. He must be afforded the right to propose other physicians for an additional expert appraisal.

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Chapter 15
Clinical Legal Medicine and Toxicology in Sexual Assaults

Mete K. Gülmen, Nebile Daglioglu, Ayse Serin and Dilek Battal

Abstract It is important for forensic centres to be appropriately accredited and to use cutting-edge technology and scientific knowledge for the benefit of their clients. This is a new phase for technology, science, medicine and justice. Daily clinical forensic practice needs to be more specific and clear in adopting objective criteria, especially considering human rights violations. In today's technologically and scientifically advanced world, justice needs to be supported much more by qualified, standardized expertise. Clinical forensic examination includes systematic documentation of physical injuries and sampling of biological evidence for legal purposes. Clinical evaluation and reporting should be submitted to the clinician by an authorized clinical legal medicine specialist and/or competent forensic scientist, fully responsible for the results and the advice provided. Forensic experts should be independent—should only use scientific knowledge and nothing else through the standardization/accreditation of their works and quality assurance, as noted by all international regulations.

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15.1 Introduction

Humankind has been on a quest for truth and justice for over 6000 years. Forensic scientists collect evidence in order to raise the profile of humanism and improve awareness of justice. In the 17th century, science and technology started working together to protect society from crime. During the second half of the 20th Century, we started noticing the direct effects of scientific knowledge and technology on justice, especially the establishment of many International Academies of Forensic Sciences.

Academies, scientific knowledge, technology, as well as cultural and social systems change the global judiciary system every day. Today, advanced technology allows us to find traces of evidence from the crime scene until the reporting phase. Technology will continue to evolve more rapidly every year and this will change the understanding, practice and attitude towards the forensic field, but also to the judicial system. It is obvious that Justice will become more personalized through the use of technology and science.

Violence against women (VAW) is one of the oldest issues worldwide. Despite being controversial, it is finally accepted and recognized as a violation of women's human rights, thanks to global women's movements. We have to examine the abundant existing literature related to VAW, and various approaches to its causes and consequences, in order to draw attention to the struggle of women to be safe from violence. Basic findings of existing research on the statistical prevalence and awareness of global efforts for fighting VAW lead to progressive changes in social attitudes, legal frameworks, and governmental policies. The İstanbul Convention, CETS 210, with its holistic approach to the issues, promotes gender equality, prevention of VAW, protection of the victims and interventions for good practices, and is thus an invaluable policy document. Today, violence against women is well recognized as a public health issue and human rights violation of worldwide significance. But over long periods, violence against women, especially domestic violence, has gone unnoticed and undocumented, partly due to its taboo nature. The last two decades have highlighted the problem of intimate partner violence and domestic violence as a major cause of intentional injury to women. The women's movement and female lawyers have brought to public attention the extent and severity of violence against women in society, and raised awareness thereof. Efforts in preventing and combating domestic violence against women at the level of international and national laws are progressing; particularly, the Council of Europe Convention on Preventing and Combating Violence Against Women and Domestic Violence (Istanbul Convention, came into force on August 1, 2014) and the New Turkish Law for the Protection of the Family and Prevention of Violence Against Women (March 8th, 2012). In September 2015, the UN adopted Sustainable Development Goals, and combating violence against women is one of the main strategies for achieving the gender equality target by 2030.

Violence against women has been a subject of research for the last 50 years, and all of the International Conventions were established very recently in the last millennium. The examination of the status of women in the last 4000 years -from the Sumerian Civilization to various Anatolian and European civilizations-reveals that women were more powerful and valuable in both their familial and professional lives.

Women all around the world are falling victim to various levels of violence. These violent actions mostly take place at their own homes. Unfortunately, this used to be considered an internal family issue, and caused the authorities to act carelessly.

The effects of local and international immigration on domestic violence against women are also serious personal issues, and fall within the responsibilities of medical experts. Despite being perceived differently by different people, it has been observed that the most common trend is to consider women, their bodies, their sexuality, and their control, as elements of honour. In addition to the responsibilities of forensic medicine specialists, other physicians should also be aware of these cases, and handle them with care. In particular, emergency care medicine specialists and family medicine practice specialists are usually the first responders in these cases. Early marriages are also a global problem, which should be acknowledged and addressed by the medical and legal professions. Serious physical and psychological traumas and damage caused by early and forced marriages should also be carefully examined by the medical and legal professions, without any exception or excuse. An important deficiency is the lack of services for victims of sexual violence in terms of both medical and legal processes. Rather than individual interventions against childhood abuse and neglect, and gender based violence, there is a need for social and legal arrangements, as well as national and international policies.

Throughout the world, perpetrators of violence against women are often the spouse/ex-spouse, partner or ex-partner. Therefore, to be able to obtain both medical and judicial aid, diagnosis of these cases is of vital importance. For many reasons such as fear, embarrassment, stigmatization worries, economic-social requirements, and the need to protect children's health and safety, women hesitate to make a formal complaint, or even to explain their concerns. Women who have suffered violence may be admitted in healthcare centres with varying complaints and symptoms such as psychosomatic pains, and gastro-intestinal complaints. Therefore the physician's habit of keeping in mind the possibility of violence during their clinical service plays a key role in diagnosis and reporting. While conducting the physical examination of women, the physician should question her clearly, face-to-face, and in an environment that is removed from the spouse/partner, regardless of whether she has suffered violence, been injured or feels safe in her current or previous marriage or relationship. It must not be forgotten that there may be various findings including redness, ecchymosis, abrasions, swelling and blunt force injuries, primarily in the face, head and neck regions, and serious injuries that can be life-threatening or lead to disability, fractures and marks of strangulation or burns. Therefore, a forensic report has to be made for each and every case, and the woman's complaints, anatomic location, size, extent and form of the injuries have to be recorded in detail.

Sexual violence is also an important problem around the world. In the last two decades, the literature that focuses on Medical and Forensic approaches in sexual violence cases has significantly changed. Recently, a holistic individual approach that aims to provide both medical and psychological assistance for the patient, and to collect and preserve the evidence in a proper way at the same time has emerged. Thus, the victim of sexual violence becomes a patient with psychological, medical and forensic needs, instead of just a forensic case. Consequently, significant changes have occurred concerning the evaluation of the patients and forensic documentation. Many countries have developed new standards for medical and forensic approaches to sexual violence cases, and the general consensus is that the physical examination, forensic examination, and treatment in such cases should be performed by trained, experienced experts, who can assess and satisfy all the needs of a patient and who regularly follow the standards and literature.

It is important for forensic centres to be appropriately accredited and use cutting-edge technology and scientific knowledge for their clients. This is a new phase for technology, science, medicine and justice. Daily forensic practice needs to be more specific and clear in adopting objective criteria, especially considering human rights violations. In today's technologically and scientifically advanced world, justice needs to be supported by much more by qualified, standardized expertise. Yet, the most important issue is that the expertise should be independent and reliable. The most controversial and problematic ethical issue is the independence and reliability of the experts. One of the main problems seems to be the adequacy of Forensic Sciences expertise and laboratory systems, such as drug testing laboratories in the system. Therefore, the question arises as to who should be authorized to enter those laboratories, as well as questions about standardization, quality assurance and acquainted scientists. It is well known in the International scientific community that a forensic case should only be handled by authorized, acquainted forensic scientists. Usually, all of the criminal and civil codes require multidisciplinary, forensic scientific approaches. Any case that will lead us to a legal problem should be handled and carefully evaluated by the forensic scientist, who is an expert in a certain field e.g.; Forensic Toxicology, Forensic Genetics, Forensic Pathology, Forensic Odontology, etc. According to all of the supranational/international agreements to which most countries are signatories, a non-forensic centre or a laboratory cannot be admitted as an institute of forensic expertise if they are not equipped with experienced Forensic Scientists, either Masters of Science in the field, preferably Forensic Experts in possession of a Ph. D., and with a minimum experience of 5 years in the field, [1–12].

15.2 Clinical Legal Medicine

Sexual assault may exert a negative impact on physical and mental health as well as reproductive function [13]. Sexual Assault cases should preferably be handled by a centre that houses all the relevant care units. The victim needs immediate medical attention regarding putative physical, genital and extra-genital injuries, prophylaxis against sexually transmissible infections and, potentially, post-coital contraception [14]. Clinical forensic examination includes systematic documentation of physical injuries and sampling of biological evidence for legal purposes. The examination should be performed as soon as possible after the assault to avoid loss of important trace evidence [15, 16].

15.2.1 Physical Evidence

The physical evidence in these cases is mainly of two types: medical evidence, and physical evidence for subsequent laboratory analysis. "Medical evidence" generally consists of histories and observations made or taken by medical experts examining complainants in medical settings. The histories and observations may be documented in various ways, including notes, reports, or photographs. New multi-spectral imaging colposcopy technologies give us an opportunity to prove vulvar—vaginal traumas, as well as penetrating traumas in anal/vaginal areas, in addition to traumas throughout the body that are not clear or distinctive. The new technology gives us the opportunity to understand and report an assault case with much more evidence than through regular, conventional methods. Each and every case has its own findings that should be evaluated and marked by using various technologies. Medical experts are also responsible for collecting evidence during their examination of a complainant [17, 18].

Biological evidence of forensic interest may be found in several cases of assault, being particularly relevant for sexually related ones. Typically, sexual assault patients fall into two categories: acute and non-acute. Acute cases (up to 72 h) occur within the evidence collection window, while non-acute rarely involve evidentiary collection, but may require an immediate evaluation [19–23].

The proper handling procedures during selection, collection, packaging, labelling, storing, and transportation of evidence to the laboratory are key steps in aiming to achieve valid and reliable results [24, 25]. Oversights or faults in these procedures can call into question the production of the proof, namely regarding evidence preservation (loss or contamination) and chain of custody [26].

In every crime against a person, as in sexual assault, the contact between the perpetrator and the victim, his/her environment, or both, always leaves evidence which is transferred from the perpetrator to the victim, to the scene, and vice versa [23, 27].

The collection of biological evidence for DNA analysis is particularly useful in these cases to establish the occurrence of sexual contact and to proceed with suspect identification.

15.2.2 Collecting and Packaging Evidence

Goals of biological evidence collection:

- Consent must be obtained in writing prior to any exam or evidence collection;
- The examiner should always wear powder-free gloves when collecting and packaging evidence;
- The examiner should always change gloves between specimen collections;
- Clothing and other evidence specimens must be sealed in paper or cardboard containers;
- All wet evidence should be dried prior to packaging whenever possible;
- In the event that the evidence is wet, the items may be first placed in paper bags then into plastic bags, provided that holes for ventilation are made in the plastic bag;
- Envelopes containing evidence should never be sealed with the examiner's saliva;
- Self-adhesive envelopes or tape should be used;
- Paper bags should be sealed with tape, never staples;
- All evidence collected and sealed should be labelled with the date and time of collection, as well as with the collector's initials.

15.2.3 Swab and Smear

Depending upon the type of sexual assault, semen may be detected in the mouth, vagina, anal cavity or on the body surface. However, embarrassment, trauma, or a lack of understanding of the nature of the assault may cause a patient to be vague or mistaken about the type of sexual contact that actually occurred. For these reasons, and because there may also be leakage of semen from the vagina or penis onto the anus, even without rectal penetration, it is recommended that the female patient be encouraged to allow examination and collection of specimens from both the vagina and anus.

15.2.4 Chain of Custody

Forensic evidence must be collected, preserved and documented in a manner that ensures its admissibility at a later date as evidence in court. The custody of the evidence, as well as any clothing or other collected items, must be accounted for from the time it is initially collected until it is admitted into evidence at trial. This is accomplished by establishing a "chain of custody." It is important to emphasize that the documentation of the chain of custody includes the receipt, storage, and transfer of evidence.

15.2.5 Collection Steps

15.2.5.1 Clothing

Clothing frequently contains the most important evidence in a case of sexual assault. Whenever possible, any wet stains should be allowed to dry before being placed into paper bags. After air-drying items when necessary, appropriate articles of clothing (i.e. underpants, hosiery, slips, or bras) should be put into individual small paper bags. It is important to remember that sanitary napkins, tampons, and infant diapers may also be valuable as evidence, since they may contain the semen or pubic hairs of the perpetrator. Items such as slacks, dresses, blouses, or shirts should be put into larger paper bags [28–30].

15.2.5.2 Trace Evidence

When caring for a sexual assault patient there may be material or fibres that are found related to the assault. This is identified as trace evidence. These materials can help to corroborate circumstances and provide evidence beyond DNA. As with all steps, be sure to wear gloves in the collection of trace evidence, changing between samples. Place any hairs, fibres, or other materials, if found on the victim or examination table, in the bindle [28–30].

15.2.5.3 Oral Swabs and Smear

In cases where the patient was orally penetrated, the oral swabs and smear can be as important as the vaginal or anal samples. The purpose of this procedure is to recover seminal fluid from recesses in the oral cavity where traces of semen could survive [28–30].

15.2.5.4 Foreign Stains on Body Swabs

Semen is the most common fluid deposited on the patient by the offender. There are also other fluids, such as saliva, which can be analyzed by laboratories to aid in the identification of the perpetrator. It is important that the provider ask the patient about any possible foreign material left behind and examine the patient's body for evidence of foreign matter.

If fluids, such as saliva, seminal fluid and dried blood, are observed on other parts of the patient's body during the examination, the material should be collected using a set of swabs. A different set of swabs should be used for every fluid collected from each location on the body [28–30].

15.2.5.5 Bite Mark

Bite marks may be found on patients as a result of sexual assault, and should not be overlooked as important evidence. Saliva, like semen, may demonstrate the DNA profile of the individual from whom it originated. Bite mark impressions can be compared with the teeth of a suspect and can sometimes become as important for identification purposes as fingerprint evidence. Saliva is collected from the bite mark area by moistening two sterile swabs with a minimum of sterile water/saline and gently swabbing the affected area [28–30].

15.2.5.6 External Genital Swabs

If the circumstances of the assault suggest there has been contact between the victim's genitalia and the offender's mouth or penis within 5 days of the examination, there exists the possibility that saliva or seminal fluid may be found on the patient's external genitalia. In this instance, the two cotton tipped swabs in the envelope should be moistened slightly with sterile water/saline and the entire pubic area should be swabbed, the swabs dried and packaged appropriately [28–30].

15.2.5.7 Hair Combings

Pubic hair can retain trace evidence from a sexual assault. For this reason, collection of pubic hair combings may be beneficial. If there is an unknown offender where a scene investigation by law enforcement is expected or, if there is a scene investigation where evidence collection reveals a hair sample in need of the victim's hair for comparison purposes, pubic hair standards are a pulled sample of the patient's pubic hair (a minimum of 30 hairs) [20, 28–30].

15.2.5.8 Anal Swabs and Smear

After putting the patient in either a supine or a prone knee-chest position and applying gentle bilateral pressure with the examiner's hands to the patient's buttocks, allow approximately 2 min for anal dilation to occur. Swab the anal cavity using the four swabs. To minimize patient discomfort, these swabs may be moistened slightly with sterile water/saline. Prepare the smear by wiping swabs across the top, labelled surface of the microscope slide. The smear should not be fixed or stained. Allow all swabs and smears to air dry.

15.2.5.9 Vaginal/Penile Swabs and Smear

Vaginal swabs should only be obtained in the adolescent (pubertal) and adult population of female patients. Prepubescent patients would undergo external genital swabbing only. When collecting the vaginal specimens, it is important not to aspirate the vaginal orifice or to dilute the fluids in any way. Prepare the vaginal smear by wiping the four swabs across the middle surface of the labelled glass slide. The smear should not be fixed or stained. Allow all swabs and smears to dry.

For the male patient, both adult and child: the presence of saliva on the penis could indicate that oral-genital contact was made; the presence of vaginal fluids could help corroborate that the penis was introduced into a vaginal orifice; and faeces or lubricants might be found if rectal penetration occurred.

15.2.5.10 Cervical Swabs and Smear

As with vaginal samples, cervical samples are only collected in patients who are past onset of menstruation. The cervix provides an excellent source for sperm and DNA collection. The cervix serves as a reservoir for sperm as the flow of cervical mucus creates strands that direct the sperm upward. Prepare the smear by wiping the four swabs across the middle surface of the labelled glass slide. The smear should not be fixed or stained. Allow all swabs and smears to air dry.

15.2.5.11 Fingernail Clippings/Swabbings

Fingernail clippings are commonly collected on patients who may have been in a physical altercation during an assault. They may contain skin cells of the suspect and are simple to collect.

Use clippers from the kit. The nurse or patient may cut the fingernails onto the enclosed bindle. Nails from both hands should be included.

15.2.5.12 Buccal Swabs

The purpose of collecting DNA Sample/Buccal Swabs is to determine the patient's DNA profile for comparison with such deposits. Prior to collection of the buccal swabs, have the patient rinse their mouth and wait 30 min before collecting the samples [28–30].

15.3 Toxicology

Drug-facilitated sexual assault (DFSA) today constitutes an important clinical and forensic issue, where the impact is far from well-defined and difficult to quantify, since not all victims of sexual violence are identified or undergo the necessary toxicological investigations in good time [31]. The list of xenobiotics found in DSFA victims, taken alone or in combination, is constantly increasing, and among the xenobiotics most present are alcohol, benzodiazepines, antihistamines, antidepressants, marijuana, cocaine and gamma-hydroxybutyrato (GHB) [32].

DFSA occurs mainly as a result of voluntary assumption by the victim, but involuntary administration, associated or not with voluntary intake, is a widely reported phenomenon in international case studies, and receives increased coverage by the media [33, 34]. In both cases, which are often difficult to distinguish, the victim becomes aware that violence has occurred at a time that is no longer useful in relation to the detection of the substance or of the metabolites in the biological fluids, so as to render necessary an official collection protocol according to the specific case [35]. A proposed operating protocol that has already been advanced concerning the timing of the collection of biological samples refers to a period of less than 4 days elapsing between the event and sampling for the collection of blood or urine. For longer periods, the analysis of the keratin matrix can provide useful information for forensic purposes, with different modes of interpretation which must necessarily be integrated with the account of the examinee, from the medicolegal ascertainment and the type of drug detected [36, 37].

A toxicological screening in all victims of violence is not indicated, since it constitutes a factor of emotional distress. The decision to collect biological samples with the objective of effectuating a toxicological screening must be taken whenever there is the obvious suspicion of DFSA, and the method of approach must be differentiated in case of ante or post-mortem ascertainment. In any case, the clinical activity of the laboratory must be developed and implemented in accordance with standardized procedures, both in relation to the modality and typology of samples collected, and in the toxicological analysis.

15.3.1 Laboratory

Many acutely poisoned patients are treated with no laboratory help other than general clinical chemistry (blood glucose, blood gases, etc.) and haematology [38]. This being said, emergency toxicological analyses (24 h availability) that could influence immediate patient management should be provided at regional hospitals, i.e. those with large accident and emergency departments. For example, in Turkey, almost all methanol poisoning-related deaths are caused by use of methanol containing illegal alcoholic beverages [39–41]. In case of methanol ingestion, the patient should immediately be transferred to a facility in possession of equipment for measuring alcohol levels in blood and the available antidote therapy. Methanol in the blood is measured by Gas Chromatography-Mass Spectrometry in special laboratories that are not accessible in most hospital or poisoning centre settings. It is also important to monitor blood methanol levels during the treatment.

Laboratories that provide analytical toxicology analyses to assist with cases of acute and chronic poisoning often offer additional services in the area of drug abuse. Drugs of abuse may be taken intentionally or accidentally in overdose. Laboratory personnel should be aware of the legal implications that might arise from any case that involves drug abuse, and make sure that full documentation is collected and retained. Forensic toxicologists include drugs of abuse screening as part of their test portfolios to aid diagnosis and treatment, and blood and urine are the samples of choice for this purpose.

An experienced forensic toxicologist is expected not only to provide valid analytical data, but also to assist the clinician in relating the findings to a particular case of poisoning. This may be quite straightforward when the presence of a high concentration of drug or poison is consistent with the patient's symptoms and the circumstantial evidence. Other factors such as age, sex, health and previous exposure have to be taken into account. For example, addicted patients may have developed a tolerance to extremely high concentrations of opiates, benzodiazepines and ethanol, and exhibit relatively mild toxicity. The route of administration (inhalation, oral ingestion, intravenous injection, etc.) can have a very significant effect on the subsequent toxicity, which must also be taken into account when interpreting plasma concentrations. Mixed overdoses of drugs and alcohol are common, and synergistic reactions can confuse the clinical picture. The forensic toxicologist must therefore develop a good background knowledge of drug interactions.

Reports should be submitted to the clinician by an authorized forensic toxicologist who is fully responsible for the results and the advice that is provided. If the methods that are used are not validated, this should be indicated to the clinician so that he or she can judge the possible margin of error.

15.3.2 The Omic Approach

Recently, pharmacogenomics as molecular autopsy has been used for the assessment of the genetic contribution to drug toxicity in post-mortem forensic toxicology. The findings, as well as other applications of clinical and scientific findings in forensic science, might add to the understanding of disease mechanisms, and optimize treatments, including drug therapy. And the use of pharmacogenomics in forensic toxicology may add to the understanding of drug toxicity due to genetically predisposed impaired drug metabolism, and may provide findings which could be back-extrapolated for the benefits of optimization of ante-mortem drug therapy. In doing so, pharmacogenomics in forensic toxicology would thus provide better interpretation, indirectly enabling the emergence of personalized medicine [42–46].

The full use of post-mortem PGx is only possible by integrating forensic pathology, toxicology and genetics [43].

The more common pharmacogenomics methods are readily performed either by home-brew assay or a commercially available test or platform. The treatments include non-amplification, e.g., real-time PCR, signal amplification methods including endpoint polymerase chain reaction (PCR) detection, fluorescent in situ hybridization (FISH), target and, allele specific primers, length analysis using restriction fragment length polymorphism (RFLP) and oligonucleotide ligation assay (OLA), and new methods including solid phase microarrays and fluorescent-based bead assay (liquid microarray) [46].

This relatively new research area, combining forensic toxicology with pharmacogenomics, is interesting and challenging. There is still a clear need for fundamental research combining genetics, toxicology and pathology before the field can be reliably used in clinical cases [47–53].

15.3.3 Training

The processes of training, quality assurance and standards of practice should be rigorous, unanimously shared and applied in all steps of the assessment Sexual Violence, including the clinical forensic assessment, the collection of evidence, the chain of custody and the laboratory testing, in order to obtain and collect all the elements to obtain scientific evidence, which is so important within the criminal justice world.

The main problem seems to be the sufficiency of drug testing laboratories in the system. Questions arise over who should be authorized to enter those laboratories, and issues centred around standardization, quality assurance and acquainted scientists also come up.

It is well known in the International scientific community that a forensic case should be handled by authorized, acquainted forensic scientists. This should apply to all forensic cases, from engineering to pathology, or from chemistry to document analysis with all the aspects of the scientific spectrum. The autopsy is the most well-known issue to the lay person when the words “forensic” or “medical examiner” come up. We know that an autopsy can only be performed by those two scientists of the forensic team.

Most clinical cases are more serious forensic issues that should be handled by a large team of forensic scientists. The judicial systems in many parts of the world are mainly interested in crime and criminals. Usually, all of the criminal and civil codes require multidisciplinary, forensic scientific approaches.

According to SOFT/AAFS Forensic Laboratory Guidelines (2006), the forensic toxicology laboratory should be directed by a person who is qualified by reason of appropriate education and experience to assume the required professional, organizational, educational, managerial and administrative responsibilities. Alternative acceptable qualifications include a doctoral degree in one of the natural sciences and at least 3 years of full-time laboratory experience in forensic toxicology; or a Master's degree in one of the natural sciences and at least 5 years of full-time laboratory experience in forensic toxicology; or a Bachelor's degree in one of the natural sciences and at least 7 years of full-time laboratory experience in forensic toxicology [54].

15.4 Conclusions

The philosophy of today's scientific knowledge and advanced technology is inadequate. The judiciary system applies science to law through philosophy, and justice needs more ethical and philosophical values than ever, which should be equally and freely applied. Ethical values and Universal Scientific Ethical Perspectives should be widely adopted in all countries, no matter what the conditions are, because they are fundamental for each country. Throughout history many religious functionaries and philosophers talked about a "middle way" concept. Although the "middle way" was initially voiced as a philosophy of life, it gradually gained a moral connotation. Philosophers started to address the way it affects people's lives and behaviour. Personal rights are the rights that a person has over their own body. Among personal rights are the associated rights to protect and safeguard the body, most obviously protected against the torts of assault and battery. Furthermore, aspects of the personality are protected, such as a person's reputation, by the tort of defamation and legislation protecting the privacy of individuals, and freedom of movement. Freedom is a personal right. All people should have freedom. A person cannot waive a personal right. These are parts of human dignity. We can neither limit nor suspend any of these freedoms. As experts, we should respect confidentiality. The right to life is the first personal right, and we cannot waive nor neglect it. Capital punishment cannot be accepted. A person's name is also his/her personal right. All of the National and International Declarations and/or Conventions are consistent with all of them. People have and keep the right to resist against authority in cases when the state violates personal rights. And this is the safety system of all of Legal and Judicial administrations. These are all parts of Natural Law. Justice leads us to unwritten legal rules. As scientists we are to seek Justice beyond written ones. We should search for an unwritten one to achieve justice. Justice is an integral part of natural law, and it is the purpose of the law. Jurgen Bauman says that "the Absolute value of Justice is that, without thinking of that value, Law is a clown show or just a chatterbox." We all have to act in accordance with the Declaration of Human Rights, as well as the Declarations of the World Medical Association. Supremacy of Law can only be protected by independent judgement and expertise. All countries should be Just.

The golden rule is to have independent, competent, adequate, controllable, internationally standardized and accredited experts and expert institutions. This means that we have to develop a system with the triangle of Justice: Judgement, Prosecution, and Defense. All parties should and must have their own experts or expert institutions. This is a must for true Justice and Law. We know very well that when we take out Justice and Law from the state, the state falls apart; on the other hand, if we can protect our society through Justice, only then we can talk about Freedom, Equality and Peace for the people. To protect Law & Justice, we have to work within the bounds of science and scientific knowledge, and ask for Justice for all parties, even for persons that we do not approve of. Higher awareness will be built on the shoulders of Forensic Scientists and practitioners of Legal Medicine, and we do need more Freedom, more Law and more Justice. The famous philosopher Ioanna Kucuradi asks for a state with a constitution that completely relies on the International & Universal Declaration of Human Rights. And this will be a must for all states.

Our Universal Scientific Knowledge and Ethics will lead us to a more humanist, more secular and more prosperous state. We will only live and survive through scientific enlightenment, science, Law & Justice under Universal Ethical Values. Therefore, forensic experts should be independent and should only use scientific knowledge and nothing else through the standardization/accreditation of their works and quality assurance [55].

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Chapter 16
Current Evidence in Personal Injury and Torture Medicine

Miguel Lorente

Abstract Why are both medicine and doctors involved in torture? If the goal of torture is to produce damage (physical or mental), pain and suffering with the aim of obtaining information, such information can be obtained without any medical involvement. However, the reality is that medicine has been an active part of torture, especially during these last decades. Modern torture requires the assistance of medicine to avoid the infliction of scars, to design procedures and tactics to exploit prisoners' weaknesses and vulnerability, as well as to falsify certificates and reports when cases are investigated. Medical torture is a question of ethics, but it is not only an ethical question. It is also a question of responsibility and professionalism, and we should act in consequence and begin with prevention from Schools of Medicine. Accountability and prevention are two key actions to eradicate medical torture, and the role of IALM and Forensic and Legal Medicine Associations is essential to achieve both, especially in the present epoch, where new methods of torture render the investigation of cases more difficult.

16.1 The Strange but Understandable Relationship Between Torture and Medicine

If we take a look at crime and the role that Medicine has played in combatting it, we see that it is not very different to the role of Medicine in relation to torture.

Through history Medicine has been a key element in crime investigation, especially when a person suffered serious injuries and damage. This situation forced criminals to adapt their behavior to medical investigation. In this way, for example, as Medicine and Toxicology discovered new techniques to identify poisons used in criminal intoxications, poisoners responded by using other toxic substances that were more difficult to detect. When fingerprints were incorporated into human

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identification protocols, they started to use gloves, and when DNA permitted the identification of the suspect from a minimal amount of biological evidence, rapists began to use condoms in their sexual aggressions.

Something similar has happened with torture given that, as Legal Medicine and the Forensic Sciences have come to understand the methods and procedures used by torturers, the latter have changed their own methods and tools in order to conceal their actions from society.

However, at some point this situation and the roles regarding torture changed, and Medicine [1, 2] moved from being "the eye that looks upon criminality" to be part of "the bandage that hides it".

To understand this new situation, it is necessary to grasp the meaning of torture and its current circumstances.

Torture is defined in the United Nations Convention against Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment (1984) as *"any act by which severe pain or suffering, whether physical or mental, that is intentionally inflicted on a person for such purposes as obtaining from him/her or a third person information or a confession, punishing him/her for an act that he/she or a third person, has committed or is suspected of having committed, or intimidating or coercing him/her or a third person, or for any reason based on discrimination of any kind, when such pain or suffering is inflicted by or at the instigation of or with the consent or acquiescence of a public official or other person acting in an official capacity. It does not include pain or suffering arising only from, inherent in or incidental to lawful sanctions*".

The goal of torture is to obtain information or a confession through punishing a person, or intimidating and coercing that person, or some persons close to him or her. Usually, when a case is reported, the circumstances of the person experiencing torture are so serious that the goal is forgotten, and torture seems an act of violence with no sense, or carried out by a sadistic person. Investigation and prevention can be affected under this idea, and at the same time, make it difficult to comprehend how doctors can be involved in such acts.

To understand the role of Medicine and the Health Sciences in torture it is necessary to grasp that the goal of torture is not damage to the person, but the obtainment of information and the coercion of the person. The infliction of damage is the instrument for achieving these goals.

Under these references, Medical Torture is defined as *"the involvement of medical personnel in acts of torture, either to judge what victims can endure, to apply treatments which will enhance torture, or as torturers in their own right. It is also related to the using of medical expertise to facilitate interrogation or corporal punishment, to conduct torturous human experimentation or in providing professional medical sanction and approval for the torture of prisoners".*

The involvement of medical doctors in torture is a consequence of its meaning and circumstances. Torture is not an isolated behavior, but neither is it an individual decision. Torture does not happen outside of a system that aims to achieve the same objectives as torturers, while using of different procedures. This context explains why one of the arguments used to present torture, and medical torture as a part of it, is the "historical one" ("things always happened this way"), presenting the idea that such conduct is "irremediable" and that attempting to combat it is "hopeless", in this way justifying torture and creating a context of passivity that make it difficult to prevent and investigate.

Many cases of medical torture have been reported from the Second World War, especially after the investigation of the Nazi regime, revealing how a number of doctors conducted human medical experimentation, among other kinds of cruelty, on a large number of people held in concentrations camps.

In response to torture, and especially to medical torture, some international organizations developed documents against medical torture, based on the breaching of medical ethics and the Hippocratic Oath. Among these documents can be listed:

– Declaration of Geneva (World Medical Association, 1948),
– Nuremberg Code (1947),
– Geneva Conventions (1906, 1929 and 1949),
– Declaration of Tokyo (World Medical Association, 1975),
– UN Principles of Medical Ethics (1982),
– UN Convention Against Torture (1984).

In spite of all these documents and the terrible history of medical torture behind us, reality has not changed enough, and the involvement of doctors and health practitioners in torture has continued, as can been seen in recent cases, such as Abu Ghraib Prison and Guantanamo Bay.

Even within this context, the consequences for the perpetrators have been minimal, and most of the doctors involved were not punished, creating a context of impunity.

It is difficult to understand and accept, but we should understand that torture is, in some way, part of the system. If we admit it, it will be easier to understand why physicians are involved in torture, and how their role is currently more necessary.

Our society is built within particular power structures. Medicine has been developed, and acts, within these structures. That does not mean that all or most medical practitioners are destined to breach their code of ethics, although medicine is just as likely to be used as a method of implementing power as any other professional activity [3].

The instrumentalization of Medicine has been used throughout history to maintain a social order established under certain ideas, values and beliefs, and thus it is not a new phenomenon. Up until 1973, the American Psychiatric Association considered homosexuality a mental illness, and the WHO kept it in its International Classification of Diseases until 1997. This is part of what Michael Foucault described as “scentia sexualis”, or the “scientification of sexuality” that made it possible to medicalize “divergent sexualities”, with doctors conducting “conversion therapy” to cure people of these illnesses, including forced hospitalization, electro-shock therapy, castration torture, drugs and lobotomies.

Torture is part of the structure of power and is conducted from inside so as to “protect” society from external enemies and threats, and this organization has two components that reinforce each other simultaneously: the social structure built under certain ideas, values and beliefs, and its own institutions created to protect and defend them.

It is part of what Dr. Steve Miles, Professor at the University of Minnesota Medical School, a board member of the Center for Victims and Torture, calls the "structured system of complicity". This system can explain why the evolution of torture permits it to adapt to new circumstances instead of disappearing, and why the punishment of doctors who participate in these practices is so rare.

All these circumstances should be considered when approaching the issue of torture and medical torture.

16.2 Torture Medicine from Evidence to Incidence

Why are both medicine and doctors involved in torture?

If the goal of torture is to produce damage (physical or mental), as well as pain and suffering, in order to obtain information, it can be achieved without any medical involvement. However, reality shows that medicine is an active part of torture, especially during these last decades.

Traditional torture methods were based on damage, pain and suffering in different degrees, in this context the role of medicine was secondary and its participation was limited to some actions within these procedures as part of illegal torture or judicial punishment, and in some cases, to conducting experiments on prisoners. However, current circumstances have changed as well as the nature of torture.

Steve H. Miles [4, 5] affirms that doctors are integral to practice of modern torture, and describes their participation in different ways: some devise torture techniques (like rectal water infusions) in order to minimize incriminatory scars. Some monitor and treat prisoners undergoing torture in order to prevent them from unintentionally dying. Some falsify medical records and death certificates to assist regimes concealing injuries and deaths from torture. Around these behaviors there are a wide range of other actions that range from their involvement in hunger strikes to human organ trafficking (Fig. 16.1).

Under these new circumstances, medical doctors and other health practitioners have become a necessary part of the process to render torture invisible, and to keep society blind to it. Medical torture has made it possible to pass from evidence to incidence, that is, to reduce evidence of torture and to present these aggressions as "incidents", as if they were outside the system. And it has been done especially in countries where scars and evidence are a problem for governments and institutions; in other countries the traditional torture methods are still used.

Modern societies are built on new elements related to globalization, population diversity, individualism, use of ICTs, materialism and immediacy. Under this context the concept of conflict, traditionally established on objective factors, has changed so as to be considered according to subjective perceptions, generating the idea of risk and threat.

Within these circumstances any suspect can be considered as an enemy, not because of his or her objective features, but because he or she can be potentially dangerous based on the theoretical framework used to consider him or her as "suspect". Due to this virtual potentiality, suspicion is provided by circumstances, not by persons, and when the social context is considered risky anyone can be classified as "suspect" and, consequently, as an "enemy". Under this atmosphere

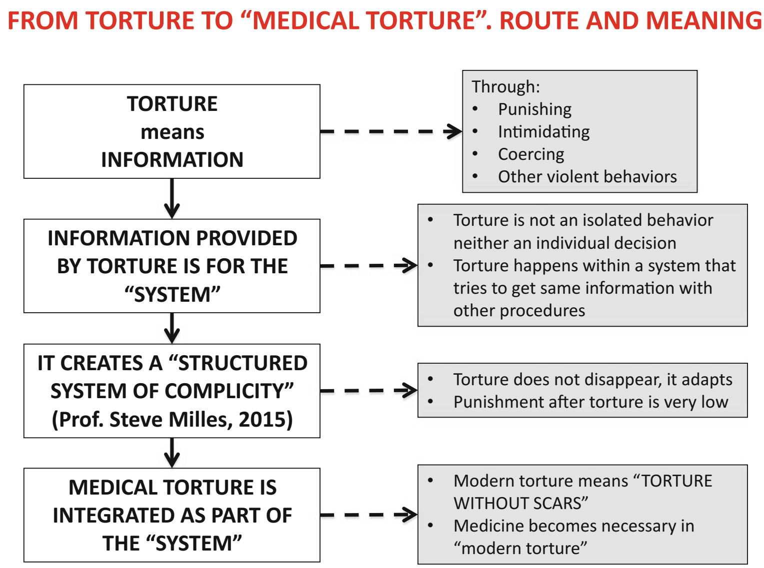

Fig. 16.1 From torture to "Medical Torture". Route and Meaning

torture is presented by a number of people as a necessity to combat the unknown threat, because it can change and become a real danger at any moment.

This approach provokes many mistakes, since it is based on an initial error that considers that the perception of certain circumstances is enough to conclude that there is a real risk situation. To conduct an investigation under these references, when there are no objective elements or evidence, interrogatory techniques aimed at proving what investigators suspect only is in the suspect's mind need to be carried out, and these interrogations often become torture procedures. In this context, to avoid criticism from society and conceal the mistake, it is necessary to erase the signs of these behaviors, which entails hiding torture through new strategies and procedures that make it possible to achieve the related goals without leaving evidence.

Modern torture requires the assistance of medicine in order to avoid scarring, to design procedures and tactics to exploit prisoners' weaknesses and vulnerability, and to falsify certificates and reports when cases are investigated. The consequences of medical torture are twofold:

1. The involvement of doctors in torture allows it to be conducted using methods that are difficult to discover and investigate.
2. Medical authority helps to sustain torture. The participation of doctors usually confers an aura of legitimacy and can create an illusion of therapy and healing. The presence of doctors can transmit the idea that "everything is under control", and that nothing serious is going to happen with the prisoners. And at the same time, it can give the prisoner a feeling of confidence and trust in the doctor that will finally be undermined and contribute to a deeper mental impact.

It is necessary to break this context of justification and impunity towards torture and torturers, and this means working on accountability and prevention (Fig. 16.2).

BEHAVIORS AND CONSEQUENCES OF "MEDICAL TORTURE"

1. BEHAVIORS

1.1. The involvement of medical personnel in acts of torture, either

- To judge what victims can endure
- To apply treatments which will enhance torture
- To devise torture techniques (i.e. "rectal water infusions")

1.2. It is also related to the using of medical expertise

- To facilitate interrogation or corporal punishment
- To conduct torturous human experimentation
- To provide professional medical advise
- To approve the torture of prisoners

2. CONSEQUENCES

2.1. Difficult to investigate

- Due to the absence of scars and signs
- There is a "certificate" that says *"everything is correct"*

2.2. Doctors participation confers

- Aura of legitimacy
- The idea that *"everything is under control"*
- Deeper mental impact on victims

Fig. 16.2 Some behaviors and consequences of "Medical Torture"

Torture is possible because of impunity, and impunity in torture is possible because it is part of the structured system of complicity and power.

In the words of Steven H. Miles [4, 5] the partnership between torturers and physicians can be summarized as follows.

- Physician involvement in torture coextends with the global practice of torture.
- Physicians play key roles in designing, implementing, monitoring, and concealing torture.
- Lack of accountability for physician torturers is the norm. Licensing boards rarely revoke or suspend licenses, medical associations rarely censure, and courts rarely convict the torturing doctors.
- Major medical associations do not offer standards or model procedures for holding torture doctors accountable.
- Accountability, although rare, is becoming more common because of pressure arising outside of the medical profession.

The field of medicine and doctors should not be part of this system. It is true that most doctors are not part of it, but it is not enough. To know that some doctors use medicine to torture demands a clear positioning of medical associations and medical institutions against it, and a consequent response in these terms.

Since Hippocrates, one of the principles of medicine is "do no harm", and it should be understood also as "do no harm, and do not allow others to inflict harm in the name of medicine".

Reality is a result, not an accident. Torture and medical torture is part of reality, not an accident. It means that it is the result of persons making decisions within the system, taking all the measures needed to achieve their goal and avoid being discovered, by changing and adapting torture procedures and involving medical doctors and other health practitioners.

It is therefore necessary to work on accountability and prevention in order to change this context and to contribute to the eradication of medical torture.

16.2.1 Accountability and Torture

- National medical associations must endorse strong standards against physicians complicity, such as WMA's Declaration of Tokyo and others [6, 7].
- Medical organizations and institutions should work to facilitate and implement documents, instruments (guidelines and casebooks to show courses and medical boards how to convene and conduct cases against doctors who are alleged to have tortured, and web portals to enable persons to report allegations of physicians complicity with torture to the authorities…) [8–10].
- National medical associations should support legislation and policies to ensure that state-licensing boards may restrict or revoke licenses for war crimes and torture even without criminal conviction, because they constitute unprofessional conducts.
- International Academy of Legal Medicine (IALM) and national forensic and legal medicine associations should develop a strategy and training on torture medicine, the procedures to investigate it under the Istanbul Protocol references, and create teams and protocols to investigate reported cases in the field [11–14].

16.2.2 Prevention and Torture

Accountability and punishment are part of prevention, but prevention needs to go further and start earlier (Fig. 16.3).

There are many things that must be done. One of them is to change the mentality of doctors and their ideas about their role in society, and it implies a different approach in Schools of Medicine learning.

Traditional teaching of medicine is focused on individual medicine and addressed to approach clinical questions. This approach leaves out or places in a secondary position the role of social medicine and all the issues related to the commitment of medicine with society in terms of public health, but also that of social wellbeing.

Medical students and doctors, independently of their clinical specialty, must learn about the social dimensions of medicine and how medicine is included in the social structures of power, and can be used in different ways to maintain these structures, as we saw when we talked about "scientification of sexuality".

In this sense, medicine can be used to conduct illegal behavior, such as torture, or can be used to contribute and reinforce the preservation of democratic freedom and values through the responsibility of doctors for Human Rights. If health means

PREVENTION OF "MEDICAL TORTURE"

- Approach and teach Social Health and Human Rights in Medical Schools
- Implement prevention programs with institutions where Medical Torture could be used
- Develop protocols and guidelines for approaching Medical Torture cases
- Provide specialized training to investigate Medical Torture
- Create international interdisciplinary teams on Medical Torture investigation

Fig. 16.3 Prevention of "Medical Torture"

public health, and "public" refers to society, then medicine has a responsibility for social health and Human Rights that need to be taught in the Schools of Medicine. Later on, it will be too late to learn such values and to understand their meaning and importance. In these circumstances it is possible to transmit knowledge and concepts, but not values; and democracy is based on concrete values, such as Liberty, Equality, Dignity, Justice… not only in their expression.

Medical torture is a question of ethics, but it is not only an ethical question. It is also a question of responsibility and professionalism, and we should act in consequence and start with prevention from our Schools of Medicine.

16.3 Conclusions

1. Medical torture has been part of torture through history, especially during the last decades.
2. Torture is not an accident neither an uncontrolled behavior, it is part of a strategy developed from "social structures of power", and medical associations and medical institutions should approach it firmly and proactively.
3. Modern torture needs a close involvement of doctors to get its goal ("information without scars"), especially in developed countries.
4. Accountability of medical torture and punishment of the doctors involved is a key element to eradicate it.
5. Prevention should be approached globally. It implies to increase and improve the teaching of social medicine in the Schools of Medicine, making the students understand the role of medicine in the preservation of democratic values and their responsibility for Human Rights.

6. Prevention also needs to act on society and State institutions to make disappear the circumstances and context used by torturers. Without transparency torture will continue acting from darkness.
7. IALM and Forensic and Legal Medicine Associations should elaborate documents on medical torture, to perform specialized training, and create international teams and protocols to approach and investigate the reported cases under national or international organizations.
8. IALM should work and collaborate to establish an interdisciplinary forum and space to deal with medical torture and other ethical issues related to Legal and Forensic Medicine.

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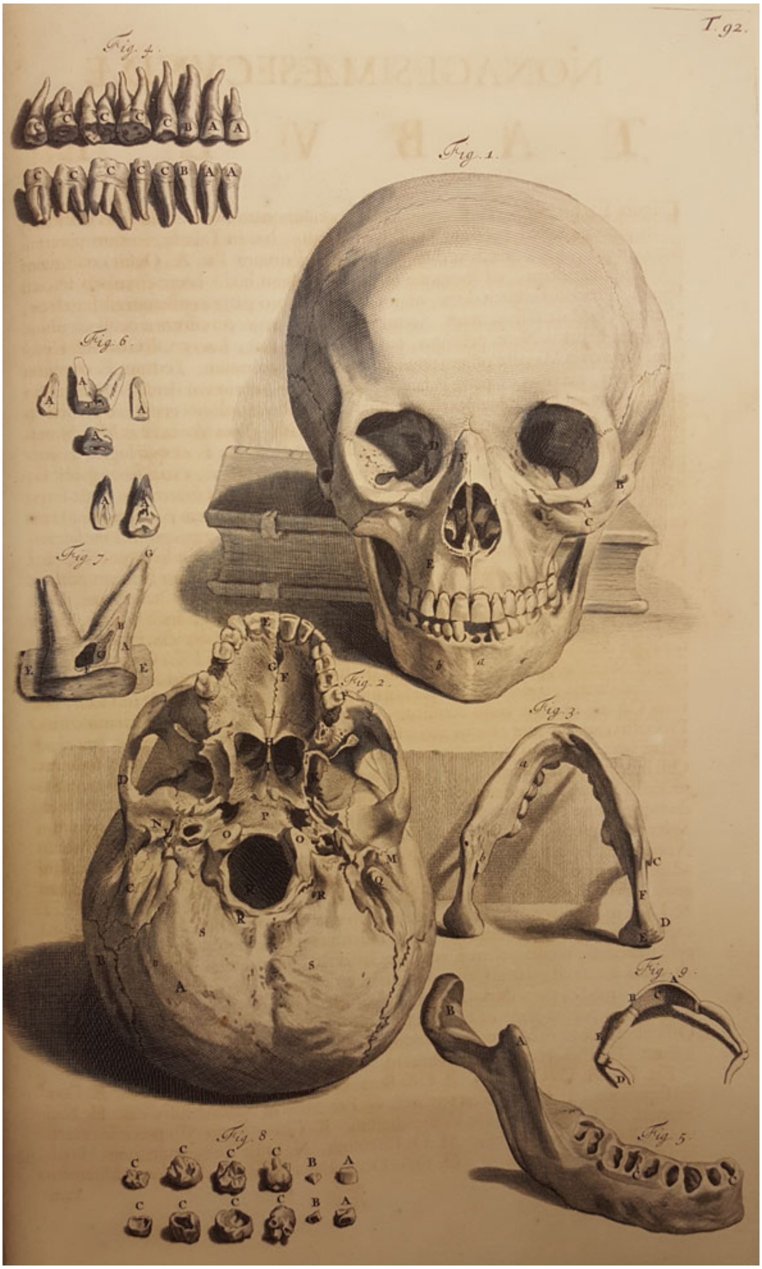

Bidloo, Govard, Anatomia humani corporis centum & quinque tabulis, per artificiosiss. G. de Lairesse ad vivum delineatis, demonstrata, veterum recentiorumque inventis explicata plurimisque, hactenus non detectis, illustrata Amstelodami: Sumptibus viduae Joannis à Someren, haeredum Joannis à Dyk, Henrici & viduae Theodori Boom, 1685

Chapter 17
Asylum Seekers and Methodology of Ascertainment

Sindi Visentin, Guido Pelletti, Thomas Bajanowski and Santo Davide Ferrara

Abstract The protection of the right to seek asylum is one of the core functions of the United Nations, and a common International Asylum System is a constituent part of the United Nation's objective. Forensic medicine plays an important role in this field, and in particular in the medical assessment of vulnerable asylum seekers. Therefore, there is the need for a shared medico-legal algorithm, based on the national and international regulatory framework and the protocols currently used for the medico-legal assessment of asylum seekers. After a systematic overview of the relevant and multi-disciplinary scientific evidence, the flow-chart on the Methods of Ascertainment and the Criteria of Evaluation has been set out in this chapter and proposed for the application of the International Medico-Legal community.

17.1 Introduction

In the last few years, global forced displacement has progressively grown, reaching the highest level on record in 2016, when it was estimated that 65.3 million individuals were forcibly displaced worldwide as a result of persecution, conflict, generalized violence, or human rights violations [1]. Most of these people apply for international protection in a foreign country, thus becoming *asylum seekers*.

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International attention towards these people progressively increased after the Second World War, as testified by the United Nations adoption of the Universal Declaration of Human Rights [2] in 1948, which was the first global expression of rights inherent to all human beings.

17.1.1 Refugee Status and International Protection

The 1951 Convention relating to the Status of Refugees and the subsequent 1967 Protocol [3] stated that a *refugee* is a person who is unable or unwilling to return to his country of origin owing to a well-founded fear of being persecuted for reasons of race, religion, nationality, membership of a particular social group, or political opinion. One of the fundamental principles contained therein, which is now embedded in customary international law, is the principle of non-refoulement, according to which refugees should not be returned to a country where they face serious threats to their life or freedom. As a consequence, state parties ensured that laws and policies concerning asylum seekers adhere to the principles contained in the Refugee Convention, through National [4, 5] and/or International legislation [6–8].

State parties can also confer complementary forms of protection, on a discretionary basis, to applicants who do not meet the definition of a refugee, but whose life or freedom would be in danger if returned to their country, such as subsidiary protection [7], withholding of removal, and temporary protected status [9].

For the granting of international protection, the fact that the applicant has previously been subjected to torture and/or other traumatic events is fundamental for the outcome of the asylum request, as highlighted in the EU directives, which also specify that the personnel examining asylum seeker applications, whenever it is deemed relevant, shall "arrange for a medical examination concerning signs that might indicate past persecution or serious harm" [6].

17.1.2 Conditions of Vulnerability and Humanitarian Protection

Asylum-seekers often experience situations of vulnerability, since they are frequently exposed to heightened risks of harm and require special care, support and protection. The concept of vulnerability is more inclusive than being at risk of persecution or refoulement and the identification of the nature of the applicant's position of vulnerability is important for the decisions related to the most appropriate placement and support options in each case [10, 11].

The categories of "*vulnerable persons*" are broadly recognized by International Agencies, and have been well classified by the European Legislation [12] as follows: minor age; unaccompanied minors; disabled people; elderly people; pregnant women; single parents with minor children; victims of human trafficking; persons with serious illnesses; persons with mental disorders; persons who have been subjected to torture, rape or other serious forms of psychological, physical or sexual violence; victims of female genital mutilation. For most of these categories, requiring specific support and special procedural safeguards, the investigation of vulnerability, to be performed in the earlier stage of the asylum procedure, has important implications in the decisional phase (juridical/administrative aspects), and in the identification and planning of urgent and non-urgent therapeutic needs (i.e. medical care, physical and psychic rehabilitation) [11–13].

The existence of episodes of torture or other traumatic events, in themselves entailing the recognition of refugee status according to the international regulations in force, is not essential to the recognition of other forms of protection, or more generally of a residence permit, regulated at national level. European states legislation allows the recognition of a residence permit on the basis of "serious humanitarian reasons", not otherwise specified, and, therefore, potentially inclusive of different conditions, partially overlapping with "situations of vulnerability", such as those of "family reunification", "state of motherhood" and "debilitating medical conditions" or "pathologies that cannot be treated in the country of origin". In 2015, twenty-two thousand authorizations to stay for Humanitarian Reasons at the first instance were recognized in the European Countries [1].

The methods of identifying vulnerable asylum-seekers and assessing their specific needs are not standardized and commonly approved [14]. A screening tool with this purpose has recently been developed by UNHCR and the International Detention Coalition (IDC) [10], but it is primarily designed to assist front-line decision makers and other practitioners with the responsibility for making decisions concerning the migration process, and it recognizes that vulnerability factors often require expert evaluation, such as medical and medico-legal assessment.

17.1.3 The Medico-Legal Assessment

The assessment of asylum seekers should consider the ascertainment of all the medical and medico-legal conditions that can influence both the migration process and the decision of the authority analysing asylum requests.

The Istanbul Protocol [15] is the most used protocol for the assessment and evaluation of asylum seekers, and has provided the first set of international guidelines for the investigation and documentation of torture and other cruel, inhuman or degrading treatment or punishment. Its medical assumptions are also reported and elaborated by the International Rehabilitation Council for Torture Victims (IRCT), in a practical guide for medical doctors engaged in the investigation of alleged torture [16]. The medico-legal standards proposed by the Istanbul Protocol are currently supported by the United Nations and international healthcare umbrella organizations, including the World Medical Association and the World Psychiatric Association [17]. In addition, several excerpts from the Istanbul Protocol have also been reported by the US Physicians for Human Rights (PHR), in their document "Examining Asylum Seekers" [18], with the aim of providing an international "gold standard" for the medico-legal documentation of torture and ill treatment, specifically addressed to asylum seekers.

The methods proposed in these documents are applicable in several contexts (i.e. human rights investigations and monitoring, political asylum evaluation and others), but they do not specifically entail a complete medical assessment of asylum seekers, since the protocols do not consider all the vulnerable conditions.

In fact, there are no international guidelines or protocols that provide a flowchart for the medical and medico-legal assessment of conditions of vulnerability not resulting from torture or other traumatic events. The existing tools were in fact created just for the initial screening made by non-medical personnel.

Therefore, there is the need for a shared medico-legal protocol complying with International and National requirements, which takes into consideration all the medical issues that could be relevant for the decision concerning the applicant, both in the reception procedures and in the result of the asylum application, namely:

– the medico-legal and forensic analysis of any injury, disease or impairment and/or other conditions of vulnerability;
– the evaluation of the degree of consistency/support between the events reported by the applicant and the injuries, diseases or impairments objectively detected;
– the current state, prognosis, therapeutic and healthcare necessities.

17.2 The Methodology

The proposed methodology is the result of an overview of the International and National regulatory framework and the analysis of the protocols currently used for the medico-legal assessment of asylum seekers at international level, which represent the milestones in this field (i.e. Istanbul protocol and related documents), and the medico-legal principles highlighted at National level [3–19].

After a systematic bibliometric overview of the relevant scientific evidence published on this topic, performed on the main bibliographic databases (MEDLINE, Scopus and ISI-Web of Science), multiple inter-disciplinary meetings of medico-legal experts, clinicians, staff of reception centres for asylum seekers and members of the territorial commission were performed, so as to identify the final objectives of the evaluation process, develop a common lexicon, and draft the flow-chart on the *Methods of Ascertainment* and the *Criteria of Evaluation*. The flow-chart has been subdivided into steps, following the systematic methodology used by the working groups of the European Academy of Legal Medicine (EALM) and the International Academy of Legal Medicine (IALM), also applied in other relevant field of Legal and Forensic Medicine [20–23], and the Guidelines published by the European Council of Legal Medicine, regarding Forensic Medical Examination on Living Persons [24].

The methodology, inclusive of the ascertainment methods and evaluation criteria set out below, must be applied by physicians with expertise and training in national and international regulatory frameworks concerning refugee status and other forms of "international and national protection", theoretical and practical experience in medico-legal semeiotics and evaluation of psychophysical status, and confident with the methodology for the documentation of torture and other cruel, inhuman or degrading treatment or punishment. The appointed physician should be a component and coordinator of a multidisciplinary team which includes trained medical personnel (e.g. internist, infectious disease specialist, psychiatrist, radiologist, paediatrician, neuropsychiatrist) and non-medical personnel (e.g. cultural and linguistic mediators, psychologists, developmental psychologists, nurses, obstetrics, social assistants, and legal experts), to be involved, if necessary, in the different ascertainment or evaluation phases. The team activities should be aided by an interdisciplinary and holistic approach in relation to the individual asylum seeker, and must aim at clinical diagnosis and the protection of the examinee.

17.2.1 Methods of Ascertainment

The Steps relating to the ascertainment methods presented in Fig. 17.1, summarized in Flow-Chart 1, are applicable in adequate *clinical settings* with the presence of a *linguistic and cultural mediator*. All Steps of the ascertainment should be documented.

Before commencing with the ascertainment, the personal information of the examinee, residence, sending institution, and the reason for the request of the ascertainment must be collected.

17.2.1.1 Step 1—Consent Acquisition

The medical examination is subject to the applicant's valid consent, which must be obtained in written form or in the presence of witnesses, who must be informed about the purpose and methods of the ascertainment, and the persons to whom the report will be made available.

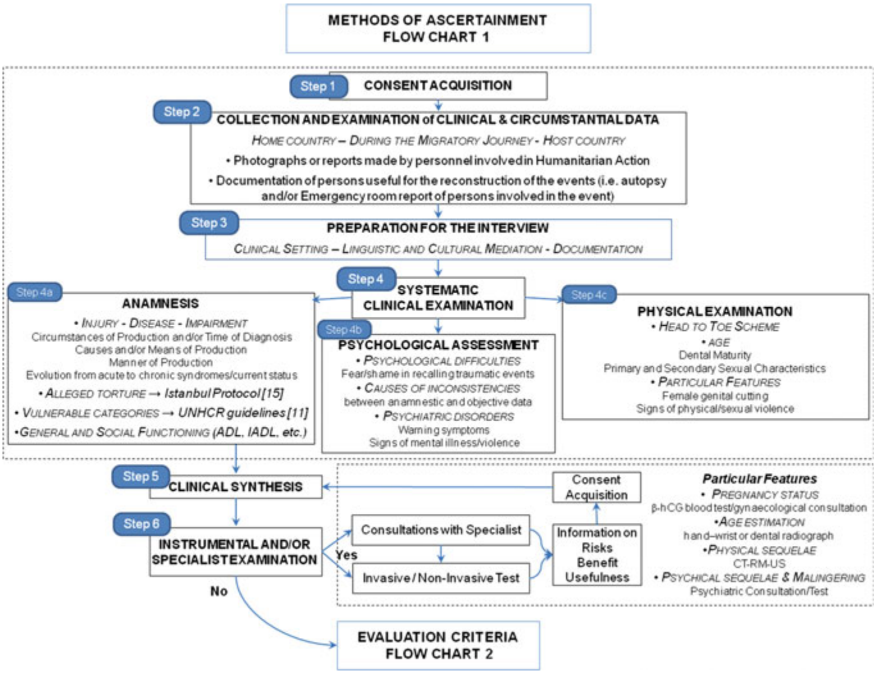

Fig. 17.1 Flow chart 1. Methods of ascertainment. Steps 1–6

17.2.1.2 Step 2—Collection and Examination of Clinical and Circumstantial Data

The circumstantial and clinical reports produced in the home country, during the migratory journey and in the host country, should be collected and examined.

Circumstantial data include police and/or authority reports and lawsuit documentation, photographs or reports made by personnel that provided aid to the applicant in the country of origin or during the migratory journey, such as those working in associations involved in humanitarian action.

Clinical data consists of hospital and emergency room reports, and any other document that could be useful for the identification of the health status of the applicant prior to and following the migratory journey.

Clinical documentation of relatives and/or persons useful for the reconstruction of the events (i.e. autopsy and/or Emergency room report of a relative or other persons involved in the same traumatic event) should be collected.

All of the collected documentation must be examined before the start of the ascertainment.

17.2.1.3 Step 3—Preparation for the Interview

Clinical Setting

The ascertainment phase must be performed in a location that respects the privacy of the examinee and in centres recognized by the National and/or International Healthcare System, which permit:

- a rapid and straightforward recourse to instrumental and/or working methods and to specialists in the field of medicine and surgery;
- reasonable duration of transfer periods from reception centres.

The examiner must take account, in the interview mode, of the age, gender identity, sexual orientation and cultural origin of the applicant in each stage of the ascertainment.

During the ascertainment, examiners should use care in the phrasing of questions, being sensitive and emphatic at the same time. They should adopt the right posture and listen with courtesy and genuine empathy to the answers of the applicants, avoiding:

1. questions that could embarrass or mislead the applicant, such as *leading questions* (questions that can implicitly suggest a specific answer), *closed questions* (questions that require a choice between two options) and *too many questions at the same time*;
2. technical or stigmatising terms;
3. subjective diminishment of the experience of the interviewed person, which may arise from habituation to accounts of torture.

The examiners should observe body language, tone of voice and facial expression, in order to obtain as much detail as possible, without it being invasive and traumatic for the victim [15, 25].

Linguistic and cultural mediation

Linguistic and cultural mediation are indispensible, in order to:

- guarantee adequate communication with the applicant;
- acquire a complete awareness of the meaning that the particular pathological state assumes, according to the culture of reference;
- facilitate and encourage potential social integration in view of the protection of bio-psycho-social well-being in the exercise of fundamental rights.

In the event that more than one interview takes place, the applicant should be followed by the same interviewer, where possible, in a shared path which includes the previous, contemporary and successive phases of the ascertainment [26].

When written in the original language, the linguistic mediator should translate the circumstantial/clinical documentation.

Mediation is the preferred solution for providing help and support to victims of torture, without, however, being influenced by the subjectivity of the cultural mediator. The ascertainment conducted in a language different from the mother *tongue can lead to an incomplete or distorted assessment of the conditions of the examinee, especially in the field of psychiatry.*

Documentation

All of the ascertainment phases must be collected and documented, preferably via information technologies (IT) systems with access to the national and/or international healthcare telematic network, keeping track of professionals who have intervened in the assessment process, of prescribed clinical and/or instrumental ascertainment and of any therapeutic remedies effectuated, all with the complete maintenance of privacy.

17.2.1.4 Step 4—Systematic Clinical Examination

This Step involves the collection of the anamnesis, a psychological assessment and an objective psycho-physical examination.

Step 4a—Anamnesis

Anamnestic data to be collected includes identification of the subject, family medical history and familial information, physiological and pathological anamnesis, as well as remote and recent disease.

For every injury, disease or impairment, the following information should be solicited:

- the circumstances of production and/or the time of diagnosis;
- the causes and/or means of production;
- in case of traumatic event, the manner of production, and whether it was accidental, self-inflicted or produced by third persons;
- the evolution of each injury or disease, from acute to chronic syndromes, their related symptoms, and the current healthcare conditions.

The clinical interview should follow, in case of alleged torture, the methodology proposed by the Istanbul Protocol [15] and, in case of specific vulnerable categories (i.e. Lesbian, Gay, Bisexual, Transgender, Intersex and gender-related persecution) the UNHCR guidance [10, 14]. As the functioning and disability of an individual occurs in a given context, the anamnesis should also include the environmental factors useful for the classification of health and health-related domains in accordance with the "International Classification of Functioning, Disability and Health" (ICF).

The ascertainment of the functional status of people with disability could be completed by the assessment of the basic and instrumental activity of daily living (ADL and IADL).

The anamnesis should include all psycho-physical impairments referred by the applicant as a consequence of single or multiple traumatic event(s), or due to other pathological causes, such as serious/terminal illness.

In case of doubts concerning family ties, familial information should be collected, in order to evaluate the necessity of performing genetic tests.

Step 4b—Psychological Assessment

The *Psychological Assessment* of the examinee must be carried out beginning from the initial phases of the ascertainment, with the help of a psychologist when deemed necessary. Psycho-pathological issues should be identified in the early stages of the ascertainment, especially in victims of torture and/or sexual violence.

– *The close relationship between the doctor, the psychologist and the cultural mediator is essential as it is difficult or impossible to trace suffering to one psychophysical source, since it is also conditioned by cultural and religious values.*
– *Early detection is a valuable tool for the prevention of the development of psycho-pathological problems, and constitutes a necessary step for the activation of programs dedicated to the psychophysical health of the person and social integration.*
– *Given the difficulties of a "psychological" nature derived from the fear and shame of recalling traumatic events, during the anamnesis inconsistencies and contradictions in the narration of the facts and/or discrepancies between the account of the examinee and objectively measured data (which will be dealt with in the successive phases) can emerge, due, for example, to memory disorders or dissociative episodes, as well as the refusal by the applicant to describe specific episodes. These findings could be interpreted erroneously, in the absence of an adequate psychological examination, as attempts at simulation by the applicant.*
– *Attention should be paid to disturbing thoughts, feelings, alterations in how a person thinks and feels, and other warning symptoms and signs of mental illness, in order to request a psychiatric consultation whenever necessary.*
– *Situations of* psychological distress *that may lead to Post Traumatic Stress Disorder (PTSD), depression and anxiety* [27–30]*, the presence of earlier somatic and mental health disorders, substance abuse, previous suicide attempts and other indicators of suicide risk, trauma and social isolation* [31]*, should be investigated.*
– *Women and children represent a particularly vulnerable and delicate group as they are more subject to harassment and violence.*

Especially in this phase, the Clinical Setting *is necessary in order to be as accurate as possible.*

Step 4c—Physical Examination

A full medico-legal examination, including internistic, osteoarticular-musculoskeletal, neurological, and detailed local examination of the injured/damaged areas, should be performed, taking photographs and describing all of the external injuries, sequelae or distinguishing marks. Photographs should always be taken using metric and colorimetric scales, as well as a reference point.

In case of doubts regarding the age of the applicant, the dental maturity and the evolution of primary and secondary sexual characteristics should be reported, preferably using the *Tanner Scale* for the classification of physical development.

In case of a traumatic event, the characterization of each lesion should be integrated with the time and the manner of execution reported by the applicants, especially in case of multiple lesions as a consequence of a single traumatic event.

Lesions identified should be classified as injuries produced before, during and after the traumatic event(s).

In case of alleged torture or other cruel, inhuman and degrading treatment, the methods of ascertainment provided by the Istanbul Protocol [15] for each organ and/or systems should be followed.

The physical examination must follow the commonly used “head-to-toe” scheme, and the physician must pay particular attention to some peculiar aspects, such as genital mutilation, gender persecution, sexual violence, signs of psychological distress or suicide attempt.

The number of girls and women seeking political asylum due to actual or feared female genital cutting [32], *which is considered a human right violation, a form of* persecution *and* gender based torture [33, 34], *is increasing, and gender persecution and sexual violence are commonly described* [35, 36] *in the home country, in the migratory journey and in the destination country.*

17.2.1.5 Step 5—Clinical Synthesis

A synthesis of all collected data must be performed, aimed at summarizing the circumstantial and clinical data obtained from Step 2 and 4.

The clinical synthesis has the function of assessing the necessity of acquiring more relevant data through the consultation of a specialist or the performance of further exams.

17.2.1.6 Step 6—Instrumental and/or Specialist Examination

If the examination of the documentation (Step 2) and the systematic clinical examination (Step 4) are not sufficient in order to obtain a proper diagnostic picture, or in case of discrepancies between the two phases, further invasive and/or non-invasive diagnostic tests must be evaluated. Diagnostic tests and/or specialist consultations can be prescribed, after properly informing the applicant of the risks, benefits and usefulness of any exam and obtaining their consent [15].

Genetic tests should be considered when there are doubts regarding family ties and the exploitation of a minor to obtain international protection.

Instrumental and/or Specialist examinations are recommended in the event that the assessment of the current status of the applicant requires specialized knowledge both for the diagnostic test and for the subsequent evaluation of the findings, and they should aim at the further study of common issues, set out as follows.

- *Pregnancy status,* through the beta-hCG blood test and, in the event of a positive value, a gynaecological evaluation.
- *Age estimation,* through the assessment of *skeletal maturity* and of dental development. The techniques (mainly radiographs, orthopantomograms and CT, but also MRI and US when available and reliable) should be applied to analyse

the bodily area (i.e. hand–wrist, clavicle, teeth) able to provide the most accurate estimation of the age on the basis of the expected/assumed age of the applicant.

- *Physical sequelae,* especially through imaging tests, such as radiography, Computed Tomography (CT), Ultrasonography (US), Magnetic Resonance Imaging (MRI) and others, taking into consideration the availability, the clinical purpose and the radiological exposure.

  – *For the detection of fractures,* radiographs*, which are readily available, are currently the most used technique in all the injured areas, except for that of the head, where* CT *is superior in detecting bone fractures, alignment and bone displacement. CT can also reveal hemosiderin deposit consequent to central nervous system haemorrhage, even years after trauma* [15, 37]. *In the assessment of victims of torture or other traumatic events, the physician could have the task of assessing and evaluating injuries that are the result of a crime which occurred months or even years before the examination. Diagnostic techniques, such as functional imaging, able to detect healed fractures that generally return to normal after a number of years, can also play an important role* [38]*; it has to be considered that* CT, X-Rays *and* Scintigraphy *utilise ionizing radiation, so their use should be limited to cases where they are strictly necessary; on the contrary,* MRI *poses no risk of radiation exposure, so that, since radiation protection is unnecessary, an investigation can be carried out purely on the basis of a forensic indication, and it allows a good visualization of soft tissue structures and of hematomas in subcutaneous fatty tissue, measuring its depth and extent, and a sensitive detection of brain abnormalities. In the absence of* MRI *and in cases of suspension torture,* ultrasonography *could provide information relating to subcutaneous injuries, testicular abnormalities and tendinopathy.*

- *Psychical sequelae,* in case of warning symptoms and signs of mental illness detected in the *Psychological Assessment* (Step 4b), through screening tests such as the *Harvard Trauma Questionnaire* [39, 40] and the *Kessler Psychological Distress Scale* (K10) [41]; or through the *consultation of a trained psychiatrist.*

  – *The frequent clinical manifestations of complex post-traumatic stress disorders are multiform, insidious and difficult to detect, particularly for doctors and psychologists without specific training and experience.*
  – *Due to the high prevalence of mental illness* [42] *in asylum seekers and refugees, screening tests for mental illness are highly encouraged* [43]. The Harvard Trauma Questionnaire [39, 40] *for the assessment of PTSD; the* Kessler Psychological Distress Scale *(K10)* [41], *a simple 10-item measure of psychological distress (particularly symptoms of anxiety and depression) based on a person's emotional state during the previous 30 days. Besides the common symptoms of PTSD, extreme traumatic experiences can determine other specific and complex psychopathological consequences, such as psychic and somatic dissociative disorders, tendency to re-victimization, loss of sense of security, hyperarousal disorders, as well as affective and*

relational disorders. This particular syndromic framework, defined as "Complex PTSD", must be taken utmost account of.

- The presence of *Malingering* should be assessed as follows:
  - *traditional approach,* applying clinical and epidemiological rules for the detection of discrepancies between the case history of the applicant and the natural clinical evolution of the injuries described;
  - *psychological assessment,* in order to avoid the misidentification of the manifestation of a psychiatric disease as a malingering attempt;
  - *neuropsychological tests*, such as *Symptom Validity Testing (SVT), Test of Memory Malingering, Word Memory Test, Rey15-Item Visual Memory Test, Minnesota Multiphasic Personality Inventory-2, Structured Interview of Reported Symptoms, Structured Inventory of Malingered Symptomatology, Computerized Assessment of Response Bias,* etc.) [44–50]; education and experience in the field and the use of a combination of tests are of key importance for improving accuracy in the detection of malingers.
- *Psychiatric symptoms are easy to exaggerate and simulate, but, at the same time, they can be responsible for discrepancies in the history provided by the applicant, and often interpreted as signs of a lack of sincerity or credibility* [51]. *The detection of malingered symptoms should be performed by taking into account the need in each particular case. The application of clinical and epidemiological rules for the detection of discrepancies between the case history of the applicant and the natural clinical evolution of the injuries described, is important (Step 8 and 9), but the discrepancy symptoms regarding traumatic events and the violence suffered should not automatically be taken as an attempt at malingering* [52]. *Changes in some responses between different interviews have been reported frequently in asylum seekers with higher levels of Post Traumatic Stress Disorder (PTSD)* [53]. *It is therefore extremely important to identify these subjects by performing an accurate psychological assessment whenever suspicion arises, in order to avoid the misidentification of the manifestation of a psychiatric disease as a malingering attempt. In addition, other traditional techniques can be applied for the qualitative analysis of symptom characteristics and the evolution of rare symptoms. Each test among those proposed has its particular limitations and in more complex cases specific neuropsychological tests should be applied, such as Symptom Validity Testing (SVT), a test that makes use of Floor Effect strategy and/or tests adopted for psychiatric or psychopathological diagnosis, which include strategies for malingering detection.*

17.2.2 Evaluation Criteria

Flow chart 2, shown in Figs. 17.2 and 17.3, illustrates the Evaluation Steps, numbered in continuity with the antecedent stage of the ascertainment.

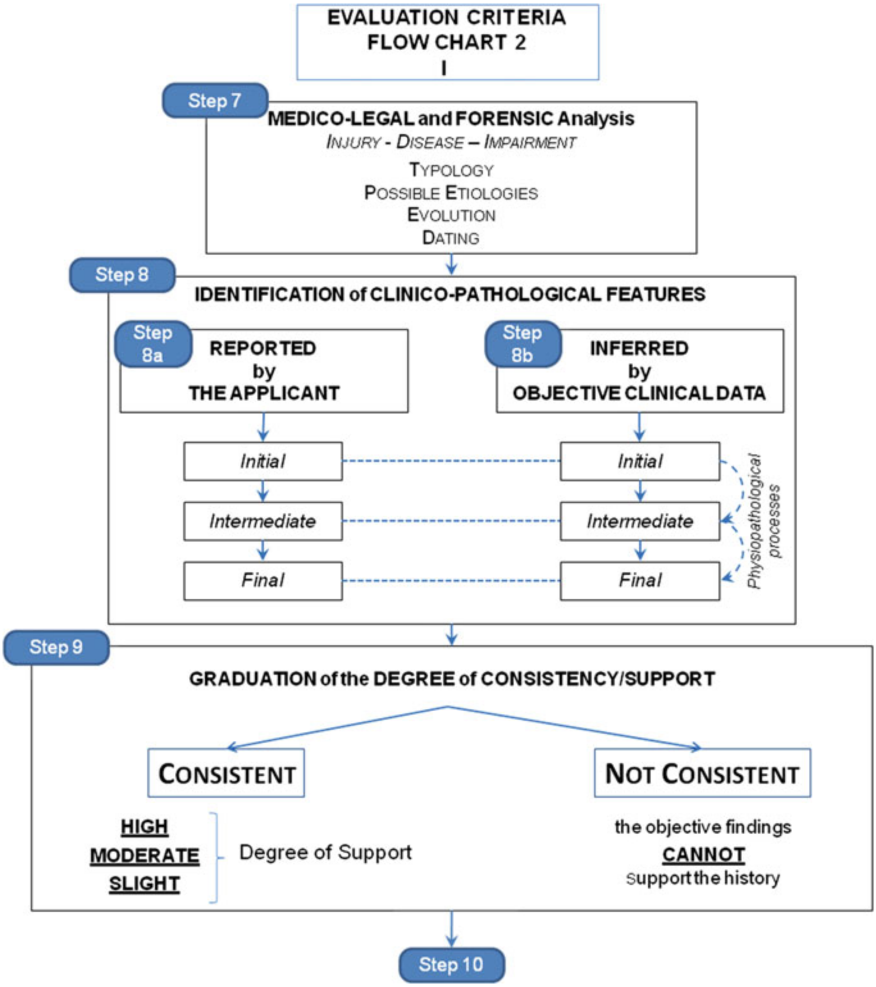

Fig. 17.2 Flow chart 2. Evaluation criteria. Steps 7–9

17.2.2.1 Step 7—Medico-Legal Epicrisis

An integrated epicrisis of all of the ascertainment data (Step 1–6), aiming at the evaluation of the relevant medico-legal aspects of the pathological features identified, should be performed. Every injury, disease or impairment should be characterized as follows.

- *Typology*. Identification of the type of injury, disease or impairment, detailing the diagnosis, in accordance with the nomenclature provided by the WHO in the International Classification of Disease (ICD-10).
- *Possible causes.*

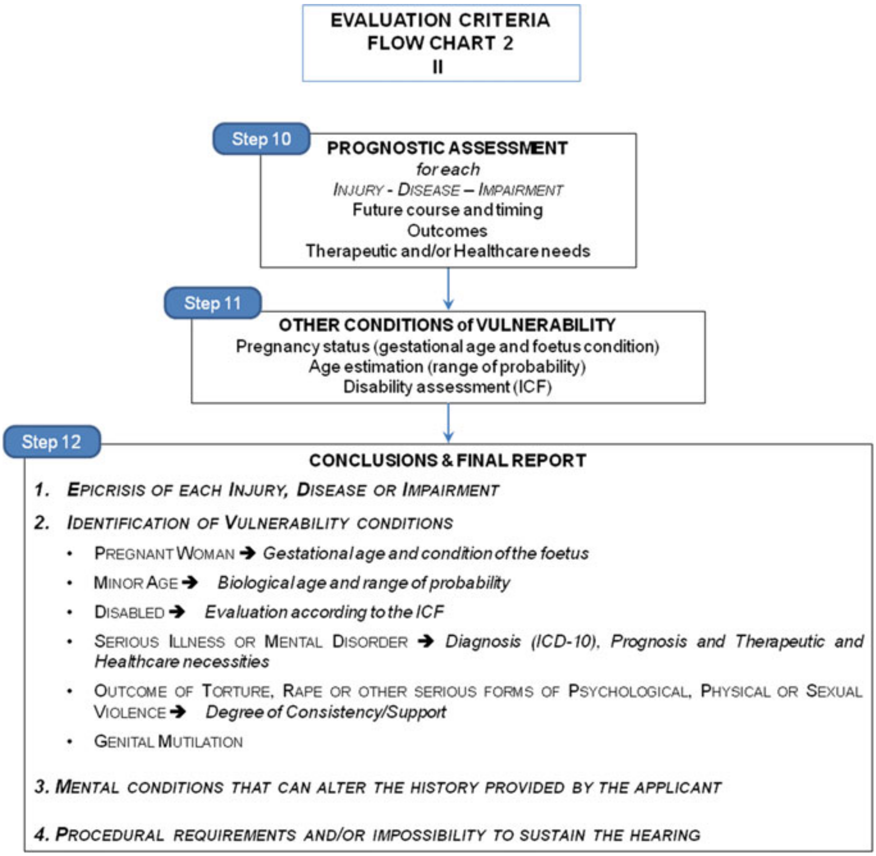

Fig. 17.3 Flow chart 2. Evaluation criteria. Steps 10–12

  – *Aetiology* (i.e. multifactorial, infective, etc.), in case of non-traumatic events.
  – *Means of production* (blunt force trauma; pointed, sharp, semi-sharp trauma; gunshot and blast wounds, thermal, electricity and gas lesions) and *manners of production* (i.e. accidental, self-inflicted or produced by third persons), in case of traumatic event.

- *Evolution*. The natural history of the injury or disease, detailing events that may have had an effect on it (i.e. medical and surgical therapies, complications, etc.).
- *Dating*. Age of onset of a disease or timing of production of an injury.

The damaging event(s) should be described on the basis of the circumstantial and clinical documentation, the medical history and the clinical data. The energy and the mechanism of injury should be reconstructed.

17.2.2.2 Step 8—Identification of the Clinicopathological Features

The identification of Clinicopathological Features, divided into *Initial*, *Intermediate* and *Final*, outlines the sequence of the physio-pathological Steps following the damaging traumatic event or the onset of the disease.

The *Clinicopathological Features reported by the applicant* (Step 8a) should include the anamnestic data, with the accurate description of symptoms and temporary impairments, the evolution from an acute to a chronic phase or to the current healthcare status, specifying the chronology of the events; an accurate description of the therapies performed (medical and/or surgery) and further complications, should be detailed.

The *Clinicopathological Features inferred by objective clinical data* (Step 8b) should be characterized according to the Medico-Legal Epicrisis (Step 7) and scientific sources, and graded according to the hierarchy source (evidence-based publications, treatises and articles published in peer-reviewed journals).

The Clinicopathological Features permit the analytical comparison between the history reported by the applicant and the objective reconstruction of the events.

17.2.2.3 Step 9—Graduation of the Degree of Consistency/Degree of Support

The comparative evaluation of the Clinicopathological Features (Step 8a) and the objective clinical data (Step 8b) should be performed.

The degree of consistency/support between the physical or psychological sequelae and the history of the applicant should be evaluated following the modulation proposed by the Istanbul Protocol [15], in terms of “high”, “moderate”, “slight” or “exclusion” of support, to be expressed at each level of the Clinicopathological Features (Initial—Intermediate—Final).

Two different judgments on the physical and psychological sequelae will have to be expressed. The global assessment of all of the injuries, rather than just the compatibility of each lesion with a particular kind of torture, is fundamental.

The data relevant for the purpose of a future socio-cultural integration emerging from the interview must be specified with the aid of a cultural mediator and a psychologist or sociologist.

In case of alleged torture the referred clinical-pathological features should be matched with the evolution of all the other possible differential diagnoses (accidental trauma, self-inflicted injuries, skin disease, iatrogenic lesions, etc.*).*

In cases of medical conditions that could be worthy of humanitarian protection, the eventuality that the applicant simulates or misrepresents his/her symptoms and impairment, or provides altered medical certificates, should be excluded.

17.2.2.4 Step 10—Prognostic Assessment

A forecast of the most probable course and outcome should be envisaged for each injury, disease or impairment, including the applicant's chance of recovery and the expected time of resolution (or stabilization in case of permanent impairments). Future therapeutic and/or healthcare needs, with an evaluation of the results to be achieved after correct treatment, should be specified. Particular attention must be dedicated to the multidisciplinary approach of "taking charge", as a prerequisite for the development of features designed to support appropriate pathways of care and rehabilitation for victims of violence and/or torture.

17.2.2.5 Step 11—Other Conditions of Vulnerability

The presence of maternity status, minor age of the applicant and disabilities, should be reported.

- *Pregnancy status,* gestational age and condition of the foetus must be specified.
- *Biological age* should be estimated in case of doubt regarding the *minor age* of the applicant, through the integration of data obtained by the physical examination, X-ray of the hand, dental examination and orthopantomogram, taking account of ethnic origin and socio-economic status.
- *Disability*, which is the complex phenomenon reflecting the interaction between a person's body and the society in which he or she lives, according to the indications provided in the International Classification of Functioning Disability and Health (ICF), covers impairments, activity limitations, and participation restrictions.

17.2.2.6 Step 12—Final Report

The final report includes the *epicrisis of all the injuries, diseases* and *impairments,* in addition to one or more of the following *categories of vulnerabilities*.

- Pregnancy status (gestational age and condition of the foetus).
- Minor age (biological age and range of probability).
- Disability (evaluation according to the ICF).
- Serious illnesses or mental disorders (diagnosis according to the ICD-10, prognosis and therapeutic and healthcare necessities).
- Outcomes of torture, rape or other serious forms of psychological, physical or sexual violence (degree of consistency/support between the events reported by the applicant and the objectively detected injuries, diseases or impairments).
- Genital mutilations.

Additionally, it is necessary to report:

- Mental conditions that can alter the account of the applicant or alter his memories;
- The need for specific procedural requirements on the part of the applicant and/or the impossibility to sustain, due to his clinical and mental conditions, the hearing with the ascertaining organism and the possible alternative solutions.

The medical report including all of the relevant medical items should be reported concisely and delivered to the Asylum Applicant. When it is deemed necessary, in case of trauma and/or torture which might entail difficulties in the reconstruction of the trauma, in the final report the need for the presence during the visit of the ascertaining body, the ascertaining physician or the personnel of protection/reception centres with which the applicant has built a relationship of trust (indispensable in the case where an alteration of the planes of reality has been detected) must be indicated.

17.3 Conclusions and Future Perspectives

The proposed methodology offers a framework of the basic steps for all of the medical information needed for an adequate evaluation of the asylum seekers.

This methodology, because of its inclusion in the wider category of personal injury and damage, retraces the fundamental steps of the methods of ascertainment recently depicted by the IALM Working Group for the ascertainment of Personal Injury and Damage under Civil-Tort Law [21]. Some relevant aspects have been emphasized, such as the figure of the forensic expert, who should be specifically trained in Forensic Humanitarian Action, the frequent absence of circumstantial clinical reports and the difficulties of the applicant in re-evoking traumatic events, the particular clinical setting, the detection of specific physical or psychological sequelae that could be the results of traumatic events and/or episodes of torture, the interpretation of malingered symptoms, which is particularly challenging in these category of subjects, and the evaluation of all the conditions of vulnerability related to the "status of asylum seeker".

The main difference between this proposal and the already existent protocols and guidelines for the assessment of asylum seekers is based on the focus on medical aspects that can require specific support and special safeguards or be worthy of protection, even if refugee status is not granted. The principal documents in the field of the asylum seeker's medico-legal assessment [15–18] are mainly aimed at the documentation of torture and other traumatic events, which are undoubtedly fundamental aspects that must be investigated.

Two protocols in particular have been drafted in order to provide an exhaustive guideline on the assessment of individuals who allege torture and ill treatment: the Istanbul Protocol [15, 16] and the US PHR document [18].

- The Istanbul Protocol is endorsed by the UN for the effective documentation of torture both in countries where this reality is endemic [54, 55] and in relation to asylum seekers who allege ill treatment.
- The PHR document is specifically addressed to the documentation of torture in asylum seekers, but is mainly focused on the US asylum procedures.

These documents accurately describe all the relevant aspects (i.e. legal, ethical, physical and psychological) related to the victims of torture and ill treatment, delineating all the steps which should be followed in each phase, including the preparation for the interview and the documentation of physical and psychological evidence of torture.

The “plan of action” defined by these documents, to which this methodology is often referred in the ascertainment phase, needs to be expanded with all the aspects that can represent conditions of vulnerability. In fact, the complex of pathologies reported by asylum applicants is nowadays not only related to the results of torture and other inhuman physical/psychical acts, but also to other conditions which could represent a heightened risk of harm or, in case of return to the home country, reduce the individual’s life expectancy or imply acute or chronic physical and mental suffering. Furthermore, the presence of vulnerable situations [12], which require specific support and special procedural safeguards, may result in different reception procedures.

Another innovation of the methodology relies on the evaluation process, which follows the basic principles already applied in other fields of legal and forensic medicine. These latter have been adapted to the area of asylum seeker ascertainment. It consists in the evaluation of the Degree of Consistency/Support between the pathological features reported and those assessed by the examiners.

As a consequence of the above, it is important that the certification of asylum seeker clinical conditions is made by a forensic expert, following a proper medico-legal methodology, in order to provide the adequate protection, safety and assistance to the applicant, and to avoid decisions misguided by malingering or false certifications. The authority competent for taking a decision on the asylum application, in fact, may not possess specific medical and/or forensic skills and misrecognize vulnerable situations or underestimate the health and life risks related to a return to the country of origin.

The outlined methodology could be enriched in the future by new diagnostic tools of imaging and bioanalysis [56, 57], by clinical and/or computerised testing, and the developments could be based on evidence derived from extensive migratory case histories.

1. The development of diagnostic imaging techniques, implemented through quantitative and biomarker approaches, free from ionizing radiation or other biological risk factors, will contribute to the identification of past violence and/or torture, their time of production, healing processes and residual outcomes [58].
2. Studies based on ethnic and geographical origins will help to reduce the confidence interval of the estimated biological age and increase the accuracy of identification of vulnerable minors.
3. The "omics sciences" will entail "diagnostic algorithms" of somatic and psychic diseases, based on new markers of disease, prognosis and damage, capable of achieving superior evaluative accuracy [58].
4. The diffusion of the current recognition techniques in relation to "malingering", and the development of new tests/technologies for the identification of "simulating" applicants, will eliminate "operator-dependence".
5. The implications of severity underlying the term "serious illness" and "serious forms of psychological, physical or sexual violence", currently subject to uneven assessment, could be based on shared tabular values or "barèmes", specific to the category of asylum seekers.
6. The use of data from imaging and bioanalysis platforms will permit the registration of the incidence of somatic-psychic pathologies, methods of torture and violence implemented in different socio-cultural contexts.

In order to guarantee adequate and effective "assistance" and "protection", the medico-legal methodology proposed here should be applied systematically, from the reception phase to that of the conclusion of any care pathway, consistently maintaining the objectivity and accuracy typical of "evidence medicine", designed to achieve the value of proof and resistant to the scrutiny of the judicial hearing.

The future aim is to share at international level a systematic methodology in the ascertainment and evaluation of Asylum Seekers, following the well-established scientific background derived from the Istanbul Protocol and related documents and from international experts' opinions. Therefore, this proposal should be part of a more extensive project requiring the creation of an International Working Group composed of medico-legal experts, and the organization of a *consensus conference,* in order to establish which are the critical aspects and the specific needs of each Nation, based on their National standards and legislations, and to design an adequate and exhaustive international medico-legal methodology for the ascertainment and evaluation of asylum seekers. This initiative could be realized under the guidance of the International Academy of Legal Medicine, as already performed in other fields of the discipline (i.e. Medical Liability [20], Personal Injury and Damage [21, 22], Psychic and Existential Damage [23]).

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Chapter 18
Forensic Trends in Forensic Anthropological Humanitarian Action

Douglas H. Ubelaker

Abstract The recent application of the anthropological sciences to forensic humanitarian action have made it necessary to develop methodologies and constitute multi-disciplinary teams that should take into account the availability of experts, the collaboration of the family, the time limits and the given political context in which it operates. The entry into a team of a “forensic anthropologist” with knowledge of archaeology is important and common practice, also for the fundamental role in the finding of adequate samples for successive and innovative bio-molecular analysis (DNA analysis), aimed at the identification of the person and post-mortem interval estimation, subject to the costs and the availability of certified laboratories. The future success of forensic humanitarian action thus entails the constitution of teams dedicated to particular countries, capable of supporting the contribution provided by universally credited international scientific organizations.

18.1 Introduction

Considerations of the possible future of forensic humanitarian action involve the assessment of the nature of the forensic sciences and discernable patterns of change. Essentially, the forensic sciences involve applying the very best science available, in whatever field to the problems that are presented to us in case work. This process has driven research, in the sense that, when particular problems are encountered, practitioners must examine what methods are available on the “scientific shelf” to resolve them. Sometimes we are unable to find the necessary techniques since case applications can be quite unique. In my experience and in that of many of my colleagues, such a predicament has proven to provide a powerful stimulus for new research specifically designed to solve these particular problems that are presented by our forensic casework.

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Looking back at the presentations in this symposium, we can recognize new problems that have surfaced and that need to be resolved. These issues drive new research and stimulate progress in our area of the forensic sciences. Indeed, in the last few years, a lot of what we call progress is directly related to the growing numbers of colleagues engaged in this type of work.

The sub-discipline of forensic anthropology relating to humanitarian action has recently emerged revealing a broader sense of the need for special techniques, methodologies and research in order to face the specific types of problems encountered in these applications.

There are many perspectives from which we can address this topic. These include the involvement of non-professionals, such as the families and relatives of victims, who are needed to bring aspects of these cases to a positive conclusion. Of course, there are robust certification programs for professionals working in most fields of forensic science. These programs are important to ensure quality in forensic applications. However, often there are not enough certified professionals available to do the necessary work, especially in the context of global forensic humanitarian action. Utilized in proper ways, many individuals other than the certified professionals can help and are needed to address the volume of problems encountered in the process of forensic humanitarian action. These individuals can include family members and related individuals, who have information and skills that can be incorporated into the overall effort.

It is possible to recognize trends that have emerged in this type of work, which I believe will strengthen and continue into the future. Although we have no way of predicting what is going to happen in the long term, we can recognize several themes emerging from recent practice that are likely to be reinforced in the near future within the field of forensic science and global humanitarian action.

18.2 Recovery of Human Remains. The Importance of Documentation

Detection and recovery of human remains represents a traditional and important part of the work of the forensic anthropologist, especially those with archeological experience, and plays a large role in the overall investigation.

Basically, if recovery is not performed correctly, there is the risk of compromising the analysis downstream. The problems encountered when remains and related evidence are not recovered properly provide powerful lessons on why it is so necessary to have the correct professionals involved in the recovery and to take the necessary time, not only in the recovery of the evidence, but also to employ methods to document it in a way that limits possible compromise later on in the analysis.

18.3 Important Value of a Multidisciplinary Team Effort

A trend in recovery efforts can also be recognized involving the growing team approach. Past practice involving a single individual with his/her shovel and excavation tools has evolved appropriately into the collaboration of large teams of experts comprised of anthropologists with archeological training, but also other specialists, all working together at the site to deal with whatever type of evidence is encountered and recovered in a practical and useful way.

Usually, these efforts do not involve unlimited resources in recovery efforts. Considerations have to be made regarding what expertise is available, as well as time constraints and the political context that frames the effort. Every practitioner has to manage those factors effectively, but the central goal is to recover all the evidence possible and to document it in a way that is not compromised later on.

Within those constraints, a team of specialists working together in a multidisciplinary effort can recover the evidence in the manner needed to facilitate later identification efforts. The inclusion of an anthropologist with archeological training in this effort is extremely important and is becoming common practice. Anthropologists with archeological training have proven their worth at many scenes and are increasingly included routinely as part of the recovery efforts. Particularly useful are anthropologists skilled in skeletal analysis who can also recognize the presence of human remains and make key observations at the site that cannot be made by others.

The following are some examples of the importance of these issues in my experience. They help us understand why recovery efforts continue to be strengthened through the inclusion of appropriately trained anthropologists.

– The survey of a possible burial site may reveal a pattern of vegetation with particular plants growing in an area where they would normally not be expected to be present. Their presence may suggest that the soil was disturbed in that area facilitating the growth of those plants. These are clues that require experience to detect. Other factors such as topographic features, soil depressions or mounds also can offer clues to consider in the research recovery effort. Using archeological techniques, patterns of soil coloration can reveal that a pit had once been present, likely related to a burial. Those color contrasts can be very clear. However, in other cases they can be subtle. In either case, a trained anthropologist-archeologist is needed to discover and interpret these soil patterns. Properly conducted, this work reveals extremely important information that not only may lead to discovery of human remains, but also provide detail on the dimensions of the burial pit and how it was constructed.
– Recovery efforts can be extremely complicated and difficult following fires and structural fires in particular. Mistakes can easily be made in recovery efforts following fires when recovery teams lack the necessary anthropological expertise. Those mistakes can involve untrained recovery specialists entering into the scene searching for a victim, expecting to find a complete charred body, perhaps in the classic pugilistic pose, involving flexed arms and legs due to

heat-related muscle contraction. Such remains of course might be found if the fire was localized and of short duration. However, if the structural fire was of long duration with intense heat, the soft tissue burns off. When the bones are directly exposed to the heat, they fragment, often extensively. The resulting small fragments can be difficult to recognize and recover since they may appear very similar to other burned material in that context.

It is easy for an untrained investigator to overlook this type of material or to confuse it with other building materials present at the scene. Current research is addressing many of the key issues involved with burned fragments [1]. These include assessing the pattern of bone fractures and other characteristics to determine if the individual was burned in the flesh or as dried bone, the temperature and nature of the fire and the exposure of the individual within the fire. A key identification problem sometimes involves very tiny bits of evidence. If the fire continues for a long period of time, small particles of the individual may be recovered, and we know through DNA analysis that identification may be possible even with such fragmentary and incomplete evidence. However, first of all investigators must recover the fragments and then differentiate them from the other materials. Two techniques are very useful in that regard.

- SEM-EDS (scanning electron microscopy-energy dispersive spectroscopy) is a type of routine analysis frequently conducted in laboratories to identify elements or profiles from material evidence [2]. Bone and tooth present a specific calcium/phosphorus ratio, which usually can be distinguished using that technique from most of the other materials also likely to be encountered in such scenes. If it turns out to be bone or tooth you can try to figure out if it is human or not through DNA analysis.
- Another technique that has proven to be very useful is protein radioimmunoassay PRIA, which looks at a specific species antibody response and is highly effective with tiny bits of material [3]. It will indicate if the sample is of human origin or not and if it is not human it will also indicate what animal is represented. If human status is revealed there is normally sufficient sample remaining to proceed with DNA analysis.

– In the proper excavation of a primary skeleton, the goal is basically to take the necessary time to properly remove all of the soil around the skeleton documenting all of the artifacts or the material that may be relevant. Basic documentation involves recording the spatial relationships of all evidence using mapping and photographic techniques. The goal during excavation is to leave the material in situ (in the original position) while removing the soil to reveal the pattern of the bones. If done properly, the pattern of the bones provides much information about the condition of the body when it was placed at that site. In a primary burial, the bones are articulated in normal anatomical order. This indicates that this individual was buried as a whole body relatively soon before death and before extensive decomposition took place.

– Another careful excavation might reveal a different pattern in which only the lower half of the skeleton is articulated. This might indicate that it was once a primary skeleton, a complete body placed at that site with the bones articulated, but a subsequent excavation disturbed the upper half. Careful examination of soil patterns may suggest a sequence of the events that produced the pattern of articulation of the bone. That sequence might involve an initial primary skeleton with a later disturbance of the upper portion of the skeleton and removal of the skull. All of that provides useful information to determine what happened and the sequence of events that lead to the discovery.
– Another example of a well excavated skeleton may reveal a distinct pattern. In a complete primary skeleton all the bones are in articulation except for the left leg, which is separated from the body. The leg bones are articulated, indicating that the isolated leg was placed there as a complete leg, but detached from its normal articulation in the pelvis. This reveals that it was buried before decomposition, but the leg was somehow forcefully removed prior to the burial and placed in an unusual position.
– Mass graves usually present complex histories and careful excavation may reveal the key patterns. For example, a deposit may contain skeletal remains that for the most part are disarticulated. That pattern suggests that they were originally placed somewhere else while the soft tissue decomposed; important information needed to reconstruct the history of the site. In addition, some articulation may also be present. Lying on top of the disarticulated assemblage described above, there may be a completely articulated skeleton. This suggests that while most of the remains were placed somewhere else and transferred to this place for burial, at the time of the burial there was an additional individual who had died shortly before the burial event. This useful information is only going to be captured if the necessary time is taken for proper excavation. In contrast, this information would be lost if the remains were simply removed without careful excavation.
– Unusual burial patterns of some individuals may also be present. A primary skeleton may suggest the individual was resting on their stomach, but with the articulated lower legs unnaturally flexed with the feet located in the stomach area. This pattern would suggest that soft tissue in the knee area was likely cut to enable relocation of the lower leg.
– Sometimes articulated groups of bones can be found within large deposits of disarticulated remains. For example, the articulated metatarsals of the foot indicate that at the time of the transfer of the secondary remains, most of the decomposition had advanced to the point that the soft tissue was gone, but in a few areas the soft tissue was preserved, maintaining the articulation of the bones. This provides information on the condition of the bodies when they were buried, information that can only be obtained with proper excavation.
– A similar example would be an articulated spine. The thoracic and lumbar vertebrae are maintained in articulation, but with neither the ribs nor the bones

of the pelvis. Such an example represents a simple articulated vertebral column in an advanced stage of decomposition for which the ligaments and the soft tissue around the column were still present maintaining the anatomical relationship of the vertebrae. This pattern is frequently seen in recent forensic cases in which advanced decomposition has resulted in disarticulation of most bones of the skeleton, but soft tissue remains to preserve articulation in the spine.

18.4 Cost of Specialized Techniques DNA

Other trends can be recognized in the development of humanitarian forensic science. The costs of DNA analysis represent factors that affect its use. With more efficient technology available in the future and the increased availability of certified laboratories, this issue will likely improve. However, molecular analysis needs to be considered in context. DNA analysis represents a powerful and important DNA methodology. However, its use still needs to be integrated into an analysis with the approaches of other forensic specialties. Errors involving DNA analysis can be reduced when that context is preserved and properly integrated into the overall analysis and interpretation.

18.5 Time Since Death

Estimation of time since death represents an important and challenging issue in forensic anthropology and with humanitarian cases. For example, assume that a particular event that occurred in 1973 is being investigated. Recovery efforts may result in excavation and retrieval of many skeletons. The question is: are all of these skeletons related to the 1973 event or could at least some of them reflect more recent or more ancient death events? Usually, that problem is resolved with clear contextual information. Careful excavation may reveal details that connect it to a particular date, but sometimes not. In some cases remains are found and the context is not clear or has been disturbed by previous activity. These human remains are available for analysis, but it is not clear as to which time period they relate. Samples can be analyzed for DNA, but if a link to a missing person is not revealed then the investigator is left wondering if the remains represent a missing person not present in the comparative database or if the individual does not relate to the death event (more recent or more ancient). We know that traditional methods trying to estimate time since death using tissue preservation are problematic. There are many factors that affect soft tissue decomposition. In both extremely wet and dry environments soft tissues may be preserved for extended periods of time. Contrasting environments can lead to the very rapid decomposition of soft tissue and hard tissue alteration and destruction. Since many of the contributing factors remain unknown in forensic analysis, accurate estimation of time since death remains elusive based on traditional approaches.

Radiocarbon analysis using the isotope carbon-14 with reference to the modern bomb-curve provides an opportunity to clarify issues surrounding time since death. The atmospheric testing of thermonuclear devices conducted in the 1960s by many countries generated a huge amount of artificial radiocarbon in the atmosphere. Through the food chain, these elevated levels were incorporated into the tissues of all living organisms, including humans. Thus, humans who were alive after the late 1950s developed higher levels of radiocarbon in their tissues than the previously stable levels that were present prior to that date. If analysis of tissues of recovered remains shows elevated levels of radiocarbon, the investigator knows that the person had to have been alive in the modern period, after the early 1950s. The atmospheric levels of radiocarbon increased dramatically until about 1964. Following the cessation of such testing at that time, these levels have gradually decreased. In the analysis of recovered human remains, if modern elevated levels are encountered, the analyst must determine if the values relate to the earlier ascending portion of the curve or the more recent descending portion. Recent research indicates that such determinations can be made in consideration of the dynamics of the formation and remodeling of different body tissues and components and the approximate age at death of the specific individual. In the analysis of blood, the turnover rate is about one year, thus the radiocarbon levels will be within one year of the death date. A similar range is present for hair, depending upon its length, of course. Tissue turnover in the lungs and other organs is between one and two years. The turnover of collagen and bone mineral is much longer and affected by age. Turnover in cortical bone is especially slow. Dental enamel is formed during the early developmental years and does not remodel; thus it retains the radiocarbon dietary values captured early in life. The contrast between the slow remodeling cortical bone, more rapidly remodeling trabecular bone and other tissues can locate the values on the bomb-curve. Since bone remodeling slows with advancing adult age, the age at death must be estimated and considered as well [4, 5].

Consider the example of an individual born in 1926 and who died in 1959 at the age of 33. Radiocarbon analysis of a sample of femoral cortical bone and cancellous bone from the body of a vertebrae and the enamel from a permanent tooth present contrasting values. The dental enamel and cortical bone reveal pre-bomb curve values. However, the more rapidly remodeling trabecular bone value falls within the initial years of the modern bomb-curve. The values are consistent with known information about this decedent and illustrate how the approach can be used to estimate time since death in an unknown case. Associated preserved remains of the larval stage of flies can also be used, since the represented larvae consumed the rapidly remodeling soft tissue of the decedent. The fly larvae puparia retain radiocarbon values within two years of the death date [6]. New information on the dynamics of radiocarbon retention, as well as other such techniques will probably continue to evolve and improve our ability to establish the death date in recovered remains.

18.6 Capacity Building

Effective capacity building within local facilities represents a key element in future progress in global humanitarian forensic action. A board-certified internationally-recognized expert can provide assistance in a short-term visit to a locale in need, but impact is much greater if the in-country team can develop the internal capacity for high-quality analysis on a routine basis. Robust training programs can effectively promote improvement in long-term success in case resolution. Quality can be augmented when such efforts are coupled with effective communication with forensic units, awareness of the growing scientific literature and new technological developments.

18.7 International Organizations

The future success of global humanitarian efforts in the application of forensic science is augmented by the growing recognition and assistance provided by international science organizations. For example, in 2015 the American Academy of Forensic Sciences initiated the Humanitarian and Human Rights Resource Center with funding provided by the (AAFS) and the National Institute of Justice in the United States. In its first year, the Center developed resources on publications, equipment, documents, protocols and educational information, available to all forensic scientists interested in becoming involved in global humanitarian and human rights applications (for details, see aafs.org). Funding was provided to support research on the use of stable isotope analysis to assist in human identification relating to unidentified border crossers from the Texas-Mexico border, improvement of osteometric sorting in large skeletal assemblages, detection of nerve agent exposure in human bone tissue and capacity building in the Philippines, Mexico and Cambodia. As other major forensic science organizations follow AAFS's lead in focusing resources on humanitarian issues, progress is likely to be augmented.

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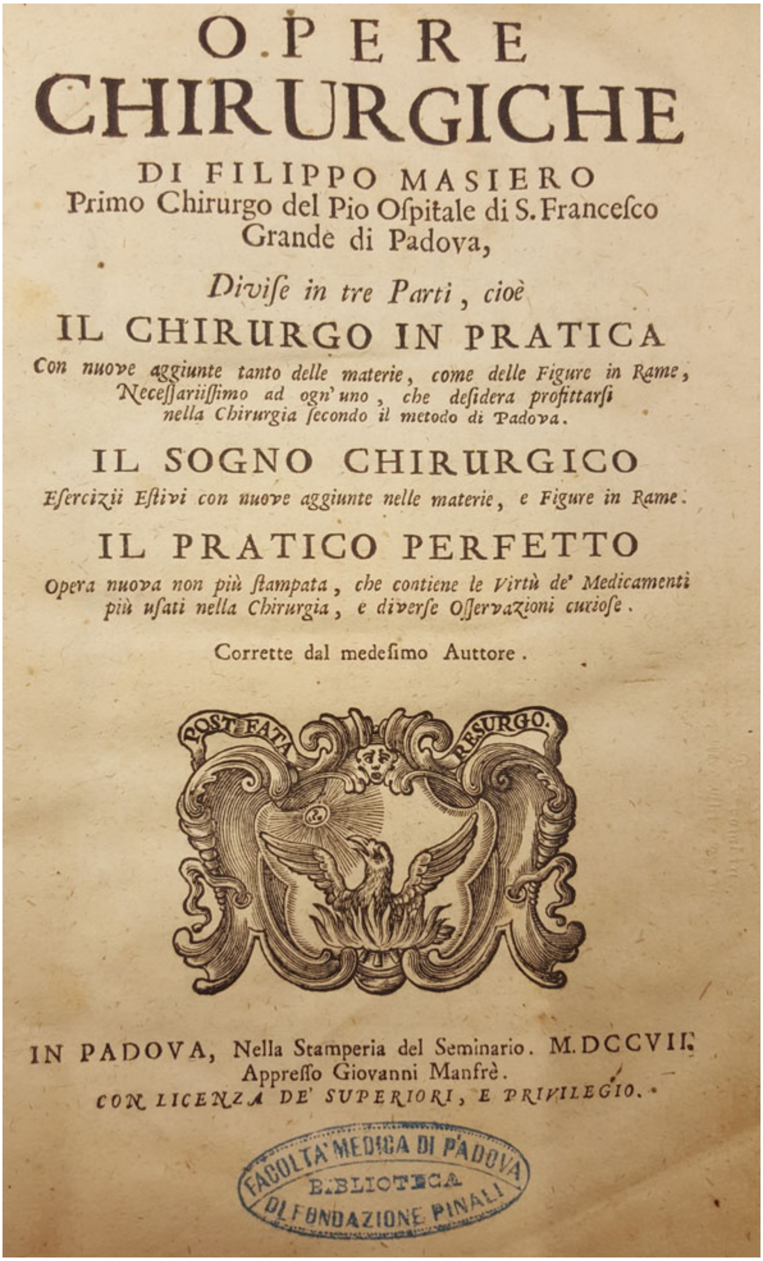
Part V
Innovation, Unitariness and Evidence. Clinical Legal and Forensic Medicine in Living Person Personal Injury and Damage Evaluation Under Civil Law

Schultes, Johann 1595–1645 Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666



OPERE CHIRURGICHE

DI FILIPPO MASIERO
Primo Chirurgo del Pio Oſpitale di S. Franceſco Grande di Padova,

Diviſe in tre Parti, cioè

IL CHIRURGO IN PRATICA

Con nuove aggiunte tanto delle materie, come delle Figure in Rame, Neceſſariiſſimo ad ogn' uno, che deſidera profittarſi nella Chirurgia ſecondo il metodo di Padova.

IL SOGNO CHIRURGICO

Eſercizii Eſtivi con nuove aggiunte nelle materie, e Figure in Rame.

IL PRATICO PERFETTO

Opera nuova non più ſtampata, che contiene le Virtù de' Medicamenti più uſati nella Chirurgia, e diverſe Oſſervazioni curioſe.

Corrette dal medeſimo Auttore.

IN PADOVA, Nella Stamperia del Seminario. M.DCCVII.
Appreſſo Giovanni Manfrè.
CON LICENZA DE' SUPERIORI, E PRIVILEGIO.

Masiero, Filippo 1660–1740, Opere chirurgiche di Filippo Masiero primo chirurgo del pio ospitale di S. Francesco grande di Padova, divise in tre parti, cioé Il chirurgo in pratica … Il sogno chirurgico … Il pratico perfetto … In Padova : nella Stamperia del Seminario: appresso Giovanni Manfrè, 1707

Chapter 19
International Juridical Overview on Personal Injury Compensation

Giovanni Comandè

> *A man's rights multiply as his opportunities and capacities develop. The more civilized the nation the richer he is in rights. The idea here is that interests -that is demand of individuals- increase with increasing civilization, and hence, the pressure of the law to meet these interests increases the scope and character of legal rights* [1]
> *Quite apart from its unfairness, […] variability [of damages] has undesirable effects on the behavioral incentives of primary actors and on settlements. If it can be reduced without unduly sacrificing other important values, justice requires that we try to do so.* [2] (The first quotation is derived from R. POUND, *Interest of Personality*, 28 *Harv. L. Rev.*, 343 (1915) (emphasis added). The second one is taken from P. H. SCHUCK, *Mapping the Debate on jury reform*, in *Verdict: Assessing the civil jury system*, 306, 325 (1994) (Litan ed.)(emphasis added).)

Abstract In the last decades there has been increasing attention given to non-pecuniary component of damages. In a rich society it is possible to grant more legal rights, because there are more resources to dedicate to their protection. If the theory that the richer the society the wider the scope of compensation for non-pecuniary losses has some truth in it, it can certainly be found historically in the evolution of the assessment in case of ascertainable illnesses. This chapter explores the Anglo-American, the French and the Italian experiences in non-economic damages compensation, against the idea that non-pecuniary damages compensation is somehow a societal response to emerging legally protected interests. As far as compensation for non-economic harms in other instances, further research and analysis are required, although the growing societal explanation seems to be in line with an expanding attention to the mental state of the victims.

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19.1 Introduction

Personal injury damages have been part of every legal order since the beginning of human history. The above quotations clearly depict a historical perspective on personal injury assessment. The growth of non-pecuniary losses is, at least to a significant extent, the response of the legal system to a demand of protection for individual interests originating in society. The different level at which demand and offer of legal protection match reflects the legal and economic development a country has reached. One of the responses of the legal system was a deep change in the contents of non-pecuniary losses. How does this insight reflect on todays' assessment?

In a rich society it is possible to grant more legal rights, because there are more resources to dedicate to their protection [3]. When there are no resources to devote to a given individual interest the attempt to safeguard it will remain a wishful attempt or a hot political issue, but it will not achieve very effective results. On the contrary, where a society can devote resources to them, there is a tendency to characterize "new" interests as legal rights and to develop some tools to protect them by building on the existent legal framework. In a way, both the demand of protection for individual interests and the supply of protection depend greatly upon the amount of resources available to a society. However, not all societies recognize compensation for non-pecuniary harms as it is historically denied, for instance, in some socialist and Islamic jurisdictions [4, 5]. Thus, we are aware that our claim that the assessment of non-pecuniary damages expresses the will of societies to protect several paramount values, such as life, health and bodily integrity, according to the actual economic possibilities of a given society, can only capture part of this difficult phenomenon.

Yet, as a matter of fact, in the last decades there has been increasing attention given to (and in the amounts awarded for the) non-pecuniary component of damages [6, 7], at least for most western countries to which we must confine ourselves here [8]. In most of these countries there is a harsh and ongoing debate on how to liquidate these damages and on what interests are really targeted by non-pecuniary damages. In fact, the kinds of losses restored in cases of personal injury have also changed, fracturing legal frameworks and justifications in most of those countries.

It was not a caprice of the Legislature that, since the end of the XIX century, statutes have been created to "protect" workers, *and only them*, *in relation to physical impairment that diminished their capacity to work*[1] *and only that*. In an earlier stage, in most countries, statutes were enacted to protect only the physical capacity of soldiers. At that time, economic developments barely allowed only the protection of the producing capacity and the ability to serve as a soldier. This was the primary exigency that society could recognize and, indeed, the only one which rich societies could afford at that time. It was the only one for which resources were

[1]It is worth mentioning that the *Restatement of the law of torts*, I, St. Paul. (1934), 4, says "the entire history of development of tort law shows a continuous tendency to recognize as worthy of legal protection interests which previously were not protected".

available and for which the answer the legal system gave was acceptable both economically and socially.

Today society's demands push toward the protection of non-lucrative investment possibility. The increase both in awards and in interest in non-pecuniary losses reflects the different level of richness of our societies.

Against this background we will focus on some legal systems clearly in line with this reasoning so as to illustrate their state of the art and perspectives [9–12].

19.2 The Category of Non-Pecuniary Losses

It is relatively easy to chart the types of non-pecuniary losses awarded nowadays [13–16]. However, it is imperative to first define more precisely the meaning of the main ones in order to identify the targeted interests.

It is interesting, for our purposes, to note that the history and developments of non-pecuniary losses have not followed the same path in the same legal family. For instance, in common law different countries have, at least slightly, distinctive notions of the various heads of damages for non-pecuniary losses.

Although, in every country, but in different periods of time, we will find completely dissimilar conceptions of each type of damages, it is possible, however, to identify some overall trends in common law systems. Therefore, the most useful way to describe and to explain (the actual notion of) them is to follow their historical development, making the differences from legal system to legal system as clear as possible. Our focus, however, will be on the two major common law systems, the USA and the UK, in this part.

Moreover, despite the different kinds of damages known in dissimilar common law systems, there are some predominant ones. We will concentrate our attention on these.[2]

19.2.1 The Anglo-American Common Law Tradition

Loss of amenity of life and loss of enjoyment of life came out of pain and suffering in the UK and in the USA, respectively. They had a different evolution in each legal system [13, 17, 18].[3]

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[2]We will not devote great attention to "loss of expectation of life", because it is clearly present only in the -British Commonwealth and there were even several attempts to eliminate it by statute. Indeed, it was short-lived and eliminated in Great Britain in 1982.

[3]Among the other types of non-pecuniary damages it is worth mentioning physical inconvenience and discomfort. It is more frequently assessed in England for breaches of contract (*Hipkiss v. Gaydan*, [1961] C.L.Y. 9042; *Elemcraft Developments v. Tankersley-Sawyer*, [1984] 15 H.L.R. 63 (C.A.)) and only rarely as a different damage for deceit (*Saunders v. Eduards*, [1987] 1 W.L.R.

It is also useful to mention here mental distress or mental anguish, nervous shock and neurosis. The first (mental distress or mental anguish) is loss that does not follow from a physical injury [17] and can sustain an action for damages. Therefore it is different from mental suffering that cannot sustain an action for damages by itself [13].[4]

There is still strong resistance to award damages solely for mental suffering. In one way or another, most of the ancient arguments against it still persist. The first of these is the fear that equalizing the mental suffering arising from physical harms and from non-physical harms could widen the field to imaginary claims.[5] In fact, when mental shock manifests itself with some physical symptoms there is no real problem in redressing the mental suffering[6] endured by the victim. In reality, in the last thirty years there have been such significant changes in these legal constructions that judges, especially in the USA, have practically turned the aforementioned rules upside down. Pain and suffering, for example, has entirely attracted the notion of mental suffering in its conceptual orbit. Therefore, many claims are no longer based on physical injury. This raises a lot of controversial problems that require a closer inquiry into each kind of non-pecuniary loss, which is impossible here. Similarly, the scope of non pecuniary damages was expanded under Italian law with the different aim of compensating for the infringement of constitutionally protected legal interests pertaining to the person, while other legal systems (e.g. France) in which there was no formal limitation have tried in various ways to govern the expansion of non-economic losses compensation.

1116 (C.A.)); nuisance (*Bunclark v. Hertfordshire C.C.*, [1977] 234 E.G. 381). Sometimes it is awarded when a plaintiff shows some physical discomfort without any actual physical lesion. Sometimes it is awarded for discomfort other than physical. *Piper v. Daybell Court-Cooper and Co.*, [1969] E.G.D. 535. The ancient "social discredit" is sometimes given for malicious prosecution and false imprisonment (*Saville v. Roberts*, [1699] 1 Ld. Raym 374; *Walter v. Alltools*, [1944] 61 T.L.R. 39 (C.A.)), but never for breach of contractual relationships (*Addis v. Gramophone Co.*, [1909] A.C. 488; *Bailey v. Bullock*, [1950] 2 All E. R. 1167). In the UK, loss of society and relatives (loss of consortium) was substituted by a fixed amount for bereavement by the Administration of Justice Act of 1982 $3, whereas in the USA it is sometimes awarded as an amount separated from pain and suffering.

[4]"The general principle embedded in the common law is that mental suffering caused by grief, fear, anguish and the like is not assessable" or "mental pain or anxiety the law cannot value, and does not pretend to redress, when the unlawful act complained of causes that alone". *Lynch v. Knight*, [1861] 9 H.L.C. 577. However damages were given "for the mental suffering arising from the apprehension of the consequences of the publication" (*Goslin v. Corry*, [1844] 7 M. & g. 342, 346); "[for] the insult offered or the pain of a false accusation" (*Ley v. Hamilton*, [1935] 153 L.T. 384, 386); "injury to the feelings" (*Mc Carey v. Associated Newspapers*, [1965] 2 Q.B. 86, 104) for deceit (*Saunders v.Eduards*, [1987] 1 W.L.R. 1116 (C.A.)) or trespass to property (*Millington v. Duffy*, [1984] 17 H.L.R. 232 (C.A.)).Damages are also historically denied for disappointment of mind. See *Hamlin v. G.N. Ry.*, [1856] 1 H. & N. 408, 411.

[5]*Vict. Ry. Camrs. v. Coultas*, [1888] 13 App. Cas. 222, 226.

[6]See *Dupey v. T.K.Maltby Ltd.*, [1955] 2 Lloyd's Rep., 168.

The definition of loss of enjoyment of life could be a physical modification of the capacity to enjoy life[7] as clearly distinguished from the capacity to work. All the precluded activities are not profitable, in the sense that they do not allow money earning as such. Loss of enjoyment of life is different from loss of earning capacity as well as from pain and suffering. Hence, it is a new kind of loss or a specification of one of the three main types of reparable losses usually awarded in a personal injury lawsuit: pain and suffering, loss of earning capacity, out of pocket expenses [19].[8]

The impaired activities are all expressions of one's individual personality and pursuits; these impairments are "limitations on the person's life created by the injury" [20]. It is the impairment of hedonic/non-lucrative activities' investment we discussed before.[9] Although, loss of enjoyment of life cannot be subsumed under either of the notions of "pain" or "suffering", it is often awarded together with pain and suffering.

They may arise from the same injury, but they are different. It is not just a "semantic bifurcation of non-economic losses" [21]. It is a deep change, too often misread, or not read at all in the assessment procedures.

Pain and suffering and loss of enjoyment of life seek to redress different losses, despite the fact that they are often awarded together. Moreover, their jurisprudence has a different meaning and it corresponds to different eras.

The way of proving them is also different. To prove loss of enjoyment of life it is enough to prove the victim's previous way of life and how the injury impaired it—the extent of the health impairment. The proof for pain and suffering is theoretically more difficult, since the physical injury, the suffering endured and the emotional responses to it [20] have to be demonstrated—an extremely subjective basket of evaluation to be addressed. In this way, damages for loss of enjoyment of life correspond to the extent of the injury more objectively.

Nevertheless, this is not entirely the actual situation since comprehensive awards for non-pecuniary losses are still the most common ones and several States[10] [22] (in the USA) do not expressly recognize loss of enjoyment of life.

However, loss of enjoyment of life is growing out of pain and suffering, as *pretium doloris*, as a different kind of recoverable loss; it is a loss that looks at the human body as a whole. It is a form of damage that looks at bodily integrity as such, instead of as an instrument to produce. But note that since non-pecuniary

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[7]*Huff v. Tracy*, [1976, 3d DST] 57 Cal. App 3d 939, 129 Cal. Rptr 551 553.

[8]*Restatement (Second) of Torts*, $924. *Kozlowsky v. Briggs Leasing Corp.*, 96 Misc. 2d, 337, 340, 408 N.Y.S. 2d 1001, 1003 (NY Sup. Ct. 1978) is a clear case on this distinction.

[9]They include so many different activities. See *Scolly v. W.T. Garratt & Co.*, [1909] 11 Cal. App. 104 P. 326, 328; 22 *Am. Jur., Damages*, 4th.

[10]It is the law in West Virginia (*Flannery v. United States*, [1982, W.Va.] 297 SE 2d 433); Pennsylvania (*Boggavarapu v. Ponist*, [1988] 518 Pa. 162, 542 A. 2d 516); Wyoming (*Smith v. Ulrich*, [Wyo. 1985] 704 P. 2d 698, 701 and n. 4); 'Alaska (*Corp. v. Horned*, [Alaska 1985] 703 P. 2d 396, 412); Montana (*Walls v. Rue*, [Mont. 1988] 759 P. 2d 169, 170, 173); Maryland (*Nemmers v. United States*, [1988, CD Ill.] 681 F Supp 567 (CA 7 Ill.) 870 F. 2d 426).

losses are often awarded all together in the form of pain and suffering, this title of damages is also changing its meaning, thereby creating some confusion. The legal concept of pain and suffering is forced to bear new goals and different meanings. It is no longer just redress for mental suffering or moral reprobation.

As anticipated, the Anglo-American expression ‘pain and suffering’ often subsumes all the above damages for non-pecuniary loss [23–25],[11] while historically it is used extensively with reference to non-physical subjective intangible losses (e.g. privacy infringements or defamation). Loss of amenity of life (enjoyment of life in the American language) could be defined as a material modification of the capacity to enjoy life as distinguished both from the loss of earning capacity (often conceived as an economic loss), as well as from pain and suffering (sometimes restricted to so-called moral suffering).[12] In short, loss of amenities refers to the victim’s inability to do the things which he was able to do before the accident, such as pursuing specific hobbies.

Thus, several classifications apply to the common law tradition [26, 27] yet the situation might appear clearer if we focus further on the English experience.

In England the Court of Appeal,[13] in announcing a 10% uplift in the level of damages for non-pecuniary losses both in tort and contract, in line with in the Sir Rupert Jackson’s *Final Report on Civil Litigation Cost* (the so called Jackson Report December 2009), endorsed a particular taxonomy of non-economic losses, developing the four fold classification used by McGregor [9] and somehow historically common to the entire common law world we briefly described *supra*. Accordingly, the main titles of recoverable damages are:

- pain and suffering,
- loss of amenity, physical inconvenience and discomfort,
- social discredit,
- mental distress.

The only compensatory [28] title of non-economic damages not subsumed in this taxonomy are ‘aggravated damages’ that are normally awarded in tort (e.g. trespass to the person, malicious prosecution, deceit, misfeasance in public office) and are intended to cope with mental distress and the injured feelings of the victim resulting from the manner in which the wrong was inflicted.[14]

In the English system as well, pain and suffering and loss of amenity are awarded in a single sum and often referred to as general damages. In Italy they are normally awarded separately although the Supreme Court in 2008 stressed the unitary nature of the category of non-pecuniary harm (*danno non patrimoniale*).

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[11] Restatement (Second) of Torts § 924 (1977).

[12] See *Boan v. Blackwell* [2001] 541 S.E.2d 242, 244.

[13] *Simmons v Castle* [2012] EWCA Civ 1288.

[14] See in Canada per McLachlin CJ and Abella J in *Fidler v Sun Life Assurance of Canada* [2006] 5 LRC 472 at [51] (Supreme Court of Canada).

While in Italy several medico-legal baremes are used and more than one scheduling system (although the Supreme court has praised as a clear expression of an equitable evaluation the one produced by the first instance court in Milan _ Tribunale Milano), in England the main reference for practitioners in computing the appropriate level of damages is the 'official' *Guidelines for the Assessment of General Damages in Personal Injury Actions.*[15] Such a general guidance is missing in the USA's State laws.

In it, for instance, a case of Tetraplegia is assessed with a value in the bracket between £262,350 and £326,700. Within this bracket the following elements will play a role:

- awareness of the injury;
- age and life expectancy;
- the presence and extent of pain;
- affect upon senses and ability to communicate;
- age;
- the extent of any residual movement;
- the degree of independence;
- presence of respiratory problems;
- depression.

Against this bedrock it is useful to further describe each other item of compensation under the English classification mentioned before.

Physical inconvenience and discomfort: this is generally included under pain and suffering and loss of amenities. If the inconvenience is not a result of a physical injury it can, in those circumstances, be assessed separately, (e.g., false imprisonment, lost luggage, breach of contract and being forced to walk home for kilometres). As the requirement of a "physical" inconvenience suggests, it does not allow compensation for mere disappointment, for instance, at the failure of the other party to honour his contractual undertaking. As has been summarized in one case: '… the critical distinction to be drawn is not [that] … between the different types of inconvenience and discomfort of which complaint may be made, but a distinction based upon the cause of the inconvenience or discomfort. If the cause is no more than disappointment that the contractual obligation has been broken, damages are not recoverable even if the disappointment has led to a mental breakdown. But, if the cause of the inconvenience or discomfort is sensory (sight, touch, hearing, smell etc.) experience, damages can, subject to remoteness rules, be recovered.'[16]

Social discredit: injury to reputation mainly recoverable under the tort of defamation, but it has also been extended to malicious prosecution and false imprisonment.

Mental distress, as a distinct claim, was not recoverable (Lynch v Knight 1861). However, in instances where it is claimed in addition to other elements of damage,

[15]Now in its 12th edition as of 2013.

[16]Fairley [2001] UKHL 49 at [85], [2002] 2 AC 732, [2001] 3 WLR 899 at 928.

then it may be recoverable, for instance, as injury to feelings as a result of libel or slander. Moreover, it has been associated and awarded in cases of psychiatric illness or actions based on discrimination. Yet in 2008 the House of Lords denied mental distress damages, in absence of other symptoms sufficient to constitute a cause of action, to individuals having pleural plaques due to exposition to asbestos.[17]

19.2.2 The Italian and French Approaches

Both in Italy and in France non-pecuniary harm can normally be compensated, both in tort and in contract. In France this has always been undisputed while in Italy it is a more recent achievement.[18] Of course, general recoverability in principle does not automatically mean that there is not a strong debate over the extent to which non-pecuniary harm deserves compensation. While in France "the mere fact that harm is of a non-pecuniary nature has never been regarded as being, in itself, a bar to compensation", in Italy the limits imposed by art. 2059 c.c. permit the award of non-pecuniary damages only when expressly provided for by the law. Accordingly, In Italy non-pecuniary losses are not expressly mentioned under contractual liability and their availability has been open to question for a long time.

19.2.2.1 France

While classifications and taxonomy have also been regular in France, only recently have some clear distinctions been made widespread and "officially" endorsed. Recent reform proposals would make them both official and formally binding.

Recent legislation[19] made it useful to distinguish clearly between *dommage* and *préjudice*: the first *(dommage)* is an interference with a person, a thing or a situation while the second (*prejudice*) refers to the actual consequences of the tort for the victim. Here we refer only to those non-pecuniary consequences. Accordingly, to continue the parallel with the other scrutinized legal systems the equivalent notion of non-pecuniary damage is non-pecuniary *préjudice* in France.

Traditional, although not official, descriptive distinctions are made according to the victim (immediate or others; so called victims *par richochet*) or the fact a *préjudice* follows from a physical interference or not.

Furthermore, due to the absence of limitations, such as those in art. 2059 Italian c.c., French courts have registered a variety of compensable non-pecuniary

[17] *Rothwell v Chemical & Insulating Co Ltd* [2008] AC 281.

[18] Cass. Sez. Un. 11 november 2008, nn. 26,972–26,975 in *Diritto e Giurisprudenza,* 2008, 526..

[19] Art 2226 of the Civil Code on prescription of damages claims for bodily damages.

damages. It was only with the so-called *nomenclature Dintilhac*[20] (from the name of Mr Jean-Pierre Dintilhac, the president of one of the *Cour de cassation*'s chambers, chairing an ad hoc committee) that France adopted a typology of damage (both pecuniary and non-pecuniary) ensuing from a bodily injury. Albeit not official, the taxonomy is "forced" on lower courts by the *Cour de cassation* that requires them to use it.

The *Dintilhac* report distinguishes between temporary and permanent damage (once health conditions have stabilized) and recognizes six main types of non-pecuniary damages.

Pain and suffering, whose understanding is not very dissimilar from the one described above.

Deficit (prejudice) fonctionnel seeks to compensate a non-pecuniary damage resulting from a medically certified disability that establishes that the damage suffered affects the functions of the human body of the victim. It aims to repair the effects of the damage affecting exclusively the personal sphere of the victim. It attempts to indemnify, as such, not only the abuses of the physiological functions of the victim, but also the constant pain she feels, loss of quality of life and disruption to the daily life subsequent to consolidation. This damage must repair the loss of personal autonomy that the victim experiences in daily activities, as well as all the specific functional deficits that remain after consolidation of the injury. Because of its general nature, the permanent functional deficit is not confused with the loss of amenity, which for its part has a specific target in that it involves the deprivation of a particular leisure activity.

Préjudice d'agrément, indeed, aims solely to repair the damage specifically related to the inability of the victim to practice a sport, specific activity or leisure activity regularly. This item of injury must be assessed *in concreto*, taking into account all of the individual parameters of the victim (age, etc.).

Préjudice esthétique permanent (or aesthetic damage) seeks to repair physical damage and generally the elements of harm able to alter the physical appearance of the victim, such as a permanent scar on the face.

This prejudice is strictly personal and experts generally assess it using a scale of 1 to 7 (from very light to very important).

Préjudice sexuel, or sexual damage, targets compensation for damages related to the sexual sphere. There are three different types of sexual prejudice:

- Morphological damage which is related to the infringement of primary and secondary sexual organs resulting from the injury suffered;
- The damage linked to the sexual act itself based on the loss of the pleasure associated with the completion of the sexual act (loss of desire or libido, loss of physical ability to perform the act, loss of the ability to access the pleasure);
- Damages related to an inability or difficulty to procreate (this prejudice can, in particular in women, result in various forms such as obstetric injury, etc.).

[20]http://social-sante.gouv.fr/ministere/acteurs/partenaires/article/nomenclature-des-postes-de-prejudices-rapport-de-m-dintilhac.

Again, this damage must be assessed *in concreto*, taking into account the personal characteristics of each victim.

Préjudice d'établissement seeks to compensate the loss of hope or any opportunity to realize a project of "normal" family life because of the seriousness of the suffered permanent disability, which the victim still suffers after consolidation: it is the loss of an opportunity to get married, start a family, raise children and general upsets in the life projects of the victim.

Préjudices permanents exceptionnels offers a general open clause for compensation targeting atypical damages, which are directly linked to permanent handicaps and which it may legitimately call for compensation. In short, it is an item that will, if necessary, make it possible to indemnify, as exceptional, any particular permanent non-pecuniary harm that is not compensable by other means.

The same Dintilhac taxonomy distinguishes damages suffered as a consequence of somebody else's death or injury (*préjudice par ricochet*) offering no significant restriction for compensation. The titles acknowledged are two:

Préjudice d'affection (injury to feelings) repairs the injury to feelings suffered by some close relatives after the disabled survival of the direct victim. It compensates almost automatically the affliction of the closest relatives of the direct victim (parents, etc.). Yet it covers persons without relationship to the direct victim, once they establish, by any means, that they had a real emotional bond with the deceased.

Préjudices extra-patrimoniaux exceptionnels (exceptional non-pecuniary loss) targeting the change in the conditions of existence experienced by relatives of the direct victim for her disabled survival. It is intended to compensate the painful upheavals that survival of the direct victim has on the daily lifestyle of her family.

The description of non-pecuniary damages not resulting from a bodily injury (either directly or indirectly) or to any physical interference with a thing or animal is more complex, since it is not clearly mapped and covers non-economic damages ensuing from an interference with a right or protected interest as well as anxiety due to exposure to a risk or the consequences of wrongful birth and wrongful life.

Last April 29th 2016, the Ministry of Justice issued a consultation of a proposed reform of tort liability.[21] The proposed reform subjects personal injury damages to tort liability, also in case it arises from a breach of contract (Art. 1233). In artt. 1267–1277 several rules are proposed for personal injury damages assessment and compensation, also applicable in case of settlements (1267). It requires assessing personal injury damages without considering the pre-existing health states of the victim (1268). It asserts a duty to assess damages specifying which item is compensated with which amount and according to an open taxonomy of prejudices to be adopted by the *Conseil d'Etat*. (1269).

The above described *deficit functionelle* is supposed to be assessed according to an indicative unified medical scheduling system, to be revised regularly according to a

[21] http://www.textes.justice.gouv.fr/textes-soumis-a-concertation-10179/consultation-publique-sur-la-reforme-de-la-responsabilite-civile-28936.html.

database of all final judgments of the courts of appeal for car accidents. Recourse action is limited to an enumerated list of occasions (art. 1273–1274) [29–31].

19.2.2.2 The Italian Search for a Synthesis

The only Italian norm referring to non-pecuniary losses in the civil code (art. 2059) was originally read as having a predominantly 'punitive' aim, because it limits non-pecuniary losses awarding them only when expressly provided for by the law. Non-pecuniary losses are not expressly mentioned under contractual liability and their availability has been open to question for a long time.

The system experienced a fundamental revolution through the judicial introduction of damage to health (*danno alla salute*).[22] Judges followed a constitutional approach to personal injury damages. They started from the assertion that everyone is entitled to the protection of health, a fundamental right protected by art. 32 of the Constitution. As the reasoning went, violation of the fundamental right to health triggers compensation to the victim from the wrongdoer. Thus, if the legal system does not provide for the requirement of compensation, its rules are contrary to the Constitution if not interpreted in a way compatible with the protection guaranteed to this fundamental right: acknowledging compensation for damage to health as such. A distinction between damage to health ('danni alla salute', always fully recoverable) and subjective *pretium doloris* (recoverable within the limits of art. 2059 c.c.) resulted. It was confirmed by Constitutional Court decisions,[23] while the Supreme Court expanded the scope of non-pecuniary losses in 2003 along the lines of the growing theory illustrated in the introduction.[24]

A subsequent quartet of decisions by the Italian Supreme Court (joint chambers) finally linked non-pecuniary damages to the idea of full compensation and to constitutional protection of fundamental rights,[25] confirming their recoverability also in case of breach of contract. These decisions opted for a more open category of non-patrimonial damage, the very idea of which lends it to further scholarly debate and judicial experimentation. This remains the most problematic feature of the Italian approach.

Contrary to the most recent French approach, the Supreme Court concluded that there is not an open list of labels for non-pecuniary damages but only one category ('danno non patrimoniale'). They: (a) are compensable when provided for by the law and (b) should be proved according to the characteristics they assume on a case-by–case basis. For instance, when such damage assumes the form of damage

[22] *Danno biologico* or *danno alla salute*: literally 'biological damage' or 'damage to health', nowadays the two expressions are used as synonyms although technically they are not.

[23] Corte Cost. 14 July 1986 n. 184, in Foro italiano (FI). 1986, I, 2053; Corte Cost. 27 October 1994, n. 372, in Giustizia Civile (*Giust. Civ.)*, [1994], I, 3035.

[24] Cass. 31 May 2003 nn. 8827 and 8828, in Danno e Responsabilità (*Danno e resp.*), 2003, 816.

[25] Cass., S.U. civ., 11 November 2008 n. 26972-26973-26974-26975 (supra fn. 19).

to health, it is the psychophysical impairment that must be medically ascertainable and demonstrated to obtain actual compensation. When another fundamental personal interest protected by the Constitution is infringed, there will be different elements to be proven in court.

Following the 2008 joint chamber Supreme court decisions, 'Non-pecuniary loss [is] understood in its broadest sense of damage caused by the infringement of interests inherent in the person not characterized by economic importance' that must be demonstrated in their actual existence and seriousness. All items of non-pecuniary losses that have emerged so far (including 'danno alla salute') have a merely descriptive function of a particular understanding of non-pecuniary losses. Accordingly, the legislature is sovereign in rendering losses compensable or not, for example, by admitting compensation for the vague sense of boredom of the reader of these pages, while making non-compensable the injury of victims of crimes of danger. What the legislature cannot do, by omission (not legislating) or action (legislating), is to rule out compensation for non-pecuniary prejudices that arise from the infringement of inviolable rights, 'given that the recognition in the Constitution of the inviolable rights inherent to the person not having an economic nature, implicitly, but necessarily, require their protection, and thus constitutes a particular case [as required by art. 2059 Ital. c.c.] set by the law… of compensation for non-pecuniary damages'.

Any compensation for non-pecuniary loss is subject, both as regards *an* and *quantum*, to the criterion of tolerance (some degree of tolerance is required in living in relation with others) appreciated according to the social conscience of the historical moment. The criterion of tolerance, so understood, acts as an elastic valve for admitting or excluding compensation for non-pecuniary damages over time.

Damages for *danno alla salute* are compensatory in nature, albeit it is undeniable that a lost limb cannot literally be fully restored by any amount of money [32],[26] and health 'cannot suffer limits to the compensation for damage done to it.'[27] Yet, as anticipated, this principle clearly appealing to full compensation has not precluded the Constitutional Court from esteeming awards to be fair and full, though departing from the values expressed in litigation and despite the recent attempts of the Supreme Court (*Corte di Cassazione*) to establish one of the judicially produced scheduling as the basic values serving full compensation and equality in the absence of specific statutory interventions [33].[28]

Social perception visualizes the award of damages as capable of making the victim whole,[29] or at least this is the way the system is described in relation to its

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[26]See Cass., 6 June 1981, n. 3675, in Bargagna and Busnelli 1995, 398.

[27]Corte Cost., 14 July 1986, n. 184 (supra fn. 24).

[28]Cass. Sez. III, 7 June 2011 n. 12408 in *Diritto e Fiscalità dell'assicurazione*, fasc.4, 2011, 1568.

[29]*Wright v. British Rys. Bd.*, [1983] 2 A.C. 773, 784–785 (H.L.) (U.K.) at 777: 'Any figure at which the assessor of damages arrives cannot be other than artificial and, if the aim is that justice meted out to all litigants should be even-handed instead of depending on the idiosyncrasies of the assessor… the figure must be 'basically a conventional figure derived from experience and from awards in comparable cases.' (per Lord Diplock).

correspondence to a severity percentage and with medical evidence being the leading guide for it.[30]

Needless to say, the Courts' discretion, if coherent reasoning is offered, remains absolute when it comes to defining the final monetary value of each element according to previous awards and adapted to the pending case. Thus, individual and just compensation appears synonymous with full compensation.

The Italian model de facto tries to bring together the best features of the other models examined. The percentage point is obtained using medical descriptions applying the general schema to the individual case while the monetary translation using the indicative tables further particularize the attempt to make any given victim whole respecting general principles of horizontal and vertical justice. Nevertheless, even the Italian model only makes relative comparisons among various cases while apparently not solving the general question of when enough is enough to trigger full compensation and how the notion takes into account cultural and cognitive variations.

In addition, the Italian experience also seems to struggle in searching to find over time the possibility of expanding the scope of non-economic losses compensation beyond those cases in which an objectively assessable psychophysical injury is medically ascertainable.

19.3 Conclusions

As the Anglo-American experiences illustrate, meaningful and simple definitions have been both employed and suggested to allocate recoverable non-pecuniary losses in one or the other category [34]. In our view, these attempts at pinpointing the role of non-economic damages have resulted in a distinction within the domain of traditional non-pecuniary losses, that is, between loss of enjoyment of life [20, 35–37] and pain and suffering that has proved extremely helpful in searching for guiding criteria for awarding non-pecuniary losses involving an actual psychophysical impairment.[31]

However, the distinction, useful in the assessment in case of impairment to health,[32] does not capture the complexities of reality and the tensions to satisfy a much-extended request for compensating non-economic harm, also when a psychophysical impairment is not present. This certainly applies to the Italian and the French experiences as well.

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[30]As often stressed by the Italian Constitutional Court. See Corte Cost., n. 184 (fn. 24).

[31]See, e.g., Annotation, *Loss of Enjoyment of Life as a Distinct Element or Factor in Awarding Damages for Bodily Injury* (1984) 34 A.L.R. 4th 293.

[32]As per Lord Justice O'Connor in *Housecroft v. Burnett* [1986] 1 All E.R. 332, 337, 'The human condition is so infinitely variable that it is impossible to set a tariff, but some injuries are more susceptible to some uniformity in compensation than others'.

Similarities can be found in the role Supreme Courts play in building and maintaining the system to Asses personal injury damages. So, for instance, it is with a Court of Appeal decision *Heil v Rankin* in which, exceptionally, a five strong Court of Appeal panel was assembled to endorse an increase in the level of general damages in personal injury actions. The CA only partially followed the recommended level of increases suggested by the Law Commission's Report *Damages for Personal Injury: Non-Pecuniary Loss.* In fact, the increase only applied to awards higher than 10.000£. In Italy, the *Corte di Cassazione* (Supreme Court), in a decision immediately followed by several others that confirmed and refined its reasons, attempted to unify at the higher level awards for non economic harms endorsing the scheduling values of *Tribunale Milano.*[33]

If the theory that the richer the society the wider the scope of compensation for non-pecuniary losses has some truth in it, it can certainly be found historically in the evolution of the assessment in case of ascertainable illnesses. As far as compensation for non-economic harms in other instances, further research and analysis are required, although the growing societal explanation seems to be in line with an expanding attention to the mental state of the victims.

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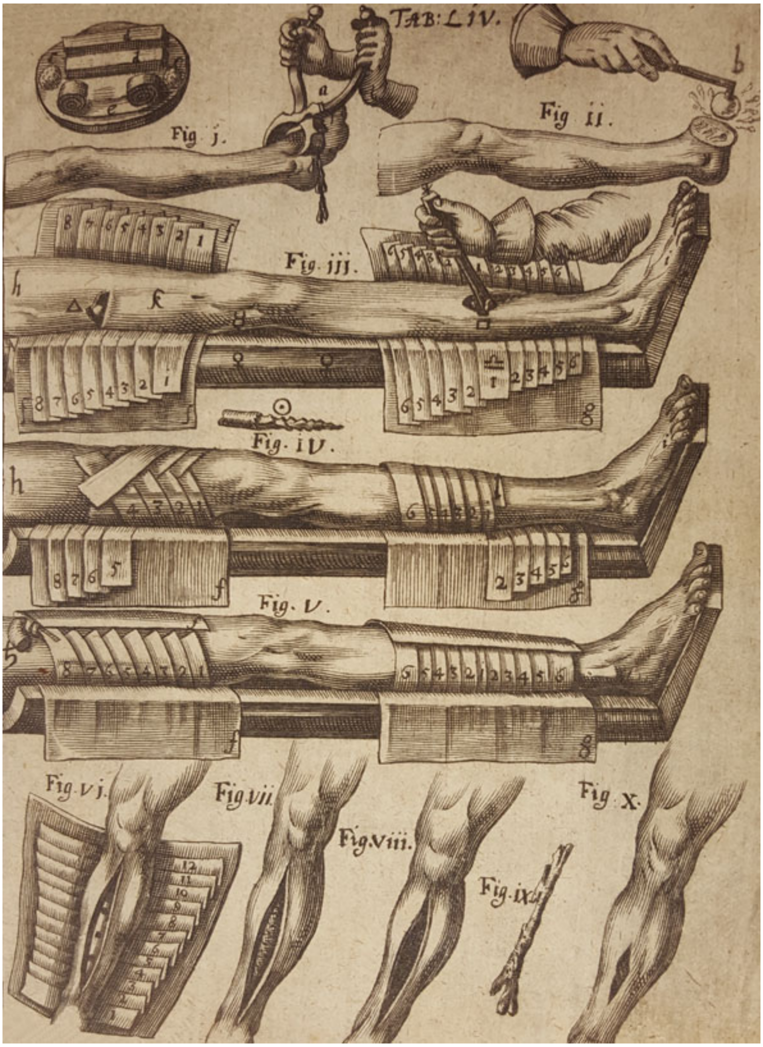

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Fig. X.

Chapter 20
Current and Future Evidence in Personal Damage Evaluation

George Mendelson and Danuta Mendelson

Abstract The most widely used methods of impairment rating at the present time are the *Guides to the Evaluation of Permanent Impairment* issued by the American Medical Association, developed from a series of articles in the *Journal of the American Medical Association* during the period February 1958 to August 1970. This chapter will discuss concerns related to the evaluation of impairment associated with pain and with 'mental and behavioral disorders'. However, before doing so the most important concerns related to the basic concepts and terminology used in this field of clinical medicine are discussed, namely the usage and meaning of terms such as "impairment", "disability" and "handicap". The chapter on the evaluation of impairment associated with "mental and behavioral disorders" in the 6th edition of the AMA *Guides* requires the use of three instruments, namely: (1) the *Brief Psychiatric Rating Scale* for the rating of symptoms; (2) the *Global Assessment of Functioning Scale* (*GAF*) from DSM-IV for the rating of 'psychological, social, and occupational functioning'—it should be noted that the GAF has been "dropped from DSM-5 for among others, its conceptual lack of clarity and questionable psychometrics in routine practice";—and (3) the mis-named *Psychiatric Impairment Rating Scale*, which incorrectly includes "Travel" and 'Employability' as ratings of psychiatric impairment. The chapter will provide an overview of the Guide to the Evaluation of Psychiatric Impairment for Clinicians (GEPIC), developed in Victoria, Australia, which in our view is the only currently published instrument that rates

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psychiatric impairment without allowing the rating to be contaminated by what the WHO would consider as aspects of disability and/or handicap using the definitions set out in the ICIDH.

20.1 Introduction

In the assessment of damages for personal injury, one of the questions asked of expert medical witnesses is to quantify the extent of permanent impairment. The assessment of permanent impairment tends to be particularly difficult where the injury is not apparent to the judge, jury or tribunal, for example in cases of chronic pain or mental illness. As a result, the evaluation of impairment and disability has been an ongoing problem in the psychiatric assessment of plaintiffs in personal injury claims and of applicants for pensions and other social security benefits [1].

According to Colledge and Krohm [2], writing in the journal of the International Association of Industrial Accident Boards and Commissions, "even primitive workers' compensation schemes had intuitive systems for cash awards for permanent injury, with amputation of extremities being the easiest cases to assess and assign specific benefits". They wrote that "Caribbean pirates in the early colonial era had developed written rules for compensating loss of hands, arms, eyes, etc. in the course of their nefarious 'trade'".

Lerner [3] referred to "medically determinable impairment" leading to cessation of work as an important factor in the determination of eligibility for disability benefits. Lerner also noted that "substantial loss of functional capacity" had to be present, but no specific method of rating psychiatric impairment was given in his article.

Many medical writers on the subject of impairment and disability have confused these concepts. Because of this, the concepts of impairment and disability are frequently misused, and the two terms used interchangeably. It is, therefore, important, in discussing the psychiatric evaluation of impairment and disability, to provide clear definitions of what is meant by these terms [4].

Impairment, according to the *International Classification of Impairments, Disabilities, and Handicaps* (ICIDH) published by the World Health Organization (WHO) [5], denotes "any loss or abnormality of psychological, physiological, or anatomical structure or function," whereas a disability is "any restriction or lack (resulting from an impairment) of ability to perform an activity in the manner or within the range considered normal for a human being".

The ICIDH also deals with the subject of handicap, which is defined as "a disadvantage for a given individual, resulting from an impairment or a disability, that limits or prevents the fulfilment of a role that is normal (depending on age, sex, and social and cultural factors) for that individual".

Additionally, handicap is defined as "a disadvantage for a given individual, resulting from an impairment or a disability, that limits or prevents the fulfilment of a role that is normal (depending on age, sex, and social and cultural factors) for that individual".

The ICIDH, in an explanatory note, states that the term “impairment” is “more inclusive than disorder in that it covers losses—e.g., the loss of a leg is an impairment, but not a disorder”. It is further noted that “Impairment is characterized by losses or abnormalities that may be temporary or permanent, and that include the existence of occurrence of an anomaly, defect, or loss in a limb, organ, tissue, or other structure of the body, including the systems of mental function. Impairment represents exteriorization of a pathological state, and in principle it reflects disturbances at the level of the organ”.

The ICIDH thus makes it clear, both in the definition of impairment and in describing its characteristics, that impairment is the objective, externalised loss or abnormality of structure or function. Impairment is what can be demonstrated, assessed, evaluated, and measured by an external observer, appropriately trained in distinguishing the normal from the pathological in relation to the specific aspect of structure or function that is the subject of the evaluation.

Impairment is therefore not a subjective complaint or symptom; it is not the complaint of inability to move a limb where physical examination reveals a near-normal range of joint movement, and it is not a statement that the person feels “depressed” where the mental state examination reveals no manifestations of lowered affect, anhedonia, depressive thought content, or psychomotor disturbance.

Equally, impairment has to be differentiated from both disability and handicap, both of which might (but not always) be the consequences of an objectively demonstrable impairment. To reiterate, disability is a loss or restriction of the ability to perform an activity, whereas an impairment is an objectively demonstrable loss or abnormality of (psychological, physiological, or anatomical) structure or function. Such an impairment must be capable of being described in terms of the specific normal basic function that has been lost or rendered abnormal.

In physical medicine, impairments are often assessed in terms of restrictions of the normal range of joint movements, for example due to pain, which can be measured. In psychiatry, impairments are abnormalities of the normal mental functions; these are commonly termed intelligence, thinking, perception, judgment, mood (or affect), and behaviour. Impairment of these basic mental functions can give rise to a range of disabilities, that is, inability to perform certain activities in a normal manner. However, such a disability must be differentiated from impairment of the basic aspects of mental functioning listed above.

Lasky [6] specifically noted that, in the final analysis, it is the responsibility of the judge—or of the administrative law tribunal—to make the definitive determination of the percentage of disability, whereas the medical expert witness can only properly determine the extent of the impairment.

Thus, while the rating of impairment—as defined in the ICIDH—is the task of the medical practitioner undertaking the assessment, the determination of disability involves legal and administrative factors in accordance with the relevant legislation. This is an important point to emphasise, as frequently conclusory opinions concerning work disability are inappropriately given by the medical practitioner as part of the evaluation and report.

Nevertheless, once the rating of impairment has been made, medical practitioners with specialised experience in occupational or rehabilitation medicine might be able to give an opinion as to the specific work disability resulting from the particular impairment in relation to the individual's work demands. However, rating of work disability requires knowledge of the particular work environment and work activities required of the individual and, for this reason, should only be undertaken by appropriately qualified and experienced practitioners.

In Australian jurisprudence, the distinction between subjective symptoms and objectively assessed impairment was emphasised in the judgment of Neilson J, in *Moran v Thomson Adsett & Partners Pty Ltd* ([1996] 13 NSWCCR 484 at 491), who held that an asymptomatic constitutional back condition might be a contributory factor causing permanent impairment. This decision also, in effect, established that an impairment might be present in the absence of a diagnosable symptomatic disorder albeit the implication that in this particular case there was a "latent impairment" introduced a concept that—according to ICIDH—"constitutes a contradiction in terms".

This chapter will review the methods used for the assessment of psychiatric impairment as well as impairment due to pain, and in particular will highlight those rating scales that purport to assess psychiatric impairment but in fact evaluate disability.

20.2 The Quantification of Damages: A Brief History

In his seminal book on tort law, Fleming [7] stated—in relation to the determination of the quantum of damages for non-pecuniary loss following personal injury—that "In the absence of any logical process for assessing such damages, courts are inevitably driven to a conventional scale or tariff acceptable to the prevailing sense of what is fair and equitable".

The Ur-Nammu law code, which is considered the oldest known legal code (c. 2050 BCE) and predates by about 300 years the more widely known Code of Hammurabi, provided for payment of a quantity of silver in compensation for having injured another by "knocking out" that person's eye.

According to Yaron [8] considerations of fairness and equity also applied in the Laws of Eshnunna, which have been dated to c. 1930 BCE. These laws provided for compensation for bodily injuries, and specifically discussed the damages that would be applicable in cases of injury or death caused by "the goring ox", "the vicious dog", and by the collapse of a "sagging wall".

The Code of Hammurabi (c. 1780 BCE), in contrast, provided for retribution rather than compensation and is perhaps best known for its principle of "an eye for an eye".

Roman Law—which was codified in the Institutes of Justinian (c. 533 CE)—provided in the *lex Aquilia* that compensation was payable for the death or injury of a slave or of a grazing animal. Pecuniary compensation was also awarded for an "outrage" committed against the person; the amount of the compensation varied according to the rank and character of the plaintiff [9, 10].

As we have indicated above, Caribbean pirates developed codes of conduct that, inter alia, provided set amounts of compensation for the injured. Pirates' articles (Articles of Agreement) thus provided standard compensation rates for the maimed and mutilated ("Table of Maims"). According to "Articles of Agreement" attributed to the buccaneer Henry Morgan, the "tariffs" were "for the loss of a right arm 600 pieces of eight, or six slaves; for the loss of a left arm 500 pieces of eight, or five slaves; for a right leg 500 pieces of eight, or five slaves; for the left leg 400 pieces of eight, or four slaves; for an eye 100 pieces of eight, or one slave; for a finger of the hand the same reward as for the eye".

The intrinsic problem of determining what is "fair and equitable" when deciding upon the quantum of damages had not been satisfactorily resolved over the millennia, and to overcome the "objective or subjective" elements in the award of compensation there emerged what Fleming called the "trend to more or less standardised awards ('flexible judicial tariffs')" that was "reinforced by the great value our law attaches to predictability, which promotes settlements and satisfies a sense of justice demanding equal treatment for equal cases".

It was to enhance such "predictability" that the American Medical Association developed the *Guides to the Evaluation of Permanent Impairment*, with the initial publication of a special issue—dated 15 February 1958—of its flagship journal titled *Guide to the Evaluation of Permanent Impairment of the Extremities and Back*, which was announced in an Editorial in JAMA of the same date [11]. That 115-page *Guide* has now expanded to almost 200 pages in the current sixth edition of the *Guides*, published in 2008, and has been split into three chapters, dealing with the upper extremities, the lower extremities, and the spine and pelvis.

That editorial made the important point that there is a distinction between disability and impairment, and that the "true responsibility" of the physician "is to do nothing more than evaluate a patient's bodily impairment" and not "to pronounce an administrative judgment on a permanent disability". As we shall demonstrate in this chapter, with respect to the evaluation of "impairment" due to psychiatric conditions that distinction was lost in the third edition of the *Guides*, published in 1988. The subsequent editions of the *Guides* have done nothing to remedy that situation, and the current sixth edition in our view has made this particular chapter unusable.

The importance of having a reliable and valid method of evaluation of psychiatric impairment has led many jurisdictions to pass legislation that specifically excludes the chapter of the *Guides* that purports to rate impairment associated with mental and behavioural disorders—as well as the chapter dealing with "pain-related impairment"—and to substitute a different method or guideline for that purpose, so as to maintain the principles of objectivity and equity in the determination of the quantum of damages discussed by Fleming.

20.3 Impairment Rating and Thresholds

Another important use of impairment rating has been in the establishment of thresholds that have to be met before legal proceedings can be initiated.

Impairment ratings are thus an essential part of common law personal injury compensation system. They are critical for determining whether the claimant can meet the statutory threshold for litigation for non-economic damages, and if the action is successful, the assessment (quantum) of damages.

Damages for non-economic loss include pain and suffering, loss of amenities of life, and loss of enjoyment of life, as well as in some jurisdictions, disfigurement, and loss of expectation of life. The High Court of Australia in *Andjelic v Marsland* [1996] HCA 55; (1996) 186 CLR 20 noted that under statutory regimes for personal injury compensation, damages in this category operate "to provide a measure of compensation for significant impairment of the ability to lead a normal life when that impairment results from one or more of those matters [pain and suffering, etc.]". The court's determination regarding the plaintiff's level of disability for the purpose of calculating the quantum of damages for past and future pain and suffering, loss of enjoyment of life, and loss of earning capacity is, to a large extent, based on medical rating of impairment.

Eight Australian jurisdictions impose statutory various thresholds, which claimants have to meet before they can obtain damages for non-economic loss.

At the Commonwealth (*Competition and Consumer 2010* (Cth) ss 87P–87S) level and in New South Wales (*Civil Liability Act 2002* (NSW) s 16), no damages may be awarded for non-economic loss unless the severity of the non-economic loss is at least 15% of a most extreme case'. Examples of "the most extreme case" include "quadriplegia, perhaps some serious cases of paraplegia, cases of serious brain damage and, perhaps, some cases of extremely serious scarring and disfigurement caused, especially to young children, by scalding or burning"(*Kurrie v Azouri* (1998) 28 MVR 406 at 413–414). As a general rule, the amount payable is determined by multiplying the maximum amount that may be awarded in a most extreme case by the percentage set out in the statutory table. While medical evidence is important, the court has the ultimate discretion to determine the severity of a claimant's injuries as a proportion of a most extreme case. For example, in *Clarence Valley Council v Macpherson* [2011] NSWCA 422, the court calculated that the plaintiff's, by then healed, "severe twisting injury to his right wrist together with a fracture of the base of the fourth metacarpal whilst using an auger drill bit powered by a chainsaw" reflected the severity and "impact upon his life as a proportion of a most extreme case" at 25%. This meant that Mr Macpherson was entitled to 6.5% of the maximum amount that may be awarded for non-economic loss damages for non-economic loss, namely, $33,800.00.

In South Australia under *Civil Liability Act 1936* (SA), the statutory threshold for damages is a significant impairment of the injured person's ability to lead a normal life for a period of at least 7 days, or a specified amount of medical expenses reasonably incurred in connection with the injury (*Civil Liability Act 1936*

(SA), s 52(1) and s 3). Once this threshold is reached, and claimant's law suit is successful, the court must be satisfied that: (a) the claimant has established, on the balance of probabilities, that his or her injury resulted in non-economic loss in terms of (a) pain and suffering; (b) loss of amenities of life; (c) loss of expectation of life; or (d) disfigurement; (b) assess the level of the injury's severity (significant, moderate, and alike); (c) allocate the value of the claimant's injury by comparing it with the gravest and the least serious non-economic loss which someone could suffer on a value scale of 0 to 60; and (d) convert the value scale into a monetary sum in accordance with statutory mathematical formula.

In Queensland, under *Civil Liability Act 2003* (Qld), ss 61 and 62, a similar four-step process applies, but on a scale running from 0 to 100.

Claimants in Victoria (*Wrongs Act 1958* (Vic), ss 28LB, 28LE and 28LF) and the Northern Territory (*Personal Injuries (Liabilities and Damages) Act 2003* (NT), Division 4, ss 22–28) have to establish a statutory threshold level of permanent impairment defined as 'significant injury' before being eligible for pain and suffering damages. The thresholds are as follows: "(a) in the case of injury (other than psychiatric injury or spinal injury), impairment of more than 5%; (b) in the case of psychiatric injury, impairment of 10% or more; and (c) in the case of spinal injury, impairment of 5% or more." (*Wrongs Act 1958*, s 28LB). Secondary psychiatric or psychological impairment cannot be included in the assessment of degree of impairment (*Wrongs Act 1958* (Vic), s 28LJ). Only impairments arising out of one incident are assessable (impairments from unrelated injuries or causes are to be disregarded). Legislation requires all relevant impairments to be assessed together, and impairments resulting from those injuries to be combined using the tables in the AMA *Guides*.

Tasmania (*Civil Liability Act 2002* (Tas), s 27) has "deductible thresholds": (a) no damages for non-economic loss will be awarded if the amount claimed is less than the minimum statutory threshold ($5000, or as periodically gazetted); (b) damages for non-economic loss are calculated as a percentage (in accordance with statutory formula) of the assessed amount if it is greater than the minimum but under a certain statutory "entry" level ($25,000, or as periodically gazetted); and (c) full damages are awarded for non-economic loss, if the amount of non-economic loss is assessed at or above the statutory "entry" level ($25,000, or as periodically gazetted).

Western Australia (*Civil Liability Act 2002* (WA), s 9 and s 10) has similar system of "deductible thresholds" and mathematical formulae for assessing claims for non-pecuniary loss that exceed the statutory threshold of $12,000 (or as gazetted).

20.4 American Medical Association (A.M.A.) *Guides to the Evaluation of Permanent Impairment*

The most influential impairment rating *Guides* have been those published by the American Medical Association (AMA), currently in their sixth edition. Although these *Guides* have been in use since the 1960s, there has been a paucity of published studies of their application in clinical practice. According to Medline only a very small number of articles dealing with the A.M.A. *Guides* have been published, and most of these discussed the *Guides* in general terms. There has been no published empirical study of the psychiatric impairment rating scale from any of the editions of the AMA *Guides*.

Indeed, it would appear that there have been almost as many articles published criticising the A.M.A. *Guides* as there have been clinical studies of its use [12–15]. An early study of the impairment rating schedule for hand impairment [16] concluded that the method described in the first edition of the A.M.A. *Guides* "showed both substantial reliability and accuracy" when compared with other tests of hand function. We are not aware of any similar study of the impairment rating methods for mental and behavioural disorders described in any of the published editions of the A.M.A. *Guides.*

A guide for the rating of impairment due to mental disorder was initially published by the American Medical Association in 1966 as one of a series of articles by the Committee on Rating of Mental and Physical Impairment; the articles were issued in book form five years later [11, 17, 18]. A second edition of the *Guides* was published in 1984 [19]. This was followed by a third edition that appeared in 1988 [20], a revised third edition in 1990, a fourth edition in 1993 [21] the fifth edition in 2000 [22] and the current sixth edition in 2008 [23].

In the first edition of the A.M.A. *Guides* the authors noted that rating of impairment was "a function that physicians alone are competent to perform," whereas the evaluation of disability was "an administrative and not solely a medical responsibility and function".

The *Guides* stated that, in undertaking an evaluation of psychiatric impairment "the primary concern is whether the mental disorder has resulted in an impairment in terms of loss of physiological, psychological, personal, and/or social adjustment". It was also noted that in some cases "the fact that motivation contains conscious elements related to exogenous social or economic factors will place the matter in the area of disability rather than impairment".

It was also stressed that "permanent impairment" can only be rated "after maximal medical rehabilitation has been achieved".

The approach to the rating of psychiatric impairment utilised by the first edition of the *Guides* was to subdivide mental disorders into six "varieties", viz., mental deficiency, personality disorders, sociopathic personality disturbance, psychoneuroses, psychoses, and chronic brain syndromes. Within each "variety" of disorder up to four "classes" of "Impairment of whole man" were defined, each "class" corresponding to a specific percentage rating of impairment.

The *Guides* provided brief descriptions and clinical examples to illustrate the appropriate impairment ratings for the various disorders and degrees of impairment.

According to Nussbaum [24] however, the A.M.A. *Guides* were considered unsuitable for use in the determination of impairment for purposes of determining entitlement to benefits under the Social Security Act in the United States. It was considered that the psychiatric diagnosis was not a reliable guide to the degree of impairment, and that disagreement as to the diagnosis could have a significant effect on the rating of impairment, for example, a diagnosis of schizoid personality disorder may be made by one psychiatrist whereas another would diagnose the same patient as suffering from schizophrenia. Using the A.M.A. *Guides*, such discrepancy would have a major effect on the impairment rating.

In the second edition of the A.M.A. *Guides to the Evaluation of Permanent Impairment* [19], the section dealing with impairment due to mental and behavioural disorders abandoned the reliance of the previous edition on diagnostic categories, and instead utilised evaluation of six specific aspects of mental functioning, namely intelligence, thinking, perception, judgment, affect, and behaviour.

The *Guides*, in the second edition, specified five principles for the assessment of psychiatric impairment, as follows:

1. In assessing the impairment that results from any mental or physical disorder, readily observed, empirical criteria must be applied accurately. Only a structured, replicable examination will result in an informed judgment.
2. Diagnosis is among factors to be considered in assessing the severity and possible duration of the impairment, but it is by no means the sole criterion.
3. In a way that is dissimilar to the evaluation of other organ systems, factors related to the individual's family, education, financial and social situations, and occupation must be taken into consideration, as well as the individual's existing level of functioning.
4. The underlying character and value system of the individual is of considerable importance in the outcome of the disorder, be it mental or physical. Motivation for improvement is a key factor in the outcome.
5. A careful review must be made of the treatment and rehabilitation methods that have been applied or are being used. No final judgement can be made until the whole history of the illness, the treatment, the rehabilitation phase, and the individual's current mental and physical status and behavior have been considered.

It will be apparent from these five principles that it was intended that rating of psychiatric impairment using the A.M.A. *Guides* should take into consideration the individual's emotional reaction to the disorder, including factors such as regression, illness behaviour, and entrenchment in the "sick role" which may be reinforced, albeit unwittingly, by well-intentioned family members. However, the table provided for the evaluation of psychiatric impairment made no provision for such factors to be taken into account.

The *Guide to the Evaluation of Psychiatric Impairment for Clinicians* (GEPIC), in use in Victoria, Australia, which is discussed below, has adopted these five principles with relatively little modification.

It should also be noted that the A.M.A. *Guides* refer to "permanent impairment". The second edition of the *Guides* stated that "impairment should not be considered 'permanent' until maximum medical rehabilitation has been achieved, and until, in the physician's best clinical judgement, the impairment is static or well-stabilized" (at p viii).

Rating of psychiatric impairment was made in one of five "classes", which correspond to a specified percentage of impairment as follows: Class 1—0–5%; Class 2—10–20%; Class 3—25–50%; Class 4—55–75%; and Class 5—over 75%.

The second edition of the A.M.A. *Guides* provided a "combined values chart", which allowed for the calculation of a "whole person" impairment rating, in cases where impairment was present in more than one body system.

The second edition of the *Guides*, in Appendix B, set out examples of what was meant by "Activities of Daily Living". Included in the list were self-care and personal hygiene, communication, normal living postures, ambulation, travel, nonspecialised hand activities, sexual function, sleep, and social and recreational activities.

The third edition of the A.M.A. *Guides* [20] provided a scale of the five "classes" of what was termed "impairment" due to mental and behavioural disorders, ranging from "no impairment" (Class 1) to "extreme impairment" (Class 5) but based these on activities of daily living, social functioning, concentration, and adaptation. The third edition of the *Guides* thus rated disability and not impairment. That edition also failed to provide percentage ratings associated with the "classes" of "impairment".

In a strong critique of the way in which the third edition of the A.M.A. *Guides* dealt with impairment due to mental problems, Spaulding [25] noted that whereas the previous editions seemed "to have promoted uniformity among jurisdictions and greater acceptance of mental disability claims", the fact that "the recent revision does not contain a percentage rating system for mental impairment" would create problems for courts and that "it has undermined the AMA's goal of objective evaluation".

Similarly, the fourth, fifth and sixth editions of the A.M.A [22–24]. *Guides* similarly have not provided any method of percentage rating of psychiatric impairment, and therefore when the fourth edition was adopted by legislation as the prescribed method of impairment rating in Victoria it became necessary to develop a different method of rating psychiatric impairment.

The legislative amendments that prescribed the use of the fourth edition of the A. M.A. *Guides* for rating permanent impairment in the transport accident and workers' compensation jurisdictions in Victoria also provided for the use of the *Clinical Guidelines to the Rating of Psychiatric Impairment* [26] [developed by members of the Medical Panel (Psychiatry), 1998] which has now been replaced by the *Guide to the Evaluation of Psychiatric Impairment for Clinicians* [27].

In January 2000, before the fifth edition of the A.M.A. *Guides* was released, a review of some of the problems in relation to the fourth edition was published, which specifically commented that the *Guides* "blurs the line between impairment and disability by including disability-related roles (such as 'occupation' and 'social and recreational activities') in the lists of activities relevant to impairment" [28]. A response to that article, co-authored by editors of the A.M.A. *Guides*, was published in the same issue of JAMA [29]. Although the editors stated that "The *Guides* does not directly address disability", nothing was done in the fifth edition to revise or modify the chapter dealing with psychiatric impairment, which continues to evaluate disability while calling it impairment.

While the A.M.A. *Guides* continue to be used in most states of the U.S., there are several states—including California, New York and Utah—where state-specific "supplemental" impairment-rating guides are used [30].

Although the approach to the evaluation of psychiatric impairment of the second edition of the A.M.A. *Guides* was preferable to those in the subsequent editions of the *Guides* referred to above, it still posed several practical difficulties. The inclusion of "Activities of Daily Living" and "Rehabilitation or Treatment Potential" within Table 20.1 at page 220 of the second edition of the *Guides* had introduced a degree of confusion in the application of that Table for impairment evaluation. The definition of "impairment" made it quite clear that, in this context, impairment refers to "loss or abnormality of psychological … function". Thus, neither "activities of daily living" (any limitation of which arising as a consequence of psychiatric impairment is a disability) nor "rehabilitation or treatment potential" (which is a prognosis) can be included in the rating of psychiatric impairment. For this reason, the *Clinical Guidelines* include only the six specific aspects of mental functioning that can be impaired, and exclude other considerations.

Indeed, it was illogical to include "rehabilitation or treatment potential" in the Table of the second edition of the A.M.A. *Guides* dealing with the rating of permanent psychiatric impairment because, by definition, if the rating was of "permanent psychiatric impairment" then the condition must be "static". If such were the case, then every subject would have a Class 4 rating for "Rehabilitation or Treatment Potential" even if all the other items were in Class 1. Because "impairment" only refers to abnormalities of psychological function, and therefore cannot include either disability or prognosis, the evaluation of psychiatric impairment could only include consideration of the six aspects of mental functioning listed in Table 20.1 at page 220 of the second edition of the A.M.A. *Guides*, namely intelligence, thinking, perception, judgment, affect, and behaviour.

The second edition of the A.M.A. *Guides* also did not provide any description as to how the overall level of psychiatric impairment was to be calculated. The "example" at page 221 of the *Guides* was not helpful; indeed, it included an error that

Table 20.1 Scheme for the rating of psychiatric impairment in the Guide to the Evaluation of Psychiatric Impairment for Clinicians (GEPIC)

Evaluation of psychiatric impairment					
Class of impairment	1	2	3	4	5
Percentage of impairment (%)	0–5	10–20	25–50	55–75	Over 75
Mental status					
Intelligence	Normal to slight	Mild	Moderate	Moderately severe	Severe
Thinking	Normal to slight	Mild	Moderate	Moderately severe	Severe
Perception	Normal to slight	Mild	Moderate	Moderately severe	Severe
Judgement	Normal to slight	Mild	Moderate	Moderately severe	Severe
Mood	Normal to slight	Mild	Moderate	Moderately severe	Severe
Behaviour	Normal to slight	Mild	Moderate	Moderately severe	Severe

would cause a candidate to fail his or her examination in psychiatry, in that it refers to "delusions" under the heading of "perception" (a delusion is an abnormality of thought content, and hence a form of impairment of thinking, as discussed below).

Statistically there are three methods by which the overall level of psychiatric impairment can be calculated, based on the individual ratings on each of the six items reflecting mental functions. The three methods are the "mean" (or average), the "median," and the "mode" [31]. Another possible method is simply to take the "highest" of the six ratings, and use that as the "whole person psychiatric impairment". It could be argued that this is the most appropriate method, especially if "impairment of affect" or "impairment of mood" is the highest rating of the six items, because disturbance of affect or mood has such a profound impact on the person's overall level of functioning.

The use of the median method was recommended in the *Clinical Guidelines to the Rating of Psychiatric Impairment* and is also used in the GEPIC. The median method is also recommended when using the PIRS adopted in NSW as part of the MAA Impairment Assessment *Guidelines*.

The "classes of impairment" in Table 20.1 provide values such as "0–5%", "10–20%", and so on. Prior to the introduction of the *Clinical Guidelines to the Rating of Psychiatric Impairment* and the "median method" there has been uncertainty whether intermediate values for "whole person" psychiatric impairment were valid or acceptable. In *Byl v VWA (Hobson Park Hospital)* (unreported, County Court of Victoria, No 205/93, Judge Williams, 10 August 1995), His Honour held that, by averaging the psychiatric impairment ratings given by two expert witnesses, the plaintiff's psychiatric impairment was 9%.

As indicated above, the evaluation of impairment for the purposes of establishing entitlement to benefit impairment in respect of a work-related injury sustained on or after 12 November 1997 resulting in permanent impairment claimed under section 98C of the *Accident Compensation Act 1985* (Vic), as amended, is to be made in accordance with the fourth edition of the American Medical Association's *Guides*, in accordance with section 91 of the Act. For the purposes of the assessment of psychiatric impairment, the *Clinical Guidelines* are to be used, in accordance with section 91(6).

The relevant Table from the second edition of the *Guides* (page 220), used for the evaluation of psychiatric impairment, has been set out below. Using the second edition of the *Guides*, it is necessary to rate affect, as compared with the *Clinical Guidelines to the Rating of Psychiatric Impairment* and the GEPIC, which rate mood. Affect refers to the individual's emotional state at the time of the consultation, whereas mood refers to a sustained feeling state, as described below.

One of the problems in using any of the editions of the American Medical Association's *Guides* is the potential for "overlap" between the chapter assessing neurological impairment and impairment associated with a mental disorder. The neurology chapter allows for the rating of impairment due to disturbances of integrated cerebral functions resulting from a brain injury, as well as organically-determined emotional disturbance. Such emotional disturbance is also rated in the assessment of psychiatric impairment, which can be used to rate psychiatric impairment due both to organic brain damage and non-organic mental conditions. The principles to be applied in resolving this problem, in relation to impairment ratings using the second edition of the A.M.A. *Guides*, were considered by Preuss SM in *Carney v Transport Accident Commission* (2002) VCAT 748. This judgment contains a useful discussion of the Court of Appeal decision in *Lake v Transport Accident Commission (No. 2)* (1997) 11 VAR 260, which considered the use of the A.M.A. *Guides* in the evaluation of the various impairments resulting from brain injury.

The current (sixth) edition of the *Guides to the Evaluation of Permanent Impairment*, published by the American Medical Association in 2008 [24], in Chap. 14 that deals with impairment due to "Mental and Behavioral Disorders", states that the "impairment rating is based on 3 scales: the Brief Psychiatric Rating Scale, the Global Assessment of Function, and the Psychiatric Impairment Rating Scale".

These three scales will be discussed below.

20.5 Brief Psychiatric Rating Scale (BPRS)

This scale, published in 1962 by Overall and Gorham [32], is used to evaluate both psychotic and nonpsychotic psychiatric symptoms. In its original form it contained 16 items; in 1967 two more items were added [33] and the "expanded" version used in the A.M.A. *Guides* is version 4.0 (24-item version), published in 1993 [34].

This "24-item iteration" of the BPRS is in part scored on the basis of the individual's self-report (items 1 to 14) albeit some of these 14 items are also rated on the basis of behaviour observed during the interview. Items 15–24 are rated by the interviewer on the basis of observed behaviour and speech.

The BPRS has been used in research with children [35] as well as in psychogeriatric settings [36, 37]. It also has been used to evaluate response to treatment during acute episodes of psychiatric illness [38] and in differential diagnosis of patients admitted to psychiatric inpatient units [39]. We are not aware of any study that had utilised the BPRS to rate psychiatric impairment, and no such study is cited in the sixth edition of the A.M.A. *Guides*.

A recent research report describing the use of version 4.0 of the BPRS in the evaluation of treatment response of patients with unipolar depression noted that this scale is useful as a "short, simple-to-administer, and informative" method to assess psychiatric symptoms in "busy psychiatric services" [40] but that "little is known about the BPRS's reliability and validity outside of the psychosis spectrum".

The editors and authors of the sixth edition of the A.M.A. *Guides* have failed to provide any rationale for the inclusion of the BPRS as an instrument for the rating of psychiatric impairment.

20.6 Global Assessment of Functioning Scale (GAF)

As stated in the sixth edition of the A.M.A. *Guides*, "The GAF constitutes Axis V of the DSM-IV. The GAF is a 100-point single-item rating scale for evaluating overall symptoms, occupational functioning, and social functioning".

It is clear that the GAF **does not** rate psychiatric impairment; at best it combines an assessment of symptom severity with the extent of related disability.

The A.M.A. *Guides* acknowledge that "One known limitation of the GAF is the result of combining functional level and symptom severity into 1 scale". It is suggested that such "limitation" can be "remedied by simultaneously performing the BPRS and the PIRS". As demonstrated in this chapter, however, neither the BPRS nor the PIRS evaluate psychiatric "impairment" in the sense in which the concept of impairment is defined by the World Health Organization. The use of two other invalid scales cannot "remedy" the fundamental flaw inherent in the use of the GAF as a measure of "impairment".

The fifth edition of DSM [41] published in May 2013, has "dropped" the GAF, and stated that it had been "dropped from DSM-5 for several reasons, including its conceptual lack of clarity (i.e., including symptoms, suicide risk, and disabilities in its descriptors) and questionable psychometrics in routine practice" (DSM-5, page 16).

20.7 Psychiatric Impairment Rating Scale

The Psychiatric Impairment Rating Scale was developed for the NSW Motor Accidents Authority. There are several methods of scoring the PIRS that are used by different Australian jurisdictions that have adopted it, and these appear to have been calibrated in a manner that makes it difficult to reach the statutory minimum impairment level for benefits.

The original version of the PIRS was included in the 6th edition of the AMA Guides; this was discussed above.

Despite its name, the PIRS does not assess impairment but disability; the important difference between these two concepts was discussed above. Thus, because the PIRS does not evaluate psychiatric impairment but provides a disability rating, its very name is misleading.

Davies [42] in an article provocatively titled "The Psychiatric Impairment Rating Scale: Is it a valid measure?" noted that "there has been significant criticism of its validity and structure, but no supporting research". His study "was undertaken to examine the validity of the use of the PIRS to assess psychiatric impairment".

The various versions of the PIRS that are used within several Australian jurisdictions use different methods of scoring, and Davies examined the version used by the workers' compensation jurisdiction in NSW [43]. He concluded that "the scoring technique in the PIRS transforms normally distributed scores to a skewed distribution with a preponderance of low scores". This is not surprising, because the original version of the PIRS—developed for the Motor Accidents Authority in NSW—was specifically designed to utilize a scoring method that would tend to produce a low Whole Person Impairment rating below the threshold of ten (10) per cent.

Davies also found that while the PIRS did produce a means of "ordering the severity of psychological disability" (that is, it is an ordinal scale), it "measures disability rather than impairment". He also concluded that "the form of scoring" used in the PIRS "does not provide a proportionate or statistically meaningful measure".

Strong criticism of the PIRS was also contained in a submission made by the Australian Lawyers Alliance (ALA) to the review of the Civil Liability Regulation 2003 (Qld) in relation to the "levels of Injury Scale Value (ISV) and injuries within s. 4 of the Regulation" [Australian Lawyers Alliance (2005)].

According to the submission: "Although titled 'Psychiatric Impairment Rating', the PIRS sets out six 'areas of function' to be rated, namely self care and personal hygiene, social and recreational activities, travel, social functioning, concentration, persistence and pace, and adaptation. All of these have been taken directly from the relevant chapter of the fourth edition of the *American Medical Association Guides to the evaluation of permanent impairment*; the first three items are included within the 'activities of daily living' head of the *Guides*. These are not issues of impairment but rather of disability".

The submission also noted that "the PIRS was essentially lifted from the NSW Motor Accidents Authority Impairment Assessment Guidelines. Chapter 7 of those Guidelines indicates that the 'mental and behavioural disorders impairment' rating was designed with a specific 10% damages threshold in mind".

Finally, the submission noted "there are now four jurisdictions in Australia that use the so-called 'Psychiatric Impairment Rating Scale", but this scale has been modified to suit every one of the individual jurisdictions. Thus, not only does the PIRS fail to assess psychiatric impairment—despite its title—if also lacks integrity, with each jurisdiction making changes to suit its own political agenda. Thus, there are four different versions of PIRS in use in Australia in four separate jurisdictions".

The ALA submission concluded that "the flawed nature of the PIRS demands its removal".

A further concern in relation to the PIRS is its availability "online" [http://www.pirs.com.au/; accessed 22 April 2016] for a fee, which means that claimants or plaintiffs can "practice" and learn what answers will produce a higher score. Because the PIRS assesses disability—the six domains listed above—it is scored on the basis of information provided to the impairment assessor by the person being evaluated: that information might or not be accurate and the assessor has no means of testing its veracity. Rating scales that assess impairment on the basis of a mental status examination, such as the GEPIC (see below), are therefore both valid and more robust than the PIRS.

The PIRS website states "The e-PIRS is a web-based questionnaire, based on the PIRS methodology and philosophy. While it is not a substitute for a thorough clinical examination, the result can help a claimant or insurer make a decision about a psychiatric injury claim. For example, many compensation systems exclude small claims by the use of thresholds. When the e-PIRS indicates a low impairment rating, significant additional expenses can be avoided. Conversely, when there is doubt, e-PIRS could help identify significant impairment and provide a basis for further specialist examinations. While the e-PIRS relies on complex algorithms, the results are explained in clear and easily understood terms".

The website also includes a pricing schedule, and 'e-PIRS vouchers are available for purchase, once you have logged in as a client". Visitors to the website are invited to "Click here to register as a new client or logon" and the "e-PIRS pricing structure is as follows:

$$
\begin{aligned}
1\ \text{voucher} &= \$8 + \text{GST}\\
5\ \text{vouchers} &= \$35 + \text{GST}\\
10\ \text{vouchers} &= \$65 + \text{GST}\\
50\ \text{vouchers} &= \$300 + \text{GST}\\
200\ \text{vouchers} &= \$1100 + \text{GST``}.
\end{aligned}
$$

20.8 Clinical Guidelines to the Rating of Psychiatric Impairment

The *Clinical Guidelines to the Rating of Psychiatric Impairment* were introduced for use in Victoria, Australia, during 1998 in accordance with the provisions of both the *Accident Compensation Act 1985* and the *Transport Accident Act 1986*. These *Clinical Guidelines* were developed because it was considered that the method for rating impairment due to mental and behavioural disorders set out in the fourth edition of the A.M.A. *Guides*, published in 1993, were unworkable and when the use of the fourth edition was adopted in Victoria from 1998 it was considered that a different method for the rating of psychiatric impairment was essential.

As noted above, the *Clinical Guidelines to the Rating of Psychiatric Impairment* were fairly closely modelled on the relevant chapter of the second edition of the A. M.A. *Guides*.

The *Clinical Guidelines* listed six factors that were to be assessed to provide the percentage rating of impairment, namely intelligence, thinking, perception, judgement, mood and behaviour. These six aspects of mental functioning can be briefly described as follows:

(1) Intelligence: refers to the level of cognitive (intellectual) function. It includes global orientation (in time, place and person), fund of general information, capacity for abstract thinking, memory functions and aspects of the use of language. Intelligence can be clinically assessed during a psychiatric consultation, and if it is considered necessary a screening test such as the Mini Mental

State Examination can be performed. Significant impairment of intelligence occurs in Dementia (e.g., following severe head injury, or due to degenerative brain disease) or may be congenital or developmental.

(2) Thinking: impairment includes formal thought disorder involving thought processes (loosening of associations, interpenetration, metonymy, thought blocking) and abnormalities of thought content (delusions, overvalued ideas), and abnormalities of the stream of thought (e.g., pressure of speech with flight of ideas, or slowed thinking due to psychomotor retardation). Delusions can be primary or secondary, and may be persecutory, grandiose, etc., or involve delusions of reference. In patients with Schizophrenia, specific delusions of thought broadcasting, delusions of influence, etc. may occur (sometimes termed first rank symptoms).

(3) Perception: disorders of perception that need to be assessed as part of the mental status examination are hallucinations and illusions. Hallucinations are subjective sensory perceptions in the absence of an actual external stimulus; these may occur in any one of the five sensory modalities. Illusions are defined as distorted perceptions of real external stimuli; they are usually visual but may involve misperception of sounds.

(4) Judgement: this refers to the ability to evaluate various situations and information, and reach an effective conclusion. Impaired judgement may affect the individual's capacity to perform certain complex tasks and to make autonomous decisions at work. Following injuries to the frontal lobes, judgement may be impaired leading to socially inappropriate behavior.

(5) Mood: this refers to the assessment of the person's sustained feeling state, which tends to be persistent and stable, and colours the total experience of the individual. During the consultation mood tends to be manifested by the subject's affect, which is the individual's immediate emotional experience. Mood is generally described along a continuum from the extreme of severe depression with suicidal ideation to that of euphoria. Affective instability (emotional lability) with marked shifts of mood may be apparent during a consultation. Another aspect of emotion that may be present during the consultation is anxiety.

(6) Behaviour: impairment of behaviour is present when the individual acts in a manner that is disruptive or aggressive; disruptive behaviour may be due to agitation or argumentativeness. Persons with an obsessional disorder may be impaired by compulsive activity. In psychotic disorders, catatonic posturing or stereotyped movements interfere with goal-directed activity.

Examples were given in the *Clinical Guidelines* for each type of impairment, allowing a rating to be made of the severity of impairment ranging among five classes, similar to those used in the second edition of the A.M.A. *Guides*. The whole person psychiatric impairment rating was determined using the median method, which was described above.

The *Clinical Guidelines to the Rating of Psychiatric Impairment* were revised in 2005, and more detailed descriptors of the various domains of psychiatric impairment were provided. The revised guide is discussed below.

20.9 The Guide to the Evaluation of Psychiatric Impairment for Clinicians (GEPIC)

Instructions for the use of the GEPIC stated that “The presence and extent of impairment is a medical issue, and is assessed by medical means. This Guide has been designed for use by medical practitioners; in evaluating psychiatric impairment in accordance with this Guide clinical information has to be obtained and assessed, together with an examination of the individual’s mental state”.

It was also noted that “The evaluation of psychiatric impairment in accordance with the Guide is meant to be informed by clinical judgement, based on appropriate training and experience, and the specific rating criteria are not meant to be used in a ‘cookbook’ fashion”.

The GEPIC provided descriptors associated with the particular classes of impairment for each of the six aspects of mental function to be evaluated. It was noted that these descriptors were intended to provide an overview of the type and severity of symptoms associated with each particular level of impairment. It was also stated that “The absence of a particular symptom in the list of descriptors does not mean that that symptom is to be disregarded. The assessor may be required to justify why that/those symptom(s) is/are associated with a particular class of severity”.

The illustrative descriptors in the GEPIC are set out below, listed under each of the six types of mental function that are to be evaluated, together with a brief definition of that mental function.

20.10 Intelligence

Capacity for understanding and for other forms of adaptive behaviour. Impairments of intelligence are a consequence of brain injury or disease. Generally, before impairment of intelligence is confirmed neuropsychological assessment should be undertaken (care has to be exercised to ensure that there is no overlap between an assessment of impairment of intelligence made during a psychiatric evaluation and an assessment of impairment of higher cerebral functions made by an assessor in accordance with Chap. 4 of the 4th edition of the American Medical Association’s Guides).

20.10.1 Guides for the Rating of Impairment of Intelligence

Class	Impairment (%)	Description
1	0–5	Normal to slight – there is no evidence of cognitive impairment on mental state examination, and the individual does not report any difficulties in everyday functioning that can be attributed to cognitive difficulties.
2	10–20	Mild – some interference with everyday functioning.
3	25–50	Moderate – a reduction in intelligence that significantly interferes with everyday functioning.
4	55–75	Moderately severe – a reduction in intelligence which makes independent living impossible.
5	Over 75	Severe – needs constant supervision and care.

20.11 Thinking

The ability to form thoughts and conceptualise. Impairment is both a matter of degree and type of disturbance, which may involve stream, form and content.

20.11.1 Guides for the Rating of Impairment of Thinking

Class	Impairment (%)	Description
1	0–5	Normal to slight – includes mild transient disturbances that are not disruptive and are not noticed by others.
2	10–20	Mild – mild symptoms that usually cause subjective distress, for example: thinking may be muddled or slow; may be unable to think clearly; mild disruption of the stream of thought due to some forgetfulness or diminished concentration; may have some obsessional thinking which is mildly disruptive; may be preoccupied with distressing fears, worries or experiences, and by inability to stop ruminating; an increased sense of self-awareness or a persistent sense of guilt; some other thought disorder that is minimally disruptive, such as overvalued ideas

(continued)

(continued)

Class	Impairment (%)	Description
		or delusions; some formal thought disorder that does not interfere with effective communication.
3	25–50	Moderate – manifestations of thought disorder, to the extent that most clinicians would consider psychiatric treatment indicated, for example: severe problems with concentration due to intrusive thoughts or obsessional ruminations; marked disruption of the stream of thought due to significant memory problems or diminished concentration; persistent delusional ideas interfering with capacity to cope with everyday activities, e.g., severe pathological guilt; formal thought disorder that interferes with verbal and other forms of communication.
4	55–75	Moderately severe – disorders of thinking that cause difficulty in functioning independently and usually require some external assistance.
5	Over 75	Severe – disorders of thinking that cause such a severe disturbance that independent living is impossible.

20.12 Perception

The individual's interpretation of internal and external experience received through the senses. Stimuli arise from the five senses—the form is relevant, not necessarily the content (refer to discussion above of the concept of perception in clinical psychiatry).

20.12.1 Definitions

Hallucinations. Abnormalities of sensory perception in the absence of external stimuli.

Illusions. Distortions of real sensory stimuli—illusions can be a normal phenomenon as well as indicating psychopathology.

Pseudohallucinations. Hallucinations that are recognised by the person as being imaginary (not real, lacking an external source or stimulus).

20.12.2 Guides for the Rating of Impairment of Perception

Class	Impairment (%)	Description
1	0–5	Normal to slight – transient heightened, dulled or blunted perceptions of the internal and external world, but with no or little interference with function.
2	10–20	Mild – persistent heightened, dulled or blunted perceptions of the internal and external world, with mild but noticeable interference with function; – pseudohallucinations.
3	25–50	Moderate – presence of hallucinations (other than hypnagogic or hypnopompic) that cannot be attributed to a transitory drug-induced state; – obvious illusions (when associated with a diagnosable mental disorder).
4	55–75	Moderately severe – hallucinations and/or illusions (as above) cause subjective distress and disturbed behaviour.
5	Over 75	Severe – hallucinations and/or illusions (as above) cause disturbed behaviour to the extent that constant supervision is required.

20.13 Judgement

Ability to evaluate and assess information and situations, together with the ability to formulate appropriate conclusions and decisions. This mental function may be impaired due to brain injury, or to conditions such as schizophrenia, major depression, anxiety, dissociative states or other mental disorders.

20.13.1 Guides for the Rating of Impairment of Judgement

Class	Impairment (%)	Description
1	0–5	Normal to slight – may lack some insight and misconstrue situations but with little interference with function.
2	10–20	Mild – persistently misjudges situations in relationships, occupational settings, driving and with finances. The misjudgements are noticed by others but are accommodated.

(continued)

(continued)

Class	Impairment (%)	Description
3	25–50	Moderate – misjudging social, work and family situations repeatedly leading to some disruption in relationships, occupational settings, living circumstances and financial reliability; – inappropriate spending of money or gambling.
4	55–75	Moderately severe – moderately severe misjudgement with regular failure to evaluate situations or implications, causing actual risk or harm to self or others; – failure to respond to any regular guidance and requirement for constant supervision.
5	Over 75	Severe – persistently assaultive due to misinterpretation of the behaviour or motives of others; – sexually disinhibited (may occur following a head injury).

20.14 Mood

Mood is a pervasive lasting emotional state. Affect is the prevailing and conscious emotional feeling during the period of the mental state examination. Affect observed during the mental state examination is a reflection of the subject's mood, and has a number of features, including:

– *Range*—Variability of emotional expression over a period of time, i.e., if only one mood is expressed over a period of time, the affective range is restricted.
– *Amplitude*—Amount of energy expended in expressing a mood, i.e., a mild amplitude of anger is manifested by annoyance and irritability.
– *Stability*—Slow shifts of mood are normal. Rapid shifts (affective lability) may be pathological.
– *Appropriateness*—The "fit" (or congruency) between the affect and the situation.
– *Quality of Affect*—Suspicious, sad, happy, anxious, angry, apathetic.
– *Relatedness*—Ability to express warmth, to interact emotionally and to establish rapport.

20.14.1 Guides for the Rating of Impairment of Mood

Class	Impairment (%)	Description
1	0–5	Normal to slight – relatively transient expressions of sadness, happiness, anxiety, anger and apathy; – normal variation of mood associated with upsetting life events.
2	10–20	Mild – mild symptoms: some or all of the below mild depression; subjective distress leading to some mild interference with function; reduced interest in usual activities; some days off; reduced social activities; fleeting suicidal thoughts; some panic attacks; heightened mood; – may experience feelings of derealisation or depersonalisation.
3	25–50	Moderate impairment – moderate symptoms: some or all of the below frequent anxiety attacks with somatic concomitants; inappropriate self-blame and/or guilt; persistent suicidal ideation or suicide attempts; marked lability of affect; significant lethargy; social withdrawal leading to major problems in interpersonal relationships; anhedonia; appetite disturbance with significant weight change; psychomotor retardation/agitation; hypomania; – severe depersonalisation.
4	55–75	Moderately severe – cannot function in most areas constant agitation; violent manic excitement; repeated suicide attempts; remains in bed all day; extreme self neglect; extreme anger/hypersensitivity; requires supervision to prevent injury to self or others.
5	Over 75	Severe – severe depression, with regression requiring attention and assistance in all aspects of self care; – constantly suicidal; – manic excitement requiring restraint.

20.15 Behaviour

Behaviour is one's manner of acting. It is considered with regard to its appropriateness in the overall situation. Disturbances vary in kind and degree. Behaviour may be destructive either to self and/or others, it may lead to withdrawal and isolation. Behaviour may be odd or eccentric. Particular mental disorders may be manifested by particular forms of behaviour, e.g., compulsive rituals associated with Obsessive Compulsive Disorder.

20.15.1 Guides for the Rating of Impairment of Behaviour

Class	Impairment (%)	Description
1	0–5	Normal to slight – transient disturbances in behaviour that are understandable in the context of this person's situation, excessive fatigue, intoxication, family or work disruption.
2	10–20	Mild – persons who generally function well, but regularly manifest disturbed behaviour under little extra pressure that nevertheless is able to be accommodated by others; – persistent behaviour that has some adverse effect on relationships or employment.
3	25–50	Moderate – occasional aggressive, disruptive or withdrawn behaviour requiring attention or treatment; – obsessional rituals interfering with but not preventing goal-directed activity; – repeated antisocial behaviour leading to conflict with authority.
4	55–75	Moderately severe – persistently aggressive, disruptive or withdrawn behaviour requiring attention or treatment; – behaviour significantly influenced by delusions or hallucinations; – behaviour associated with risk of self harm outside the hospital setting, but not requiring constant supervision; – manic overactivity associated with inappropriate behaviour; – sign regressed behaviour, e.g., extreme neglect of hygiene, inability to attend to own bodily needs.
5	Over 75	Severe – requiring constant supervision to prevent harming self or others (repeated suicide attempts, frequently violent, manic excitement); – catatonic excitement or rigidity; – incessant rituals or compulsive behaviour preventing goal-directed activity.

Table 20.1 sets out the psychiatric impairment rating scheme in the GEPIC.

20.16 Guidelines for the Assessment of Psychiatric Damage in Northern Ireland

The current fourth edition of the *Guidelines for the Assessment of General Damages in Personal Injury Cases in Northern Ireland* [44], published in March 2013 (hereinafter GAPICNI) illustrates an approach to the assessment of the quantum of damages in cases involving psychiatric injury that does not involve psychiatric impairment rating by a clinician but rather relies on general factors; it also differentiates between psychiatric damage attributed to the development of Post-traumatic stress disorder from that due to other psychiatric conditions. The GAPICNI also prescribes a "range" of pecuniary awards based on the extent of the "psychiatric damage".

These *Guidelines* state that "The factors to be taken into account in valuing claims for psychiatric damage include the following:

(i) Ability to cope with life and particularly work;
(ii) Effect on relationships with family etc.;
(iii) Extent to which treatment would be successful;
(iv) Future vulnerability;
(v) Prognosis;
(vi) The extent and/or nature of any associated physical injuries;
(vii) Whether medical help has been sought.

For "Psychiatric Damage Generally" there are four degrees of severity, namely "Severe psychiatric damage" (range of pecuniary award £70,000–£175,000), "Moderately severe psychiatric damage" (£40,000–£100,000), "Moderate psychiatric damage" (£10,000–£40,000), and "Minor psychiatric damage" (to £10,500).

Plaintiffs who claim compensation for Post-traumatic stress disorder (PTSD) are generally awarded lower damages. The GAPICNI notes in relation to this condition that "An increasingly large number of cases deal with a specific reactive psychiatric disorder in which characteristic symptoms are displayed following a psychologically distressing event outside the range of human experience which would be markedly distressing to almost everyone. Such symptoms would affect the basic functions such as breathing, pulse rate and bowel and/or bladder control. They would also involve persistent re-living of the relevant event, difficulty in controlling temper, in concentrating and in sleeping, and exaggerated startled [*sic*] response. There may be exceptional cases where consequences are so severe they equate more with the type of damage envisaged in para. A above".

Without further comment on this somewhat bizarre characterisation of the clinical symptoms of Post-traumatic stress disorder and the confused terminology that referred to "symptoms" that "are displayed" (the Acknowledgment indicates that the GAPICNI was prepared by lawyers apparently without any medical input), it is worth noting that plaintiffs with "Severe PTSD" are to be awarded

₺50.000–₺100,000, those with “Moderately severe PTSD” would receive ₺35,000–₺70,000, those with “Moderate PTSD” ₺10,000–₺40,000, and that “Minor PTSD” (where it is stated “In these cases a virtually full recovery will have been made within one to two years and only minor effects will persist over any longer period”) would receive damages of ₺3500–₺10.000.

20.17 Pain-Related Impairment

The prevailing view that pain is not an impairment was challenged by the inclusion in the fourth edition of the A.M.A. *Guides*, published in 1993, of a chapter titled “Pain” that included—for the first time since the *Guides* were published—a proposed method for rating impairment attributed to pain per se. It was argued—somewhat confusingly in our view—that because

> the Guides interprets [*sic*] the definition of impairment to involve also interfering with the individual’s performance of daily activities. In this broader context, impairment is at the level of the individual, is based on an illness model, and is viewed as being dependent on personal needs and the demands of the external milieu. In this context, pain may be viewed as an impairment that should be assessed according to the individual’s residual functional capacity. Chronic pain and pain-related behavior are not per se, impairments, but they should trigger assessments with regard to ability to function and carry out daily activities.

Until the publication of the fourth edition of the A.M.A. *Guides* it had been considered that in assessing, say, an impairment associated with joint pathology, limitation of range of joint movement due to movement-related pain is adequately taken into account in the impairment rating and that the additional or separate rating of the joint pain is not required. This consideration was implicitly recognised in the paragraph quoted above.

The fourth edition of the A.M.A. *Guides* also recognised that:

> Pain is a subjective perception. Usually no exact relationships exist among the degree of pain, extent of pathologic change, and extent of impairment.
>
> Decreased ability to carry out daily activities may be one result of pain-related impairment. This decreased ability is *not* merely a function of verbal behavior. An individual who complains of constant pain but who has no objectively validated limitations in daily activities has *no* impairment.

It was suggested that the “proper test” for pain-related impairment was “not ‘Does this daily activity cause pain?’ but rather ‘Can the patient perform this daily activity?’”.

A “Pain Intensity-frequency Grid” was provided in this chapter but there was no guidance as to the actual method for the numerical rating of pain-related impairment.

The list of references appended to the chapter included several articles about “pain-related disability” and one dealing with chronic pain and “functional impairment" but there were no studies cited that referred to pain per se as an impairment in its own right and not as a factor contributing to physical impairment rated in accordance with other chapters of the A.M.A. *Guides*.

The response to the inclusion of this chapter in the fourth edition of the A.M.A. *Guides* was that many jurisdictions that adopted the fourth edition legislatively "excised" this chapter to ensure that it did not form part of the impairment-rating schemes used in the respective workers' compensation, motor vehicle accident and other jurisdictions in which compensation for personal injury based on impairment could be awarded. The rationale for doing so was that the concept of impairment—as discussed above—was that "impairment is any loss or abnormality of psychological, physiological, or anatomical structure or function" that can be observed, evaluated and rated by an observer. As acknowledged by the *Guides*, "pain is a subjective perception" and therefore cannot be rated "objectively" and independently of the description of the subjective experience.

For example, in Victoria, Australia, when the Accident Compensation Act 1985 (Vic) was amended to make the fourth edition of the A.M.A. *Guides* the "prescribed edition" for the purpose of impairment assessment, Chap. 15—"Pain"—was excluded (as was Chap. 14, dealing with impairment due to Mental and Behavioral Disorders, as discussed above).

Similarly, Chap. 18 ("Pain") of the fifth edition of the A.M.A. *Guides* was excluded in those Australian jurisdictions that have adopted the fifth edition for rating of impairment for the purpose of determining the quantum of damages in workers' compensation, motor vehicle accident and other civil liability claims.

The *Guidelines for Evaluation of Permanent Impairment* published by the Queensland Workers' Compensation Scheme, which are based on the fifth edition of the A.M.A. *Guides*, specifically provide that "the AMA5 Chapter devoted to assessment of chronic pain is to be disregarded for the purpose of this Guide".

The reasons for "disregarding" Chap. 18 ("Pain") of the fifth edition of the A.M.A. *Guides* set out in the Queensland *Guidelines* are:

- the Chapter does not contain validated instruments that convert the rating given by an examiner into a whole body impairment rating;
- no work has been done at this time to enable such conversion to occur;
- measuring impairment for this condition is complex and requires a high degree of specialised knowledge and experience. This level of knowledge and experience is not widespread and it would be difficult to ensure consistency and equity in the assessment process.

Perhaps even more importantly, it was also stated:

> As with all largely subjective complaints in compensation systems, there is a concern that monetary compensation for non-specific conditions such as chronic pain can in some cases complicate the restorative and rehabilitative efforts of the worker and his or her health advisers. Hence the need for further investigation to determine a better and fairer system that recognises the difficulties associated with these conditions while, at the same time, promoting effective rehabilitation.

Similar concerns were in fact expressed by the authors of Chap. 18 in an article that discussed the confusion between the concepts of impairment and disability inherent in the assessment of pain [45].

In response to what were considered “many ambiguous points” related to assessment of pain-related impairment in the fifth edition of the A.M.A. *Guides*, an attempt has been made in Korea to develop a guideline to rate impairment due to pain based on complex regional pain syndrome [46].

Earlier attempts had also been made to develop impairment ratings for pain due to specific conditions, such as posttraumatic headache [47], but these have not been replicated and appear not to have been adopted by other clinicians and we are not aware that they have been introduced in any jurisdiction.

20.18 Summary

According to Biklen [48], the exercise of clinical judgement in the rating of impairment and disability is influenced by many factors. Among such factors, of which the rater may be unaware, are service traditions, economics, bureaucratic exigency, politics, and societal prejudice. Indeed, Biklen considers these factors to be so pervasive that he describes reliance on clinical judgement to be “little more than a mythology”.

In view of the statutory requirements noted above, which specify that medical ratings of impairment are mandatory to determine certain specified entitlements, there is a clear need for a wider appreciation both of the different, and specifically defined, concepts of impairment and disability, and also of the method of impairment rating if this has been stated in the relevant legislation.

The difficulties in the rating of psychiatric impairment and disability were discussed by Heiman and Shanfield [49], who noted the tendency of psychiatric evaluation to “legitimize subjective distress” that might be overemphasised during the process of psychiatric evaluation. In the absence of objective, agreed upon, impairment rating criteria this at times causes personal values to influence the psychiatric assessment.

It is to avoid the likelihood of such personal and idiosyncratic factors influencing the rating of psychiatric impairment that detailed and specific evaluation methods are required. *The Guide to the Evaluation of Psychiatric Impairment for Clinicians*, now in use in Victoria and in the workers’ compensation jurisdiction in South Australia [50] provides the most detailed method for the evaluation of psychiatric impairment among personal injury litigants and claimants for compensation benefits.

The six aspects of mental functioning specified in this particular rating method are assessed during the mental status examination, and can be readily described in the context of a forensic psychiatric report and testimony.

As described above, different methods of assessing psychiatric impairment are in use throughout Australia. Among the 22 jurisdictions that have been surveyed, only four utilise rating methods that truly evaluate psychiatric impairment: these are the workers' compensation, transport accident and personal injury jurisdictions in Victoria, and the workers' compensation jurisdiction in South Australia, which all mandate the use of the GEPIC. The four Commonwealth jurisdictions, as well as the workers' compensation jurisdictions in New South Wales, Queensland, Tasmania, Western Australia and the Northern Territory, and the motor accident jurisdiction in New South Wales, all utilise methods that rate disability rather than psychiatric impairment, although the relevant statutes refer (inaccurately) to the rating of impairment. The remaining motor accident and workers' compensation jurisdictions do not use any legislatively prescribed method of rating psychiatric impairment.

Here psychiatric impairment is assessed using the third, fourth, fifth or sixth editions of the American Medical Association's *Guides*, which do not provide a percentage rating of impairment, or guidelines based on these editions. It is a requirement that—for the purpose of calculating the amount of damages to be awarded—a percentage rating be given. However, as was made clear by Cocchiarella [51]—who described herself as "a user, an author, and an editor of the AMA *Guides* 5th"—in a discussion of the fifth edition of the A.M.A. *Guides*, which uses the same methodology for rating psychiatric impairment as the third and fourth edition, that is incorrect. In the example given in her article, of a patient with depression, it is stated that "[T]he Guides 5th and 4th do not provide impairment ratings for impairments due to a psychiatric origin". Instead, there is a comment about the impact of depression on activities of daily living, social functioning, concentration and adaptation. It is concluded that the limitations in these areas "would preclude his performance in work, unless special support or assistance was available". It is clear from this discussion by the editor of the fifth edition of the A. M.A. *Guides* that the methodology used to rate psychiatric "impairment" in the third, fourth and fifth editions fails to do what it purports to do, and similarly guidelines such PIRS, in use in New South Wales, Queensland and Tasmania, based on that methodology, do not rate psychiatric impairment.

The most objective methods of rating impairment are those that are based on an assessment of abnormality or loss of anatomical, physiological or psychological structure or function, including the basic aspects of mental function, as defined by the WHO. Indirect methods that purport to evaluate impairment but in reality assess disability, such as the PIRS, are influenced by the various factors that impact on the examiner as well as those that affect the subject. Factors that influence the examiner were noted by Biklen and have been listed above—they include service traditions, economics, bureaucratic exigency, politics, and societal prejudice.

Factors that influence the subject's level of function and disability include motivation, as well as numerous other factors related to premorbid personality, demographics, interpersonal dynamics, cultural and occupational factors, as well as societal and economic factors. It is because these various factors impact on the various aspects of functioning that is assessed by rating scales such as PIRS that it is not an appropriate instrument to assess psychiatric impairment. As emphasized above, and made clear in the WHO definition, the rating of impairment can only take into consideration that which can be directly observed by the examiner and which represents a departure from normal anatomical, physiological or psychological structure or function.

There are now several jurisdictions in Australia that use the so-called "Psychiatric Impairment Rating Scale", but this scale has been modified to suit every one of the individual jurisdiction. Thus, not only does the PIRS fail to assess psychiatric impairment—despite its title—but it also lacks integrity, with each jurisdiction making changes to suit its own political agenda. Thus, there are different versions of PIRS in use in Australia in different jurisdictions.

Although the introduction to Chap. 14 of the fourth edition of the American Medical Association's *Guides to the Evaluation of Permanent Impairment*, dealing with mental and behavioural disorders, on which the PIRS is based, specifically states that percentage ratings should not be given to assessments using the methodology set out in that chapter, both NSW and Queensland have assigned percentage ratings to the PIRS under the relevant legislation. No percentages are used in Tasmania, where the WorkCover *Guidelines* use the NSW MAA *Guidelines* version of the PIRS but without the percentage rating table.

As we indicated above, the chapters in the various editions of the A.M.A. *Guides* dealing with impairment attributed to pain are similarly unsatisfactory, and many jurisdictions that have adopted the fourth or subsequent editions have "excised" that chapter by statute. Attempts have been made to develop methods to rate pain-related impairment due to specific pain syndromes but such attempts have not met with success and have not been adopted in any legislative scheme.

The adoption of uniform methodology for the assessment of psychiatric impairment in the various jurisdictions, based on a rating instrument that truly assesses impairment rather than disability, would be an important step in eliminating the haphazard ways in which entitlements to damages for non-pecuniary loss, as well as other statutory entitlements, are currently assessed, and it would make a significant contribution to the establishment of a more equitable approach to the awarding of benefits and compensation for those who had suffered a personal injury through no fault of their own.

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Chapter 21
Detection of Malingering in Psychic Damage Ascertainment

Giuseppe Sartori, Andrea Zangrossi, Graziella Orrù and Merylin Monaro

Abstract Malingering is the intentional feigning or exaggeration of physical or psychological symptoms. Since the beginning of 1900 malingering detection has been one of the main challenges in medico-legal practice and in particular in psychiatric and cognitive assessment, as behavioral symptoms are very easy to produce, so that the need for specific tools and strategies for malingering detection is crucial. Although several tools and strategies are available, conclusions are often derived from mere subjective impressions and in many cases they lead to misclassifications. Here we present a non-exhaustive review of strategies for the detection of malingering, starting from the logic underlying a qualitative analysis of symptoms, to validated tools specifically designed to detect attempts at simulating or exaggerating psychopathological, psychiatric or cognitive diseases. Finally, we describe two recent approaches to the malingering detection problem. These approaches are grounded on the analysis of the reaction-times and on the dynamic analysis of kinematic features of mouse trajectories while an examinee is answering to double-choice questions.

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21.1 Introduction

One of the most important challenges in medico-legal practice is represented by the evaluation of malingering. Since the early 1900s, indeed, the need of specific tools and strategies to detect malingering has been present. Malingering refers to an intentional production of false or exaggerated physical or psychological symptoms, and occurs when external incentives are available. Given that behavioral symptoms are very easy to produce, the detection of malingered symptoms is particularly challenging in psychiatric, psychopathological and neuropsychological assessment. The incidence of malingering in a medico-legal setting has been estimated at around twenty-nine percent of personal injury cases [1]. Some studies [2] have suggested that 15–64% (average 40%) of mild head injury litigants produced malingered symptoms. However, given the hidden nature of deception, a precise estimate is not reachable. A realistic base-rate could be an incidence of roughly 30–40% in head injury litigations. According to some authors, this data seems higher in the criminal forensic field, in which an incidence of 54% is signaled [3].

In psychological and neuropsychological assessment, an appropriate effort is determinant for the evaluation of performance in cognitive testing. Indeed, half of the variance on neuropsychological test performance seems explained by effort, while roughly 10% by education and, globally, around 10% by age and brain injury severity [4]. Therefore, malingerers displaying cognitive symptoms often tend to offer sub-optimal effort in order to achieve a poor performance in cognitive testing. In other domains, instead, malingerers can try to feign symptoms, such as those regarding psychiatric diseases.

A number of strategies are now available for the detection of malingering, but it nevertheless remains a challenging issue for examiners.

In most cases conclusions are indeed simply derived from subjective impressions, leading to a high misclassification-rate [5]. We can identify three classes of malingering detection strategies. The first class refers to "logical reasoning" and consists in applying clinical and epidemiological rules to the forensic context. Strategies belonging to this class are based, for example, on the detection of discrepancies in the examinee's psychometric profile as well as on the qualitative analysis of reported symptoms, considering their incidence in the psychiatric population. The second class refers to the use of neuropsychological tests specifically built under the *Symptom Validity Testing* (*SVT*) perspective or the *Floor Effect strategy*. In brief, the former refers to forced-choice tests in which answering by chance can lead to 50% accuracy. So that, if an examinee's performance is not significantly above chance, this may indicate a malingering attempt. The latter refers to tests for which the mean score obtained by the validation sample is the maximum score, so an examinee who does not obtain the top score may be a malingerer. The third class refers to the use of computerized tasks in order to implicitly detect psychometric signs of deception in malingering. Tools belonging to this class consider, for example, the analysis of reaction-times (e.g., the *autobiographical Implicit Association Test*, aIAT; [6]) to determine whether the subject under scrutiny is simulating the disorder or the symptoms declared are genuine. Finally, this class includes some fresh techniques, which are still under investigation, exploiting new kinds of technologies. One promising technique consists in analyzing the kinematic response of the examinee to double-choice questions concerning the declared symptoms. The kinematic analysis of the mouse movement that the subject performs to reach the desired response seems to provide a good implicit index of deception. This methodology and other techniques of brain reading, combined with innovative systems of machine learning, may be a turning point to the challenge of malingering detection.

In what follows we present a non-exhaustive review of strategies and tests belonging to the above cited classes, focusing in particular on the most recently developed tools.

21.2 Traditional Approaches to Malingering Detection

The first two classes presented above can be considered as the traditional approach to the detection of malingering.

Several qualitative analysis strategies might be adopted for this purpose. Here we present the three most common strategies which have been adopted in clinical and forensic practice.

One of these is the *qualitative analysis of symptom characteristics*, which aims at identifying some typical features of malingering which can be easily noticed. The majority of them depend upon stereotypic representations of mental diseases. For example, "negative" symptoms such as catatonic behavior or inappropriate affect are rarely feigned, while malingerers more often feign delusions or hallucinations [7]. Moreover, malingerers often tend to report a huge number of symptoms, as they believe that reporting more symptoms will increase the probability of being identified as affected by a genuine mental disorder.

Another common strategy is the *discrepancy method*, which consists in evaluating the plausibility of the reported symptoms profile if compared to the typical findings expected with a claimed disease [8].

Furthermore, an important strategy is grounded in the analysis of *rare symptoms,* and aims at identifying the examinee's reported symptoms that are infrequently seen in the clinical population. Malingerers, for example, may report a wide range of symptoms, trying to feign a kind of unspecific psychopathology [9], given that they do not have in mind a clear representation of the pattern of symptoms typically associated with a specific disease. They may therefore display "obvious" symptoms, that is, symptoms which are usually associated with mental illness (e.g., hallucinations) even if they are not plausible for the disease they are trying to feign.

Together with these briefly summarized strategies, several conventional psychometric tests have been investigated for their sensitivity in detecting malingering (e.g., suboptimal effort, see e.g., [10, 11]). Indeed, a number of tests adopted as supporting tools for psychiatric or psychopathological diagnosis include strategies for the identification of malingering. The *Minnesota Multiphasic Personality Inventory-2* (MMPI-2; [12]), one of the most common tools in psychopathology, for example, is equipped with specific scales whose scores can be interpreted as indicating the presence of malingering attempts. In particular the F scales (F, Fb, Fp) and the consistency scales (VRIN and TRIN) are useful for this purpose. The *Structured Interview of Reported Symptoms* (SIRS; [13]) is a tool designed to assess a wide range of psychopathological symptoms and it allows the critical analysis of the examinee's answering style, classifying it as indicating "honest responding", "indeterminate", "probable feigning" and "definite feigning". A tool specifically designed for detecting psychiatric (as well as cognitive) symptoms displayed by malingerers is the *Structured Inventory of Malingered Symptomatology* (SIMS; [14]), a self-administered questionnaire consisting of dichotomous items (i.e., true–false) whose underlying logic is that in a "normal" psychiatric profile only a small amount of highly atypical symptoms should be detected. Other useful tools are the *Personality Assessment Inventory* (PAI; [15]), in particular its scale, the *Negative Impression Management scale* (NIM; [15]), which can be used to identify exaggerations in the reporting of psychopathological symptoms, and the *M-Test* [16], which has been specifically developed to detect malingering of schizophrenic symptoms.

Furthermore, several tests can be included in neuropsychological assessment to detect malingering of cognitive symptoms. Although any cognitive symptoms can be potentially malingered in order to obtain advantages, tests belonging to this category are mainly focused on the detection of feigned memory impairment, as this kind of deficit is the one most frequently feigned. One of the most useful and commonly used tests in clinical and forensic practice is the *Test of Memory Malingering* (TOMM; [17]), a forced-choice recognition test composed of three-trials (although the third is considered optional), of 50 items each, designed to discriminate between true memory-impaired patients and malingerers. This test is grounded in the aforementioned *Symptom Validity Testing* (SVT) as well as on the so-called *floor-effect*. So that a cut-off score near to maximum (corresponding to 90% of correct responding) revealed in several studies a classification accuracy of 95% of all non-demented patients as not malingering [17, 18]. Another SVT test is the *Word Memory Test* (WMT; [19]), a memory test administered through a computerized task in which first a word list is presented twice, then pairs of words are presented and subjects are later asked to recall as many of the word pairs as possible. Under the same perspective, another commonly used test is the *Computerized Assessment of Response Bias* (CARB; [20]), which is essentially a digit recognition task.

Another useful screening test is the *Dot Counting Test* (DCT; [21, 22]), in which subjects are presented with a sequence of cards on which series of dots are printed. The task consists in counting the number of dots as quickly as possible. For non-malingering patients it is expected that the time for counting proportionally increases when dots are presented in an ungrouped way, rather than when they are grouped. Finally, a very simple screening measure for the detection of malingered memory impairment is the *Rey15-Item Visual Memory Test* (MFIT; [23]), which consists of a grid with 15 items arranged in three rows and five columns. The examinee should look at the grid for 10 s and then try to reproduce the greater number of elements he can recollect. Traditionally, if less than nine items are recalled a malingering attempt is probable (different studies indicate different cut-offs, so this test can be used as a qualitative source of information).

All of the previously presented tools are tests for which validation studies have been published, thus satisfying the *Daubert* criteria which have to be considered in evaluating the admissibility of these instruments in Court as scientific evidence.

21.3 Recent Approaches in Malingering Detection

In the previous section we described techniques aimed mainly at detecting the examinee's malingering "style", although the plaintiffs in some cases may over-report very specific symptoms (e.g. shoulder pain, amnesia for the crime, whiplash, etc.). In these cases some new emerging techniques may be useful, as they permit the testing of specific malingered information.

Recently, for example, reaction-times (RTs) based techniques have been introduced. Reaction time-based techniques detect longer latencies and higher error rate, which are associated to the additional cognitive processes involved in lying compared to truth telling [24]. These methods are based on the assumption that a more cognitive demanding task would also be more time-consuming [25]. Techniques belonging to this category are the RT-based version of the Concealed Information Test [26] or the autobiographical Implicit Association Test (aIAT; [6]).

The aIAT is a novel variant of the Implicit Association Test [27] which allows us to verify whether an autobiographical memory is encoded within the mind of the respondent. The aIAT is able to evaluate which one of two autobiographical events is true and, consequently, to detect the fake event [6]. It has been tested for different purposes and on different constructs. In fact, validation studies of the aIAT have been carried out on over 500 subjects, on an array of conditions (see [28] for a review). The validity of the technique has been confirmed in a number of different labs and in different languages [29]. The validation studies, both in forensic and clinical settings, confirmed that the aIAT is a precise tool for detecting which of two autobiographical memories is true, with an accuracy rate of around 91% [6]. In particular, regarding the detection of malingering in cases of compensation for damages, evidence-based investigations have demonstrated that aIAT can efficiently detect faked whiplash symptoms [30] and faked depression. Moreover, phantom limb pain and psychogenic amnesia have been assessed in some single case studies.

Specifically, the aIAT refers to a computer-based categorization task and includes stimuli belonging to four categories: two of them are logical categories represented by sentences that are certainly true (e.g. 'I am in front of a computer') or certainly false (e.g. 'I am climbing a mountain') for the respondent. The other two categories represent alternative versions of the construct under investigation (e.g. 'I went to Paris for Christmas' versus 'I went to London for Christmas'), only one of the two being true. The aIAT is structured in five classification blocks: three simple categorization blocks (1, 2, 4), and two combined categorization blocks (3 and 5). In simple blocks, each response button is used to classify sentences related to only one category. In double blocks, each response button is used to classify sentences related to two different categories. In Block 1, participants have to classify true and false sentences (e.g., I am in front of a computer vs. I am in front of a television) using two response keys, one on the left and one on the right of the keyboard. In Block 2, participants have to classify autobiographical sentences (e.g., I went to Paris for Christmas vs. I went to New York for Christmas) with the same two response keys. In Block 3 (double categorization block), true sentences and sentences related to the first autobiographical event (e.g., Christmas in Paris) are paired on the same response key and false sentences and sentences related to the second autobiographical event (e.g., Christmas in New York) are classified with the other response key. In Block 4, only autobiographical events are reverse classified with the two response keys. Finally, in Block 5, participants have to classify both true sentences and sentences related to the second autobiographical event (Christmas in New York) with the same response key, and false sentences and the first autobiographical event (Christmas in Paris) with the other key.

The logic aIAT is grounded on basically follows the same principle of the original IAT: when two sentences, which are both true, share the same motor response, the subject shows faster reaction times in comparison to the situation in which two incongruent sentences (a true sentence and a false sentence) share the same key of response. In other words, pairing congruent sentences, such as a truthful autobiographical event with a certainly true sentence, should facilitate faster responses. Therefore, the specific pattern of RTs for the double categorization blocks indicates which autobiographical event is either true or false.

However, RTs-based techniques are not without limitations and absolutely resistant to countermeasures. In fact, they only studied the latency in the response, so the liar only has to check this unique parameter to falsify the evidence.

21.4 Innovations in Malingering Detection

In recent years, computer science and new technologies are increasingly applied in all fields, both to solve outstanding issues and to improve current methods. In particular, in forensic psychiatry, a progressive fusion is now coming between the knowledge derived from neuroscience and the tools provided by computer science and bioengineering. The ultimate goal will probably be that of establishing a computerized system which, relying on objective elements, will be able "to label" patients providing a certain diagnosis. In a similar way, as regards the ascertainment of somatic and psychiatric damage, the ideal situation would be that of an algorithm able to identify, based on collected objectivity, the malingering or the authenticity of the symptoms declared by the patient. Although today we are far from the fulfilment of this scenario, some techniques and methods that may be applied in the forensic stage with excellent results are already available. Currently, one of the most promising fields, which falls within the area of artificial intelligence, is machine learning [31]. Machine learning is a discipline closely related to computational statistics that exploits one or more mathematical algorithms to categorize elements into different classes. It deals with the creation of systems and algorithms that are based on real observations, such as, for example, the patients' data, to make some predictions on new data or to produce new knowledge. Learning takes place by capturing features of interest, which in this case are represented by objective indices of malingering, to analyse and evaluate the relationships between the observed variables and to establish automatic data classification rules. Currently machine learning is starting to be used for a wide range of purposes, for example on brain imaging data, an approach called "brain reading" or "mind reading" [32, 33]. Some pioneering studies have already shown that it is possible to identify a liar automatically by applying machine learning techniques to the mere kinematic analysis of the motor response to a double-choice task [34].

As well as RTs, which are considered reliable behavioral indices of deception [24, 26, 35], kinematic indices may provide a clue for recognizing deceits. Recently, researchers have measured hand movements during the choice tasks on a screen to understand the dynamics of a broad range of psychological processes [36]. They found that hand-motor tracking provide in real-time a good trace of mind processes underlying a task, including the cognitive processes involved in the production of a deception. Duran, Dale and McNamara [37] presented a novel and innovative study on the detection of deception by kinematic analysis. The authors compared motor trajectories while subjects were engaged in an instructed lie task. In the task participants were required to respond truthfully or not to the presented sentences, by means of a visual cue. The analysis of motor trajectories (the authors did not use the mouse for recording the response, but rather the Nintendo Wii Remote) led to interesting results. Deceptive responses could be distinguished from truthful ones using several parameters including motor onset time, the overall time required for responding, the trajectory of the movement and kinematic parameters, such as velocity and acceleration. Hibbeln et al. [38] studied the mouse movements in an insurance fraud online context. Their results suggest that being deceptive may increase the normalized distance of movement, may decrease the speed of movement, may increase the response time, and may result in more left clicks. Valacich et al. [39] proposed a pilot study to identify guilty individuals involved in specific insider threat activities. They analysed mouse movements while participants compiled an online survey similar to the CIT. Their preliminary observations showed that guilty insiders had a different pattern of continuous motion when answering the key-item as compared to the answering of non-key-items, which may be indicative of increased cognitive activity while deceiving. Finally, Monaro et al. [34] are reporting on a new method that allows the identification of false self-declared identity, based on indirect measures of the memories relating the affirmed personal details. This method exploits kinematic analysis of the mouse as implicit measure of deception, while the user is responding to personal information.

The authors analysed signatures of deception in terms of the shape of each movement trajectory and the location of the trajectory over time. They also quantified the trajectory properties on dimensions of velocity, stability, and direction. Results show that using mouse movement analysis, it is possible to reach a high rate of accuracy, around 95%, in detecting the veracity of self-declared identities.

According to this evidence, the kinematic analysis of the subject's response is a promising technique that may be applied to the forensic setting, both in cases where it is necessary to evaluate the truthfulness of the testimony and in insurance issues, where the malingering is not easy to identify.

To date, there are still no studies in the literature investigating malingering behaviour through the kinematic analysis of the responses provided by the patients about their symptoms. However, here we anticipate the results of some experiments that Monaro and Sartori are leading, which are investigating simulation of psychiatric disorders more involved in cases of compensation for damage, such as depression, anxiety and post-traumatic stress disorder. Preliminary analysis shows that, through machine learning techniques, it is possible to construct algorithms that provide good results in distinguishing between simulators and sincere people, considering both the score in response to the psychological tests and the kinematic indices of the mouse movement during the response act.

Compared to techniques based on the simple RT, pushing a key on keyboard, kinematic analysis of mouse movements show a great advantage. While button press may only permit the recording of RT, the use of a mouse allows us to capture the cognitive complexity in stimulus processing by means of the registration of a variety of indicators, not only the reaction time. For this reason, the technique is promising, also concerning resistance to countermeasures. The large number of characteristics of movement seem, in principle, difficult to control entirely via efficient countermeasures to lie detection. Moreover, this methodology may potentially achieve a high accuracy on the specific single symptom and not for a range of symptoms that compose the spectrum of a given pathology. In fact, one of the main challenges in the identification of malingering is not only that of deciding whether the patient has a particular disease or not, but also estimating the severity of the disease, identifying patients that exasperate symptoms (declaring more symptoms than actually experienced) or aggravating their intensity.

Finally, a huge advantage of both kinematic and RTs based techniques is that the collection of these indices is inexpensive and does not require any equipment in addition to that which the subject is already using during the interaction with the computer. Again, these indices are very well adapted to the detection of lies, also in the context of the web, and do not require any particular expertise of the examiner, making them fit to be applied in the screening phase.

21.5 Conclusions

Malingering detection is one of the most challenging issues in the assessment of an examinee's mental state. Psychiatric and cognitive symptoms are easy to exaggerate and feign, so that the use of appropriate strategies and tools to detect malingering attempts is crucial both in clinical and forensic context.

Successful malingering is linked to economic and societal consequences, such as increased insurance premiums and funds' assignment to undeserving people (and not to truly deserving patients). Moreover there are consequences on justice, as malingerers might be allowed to avoid prison or have reduction of their punishment.

Today we have several available tools that can be useful to detect malingerers. Although none of them can be considered without limitations and criticism-free, their combined use may increase examiner's ability to accurately detect malingering attempts. Furthermore, this "traditional" tools can be supported by the use of new methods, grounded on automatized psychometric index recording, such as reaction-times and kinematic aspects of mouse trajectories, which have showed promising abilities.

In conclusion, to accurately detect cases of malingering, examiners should consider multiple sources of independent data which must include valid, structured measurement techniques specifically designed for the detection of malingering attempts [40], eventually supported by new developing automatized and examiner-independent techniques.

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Part VI
Innovation, Unitariness and Evidence. *Clinical Legal and Forensic Medicine in Living Person* Malpractice and Medical Liability. Injury and Damage

Schultes, Johann 1595–1645 Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666

Schultes, Johann 1595–1645, Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666

Chapter 22
Current and Future Evidence in Medical Malpractice

Peter Vanezis and Stephanie Prior

Abstract An overview of the principal procedures and tools that are made available to one who intends to bring legal proceedings in order to seek recognition and compensation for damage from medical malpractice in the United Kingdom is set out. A close examination is performed of multiple aspects characterizing the professional responsibility of the physician within the Common Law System, outlining new prospects for the sharing of biomedical knowledge among healthcare professionals, also through the use of biomedical platforms, with the aim of ensuring adequate performance and the promotion of continuous professional improvement. Finally, the proposal of a predetermined damage assessment system that allows the healthcare structures to fulfill the contractual obligation in case of violation of the same is discussed. The preventive quantification of damages from malpractice contained within a certain threshold and the timely admission of liability would allow the reduction of procedural expenses and, simultaneously, the speeding up of compensation procedures.

22.1 Introduction

Medical malpractice or clinical negligence, as is the common term for claims relating to medical treatment in the United Kingdom, is a very specialist area of litigation. Essentially, if a person attends hospital or other centre providing healthcare, and receives substandard medical care which goes on to cause personal injury loss and damage, then a potential claim for clinical negligence can be investigated and pursued.

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The assessment of such a claim is complex and varies widely between different judicial systems. The overview presented here describes the situation in the United Kingdom and the current practice and future evidence in this area.

If a person receives treatment under the National Health Service (NHS) that they believe is of a poor standard such as a delay in diagnosis, poor communication from medical professionals, misleading or incorrect medical advice or just a general lack of treatment then the first step in regard to pursuing a claim for clinical negligence is to lodge a formal complaint with the NHS Trust who provided the medical care. This complaint must be lodged within 12 months of the NHS medical care or treatment and must set out all the details relating to the treatment. Quite often, a patient who has experienced poor medical care is after a full explanation as to why their treatment was substandard and what went wrong. They will also seek reassurance that it will not happen to anyone else and that there will not be a repeat of the mistakes made.

The NHS Complaints procedure covers any complaints regarding services provided by and paid for by the NHS Trust or primary care provider such as General Practitioners, Dentists, Opticians, Pharmacists and other NHS providers. The first stage of the complaints procedure is for the aggrieved patient to submit a formal letter in writing setting out the reason for the complaint and providing as much information as possible so that the treating members of staff can be identified and an investigation can then be commenced. A letter of acknowledgement is usually received within a few days with a formal response within 23–25 working days. Complaints can also be raised orally and if a patient has died then a complaint may be raised by another person on the deceased's behalf and also if the patient was a child or does not have capacity under the Mental Capacity Act, 2005, then another person may lodge a complaint on the child or incapacitated person's behalf.

Once the letter of response is received, the complainant may discontinue the complaint if satisfied with the response from the other party or if still dissatisfied may request a meeting with the complainant to discuss any outstanding issues. If a meeting does take place, minutes of that meeting will be taken and the complainant is entitled to ask for copies of the minutes.

If the patient still remains unhappy with the way in which the complaint has been dealt with they may contact the Parliamentary and Health Service Ombudsman www.ombudsman.org.uk. This is an independent body which is separate to the NHS complaints procedure and will carry out investigations into complaints made about the services provided under the NHS Trust including any private healthcare paid for by the NHS for patients in England, Wales (Public Service Ombudsmen for Wales) or Scotland (Scottish Public Services Ombudsmen). Sometimes the complainant will be interviewed by the ombudsman, but cannot investigate a complaint against an NHS Trust or body if the complainant has indicated a desire to commence legal proceedings. Once a complaint is referred to the ombudsman it can take time. Often a patient will have been through the NHS complaints procedure and this will already have taken many months. The ombudsman's investigation can also take a while and for the whole time the complaint is being investigated the deadline for pursuing a claim for clinical negligence is getting shorter. The time limit for pursing a claim for clinical negligence is 3 years from the date of the alleged negligent treatment or 3 years from the date that the patient became aware that he/she had suffered a significant injury as a consequence of negligence.

Further, the ombudsman can recommend financial recompense is made to cover financial expenses that the patient has incurred, but cannot award a sum of compensation.

The ombudsman publishes annual reports setting out the complaints that they have investigated and they name and shame the NHS Trusts and health authorities involved. They do not, however, name individual General Practitioners, but they do prepare special reports setting out particular issues that are of concern and advisors are encouraged to put forward to the ombudsman cases of general concern.

On 1 August, 2007, The Regulatory Reform Order 2007 (SI2007/1889) came into force and this amended a previous Act, namely the Parliamentary Commissioner Act 1967, also the Local Government Act 1974 and the Health Service Commissioners Act, 1993, and allows the Parliamentary Ombudsman and the Local Government Ombudsman and the Health Service Ombudsman for England to work together on cases where there are issues relating to more than one of the individual jurisdictions.

The relevant legislation governing the NHS complaints procedure is the Local Authority Social Services and National Health Services Complaints (England) Regulations 2009 (SI 2009/309 as amended by SI 2009/1768). The Regulations set out the various obligations on NHS bodies, General Practitioners and other primary care providers in relation to the NHS Complaints Procedure.

When deciding to pursue a complaint against the NHS Trust or a care provider, patients may contact the Citizens Advice Bureau (CAB), AvMA (Action for Victims of Medical Negligence) or the Patient advice and Liaison Service (PALS). PALS are usually based in the NHS Trust and many patients do not feel that they are independent as they are based within the hospital where they were treated. However, PALS will provide advice and assistance to patients in regard to the NHS Complaints procedure.

The NHS complaints procedure does not apply to care provided on a private basis, but it will cover any complaint about a member of staff employed in an NHS Trust hospital in the private paying wing of the hospital.

The NHS Redress Act 2006 is an Act that was to assist in the efficiency of the handling of clinical negligence complaints. In reality, it imposes a duty on those involved in the complaints procedure to promote resolution of the complaint. It also gives the Secretary of State power to establish a scheme of redress and the Department of Health hopes that all providers of hospital services will be required to be members of such a scheme. The National Health Service Litigation Authority is the main authority that handles all clinical negligence claims made against the NHS and the NHSLA determines whether to admit or deny liability for a claim on behalf of the NHS Trust.

Once the NHS complaints procedure has been exhausted or, in relation to a case relating to a private medical case, a complaint has been made and a response received, a Claimant can then consider seeking legal advice to pursue a claim in clinical negligence.

22.1.1 The Pre-action Protocol for the Resolution of Clinical Disputes

This Protocol came into force on 26 April, 1999, and its purpose was to encourage the parties to a potential claim for clinical negligence to share information and it provides for a timetable of steps that need to be taken to allow openness when something has gone wrong.

It sets out a "code of good practice" which should be followed by both parties and it applies to all services within the health service, including both private and NHS care.

There have been changes to the Protocol as a consequence of updates to the Civil Procedure Rules [1] and these changes were introduced on 1 October, 2010.

The first step of the Protocol is that a Claimant can apply for copies of their medical records held by the healthcare provider and a request for the records must be made in a specific format and a Hospital Protocol Authority must be completed in full and signed by the Claimant of his or her litigation friend if the Claimant is a child or does not have capacity. If the records being applied for relate to a person who has died, then the Personal Representative of the deceased can apply for disclosure of the records. The record holder must supply copies of the medical records, including all scans, medical reports, X-rays etc., within 40 days of the written request. The Data Protection Act 1998 governs disclosure of medical records relating to a person who is alive and stipulates that a maximum fee of £50 can be charged for disclosing copies of the medical records. There is no extra charge for x-rays or records from other departments within the hospital, such as physiotherapy or occupational therapy. The Access to Health Records Act 1990 governs disclosure of medical records of deceased patients and the maximum fee for providing such records under this Act is £10 plus photocopying and postage fees.

If the request for the medical records is not forthcoming, and the deadline of 40 days has passed, the Claimant is entitled to make an application to the court for Pre Action Disclosure of the records and the costs involved in making the application will have to be paid by the record holder.

The Civil Procedure Rules allow Claimants and healthcare providers to apply for copies of medical records from any other third party under Rule 31.17.

Once the medical records have been received the Claimant's lawyer will review the medical records and ensure that they are complete. If any records are missing these should be identified and copies requested from the healthcare provider. As soon as a complete set of the records is received they are then collated and paginated. The medical records are helpful, as they will allow the Claimant's lawyer to finalize the Claimant's initial witness statement and identify any potential areas of substandard medical treatment.

The medical records and any other relevant records, such as employment records, personnel records and school records will also be obtained.

Once the medical records and the Claimant's witness statement has been finalized, other witness statements may be taken from the Claimant's family or friends if they were present at the time of the alleged medical treatment and these can add further information to support the Claimant's case against the healthcare provider.

Thereafter, the above evidence is sent to an independent medical expert who is a specialist in the field of medicine or surgery relating to the potential claim for substandard care. If a Claimant is complaining about substandard treatment from a General Practitioner then the General Practitioners medical records and other hospital records, together with the Claimant's witness evidence, will be sent to an independent General Practitioner with expertise in medico-legal issues and is an approved Law Society medical expert. A full letter of instruction will be sent to the medical expert setting out the details of the potential claim and the issues that need to be addressed in relation to negligence.

22.2 Challenges for Evidence in Medical Malpractice and Principles of Clinical Negligence

22.2.1 Duty of Care

A doctor has a duty to a patient to provide reasonable care which should include a duty to investigate and initiate action in order to take all reasonable steps to attain the health of the patient. To establish negligence, the Claimant must ensure that there was an "act" or "omission" by the doctor that amounted to a breach of duty towards the Claimant.

The case of Donoghue –v- Stevenson [2] set out this duty. It was a House of Lords case and Lord MacMillan said:

> The law takes no cognizance of the carelessness in the abstract. It concerns itself with carelessness only where failure in that duty has caused damage. In such circumstances careless assumes the legal quality of negligence and entails the consequences in the law of negligence…. The Cardinal principle of liability is that the party complained of should owe to the party complaining a duty to take care, and that the party complaining should be able to prove that he has suffered damage in consequence of a breach of that duty.

The two parts to the test of breach of duty are the standard of care provided by the doctor to the Claimant and whether or not that care was substandard. The case of Bolam –v- Friern Hospital Management Committee [3] set out a useful summary of a legal definition of negligence. It is a case dating back to 1954 and concerned Mr. John Bolam, a man who had mental health problems. He was treated at the Defendant's hospital and underwent Electro-convulsive therapy and during the course of the therapy was injured as he was not restrained at the time of the treatment and fell and injured both of his hip joints and fractured both sides of his pelvis. He sued the Defendant hospital on the basis that he should have been restrained while undergoing treatment and it was negligent not to have done so. However, at this time some doctors were restraining patients during such treatment and some were not. McNair J stated:

> A man need not possess the highest expert skill at the risk of being found negligent. It is well established law that it is sufficient if he exercises the ordinary skill of an ordinary competent man exercising that particular art…. A doctor is not guilty of negligence if he has acted in accordance with a practice accepted as proper by a responsible body of medical men skilled in that particular art… Putting it the other way round, a doctor is not negligent, if he is acting in accordance with such a practice, merely because there is a body that takes a contrary view.

Many years later, in the case of Sidaway –v- Board of Governors of the Bethlem Royal Hospital and Maudsley Hospital [4] Lord Bridge held:

> The right practice accepted as proper by a responsible body of medical men must be 'rightly' so accepted.

Sidaway is no longer good law. It has been overturned by the Supreme Court, in the case of Montgomery –v- Lanarkshire Health Board [5]. The conclusion and analysis of the law in that case was "unsatisfactory". Montgomery is now seen as the leading case on issues relating to consent.

Mrs Montgomery was pregnant and was also diabetic. She came under the care of the Defendant's hospital, and due to the fact that she had diabetes, she was more likely than not to have a large baby. Therefore, the risk of shoulder dystocia was in the region of 9–10% the risk of catastrophic injury of less than 0.1%. She was not advised of the risks by the Defendant. During the delivery of her baby, shoulder dystocia occurred, leading to her baby being deprived of oxygen and being born with severe disabilities. The case was that she should have been advised of the risk of shoulder dystocia and that a caesarean section would have been a delivery option for her. Her arguments were rejected at first instance, but the Supreme Court overturned the decision in the case of Sidaway and "*that an adult person of sound mind was entitled to decide which, if any, of the available forms of treatment to undergo, and her consent had to be obtained before treatment interfering with her bodily integrity was undertaken. Doctors are under duty to take reasonable care to ensure that patients were aware of any material risks involved in any recommended treatment, and of any reasonable alternative or variant treatments*". It was accepted that, had she known the risks, the Defendants conceded, she would have opted for a caesarean section, and she succeeded on breach and causation.

22.2.2 The Level of Skill Required

The level of skill required of a doctor is related to the skills the doctor has. In other words, the more skills the doctor has, the higher the standard of skill that is expected of him/her. The case of Wilsher –v- Essex Health Authority [6] involved a junior doctor who carried out a procedure on a child incorrectly and this led to the child suffering a serious injury. Mustill LJ stated that:

> To my mind, this notion of a duty tailored to the actor rather than to the act which he elects to perform, has no place in the law of tort. Indeed, the defendants did not contend that it could be justified by any reported authority on the general law of tort. Instead, it was suggested that the medical profession is a special case. Public hospital medicine has always been organized so that young doctors and nurses learn on the job. If hospitals abstained from using inexperienced people, they could not staff their wards and theatres, and the junior staff could never learn. The longer term interests of patients as a whole are best served by maintaining the present system, even though this may diminish the legal rights of the individual patient for, after all, medicine is about curing not litigation. I acknowledge the appeal of this argument … Nevertheless, I cannot accept that there should be a special rule for doctors in public hospitals…. To my mind, it would be a false step to subordinate the legitimate expectation of the patient that he will receive from each person concerned with his care a degree of skill appropriate to the task which he undertakes to an understandable wish to minimize the psychological and financial pressures of the hard-pressed young doctors.

The test places the Claimant in the position that he/she must prove their case and it is not that the defendant can just absolve responsibility because their medical expert agrees with the defendant. The test of reasonableness is an objective test for the court to determine and the leading case in this is Bolitho –v- City and Hackney Health Authority [7], where a small boy was admitted to hospital with respiratory problems and during his admission suffered two short episodes of respiratory distress. On each occasion a doctor was called to attend to him but failed to attend, although after the first episode the child appeared to recover, but after the second episode he collapsed and suffered a cardiac arrest and brain damage. It was agreed that if he had been intubated after respiratory problems he would not have suffered the cardiac arrest. The defendant Health Authority accept that there had been a breach of duty, but even if the doctor had answered her bleep and attended to the child, she would not have intubated him and therefore there would have been no damage arising out of the breach. The Claimant argued that the failure to intubate was negligent and the defendants argued that the decision to intubate would have been in accordance with a body of responsible professionals.

This case extended the test in the Bolam case so that the defendant would have to show that the body of responsible, reasonable or respectable medical opinion for which they were relying, could be demonstrated to be such. In Lord Browne-Wilkinson's judgment he expresses the view that in most cases the expert will be able to satisfy the court that his opinion is a reasonable medical opinion, but in fact it does give the defendants the opportunity to fail where it can "*in rare cases be demonstrated that the professional opinion is not capable of withstanding* *logical analysis, the judge is entitled to hold that the body of opinion is not reasonable or responsible*".

22.2.3 The Legal Test for Establishing Clinical Negligence

To establish clinical negligence, the test that is applied is proof on the balance of probabilities and the practical application of this test reveals that the more serious the allegation of negligence then the stronger must be the evidence.

It should also be remembered that the principles in the case of Bolam cannot be applied to questions in a case that relate to fact, hypothetical questions or otherwise. Therefore, if there is an issue in relation to the breach of duty and the causal link to the damage that has occurred, i.e. causation, for example, in a case such as whether the treatment which was not given would have successfully treated the Claimant or avoided the injury, any dispute between the medical experts will not attract a Bolam analysis. Instead, the court will have to decide the issue on the basis of which evidence it prefers on balance.

22.2.4 Vicarious Liability

In most cases it is very clear that a doctor or nurse was employed by the hospital, clinic or GP practice and if the treatment is on the NHS then the hospital, clinic or GP practice will be vicariously liable for any negligence on the part of the doctor or nurse. The Court of Appeal in the case of Cassidy –v- Ministry of Health and Others [8] made it quite clear that a hospital authority will be vicariously liable for the negligence of an employee whether employed under contracts of services or contract for service. Delegation of the performance of the duty of care to give proper treatment does not remove its liability for that treatment. This is because a hospital, health authority, clinic or GP is there to provide healthcare to patients.

A recent case of interest is that of Woodland –v- Swimming Teachers Association which endorses the reasoning in Cassidy. In Woodland the Claimant was a normal healthy 10 year old child who was a pupil at a school and she attended a swimming lesson in school hours at a local swimming pool. The swimming lesson was part of the national curriculum. During the lesson she was found "hanging vertically in the water". Her lesson was conducted by a teacher and a swimming instructor both of whom were employed by an independent contractor. The independent contractor had a contract with the Claimant's school. The Claimant was resuscitated but suffered severe brain damage. The Claimant, through her father, sought a claim against the local authority. At first instance the claim was struck out as it was held that the defendant did not owe a non-delegable duty of care to the Claimant. The case was appealed to the Court of Appeal and the outcome was the same. On appeal to the Supreme Court the Claimants appeal was allowed.

The judgment in Woodland-v-Essex County Council [9] clarifies further qualifications as follows.

(1) There was an antecedent relationship between the Claimant and the Defendant, independent of the negligent act or omission itself, which placed the Claimant in the actual custody, charge or care of the Defendant, and from which it was possible to impute to the Defendant the assumption of a positive duty to protect the Claimant from harm, not just a duty to refrain from conduct which would foreseeably damage the Claimant. It was characteristic of such relationships that they involved an element of control over the Claimant, which varied in intensity from one situation to another, but was clearly very substantial in the case of schoolchildren.
(2) The Claimant had no control over how the Defendant chose to perform the relevant obligations (whether personally or through employees or third parties).
(3) The Defendant had delegated to a third party some function which was an integral part of the positive duty which he had assumed towards the Claimant; and the third party was exercising, for the purpose of the function thus delegated to him, the Defendant's custody or care of the Claimant and the element of control that went with it.
(4) The third party had been negligent, not in some collateral respect, but in the performance of the very function assumed by the Defendant and delegated by the Defendant to him.

The Supreme Court accepted that a duty of care to a patient, a child or an otherwise vulnerable person is personal and arises from an acceptance of the patient into its care or custody for treatment. This duty may not be delegated to a third party even if that party is providing the actual treatment.

When a private clinic agrees to perform a surgery or provide a treatment to a patient, a relationship between the patient and the clinic is created, placing the patient in the care of the clinic. The clinic accepts the responsibility for the patient in that they provide and assign the serviceman, locum and equipment for the surgery and offer post-surgical care. From that, one can impute that the clinic assumes a positive duty to protect the patient from harm. The clinic further exercises some control over the patient in that they (the clinic) choose and assign a clinician to the patient. Because of that, the patient is deemed to be especially vulnerable as it is outside of his/her power to check the credentials of the treating clinician and/or he/she does not have an option to decide or choose the surgeon. Considering that the treating clinician is assigned to the patient by the clinic, the onus is on the clinic to satisfy that they have done necessary and sufficient checks on the treating clinician and his insurer before offering the services of the clinician to the patient. Otherwise, one is negligent in failing to do so.

Furthermore, it has to be considered as to whether it is fair, just and reasonable to apply the test to a private clinic. Imposing the non-delegable duty on a private clinic cannot be perceived as a significant burden in that they are in control of the crucial information about the treating clinician, assign the clinician and benefit from the contractual agreement, which goes to the root of the potentially negligent treatment.

The non-delegable duty in Woodland-v-Essex County Court gives hope for patients in private clinics that their medical negligence claims would be dealt with by the clinics and not the self-employed clinicians. Whether the test will be used for such a scenario is still unknown.

22.2.5 Causation

The standard test for determining whether or not the defendant's breach caused the Claimant's loss is, as previously explained, the "but for test". The Claimant must prove that the clinician's duty correlated with the kind of loss for which she has suffered. Lord Hoffman, in the case of South Australia Asset Management Corporation –v- York Montague Limited (sub-nom Banque Bruxelles Lambert SA –v- Eagle Star Insurance Company Limited) 1997 [10] said:

> A mountaineer about to undertake a difficult climb is concerned about the fitness of his knee. He goes to a doctor who negligently makes a superficial examination and pronounces the knee fit. The climber goes on the expedition, which he would not have undertaken if the doctor had told him the true state of his knee. He suffers an injury which is an entirely foreseeable consequence for mountaineering, but has nothing to do with his knee.

If we look at the "but for test" the doctor is deemed responsible for the injury suffered by the mountaineer because he would not have suffered damage if he had been given the correct information about his knee, and he would not have gone on the expedition, and he would not have suffered his injury.

"The reason that he is not liable is that he was asked for information on only one of the considerations that might affect the safety of the mountaineer on the expedition, and there is no reason of policy for transferring to the negligent doctor all the foreseeable risks of the expedition. So, a defendant who is under a duty to take reasonable care to provide information on which the claimant will decide upon a course of action is, if negligent, not generally regarded as responsible for all the consequences of that course of action. He is only responsible for the consequences of the information being wrong. If the duty is to advise whether or not a course of action should be taken, the adviser must take reasonable care to consider all the potential consequences of that course of action. If he is negligent, he will be responsible for all the foreseeable loss that is a consequence of that course of action having been taken".

22.2.6 Material Cause

The Claimant does not have to show that the Defendant's negligence was the only or sole cause of the loss. If this cannot be established due to medical science, then the extent of the contribution of the negligence to the injury is enough that it was a "material" or "effective" cause. An interesting case on this point is Wilsher –v- Essex Health Authority 1988 [11], in which the Claimant, a baby born 3 months prematurely, developed Retrolental Fibroplasia (RLF) that caused him to go blind. It was alleged that he developed RLF as the hospital failed to monitor his levels of extra oxygen, given his prematurity. At the Court of Appeal the Defendant was found liable on the basis that a risk had been created or that there had occurred an increased risk of an existing injury. However, on appeal to the House of Lords, it was held that a number of other factors could have caused RLF including:

> Excessive levels of oxygen, and the Defendant's negligence in failing to prevent excess oxygen causing the condition, provided no evidence and raised no presumption that it was excess oxygen rather than any other four non-tortuous factors that caused or contributed to the Claimant's condition. This issue remained unresolved and the issue had be remitted for a re-trial. The case was subsequently settled.

22.2.7 Increasing the Risk

The well-known case of Fairchild –v- Glenhaven Funeral Services Limited [12] is a good example of this. Three employers of Glenhaven Funeral Services developed Mesothelioma, which had caused lung cancer. The claims were dismissed on the basis that there was no cause or link against any of the Defendants. However, on appeal to the House of Lords, it was held that the ruling in its current form can be stated as follows:

> When a victim contracts Mesothelioma, each person who has, in breach of duty, being responsible for exposing the victim to a significant quantity of asbestos dust and thus creating a "material increase in risk" of the victim contracting the disease, will be held jointly and severally liable for causing the disease". It would be impossible to bring claims against each Defendants.

22.2.8 Apportionment

In the case Dingle –v- Associated Newspapers Limited [13], Devlin L. Jones said "*where injury has been done to the Plaintiff and the injury is indivisible, any tortfeaser whose act has been an approximate cause of the injury must compensate for the whole of it. As between the Plaintiff and the Defendant, it is immaterial that* *there are others whose actions also have been a cause of injury, and it does not matter whether those others have or have not a good defence. These factors would be concerned with that; you can obtain judgment for a total compensation from any one person, it is immaterial to the Plaintiff whether they are joint tortfeasers or not. Four men, acting severally, not in concert, strike the Plaintiff one after the other, and as a result of his injuries he suffered shock and is detained in hospital and loses a month's wages, each wrong-doer is liable to compensate for the whole of the loss of earnings. If there were four distinct physical injuries, each of them would be liable only for the consequences peculiar to that injury he inflicted, but in the example I have given, the loss of earnings is one injury caused in part by all four Defendants. It is essential for this purpose that the loss should be one and indivisible; whether it is so or not is a matter of fact and not a matter of law*".

22.2.9 Loss of Chance

It is for the Claimant to show that it is more likely than not, for example greater than a 50% chance, that the negligence caused a loss. An interesting case in this regard is Hotson –v- East Berkshire Area Health Authority [14], which involved a young teenager who fell from a tree and fractured his left femoral epiphysis. The fracture was not diagnosed for 5 days, and he developed a serious condition; a vascular necrosis, and he had permanent disability in his hip. Liability was admitted, but when the matter proceeded to trial, it was found that there was a higher probability, assessed at 75%, that he would have got the condition in any event. He was only awarded compensation on the basis of a 25% chance that the condition would not have developed. This decision was reversed in the House of Lords.

Another case we can mention is Greg –v- Scott 2005 [15], which involved a Claimant who had been diagnosed with a benign tumor when in fact it was malignant. His treatment was delayed by about 9 months, in which time his condition deteriorated. He became very unwell and was diagnosed with a rare type of cancer, and his prognosis was poor. He argued "reduction of the chance of a favorable outcome" failed as his prospects of recovery were 42% at the time of his initial misdiagnosis, and had reduced to 25% as a result of the delay in diagnosis. It was shown that the Claimant would have failed to survive a 10 year period even if he had been treated earlier. His claim was dismissed, but he appealed the decision on the basis that his cancer had progressed much more quickly as a result of the delay in his diagnosis. Therefore, "the claim against a doctor who has negligently caused a reduction in the prospects of a successful recovery will fail on causation grounds if the claimant cannot prove that, on the balance of probability, he would have recovered".

22.2.10 Consent

As discussed in the case of Montgomery, a patient must be warned of the risks involved in any treatment. Treatment cannot be performed without the consent of the patient.

22.2.11 Intervening Acts

Causation may be broken in another event caused by a third party or claimant, which means the "chain of causation" is broken by a "novus actus interveniens". If this occurs, the claim will fail.

In the case of McFarlane –v- Tayside Health Board [16], a woman underwent a sterilization operation that was negligently performed. She fell pregnant and she delivered a healthy child. She could not claim financial costs of bringing up her child, but could recover damages for pain and suffering and the cost of the pregnancy.

22.2.12 Foreseeability

The Claimant must show that his or her loss was reasonably foreseeable at the time of the breach of duty.

> A standard test has been developed so that the only type of harm to have been foreseen, not the precise injury, with the result that a claimant who is affected by the injury in a way that was not itself foreseeable – the claimant with an "eggshell skull" may recover for the whole of his injury. The claimant only need show the type of harm that was reasonably foreseeable.

22.3 From Clinical to Scientific Evidence

Evaluation of clinical information for its use as scientific evidence is dependent on a number of important considerations, which includes the expert's assessment of the clinical findings, taking into account the existing evidence base for the relevant findings with a view to providing scientific evidence to the court.

22.3.1 Quality and Type of Expert

A medical expert who assesses personal injury damages is an experienced doctor or other relevant healthcare professional who has an extensive knowledge within their particular area.

The type of expert that an injured person needs to be examined by is usually the same type of doctor as the one who had been providing the care. So if an injured client has been treated by a Consultant Hand Surgeon, then a report from an independent Consultant Hand Surgeon is required. In injuries involving fractures, the client is usually examined by a Consultant Orthopaedic Surgeon.

The qualification and knowledge of the expert doctor must be established in court before evidence is accepted either in documentary or oral form. A consultant physician who is in good standing with the General Medical Council and on their Specialist register will usually only be required, in the UK courts, to submit of his/her CV so as to demonstrate experience and knowledge in a particular area of specialization. Although it may be sufficient merely to state academic and professional qualifications, the details of experts' qualifications to be given in reports should be commensurate with the nature and complexity of the case. However, where highly specialized expertise is called for, experts should include the detail of particular training and/or experience that qualifies them to provide that highly specialized evidence.

In some cases, the expert will provide evidence solely upon the basis of documentation, such as case notes, case histories, and other documentation. On other occasions, when considering issues of causation, the extent of an injury that has been caused, and a prognosis for the future, the documentation needs to be supplemented by an examination of the claimant by the expert witness and the provision of a further medical report.

If an opinion is sought on a subject in respect of which the expert does not consider that he or she has adequate experience or expertise, it may be appropriate to decline the instruction. It is vital therefore that the correct person is instructed to provide expert evidence in a case and that there is no conflict of interest between the expert and the proposed defendant.

22.3.1.1 Discussions Between Experts

The court has the power to direct discussions between experts for the purposes set out in the Rules (CPR 35.12). Parties may also agree that discussions take place between their experts at any stage. Discussions are not mandatory unless ordered by the court. The purpose of discussions between experts should be, wherever possible, to identify and discuss the expert issues in the proceedings and attempt to reach agreed opinions on those issues. If that is not possible, then it is necessary to understand if they can narrow down the issues, identify where they agree and disagree, summarize their reasons for disagreement on any issue and identify what action, if any, may be taken to resolve any of the outstanding issues between the parties. They are not to seek to settle the proceedings.

22.3.2 Access to Medical History, Assessment and Medical Examination

It is incumbent upon the claimant to ensure that the legal team and medical expert is provided with any documentation that they may have in relation to the incident and might include photographs of the accident location and of his/her injuries; all written reports from where the accident took place or if the police attended, a police report, and documentation from any witnesses. These types of documents will also be required later in the claim if liability is denied. Under such circumstances it will be necessary to provide the other party's insurance company with further documentation and evidence.

The expert, in conjunction with the medical records, will ascertain the history from the client to include:

- past medical history, in order to identify from the records the client's state of health and fitness as well as any past or more recent conditions (ongoing/unresolved) that might have a bearing on the injury that is being claimed for. Enquiry should also be made concerning significant illness or conditions, both as a result of natural disease or from unnatural conditions such as trauma that had occurred years earlier.
- conditions from which the subject is currently suffering, acute or chronic, and the treatment given, including drug medication and possible side effects.
- detailed information on the family and, in particular, any medical conditions within the family.
- description of any particular/specific daily recreational activities and an assessment of the intensity and physical impact of these.
- social interaction with friends and frequency of interaction.
- type of activities undertaken at work and specification of skills required to carry out tasks in relation to the subject's general and specific aptitudes.

22.3.2.1 Clinical Examination

The extent and type of clinical examination will be governed to a great extent by the type of injury for which the claim is being made, the objective being to assess the degree of damage and extent of temporary or permanent impairment, both physical and psychological.

22.3.2.2 Further Investigations

There may be a requirement for further clinical assessment and/or investigations involving the use of various tools both for imaging and recording purposes. This could include radiological examination and specific tests using specialized instruments and devices.

22.4 The Future and Futuristic Evolution of Evidence in Medical Malpractice

22.4.1 The Concept of Integrated Bio-Medical Platforms Towards Evidence

Keating and Cambrosio's concept of the biomedical platform [17] was defined as a specific configuration of instruments, individuals, and programs. Biomedical platforms generate routines, entities, and activities, held together by standard reagents and protocols. The notion of a biomedical platform allows researchers interested in the development of contemporary medicine to describe events and processes overlooked by other approaches. They observe that new platforms are often aligned with existing ones, are integrated into an expanding set of clinical-biological systems. As can be appreciated, the biomedical approach has, and will have, a significant role to play in modern medicine and, of course, require healthcare professionals to change the management of different conditions to comply with different approaches to diagnosis and treatment. Accordingly, this will have an effect on the expectations placed upon health professionals, to ensure that the expected standard of care is maintained. It remains to be seen whether this will ultimately have a significant effect on increasing the casework in medical litigation.

22.4.2 Medical Innovations Bill

A further recent development in the United Kingdom which could have had significant detrimental effect on the standard of care involved the attempt to introduce the so-called Medical Innovations Bill. Maurice Saatchi, following the death of his wife Josephine Hart to ovarian cancer, campaigned for a change to the UK law, which he believed held doctors back from recommending innovative treatments out of fear of litigation. Saatchi said that he believed that health provision in the UK was "innovation averse" and that the current standard treatment offered to people with cancer was "degrading, medieval and ineffective", leading "only to death".

Saatchi's Medical Innovation Bill proposed that doctors be permitted to use non-standard treatments for any medical condition. The bill was formally introduced in 2013 and was co-adopted by the government in its passage through parliament. However, the bill's proposals were criticized by most doctors and medical bodies and it failed to progress through the House of Commons after the Liberal Democrats declined to support it.

An editorial in The Lancet, Oncology [18] stated that Saatchi was promoting "precisely the type of emotional response that evidence-based practice seeks to avoid", that the current UK law already provided for medical innovation, and that the bill's provisions threatened to undermine the Hippocratic Oath.

Cancer Research UK [19] has stated that there is "no pressing need" for new legislation.

In November 2014 [20], more than 100 medical professionals signed a letter to The Times saying that the existing law did not impede innovation, as had been claimed, and that the proposed new legislation could have the unintended consequence of weakening the evidence base for research by leading to an accumulation of merely anecdotal evidence.

It was argued that the Bill was not just simply unnecessary, but as first drafted it was positively dangerous. It proposed to give clinicians legal immunity if they try new things out. As we are all aware, breakthroughs in medical research are made systematically, one step at a time, involving scrupulous trials with ethical oversight and peer review. It would thus be reckless to return to the age of freelance have-a-go heroes who experiment on their patients. This would undermine evidence-based medicine [21].

22.4.3 The Future of Clinical Negligence

At present the costs relating to clinical negligence claims are budgeted, which means that the costs at the various stages of the clinical negligence claims procedure are fixed in relation to the type of case, the number of witnesses required, the number of experts required and the number of days that the matter will require at trial. This budget must be set out in a document entitled Precedent H. This is mandatory and each stage of the investigation of a clinical negligence case must be budgeted and there is always a Costs Management Order that must be approved by the court. The rules relating to this are contained within the 83rd update of the Civil Procedure Rules which came into force from 6 April 2016 and bring significant changes to the budgeting process, such as the type of budget to be used in specific cases, the timing of filing such budgets and the content of the budget.

In addition, the government is consulting on fixing costs for clinical negligence cases as a way of promoting "better litigation" and also reducing costs in claims of this nature. The proposal follows that of Personal Injury claims and it is anticipated that if the proposals come into force a fixed costs scheme for clinical negligence cases valued up to £25,000 would in principle work. It would be easy to implement and it would work very well. However, fixing costs in higher value cases would be fraught with difficulty, especially cases up to the value of £100,000 or £250,000 as proposed.

A fixed fee scheme for cases between £1000 and £25,000 would work because these types of cases are low value cases and once liability has been issued they are relatively easy to progress and settle. Cases over that band require more medical evidence and in complex birth injury cases, for example, require experienced solicitors handling the case and other experts If not, then issues can be overlooked and unmeritorious cases proceed or, even worse, large cases are under-settled, which is likely to lead to further litigation, such as professional negligence.

The other area of contention is that the proposed fixed costs regime only applies to Claimants bringing clinical negligence claims and if the fixed fee regime is to be implemented then it should apply to both parties, the Claimant and Defendant. Further, that the cap should be set at a sensible level.

Catherine Dixon, CEO of the Law Society and ex-CEO of the NHS Litigation Authority has said "*the focus should be on reducing the amount of negligent care which is harming patients in the NHS… Given this reality (that 41%—almost half a billion pounds of the compensation paid out by the NHS—was for obstetric claims mainly paid to brain-damaged children), plus the fact that almost half of these (legal) costs are arising from brain—damaged baby claims, you would think that every action would be taken to stop damaging babies' brains. If the cost runs into billions and the result is untold misery to babies and their families, isn't it worth investing more to stop this from happening?*".

Further, the way forward is for Defendants to speed up disclosure of medical records, admit liability at any early stage, avoid trials which would cut costs not only in obtaining expert evidence, but in legal fees, and reduce the number of claims relating to brain damaged babies. Recruitment of more experienced and specialist nurses and medical staff will also prevent medical accidents from happening in the first place.

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Chapter 23
Methodology of Ascertainment in Medical Malpractice in Living Persons

Thomas Bajanowski

Abstract In recent decades, increasing significance has been placed on medical responsibility and liability. Progress in modern medicine has raised expectations that any health problem can be treated by specialists in any field of medicine. Nevertheless, depending on the circumstances and complications surrounding each particular case, a suspicion of medical malpractice can arise if the outcome is not as expected. The role played by legal medical experts has taken on increasing importance in judicial investigations. It is against this background that the first "Consensus Guidelines Document" dedicated to the evaluation process in cases of suspected medical malpractice was developed by the ECLM working group on medical malpractice in 2012. Once the qualification of the expert has been established as a prerequisite for the procedure, the methods of ascertainment can be determined. These include collecting all clinical data from the admission authorization to reports on post-surgical treatment and release from hospital. An analysis of the clinical documents may reveal a necessity to contact specialists of other fields to ensure a better definition of the case in question. A subsequent clinical examination is required to establish the clinical condition of the patient. Furthermore, the relationship between the current situation and previous medical actions must be verified. Other diagnostic procedures may be performed to ascertain the possibility of medical malpractice as a possible cause of impaired health. A clinical synthesis should summarize the main, central facts. The final evaluation begins with a comparison of data, followed by the identification of pathological features, damage or incapacities that constitute either temporary or permanent impairment, and the reconstruction of pathophysiological pathways. This evaluation should be based on scientific sources such as guidelines, consensus documents and established operational procedures. It may then be possible to determine real error, pseudo-error, conscious error or non-observance of required rules. This may also comprise a

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discussion of the direct relationship between error and damage, including the degree of probability of the relationship. All of these steps are described in detail in the ECLM consensus document. This can be seen as an example of harmonization of juridical-legislative medical malpractice procedures in European states.

23.1 Introduction

Over the last few decades, new and revolutionary techniques have been introduced in medicine. Both diagnostic and therapeutic procedures have become increasingly specific and sophisticated, which has led to enormous advantages in patient care. Nevertheless, with advancing progress in medical engineering and the increasing specialization of medical practitioners, new risks associated with new techniques have been identified. The number of cases of suspected medical malpractice is increasing in all developed countries [1, 2] and "each malpractice claim gives rise to a scientific challenge in the analysis and evaluation of the clinical case in question" [3]. Today, the phenomenon of medical malpractice is of worldwide significance, independent of political structures, affiliation to social class or cultural traditions. In the European Community, about 80% of all people believe that medical malpractice is a significant problem, while about 50% of these people believe that they have been involved in such a case [4]. Oyebode stated that about 3–16% of all hospitalized patients worldwide suffer injury as a result of medical intervention [5].

In general, the rights and responsibilities of doctors with regard to a patient's treatment are usually stated as part of the code of medical ethics and/or the Medical Association's professional code of conduct. This means that there are differences in law from country to country as well as between member states of the European community. In cases of medical malpractice, legal liability means that doctors may face consequences pursuant to the penal code, civil code or administrative legislation of the various countries [6–8].

Medical professional responsibility in the UK is regulated by the General Medical Council. This involves setting standards of 'good medical practice', assuring the quality of undergraduate medical education, administering systems for the registration and licensing of MDs, and dealing with doctors who are not fit to practice. GMC takes into account Common Law, the Data Protection Act 1998, the Human Rights Act 1998 and the Health and Social Care Act 2001 [9]. For a claim to be successful, the following components need to be proven: a duty or contract of care between the defendant and the claimant, a breach of this duty, and the fact that the breach caused the damage.

In Italy, responsibility in civil matters is based on a transaction contract under article 1218 of the Civil Code [10]. In Germany the medical doctor's responsibility is set out in Professional Ordinances, in Austria as part of the Physicians' Law 1998, and in Switzerland in specific regulations of the various cantons.

In France, principles of doctor's liability were introduced in 1835 and developed by the Mercier case in 1938 and the law of March 2002. The principles are based on the conditions that medical liability requires injury to have been caused by an error in treatment, and proof to be given by the patient [11]. In Portugal, medical professionals can be charged with malpractice at three levels (civil law, criminal law, disciplinary), even simultaneously [12]. In Lithuania, the Law on Patient's Rights and Compensation for Medical Injuries (Law 102-2317, 115-4284) and the Penal Code are specific norms that regulate medical responsibility [13].

Irrespective of differences in legislation, the definition used for damage, the use of the term "adverse event", and practical investigation in suspected cases of medical malpractice are similar in most countries. Furthermore, in most countries, the patient has to prove that damage was caused by an error made by the MD. Often, medical experts are asked to fulfill a number of important tasks or answer important questions. These experts should have specialist knowledge of the relevant field of medicine, and they should be independent of each of the parties involved.

As a first step, it is usually necessary to detect an injury or a health problem. The second step is finding a medical error, and finally it is necessary to provide proof that this error caused the health impediment.

23.2 Definitions

The first step of the investigation is to determine a dysfunction of the body, such as an injury or invalidity. The difficulty here is that different definitions may be in use to describe the disturbances. This applies in particular to the terms *error* and *adverse event*. According to the WHO definition from 2004, an adverse event is an injury which is related to medical management, in contrast to complications arising as a result of disease. Medical management comprises all aspects of care, such as diagnosis, treatment, failure to diagnose or treat, and the systems and equipment used to deliver care. Adverse events may be preventable or non-preventable (WHO 2004). Other definitions have been suggested by Fischer et al. [14], Thomas et al. [15], or Vincent et al. [16]. Furthermore, the Council of Europe has used two nearly but not quite identical definitions (Council of Europe 2006) for the generic term of patient safety:

– freedom from accidental injuries during the course of medical care; activities to avoid, prevent, or correct adverse outcomes which may result from the delivery of health care
– the identification, analysis, and management of patient-related risks and incidents, in order to make patient care safer and minimize harm to patients.

Since a large number of adverse events stem from errors, a number of different definitions of the term error are in use in scientific literature. Ferrara et al. used the terms real error, pseudo-error, and conscious error in their recommendation [3] and differentiated between non-observances and no-fault medical accidents. They defined the terms as follows.

- Real error: error due to violation of a scientific law or of consolidated rules of experience and competence
- Apparent error: error due to a lack of scientific knowledge on a specific issue
- Conscious error: an error made in full conscience
- Non-observance: non-observance of rules of scientific medicine
- No-fault medical accident: iatrogenic damages which are not caused by an error but related to therapeutic risk.

Diagnosing and evaluating dysfunctions of the body or an injury or invalidity requires specialist knowledge. Therefore, the question concerns the minimum requirements, competences and expertise that are essential for investigating a case of suspected medical malpractice. As part of a European guideline on methods of ascertainment in cases of malpractice, Ferrara et al. [3] defined the qualification and competences of such experts as follows.

- The expert should be a specialist in legal medicine or should have completed postgraduate training in legal medicine, preferably at university level. They should be recognized by the national authorities as a medico-legal expert,
- The expert should have adequate training/knowledge in:
  - criminal, civil and administrative law with reference to medical liability
  - the medico-legal evaluation of psychophysical validity in civil law and private/public insurance
  - theoretical knowledge and practical experience in judging causal links, in particular, demonstrating causal links between medical error and damage, and subsuming such phenomena under scientific points of view.

The paper published by Ferrara et al. [3] is at present one of the best and most comprehensive overviews on methods of ascertainment and should be used as the basis by which to standardize examinations in the various countries of the European community. Therefore, the following description makes primary reference to this document.

23.3 Analysis of Medical Documents

Irrespective of differences in juridical or extrajudicial conditions in the various countries and irrespective of the person suffering from the injury, the role of the medico-legal expert as a consultant for the injured person, judge, insurance company or other persons or institutions, the methods of ascertainment applied must be the same in order to obtain objective results. The investigation should include an analysis of the pre-clinical and clinical documentation and, if necessary, a consultation with specialists of other disciplines, a clinical examination of the injured person, and additional diagnostic tools (if indicated). These findings then form a basis for subsequent evaluation.

23.3.1 Analysis of Pre-clinical and Clinical Documents

The first stage of the procedure is to analyze the *pre-clinical and clinical documents*, as it is these that will form the basis of the subsequent evaluation and judgement. It is extremely important to have all of the data on hand that might facilitate reconstruction of any required detail of the medical treatment. If any important information is missing, the final assessment may be incomplete or incorrect. In any case, the medico-legal expert must have full *authorization* either from a judge or from the injured patient. The expert must be furnished with a signed document in which the patient (or in cases involving children, mentally ill or unconsciousness persons, the patient's representative) gives their consent to the investigation. The document must state explicitly which medical documents are to be included in the analysis and which additional investigations are allowed.

The first MD contacted by the patient may be a general practitioner, a registered doctor with a different area of specialization, or an emergency doctor. In all cases, a brief documentation of the consultation must be compiled. This may include a brief description of the actual health problem and the patient's current condition, as well as a diagnosis, initial treatment and decision on how to proceed. If hospital treatment is necessary, the admission has to be organized. It goes without saying that the documentation of hospital treatment needs to be much more detailed.

The *medical history* is the first information requested by the MD responsible for hospital treatment. The information given by the patient must be relevant and complete, as, together with the results of the first *physical examination*, it is what the subsequent diagnostic and therapeutic accuracy is founded on and it is therefore a prerequisite of all further diagnostic and therapeutic steps. The first diagnosis should be based on the clinical history and physical examination and may be in the form of a presumption.

The *patient's journal* records all facts relating to the medical treatment, including the patient's condition and perception, drug treatment and response to treatment, other forms of treatment, changes in clinical state, and additional diagnostic procedures and their results (laboratory tests, imaging, functional tests, etc.).

The *medical orders sheet* contains all the decisions made by the doctors involved in the therapy. All these orders have to be signed, so that a doctor making a decision can be identified.

In the case of invasive diagnostics or surgical treatment, the patient has to be informed prior to treatment to enable him to give informed consent. The *informed consent document* is mandatory by law and contains a description of the planned surgical technique or method of treatment, along with possible complications and, if possible, information on alternative therapeutic options. The document has to be signed by the doctor providing the explanation and by the patient himself, thus stating that he was able to freely decide on the treatment or test after sufficient comprehensive information was available.

The *emergency room report* may be the first clinical document to record hospital treatment. This report is usually drawn up if the patient requires immediate medical treatment due to sudden severe health problems. It contains a short overview of the clinical symptoms, the results of the clinical examination, the first therapeutic steps and the initial diagnosis. Furthermore, a decision has to be made on the subsequent procedure; for example, it may be possible to send the patient home, or the patient may have to be admitted to hospital in order for further diagnostic steps to be performed, resuscitation attempts made or a decision taken to perform immediate surgical intervention. All decisions must be well documented and in compliance with guidelines of good clinical practice, assuming such guidelines are available to suit the patient's circumstances.

Indications may sometimes necessitate consulting specialists in other medical disciplines for their opinion with regard to diagnoses or treatment, or performing specific diagnostic examinations. These consultations are usually performed on the request of the MD responsible for the patient's treatment. The results are documented in a special report (*consultation sheet*), which forms an integral component of the patient's journal.

Results of *complementary examinations* (laboratory tests, imaging, microbiology, virology, neurology, etc.) have to be documented separately on special reporting forms. The report should state a question to be answered or state the cause and the results of the examination, along with a brief assessment by the specialist performing the test. The results may have a bearing on the way the further treatment proceeds (surgical or drug administration).

If surgical treatment is indicated, the patient has to be informed on the details of the operation, the associated risks, possible alternatives and the expected prognosis prior to surgery (informed consent). Furthermore, it may be necessary to perform additional investigations to evaluate the function of the cardiac and respiratory systems, so that the patient is informed on the possible individual risks which may be present and can influence the function of both systems during anesthesiology/surgery. The *pre-surgery examination report* is the document which contains all of the information regarding the planned anesthesiology. It has to be compiled by an anesthesiologist. Compiled during surgery, the *anesthesiology report* contains all information on the patient's condition as well as on all drugs and gases administered. The *operating room report* contains a detailed description of surgical intervention.

This includes all steps of the procedure, starting with the first cut and ending with the final stitch. A reader of this document must be able to understand and reconstruct all the details of the surgery, including any surgical and postsurgical complications. Directly after surgery, the patient must be monitored by an anesthesiologist or other qualified member of the clinical staff. The aim is to avoid or diagnose any complication resulting from surgery or anesthesiology as early as possible and to treat it appropriately. The details of this monitoring process are described in the *post-surgery evaluation form*. After surgery, any removed organ or tissue specimens are usually investigated by a pathologist. He performs both a macroscopic and microscopic examination of the preparation and renders a pathological diagnosis in the course of the *pathology report*. In cases of tumor surgery, the pathological grading and staging of the tumor may influence further treatment.

Sometimes it may be advantageous to record, for example, adverse events or other unpredictable incidents in a detailed report (*witness statement*). Such witness statements can form part of the clinical documentation, or they may be written on the basis of a hearing by the police, a judge or, depending on national regulations, by the expert himself.

The *nursing journal* contains all information on vital signs (heart frequency, blood pressure, body temperature, etc.), drug administration, and disposition of the patient. Furthermore, special requests of nurses to MDs should be recorded, along with the responses. These may be questions regarding care, additional drug administration, or other diagnostic/therapeutic actions.

If the patient is discharged from hospital (either to their home or to another hospital) a *discharge report* is compiled summarizing all the details of the hospital treatment, including the cause of hospitalization, diagnoses, treatments, and recommended further therapy (drug administration, monitoring and/or surgical treatment). The report is addressed to the general practitioner who arranged the patient's admission to hospital or the MD responsible for subsequent treatment at the other hospital.

23.4 Consultation with Another Specialist

It can sometimes be useful and/or necessary to contact a specialist from another field of medicine to ask for his contribution/advice with respect to certain problems or questions arising during the clinical ascertainment phase. Such consultation may result from direct appointment by a judge, an appointment by a judge after recommendation by the medico-legal expert or by the medico-legal expert himself. In their consensus document, Ferrara et al. recommended that such a specialist be nominated by the judge if this is recommended by the medico-legal expert.

23.5 Clinical Examination of the Patient

A clinical examination can be recommended in all cases. The aim of the examination is to obtain objective information on the present condition of the patient. Furthermore, it is of interest to know whether or not the present clinical state corresponds to what is recorded in the clinical documents. Differences are of interest, because they may be due to healing processes. The examination includes internal medical tests, neurological, orthopedic, surgical or any other tests necessary for detecting any health problems or injuries. The clinical examination can be augmented by laboratory tests that may be necessary for specific diagnoses. If additional invasive diagnosis is required, the patient's consent is necessary. Extensive information is then required, to ensure informed consent. In such cases, the expert has to consider costs and benefits as well as any risks associated with the procedure. Finally, it should be clarified whether the present condition is in accordance with the clinically documented facts and actions. All the results of the expert's examination have to be recorded with regard to their nature, the location of damages/injuries, their significance and the limitation of physiological functions. The results of the expert's clinical examination are a central component of the expert's final report and form the basis for qualifying and quantifying the "biological damage".

23.6 Evaluation

The analysis of the medical documents and the results of the clinical examination form the basis by which to describe the patient's pathological features and to identify damages or incapacities, which may be either temporary or permanent. Subsequently, the expert has to evaluate the pathological features and the treatment required, using scientific sources. Such sources include national and international guidelines, consensus documents, evidence-based manuscripts published in scientific journals, generally accepted operational procedures, or other sources from the literature. Based on this process, the expert must reconstruct the ideal path of treatment that the physicians should have followed in diagnosis and therapy.

The analysis of the medical documents and results of the expert's clinical examination should allow the actual medical conduct of the case in question to be reconstructed. This conduct must be evaluated from an ex-post point of view, with regard to the completeness and correctness of the documents, appropriateness of the diagnostic examinations, indications and contraindications of therapy, correctness and adequate timing of therapy, appropriateness of treatment and care and the qualification of the MDs and other medical staff. If it is not possible to reconstruct the actual medical conduct, the evaluation process should be halted and the reasons for doing so reported.

Next, the actual medical conduct is compared with the ideal medical conduct. This should demonstrate that the MD responsible for the treatment either met or failed to meet the requirements of standard care. All steps of the treatment must be evaluated for errors that may have occurred and/or non-observances of the required rules. This may include the patient's informed consent, as well as diagnosis, prognosis or therapy. If this comparison shows that the doctor did not meet the required standards of care, an error or non-observance is evident. This error must be characterized using the above mentioned classification of error. In the next step, the error identified must be evaluated from an ex ante (the doctor's) point-of-view. The main question is now whether the error could have been avoided if the MD had met the standards of care.

Depending on national law and/or the purpose of the examination and the client, it may be necessary to state the probability of the causal link between the error and the event. This probability can be given as a verbal description or as a percentage. This may be difficult in some cases, but to do so is important since statistical calculations, means of deduction or criteria of rational credibility referring to average experiences and expertise in the medical field in question can be extremely useful.

23.7 Conclusion

The ascertainment of injury and evaluation of treatment are complex and often difficult issues in cases of suspected medical malpractice. Moreover, the clinical point of view may differ from medico-legal opinion and from juridical judgement. Further differences in evaluation are caused by the heterogeneity of legislation in various countries. However, the methodology used to identify the injury or the extent of damage should not differ substantially in the countries of the European Community or in other developed countries [17]. Therefore, the ascertainment approach should be based on similar scientific principles in all countries.

It is the merit of the members of the EALM working group chaired by Professor Ferrara that Guidelines on Methods of Ascertainment and Criteria of Evaluation in cases of suspected medical malpractice have been developed. These Guidelines may be of help in harmonizing Legal medicine in Europe. Furthermore, the Guidelines may guarantee objectivity and reproducibility in the collection of scientific evidence, and may be followed by further steps to prevent medical malpractice and increase patient safety. "A better method of case ascertainment will result in more reliable and richer data and the participation of medico-legal experts in both the assessment and the data analysis will guarantee the usefulness of the results" [18]. If the methodology described as part of the Guidelines is used for case analysis it should be possible to extend and improve the database for research in this field, so as to learn more effectively from cases identified as medical malpractice. In this way the Guidelines could be of help in improving patient safety.

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Chapter 24
Forensic Clinical Anatomy—Definitions, Methods and Fields

Andrea Porzionato, Veronica Macchi, Marios Loukas and Raffaele De Caro

Abstract *Forensic Clinical Anatomy* may be defined as the practical application of Clinical Anatomy to the ascertainment and evaluation of medico-legal problems. *Individual Anatomy* (normal anatomy, anatomical variations, age-, disease- or surgery-related modifications) may acquire relevant significance in various fields of Legal Medicine, such as Child Abuse, Sudden Death, Medical Responsibility and/or Liability, Personal Injury and Damage. Anatomical data of forensic interest may arise from the correct application of methods of ascertainment and anatomical methodologies may then be required for further comprehensive analysis. The rigorous interpretation of the anatomical data, derived from ascertainment phase and analysed on the basis of pertinent literature, may be pivotal for the correct application of evaluation criteria in various forensic contexts. Awareness of the relevance of Individual Anatomy should be one of the principles guiding the Clinician to *Personalized Medicine*; conversely, in *Personalized Justice*, medico-legal analyses cannot ignore the implications of individual anatomy in terms of ascertainment and evaluation.

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24.1 Introduction

In the very first paper of the journal Clinical Anatomy, Ger and Scothorne (1988) (‘What is Clinical Anatomy? Does it need, or deserve, a new journal?’) stressed how “developments in many branches of medical practice depend upon—and generate—greatly increased knowledge of specialized human anatomy”. They furtherly specified that “many surgical specialties rely heavily on a detailed knowledge of human topography”. In this sense, the new journal was “dedicated to the dissemination of new knowledge of all those aspects of anatomy of relevance to clinical science and practice”.

Nowadays, many different (although quite consistent) definitions of Clinical Anatomy are available also in web dictionaries:

– the practical application of anatomic knowledge to clinical problem solving, e.g., to diagnosis and treatment or in devising a surgical solution (mediLexicon International);
– the practical application of anatomic knowledge to diagnosis and treatment (Thesaurus).

The American Association of Clinical Anatomists states that “clinical anatomy is defined as anatomy in all its aspects - gross, histologic, developmental and neurologic—as applied to clinical practice, the application of anatomic principles to the solution of clinical problems and/or the application of clinical observations to expand anatomic knowledge”.

In the Intersocietal Symposium of the International Academy of Legal Medicine (Venice, June 2016), the European Association of Clinical Anatomy organized a Parallel Session entitled “Forensic Clinical Anatomy”. This event was the opportunity to discuss and define the role of Clinical Anatomy in Forensic Medicine and to define methodologies and fields of what we can call Forensic Clinical Anatomy.

24.2 Definition

Forensic Clinical Anatomy may be defined as the practical application of anatomical knowledge and methods (from ultrastructural to macroscopic aspects), endowed with substantial clinical/surgical implications, to the ascertainment and evaluation of medico-legal problems.

A first preliminary question to be considered could be the following: which is the anatomy of *relevance* in a forensic setting? The anatomy ascertained and evaluated in a forensic context must be considered with reference to the specific case, according to a definition of *individual* anatomy. Individual anatomy may be defined as the anatomy of that specific person considered in the particular moment of clinical and/or forensic relevance. In this sense, individual anatomy is given by the (statistically) normal anatomy and by all the factors which modify it, i.e., the presence of anatomical variations, age-related modifications (development, maturation, aging) and disease- or surgery-related modifications.

24.3 Methods

Consistently with the Ethos of the IALM Intersocietal Symposium—'P5 Medicine and Justice', awareness of the relevance of *Individual Anatomy* should be one of the principles guiding the Clinician to realize a truly *Personalized Medicine*; conversely, in the context of a *Personalized Justice*, medico-legal analyses cannot ignore the implications of individual anatomy in terms of ascertainment and evaluation.

In the application of the various methods of ascertainment, the identification of anatomical data of particular forensic relevance is quite frequent; be they normal or variant and/or of particular relevance for evaluation of medico-legal questions; these relevant morphological findings may require further specific analysis by the medico-legal consultant through various other methods. Conversely, we can also stress that many methods and techniques of forensic usefulness show sound anatomical/morphological bases and have sometimes been directly derived from anatomical research. In this paragraph, we will consider the relationships between methods of ascertainment and relevant individual anatomy from the point of view of Forensic Clinical Anatomy.

24.3.1 Analysis of Clinical Data and Consultation with Specialist

Analysis of clinical/documentary data must be performed in a critical way with reference to anatomical findings. In fact, relevant aspects of individual anatomy may be explicitly described in terms of certainty or only hypothesized in terms of probability or possibility. Moreover, in other cases, explicit references to relevant anatomical data may be missing, although in the presence of clinical or instrumental data of potential interest. This is the case, for instance, of an instrumental examination [Computed Tomography (CT), Magnetic Resonance (MR), etc.] with a radiological report not mentioning an anatomical variation, which is instead identifiable at imaging revision.

The analysis of clinical/documentary data in the case of dead persons does not significantly differ from the case of living persons. What are different are the following methods of ascertainment and, as a consequence, data must be examined keeping in mind which further methods of ascertainment can be applied (revision of imaging, consultation with specialist, pre-autopsy examination, autopsy, further diagnostic procedures). In this sense, there is a particular need for timely analysis of clinical/documentary data in order to permit consistent planning of the other ascertainment steps. This aspect is particularly important if implications of Forensic Clinical Anatomy are present. The potential relevance of anatomical data must be rapidly focused on the examination of clinical/documentary data that permits the planned revision of the imaging, the consultation with a specialist and the examinations before autopsy.

From the point of view of Forensic Clinical Anatomy, the consultation with a specialist may help in confirming explicit anatomical description or in the better focusing relevant anatomical questions. For instance, a radiologist and/or anatomist co-consultant may permit more accurate detailing of anatomical data from instrumental examination or a surgical co-consultant may formulate further considerations about clinical/surgical implications of the individual anatomy. Particular attention must be paid to the correctness of nomenclature used in clinical documentation in order to avoid confusion. For instance, in a judicial case of death due to haemorrhagic infarction of the cerebellum, the CT angiography report in the clinical documentation made reference to a “suspected double superior cerebellar artery”, but our imaging revision came up with a different diagnosis, i.e., the ‘double origin’ of superior cerebellar artery. This obviously permitted the planning of a focused neuropathological examination which confirmed the diagnosis [1].

24.3.2 Clinical Examination

Clinical examination is another fundamental step in which data about individual anatomy may be assessed. In the clinical examination it is possible to verify anatomical variations or other relevant anatomical data already cited in clinical/documental data; on the other hand, relevant anatomical findings may arise for the first time on this occasion. The clinical examination consists of anamnesis and physical examination. In anamnesis, it must be verified if communications have been provided to the patient regarding the identification of relevant anatomical data by the physicians.

The physical examination may permit the direct identification of anatomical variations at the level of various structures, such as skin, bones, muscles or natural orifices. Among bone anatomical variations we can cite the humeral supracondylar process (also called supra-epitrochlear, epicondylar or epicondylic process), which is sometimes palpable as a mass on the upper arm. It is a bony spine of variable dimensions projecting from the humeral anteromedial surface and frequently joined to the medial epicondyle by a fibrous band, the ligament of Struthers. Its incidence has been reported to vary from 0.1 to 5.7%. Its importance relies on the fact that, although usually clinically silent, it may cause compression of the median nerve and brachial artery [2–4]. Examination of the oral cavity and isthmus may show the presence of a high-rising epiglottis [5–7], a variant structure with possible clinical and forensic implications due to problematic intubation. Neurological examination may also permit the identification of variations in the territories of some nerves.

Apart from direct identification, anatomical variations may sometimes be hypothesized on the basis of the integrated clinical picture, derived both from medical documentation and clinical examination.

24.3.3 Further Diagnostic Examinations on Living Persons

In the general application of methods of ascertainment on living persons, if the available data are not sufficient for detailed diagnosis the possibility of further diagnostic exams can be considered. This may also be possible if the existence of relevant individual anatomy derives from the clinical/documental data and/or clinical examination (or it may be hypothesized on their basis), but further detailing is needed. In these cases, a careful evaluation of the cost/benefit ratio must be performed, with reference to invasiveness and economic costs. A detailed informed consent document must also be compiled, specifically addressing the reasons of prescription and risks connected with the procedure. Imaging techniques are obviously the most frequently involved methods permitting identification of anatomical variations or assessment of other relevant anatomical data. Electromyography analyses, usually involved in detailing iatrogenic nerve lesions, may also add important data regarding nerve territories, which quite frequently show some amounts of variability.

24.3.4 Pre-autopsy Examinations

Pre-autopsy examinations include a wide series of imaging techniques (selective X-rays, CT, MR, Post-Mortem CT angiography, 3D surface scanning/ photogrammetry, integrated CT/MR and surface scanning, etc.), which are quite frequently used to preliminarily verify the occurrence and characteristics of traumatic findings and natural pathologies. A huge amount of literature and excellent up-to-date reviews are present on the matter [8–14].

With regard to Forensic Clinical Anatomy, it must be considered that nowadays the above methods permit the acquisition of the smallest anatomical details, being particularly useful also for assessing relevant individual anatomy. For instance, multiphase post-mortem CT angiography makes it possible to follow the perfusion of even the smallest vessels. Thus, relevant anatomical variations may be identified at pre-autopsy imaging examinations.

In our experience, data concerning relevant anatomical variations or anomalies may be indirectly derived from pathological findings. For instance, a preliminary post-mortem CT performed on a putrefied body showed calcifications at the level of the aortic valve, pathological alteration, which is frequently correlated with the bicuspid valve (Fig. 24.1). This anatomic anomaly was then confirmed at the cardio-pathological examination (unpublished case).

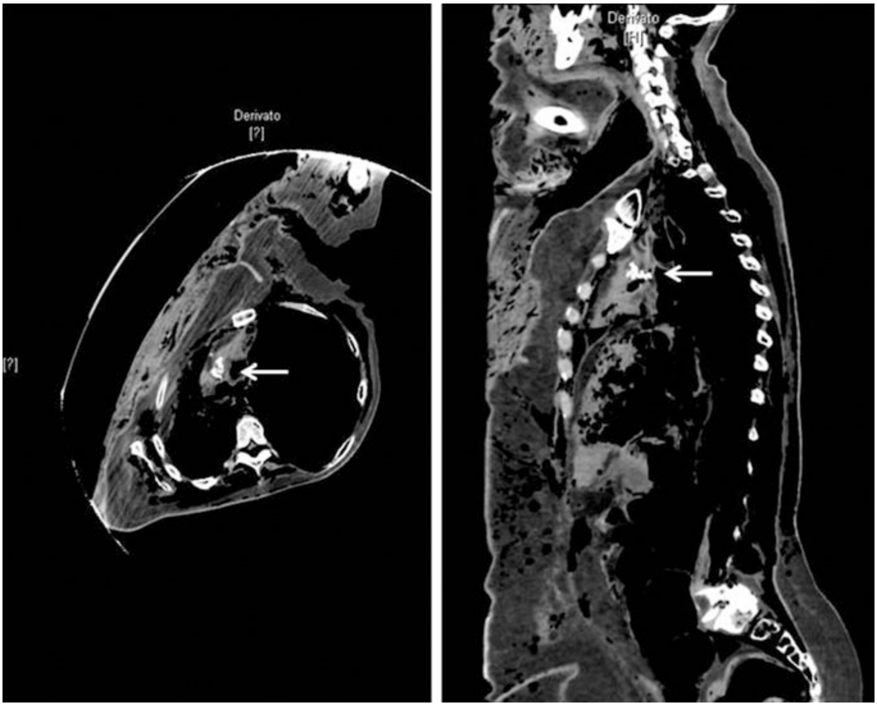

Fig. 24.1 Pre-autopsy total body computed tomography showing calcifications at the level of aortic valve (*arrows*). Cardio-pathological examination confirmed the hypothesis of bicuspid aortic valve

24.3.5 Judicial Autopsies and Anatomical Dissections

The main principles and procedures of judicial autopsy are indicated in "Recommendation n. R (99) 3 of the Committee of Ministers to Member States on the Harmonisation of Medico-Legal Autopsy Rules" [15]. Although the detailing of all anatomical data of forensic relevance is implicitly included in correct procedures of judicial autopsy, we may also consider that judicial autopsies and anatomical dissections are in general characterized by different aims and methods. Anatomical dissections, which are usually performed for anatomical education or research, are specifically focused on the analysis of normal structures, also with reference to possible variations. Identification of variant anatomical data in the course of judicial autopsies is quite frequent and does not usually require specific approaches. In other cases, instead, complex anatomical patterns, preliminarily reported/identified in clinical/documental data or pre-autopsy examinations, require the integration of the common procedures of judicial autopsy with dedicated dissections specifically aimed at anatomical analysis. In this sense, the integrated analysis of clinical/ documental data and pre-autopsy examinations assume even greater significance in view of a correct planning of autopsy procedures, although the possibility/opportunity to modify procedures in response to new relevant data must always be kept in mind, even in the course of an autopsy.

24.3.6 Post-mortem Analyses on Autopsy Samples

Many further diagnostic procedures may also be applied after autopsy to better define relevant anatomical findings. Post-mortem analysis of single organs or structures through imaging techniques, such as X-ray, CT, MR, angiography (eventually with casts) and micro-CT, is frequently involved in forensic pathology and may also give further information regarding anatomy. For instance, we have recently performed a CT analysis of a larynx *in toto*, sampled at autopsy, in order to better analyse two processes symmetrically arising from the posterior borders of the laminae of the thyroid cartilage. Also on the basis of imaging results, it was possible to definitely ascribe the findings to anatomical variation without pathological significance (Fig. 24.2). In another judicial case, micro-CT on lung samples permitted an improved characterization of the three-dimensional distribution and anatomical localization of diffuse pulmonary ossification in a case of permanent vegetative state [16].

Plastination is a typical anatomical method to preserve viscera and structures. It was originally developed for anatomical education, but in our experience it may

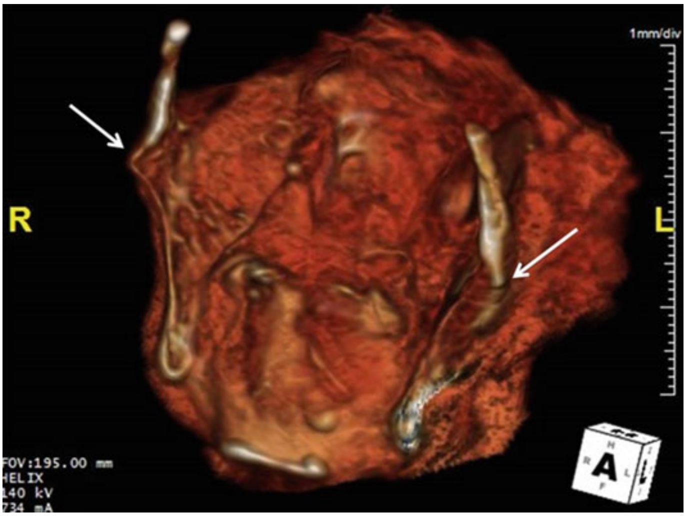

Fig. 24.2 Computed tomography of larynx *in toto* sampled at autopsy (posterior oblique view). An anatomical variation may be identified consisting in two processes (*arrows*) arising from the posterior border of the laminae of the thyroid cartilage

also be useful in forensic contexts. In a case of homicidal gun-shot wound to the head, the brain was plastinated in order to permit a better evaluation of the three-dimensional disposition of the pathway (Fig. 24.3 unpublished case). Plastinated specimens may also be used for direct demonstration in the judicial context.

Microscopic analysis of autopsy samples may also help in the identification of the nature of some findings. There are many variant anatomical findings which must be put in differential diagnosis with pathological or traumatic ones and microscopic examination is frequently useful for this purpose. For instance, microscopic examination may permit the differentiation of a Mongolian spot from an ecchymoses [17] and accessory skull sutures from skull fractures [18].

Morphometric and stereological approaches may also be useful, or even necessary, to objectively define some developmental, anatomic or pathological findings of forensic significance. These methods have usually been derived from research in normal anatomy and permit the reliable detection of small changes. In our experience, we have applied morphometric methods to demonstrate changes in the connective and parenchymal tissues of carotid bodies of opiate-related deaths or aged persons [19, 20], or to verify morphometric parameters of brainstem nuclei in normal and SIDS cases [21, 22].

Three-dimensional reconstruction of microscopic findings through image-analysis software may also permit to better detail anatomical data of forensic significance. Although not frequently used in forensic pathology, it has been widely involved in morphological and developmental research. Our research group, for instance, has applied methods of three-dimensional reconstruction to show the

Fig. 24.3 Horizontal section of a plastinated brain showing the pathway of a gun-shot wound

development of hindbrain vasculature [23], the organisation of the area postrema [24] and the disposition of the collagen bundles in fascial structures [25]. Regarding specific applications in Forensic Clinical Anatomy, contemporary reconstructions of pathologic findings (hypoxic-ischaemic lesions) and normal structures (solitary tract) have permitted the specific localization of lesions in the solitary tract nuclei along all the extension of the nuclei themselves [26]. Disorganization in the three-dimensional disposition of the connective tissue of the carotid body has also been shown in opiate-related deaths [19] and the terminal pathway of a heart stab wound has also been reconstructed in the anatomical context of muscle bundles and blood vessels, giving a detailed view of the local anatomy [27].

Genetic examinations may also play a role in the clinical and forensic assessment of some anatomical variations, which may posit the hypothesis of an underlying genetic syndrome. These analyses may be requested in the case of living persons (in the presence of detailed informed consent) or dead bodies.

24.4 Fields

Implications of Forensic Clinical Anatomy may invest various fields of Forensic Medicine, as individual anatomy frequently acquires relevant significance in Forensic Pathology and Clinical Legal Medicine and morphological methods, derived from normal anatomy research, are frequently applied. The following fields may be preliminarily identified as the richest in terms of Forensic Clinical Anatomy implications.

24.4.1 Child Abuse

In Child Abuse (in all its forms), problems of differential diagnosis between anatomical structures (normal or variant) and pathological findings frequently arise. For instance, Mongolian spot may be included in the anatomical variability of the skin in a developmental context, but this condition may be difficult to differentiate from ecchymoses, both in clinical and necroscopic contexts [17]. In Fig. 24.4, we show a hyperpigmeted area in a 1-month-old child found dead in the morning in the parents' bed; sampling of the area and microscopic examination excluded ecchymosis and permitted the diagnosis of Mongolian spot (unpublished case). Additional variant skull sutures may also be in differential diagnosis with abuse-related skull fractures [18].

In the wide field of Child Abuse, studies of Functional and/or Biomechanical Anatomy have also been performed on cadavers to verify the compatibility of various lesions with accidental dynamics. For instance, studies involving pediatric cadaver drop tests are present in literature, for better evaluation of the forces needed to produce skull fractures [28–30].

Fig. 24.4 Hyperpigmented area in a 1-month-old child found dead in the morning in the parents' bed. Sampling of the area and microscopic examination excluded ecchymosis and permitted to formulate diagnosis of Mongolian spot

Some kinds of damages following Child Abuse are also strictly anatomical in nature and require morphological/morphometric methods of investigation for adequate assessment. For instance, structural gray and white matter changes have been reported through imaging techniques in subjects who experienced childhood trauma, with and without post-traumatic stress disorders, as a result of plasticity response of the central nervous system [31].

24.4.2 Sudden Death

In general, cases of Sudden Death frequently require detailed anatomical studies aimed at verifying the possible existence of anatomical situations or abnormalities causing an underlying vulnerability to external stressful stimuli. In the triple risk model for Sudden Infant Death Syndrome, for instance, an underlying abnormality (nervous system, heart) makes the infant more vulnerable to a stressful environment (prone position, smoke exposure, etc.) during a critical developmental period (from one month to one year of age) [32].

The potential role of developmental abnormalities of brainstem nuclei and peripheral chemoreceptors, such as carotid body, has been ascertained through various morphometric approaches in the literature and also by our research group. [21, 33–35]. These studies have also stimulated the interest (also by our group) in research concerning normal morphometric parameters of brainstem nuclei across different ages [34]. This is an example of how pathological and/or forensic questions may stimulate research in normal anatomy.

24.4.3 Medical Responsibility and/or Liability

Implications of Forensic Clinical Anatomy are frequent and highly significant also in the analysis of suspected Medical Responsibilities and/or Liabilities.

Specific European Guidelines have been recently released regarding methods of ascertainment and criteria of evaluation in Malpractice and Medical Liability [36, 37]. Individual anatomy may acquire specific significance along the application of the various steps of analysis:

(1) how aspects of individual anatomy (of relevance from a forensic point of view) may arise from application of methods of ascertainment, and how they may be further ascertained through specific anatomical methodology;
(2) how data regarding individual anatomy, once fully ascertained and consistently discussed in light of pertinent scientific knowledge, may help in the correct application of the criteria of evaluation and in the final judgment in relation to identification of profiles of Medical Responsibility/Liability.

24.4.3.1 Methods of Ascertainment

Concerning methods of ascertainment in claims for Medical Responsibility and/or Liability, we can make general reference to the previous paragraph relating to Methods. A particularly critical approach would be necessary, however, to verify if the ascertainment of individual anatomy, as derived from clinical documentation, was completed or if further analyses by the medico-legal consultant are needed. It is also pivotal to verify if explicit references to anatomical variations or other relevant anatomical data are present in the informed consent documents, in terms of diagnosis of actual findings or risk of occurrence, with exposure of possible influences on the outcome of procedures/therapies.

24.4.3.2 Evaluation Criteria and Individual Anatomy

In the medico-legal analysis of Medical Liability cases, the phase of ascertainment is followed by the assessment of a series of evaluation criteria which have been widely exposed in European Guidelines [37]. In synthesis, and with reference to the analysis of cases with particularly relevant anatomical basis, the following main steps may be identified.

- Reconstruction of the Physio-Pathological Pathway
- Identification-Evaluation of Errors
- Discussion of Causal Value
- Damage estimation

Reconstruction of the Physio-Pathological Pathway

After identification of the pathological features, which can usually be subdivided into initial, intermediate and final clinical pictures, all of the physiopathological links must be derived on the basis of consistent and up-to-date literature on the matter. This must lead to a comprehensive reconstruction of the physio-pathological pathway along its entire course. This criterion is still focused on the *biologic* (more than legal) aspects of the case.

If anatomical data of forensic relevance have been ascertained, their role in the physio-pathological pathway must be specifically considered and discussed (*Anatomo*-Physio-Pathological Pathway). The question to be answered is the following: what was the role of individual anatomy in realizing the final damage (chronic pathological state, permanent injury, death)? For instance, with regard to the above case of double origin of the superior cerebellar artery, the occurrence of a haemorrhagic infarction in the corresponding vascular field was intriguing in suggesting a possible Anatomo-Physio-Pathological association. Although associated with long term compensation, the double origin of the superior cerebellar artery may have represented an anatomic situation of haemodynamic instability, also due to the small distance between the two origins and small diameters of the two vessels [1].

Iatrogenic lesions may also be favoured or biologically caused by an underlying anatomical variation or by local modification of anatomy due to preceding surgery (e.g., peritoneal adhesions after general or gynaecological surgery). In these cases, the medico-legal consultant should consider, for instance, if the iatrogenic lesion would have occurred in the presence of an usual anatomical context and in which terms the individual anatomy increased the injury risk. For instance, in a judicial case of femoral nerve injury due to trocar insertion in a too caudal position in the lower abdominal quadrant, specific considerations were formulated regarding the possible favouring role of peritoneal adhesions due to preceding surgical hysterectomy [38].

Identification-Evaluation of Errors

A further step of analysis involves the evaluation of the medical conduit, i.e. the identification and evaluation of errors. On the basis of the ascertained data and literature on the matter, the *ideal* medical conduit should be reconstructed, i.e., the conduit which the physicians should have followed in terms of diagnosis, prognosis and treatment. Moreover, the *real* medical conduit should be carefully reconstructed, mainly on clinical data, and compared with the ideal one. Errors may be mainly identified as contradictions between real and ideal conduits.

In a context of Forensic Clinical Anatomy (frequently involving the occurrence of iatrogenic lesions in normal or variant anatomical settings) typical professional errors may consist in:

- insufficient knowledge of normal anatomy,
- failure in the identification of relevant variant anatomical data,
- omitted consideration of the possibility of a variant anatomy,
- inadequacy in management of an ascertained or supposed anatomical situation.

The judgment about the above possible errors must also be formulated on the basis of *ex-ante* evaluation about the presence of eventual causes of justification (justifiable errors). For a comprehensive evaluation of the above aspects, significant information may be derived from various scientific sources, in terms of:

- prevalence of anatomical variations or modifications (meta-analysis works),
- technical possibility of their identification,
- risk-benefit ratios (complication risks, economic costs, …) of diagnostic procedures.

Discussion of Causal Value

The identification and evaluation of what we can call "anatomy-related" errors imply a following discussion about their causal value. In this sense, the medico-legal expert must evaluate what was their role in the determination of the final damage. The causal value of an error (and obviously also of an anatomy-related error) must be evaluated on the basis of a criterion of scientific probability, including universal law, statistical law or criterion of rational credibility [37]. Further evaluation of the causal value and causal link between error and damage must be performed through counterfactual reasoning, which in the specific context of Forensic Clinical Anatomy may be summarized by the following question: if the individual anatomy had been correctly identified, considered, and managed (or if anatomy-based errors had not occurred), would the damage have been prevented? And with which probability? Obviously the most useful scientific sources for this evaluation are research papers and case histories detailing the efficacy of diagnostic procedures and therapeutic approaches performed in the presence of adequate knowledge of underlying anatomy.

Damage Estimation

Individual anatomy may biologically determine the extent of damage. For instance, a nerve injury could imply a more severe damage if that nerve shows a wider territory of innervation for an anatomical variation. This aspect of evaluation, as already stated, mainly invests the biological aspects of damage, and should be included in the reconstruction of the physio-pathological pathway, with particular reference to the final clinical picture.

Further and different evaluation, instead, must address the quantification of damage (temporary and/or permanent biological injury) causally related to errors. In other words, the medico-legal consultant must specifically evaluate which part of the whole damage must be ascribed to inadequate medical conduit. With reference to anatomy of forensic relevance, it is possible that ignorance of an anatomical variation (anatomy-related error) may worsen a damage which would otherwise be lighter and (eventually) justified. The comparative evaluation must obviously be performed on the basis of specifically anatomical considerations.

24.4.4 Personal Injury and Damage

Specific Guidelines created by the International Academy of Legal Medicine have recently been released regarding methods of ascertainment and criteria of evaluation of personal injury and damage under civil-tort law [39]. Obviously, individual anatomy (anatomical variations, developmental anatomy, disease/surgery-modified anatomy) may acquire specific importance for the definition of personal injury and damage. Although injury/damage may derive from medical malpractice (see previous paragraph), in the present section we will mainly consider injury/damage from other types of traumatic events, such as traffic, sport-related, work-related, or domestic accidents.

Implications in terms of Forensic Clinical Anatomy in the methods of ascertainment have been previously considered in the paragraph entitled ‘Methods’. Concerning the field of personal injury and damage, we can stress the relevance of clinical/documental data to clarify the mechanism of injury, which is also necessary to understand the eventual role of individual anatomy in the production of injury/damage.

In the medico-legal analysis of cases concerning Personal Injury and Damage, the following evaluation criteria [39] have been identified.

- Verification of maximal medical improvement/stabilisation
- Clinical and medico-legal epicrisis
    - (a) Pre-existing health status
    - (b) Reconstruction of the damaging event
    - (c) Identification of physio-pathological features
    - (d) Identification of injury, temporary and permanent impairment
- Causal value & link
- Impairment & Disability description.

As it regards Forensic Clinical Anatomy, it must be stressed that specific definition of the individual anatomy is of particular relevance for an adequate evaluation of the pre-existing health status, which is necessary in order to detect any changes which occurred as a result of the damaging event.

Individual anatomical data are also usually relevant for the identification of the physio-pathological features, on the light of (biomechanical) reconstruction of the damaging event.

The role of individual anatomy must also be specifically considered in the evaluation of the causal value and description of impairment and disability.

24.5 The Role of Body Donation Programs in Surgical Training and Innovation—Forensic Implications

An important aspect of Clinical Anatomy includes the role of Surgical Training and Innovation with anatomical material. We have developed a specific experience on the matter thanks to our Body Donation Program [40–42]. The Body Donation Program for Anatomical Education and Research of the University of Padova was developed in the early 2000s and received formal approval from the Council of the Faculty of Medicine and Surgery in 2003. A Quality Management System has also been developed for optimal use of anatomical material. In 2011, the Body Donation Program achieved certification by an accredited third-party registrar (Certiquality Srl©, Quality Certification Body, Milan, Italy), which audited the quality management system and certified that the Body Donation Program of the University of Padova met EN ISO 9001:2008 criteria [42]. Using cadavers for medical training and scientific research is carried out according to European, Italian and regional guidelines [40, 43].

Many authors have emphasized the importance of human cadavers for training in open surgery and laparoscopy, also with respect to virtual simulators and animal models. It has been stressed that simulators obviously do not show anatomical variability or pathological alterations which represent unexpected difficulties in surgery [44]. Live animals show anatomical differences with respect to humans and some legislations greatly limit the possibility of performing surgical training on them [44, 45].

In collaboration with Surgeons of our University, we have developed in cadavers innovative approaches of Natural Orifice Transluminal Endoscopic Surgery (NOTES), such as transanal ileoproctostomy [46]. In our experience, we have also developed and verified surgical techniques on pig experimental models [47] but fresh or minimally embalmed (Thiel's method) cadavers show significant advantages in terms of anatomy [48]. The important role of cadavers with respect to animals has been stressed by many other authors [49, 50], although integration between cadaver and animal models probably represents the gold standard, allowing detailed knowledge of human surgical anatomy and the correct management of surgery on living subjects [48].

These aspects and activities of Clinical Anatomy and Body Donation Programs imply forensic implications have not yet been adequately evaluated in the literature. Claims of Medical Liability may be addressed to Hospitals also on the basis of insufficient training of (Resident or Specialized) Surgeons; the absence of training programs on cadavers may support such claims. Moreover, surgical failure or iatrogenic injuries in the application of new techniques or materials may also be ascribed to Medical Responsibility in the absence of adequate preliminary testing on cadavers. Apart from the above implications on the matter of Medical Responsibility and/or Liability, it must also be considered that cadavers of Body Donation Programs may also be used to verify and demonstrate anatomical basis of some lesions in various forensic contexts. For instance, our Body Donation Program has been involved in an analysis of possible injury mechanisms in the Turin Shroud Man on (practically verified) anatomical bases [51, 52]. In a claim for Medical Responsibility concerning duodenal perforation in the course of robotic laparoscopic prostatectomy, we have also used cadavers of our Program to demonstrate and show, with photographs and videos, the anatomical impossibility of the lesion if ascribed to direct mechanical injury (unpublished case).

24.6 Conclusions

In the present work, methodological/criteriological aspects have been considered with reference to Forensic Clinical Anatomy. The relevance of individual anatomy has been stressed for a personalized approach to medicine and justice. Insufficient awareness of anatomical variability and of clinical/instrumental potentialities in detailing individual anatomy may affect the personalization of *clinical approaches*. Conversely, the identification of relevant anatomical findings in the *medico-legal analysis* should involve the integration of methods of ascertainment with anatomical methodologies and the discussion of evaluation criteria in the light of up-to-date anatomical literature. In literature concerning *radiologic, clinical, and surgical anatomy*, methods and findings are discussed with reference to clinical/surgical implications, but forensic implications (although of potential interest) are frequently overlooked. A better awareness about the forensic relevance of some clinically-oriented anatomical data may also positively invest the research on radiologic/clinical/surgical anatomy.

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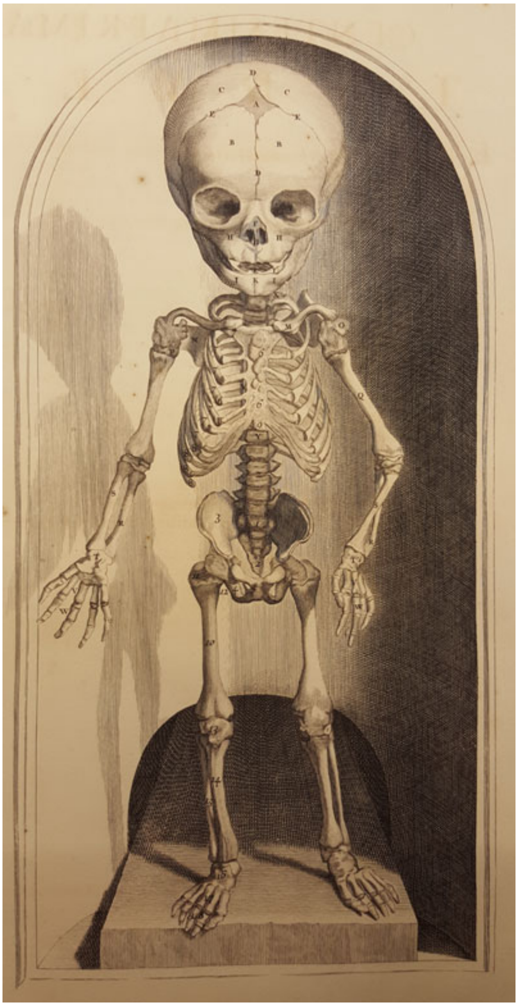
Part VII
Innovation, Unitariness and Evidence. Clinical Legal and Forensic Medicine in Living Person Personal Identification and Age Estimation



Bidloo, Govard. Anatomia humani corporis centum & quinque tabulis, per artificiosiss. G. de Lairesse ad vivum delineatis, demonstrata, veterum recentiorumque inventis explicata plurimisque, hactenus non detectis, illustrata Amstelodami: Sumptibus viduae Joannis à Someren, haeredum Joannis à Dyk, Henrici & viduae Theodori Boom, 1685

Skull *Source* Photo by ECunha; Body *Source* INMLCF, IP.

Chapter 25
Historical Routes and Current Practice for Personal Identification

Eugénia Cunha and Cristina Cattaneo

Abstract This article is about identification of cadavers and human remains, focusing mainly on the more accepted methods to establish identity. Emphasis is provided on the methods applied to bodies in an advanced state of decomposition and/or skeletonized and the contribution of each sub-discipline in the field of identification, including anthropology, odontology, genetics, imagiology and dactiloscopy. Some insights on the history of personal identification and emerging issues and future perspectives are discussed, such as the need of implementation of best practices to identification and the comparative phase of identification. To do this, is of paramount importance that all the countries adopt a same policy to collect ante and post mortem data, which has been claimed by all international agencies dealing with this subject.

25.1 Introduction

Generally, identification standards are classed as primary and secondary, for example by Interpol.

> *No man can cross the same river twice,*
> *because neither the man nor the river can remain the same*
> Heraclitus

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25.1.1 On the Historical Routes of Identification and Some Preliminary Notions About This Concept

Identification of human remains has always been a key issue for human societies. Yet, today, identification is more fundamental than ever and a proof of identity is mandatory. Criminal investigations, unexplained deaths (homicide, suicide), unidentified deceased in open investigations, accidents and mass disasters, as well as genocides and war crimes are the main events where identification is needed. These different scenarios well illustrate the impact of identifying the dead: humanitarian, administrative and judicial. Besides, the right of human beings to keep their identity after death is nowadays universally recognized [1, 2]. The enormous fleets of people who go missing is a worldwide problem [3, 4]. More recently, the increasing awareness about identification is mainly due to the unprecedented humanitarian tragedy of unidentified bodies, with emphasis on dead migrants [4, 5].

25.1.1.1 The Concept of Identification

Greek philosophers such as Aristotele (year of death: 322 a. C) and Heraclitus (year of death: 475 a. C) were probably the first to reflect about identity. The etymology of the word comes from the latin "idem" which means "the same" [6]. Much later, another philosopher, Leibniz (XVII and XVIII), proposed a law which could only be true if time did not imply any type of change. Indeed, a biological entity does not remain the same through time. Thus, as Black [7] points out, the key question is how much change an identity can tolerate. Yet, although we grow and alter with time, on the other hand there are some parameters that do not change with time. These later ones will be the most valuable ones for identification.

Historically, it is curious to mention that one of the first methods accepted for identifying criminals, within the so-called criminal anthropology, was Bertillon's anthropometric method based on body measurements which he proved to be immutable. Bertillon (end of 19th century) is known as the precursor of anthropometric identification, a method that prevailed for a while because of the absence of other successful alternatives. Anthropometry was the first scientific system used by police to identify criminals and the mug shot became well known. By the end of the 19th century the works of Galton (end of 19th), Edward Henry (early 20th) and Vucetich (end of 19th) put fingerprints definitively into the limelight [8]. Much later, a big step forward was provided with the application of DNA profiling techniques. In 1992, this technique was applied to confirm the identity of skeletal remains of Josef Mengele [9].

It is important to be aware of the difference between identification and recognition. While the first is based on scientific methods, the second is empirical. To recognize is to acknowledge that the person we are looking at is someone we know. This can, however, imply pitfalls. The well-known example of Marilyn Monroe is a good example: the recognition of her fresh cadaver would hardly have been accomplished by someone who knew her, despite her popularity, because of post mortem alterations.

Besides the clarification of the concept of identification (i.e. what is it), preliminary questions about identification are also why, how and when to identify.

There are a series of requirements, both biological and technical, that a sound identification method should meet: they should be based on features which should be unique and immutable. Besides, technically, the methods have to work and be easy to apply, i.e., practical and easy to classify.

Whatever the identification method, this process is mandatorily a comparative one, that is, no matter what, identification always implies the comparison of two types of data, ante mortem and post mortem. Without a comparison no identification can be accomplished.

Moreover, what are the possible results of an identification exercise? Since it is not always possible to achieve a positive ID (identification), the results can be categorized as follows: positive or confirmed ID, possible or presumptive ID, exclusion, inconclusive.

Collective or contextual identification are concepts discussed by Kimmerle et al. [10], which places an individual into a group.

Interpol classifies the possible results as follows:

Identification (absolute certainty the PM and AM records are from the same person).

Identification probable (specific characteristics correspond between PM and AM but either PM or AM data or both are minimal).

Identification possible (there is nothing that excludes the identity but either PM or AM data or both are minimal).

Identity excluded (PM and AM records are from different persons).

Insufficient evidence (neither PM nor AM comparison can be made).

25.2 How to Identify

Identification methods are classified in primary and secondary. Primary identifiers are the most reliable methods to confirm an identity and they include ‘Friction Ridge Analysis’, ‘Forensic Odontology’ and ‘DNA’, which are normally represented by the universal symbols on Fig. 25.1.

Secondary identifiers (Fig. 25.2), in theory, may themselves not be enough to prove identity, although this is becoming more and more heavily disputed in the scientific scenario. Yet, in combination, they may give sufficient information to ensure identification in selected cases. Moreover, in the cases where none of the primary identifiers are available they may be the only resource for identification. Again, this implies that medical findings and information on clothes and personal belongings are given for the missing persons (ante mortem data).

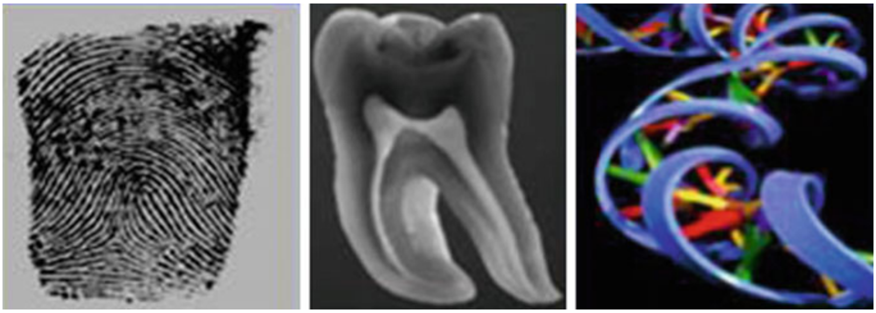

Fig. 25.1 Primary identifiers, friction ridge analysis, forensic odontology and DNA (Interpol)

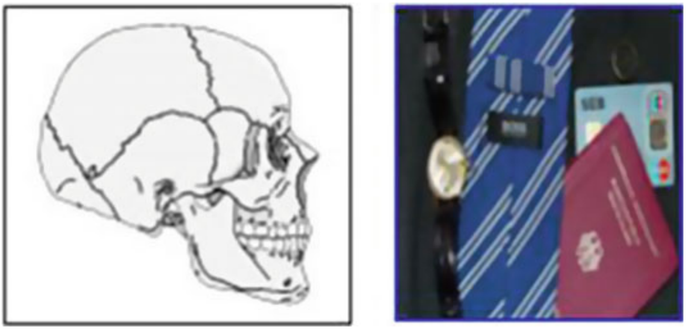

Fig. 25.2 Secondary identifiers: personal data/medical findings; evidence/clothing (Interpol)

An identity can be achieved on the basis of a combination of features and, on the other hand, an exclusion can be made on the basis of mismatching features.

Since identification is a multidisciplinary process, different types of evidence contribute to the final result. Some of this evidence is tackled in this chapter, with a particular emphasis to forensic anthropology. The state of decomposition of the remains dictates the type of methods that can be applied. In general, the longer the time since death, the more probable it is that forensic anthropology will be involved. In other words, there are techniques more adequate to fresh cadavers, such as friction ridge analysis or facial comparison/superimposition, whereas others are more suitable for decomposed bodies, like anthropology.

Another very relevant aspect which also dictates the techniques to be applied is where there is, or not, a previous suspicion of identity of the victim under forensic analysis. There are cases with suspicion and others where there is no idea at all about the identity of the victims. Since all the techniques invariably imply a comparison between ante and post mortem files, in the cases without the suspicion of identity DNA cannot work since there are no samples to compare it with.

25.2.1 Fingerprints: Dactiloscopy

The uniqueness and immutable character of fingerprints makes them one of the more widely used methods to identify cadavers which still retain the papillary skin in a reasonable state of preservation. Apparently it is the only identification method which fulfills all the technical and biological aforementioned requirements and can be applied independently of the age of the individual since prints are created during fetal development. Galloway and Charlton emphasize one of its great advantages: "they are unique even in identical twins, unlike DNA" [8].

The origins of modern finger printing go back to the last two decades of the 19th century. Sir W Herschel was the first to realize the individuality of fingerprints [8]. A publication in Nature by Faulds in 1880 is a remarkable milestone. Galton, also from the 19th century, can be considered a pioneer of personal identification, being the first researcher to prove the immutability of fingerprints. Fingerprints quickly surpassed anthropometric systems [8].

Some benefits of friction ridge are worth mentioning: they are easy to recover and are an inexpensive procedure. Moreover, there are AM databases in many countries. In some countries, the official identity document includes a record, becoming an important biometric identifier. Nowadays, they are recorded by means of digital technology, namely fingerprint scanning.

In mass disasters, dactiloscopy has been playing a major role in the identification of the victims, as in the tsunami in Southeast Asia in 2004. In the initial stages of the identification process it is indeed a very useful procedure for confirming the identity of the victims recognized by their relatives or to start the identification process itself.

25.2.2 Forensic Genetics

25 years ago Hagelberg and collaborators wrote in Nature that "There is considerable anthropological and forensic interest in the possibility of DNA typing of skeletal remains [11].

In the UK, the first time that the DNA profiling technique was applied to paternity testing was in 1985. Only after was it applied to criminal cases. In 1992 DNA was used to verify the identity of some skeletal remains from Argentina as those of Josef Mengele [9]. It introduced a period where everything could be expected from genetics: the panacea period. A quarter of a century later, one of the biggest misconceptions is still that DNA solves everything.

Above all, there are no large DNA databases which guarantee that a DNA profile from a victim can be compared with a missing person, as the majority of DNA databases include mainly offenders. Furthermore, although DNA can be taken from all kinds of remains, sometimes the quality of DNA taken from bones is not enough. Thus, it is worthwhile to devise strategies which combine forensic anthropology, odontology and forensic genetics [12]. In many cases, forensic anthropology significantly decreases the number of suspects for a possible match. “Presorting based on biological profile reduces the number of comparative samples to be obtained and analyzed” [12]. This is particularly helpful in mass disasters and mass graves with commingled remains.

On the other hand, one of the most positive aspects of this technique is that, as dactiloscopy, it quantifies the results, which makes it much more objective and accurate. It must also be said that DNA matching is the best way to identify body parts. The never-ending advances in genetics have been the accomplishment of better results from degraded and fragmented evidence, but always with the premise that ante mortem data is available for comparison.

25.2.3 Forensic Odontology

The context of forensic dentistry is becoming wider: besides teeth, the lips, by means of cheiloscopy, which is based on the uniqueness of lip prints, and the palate, specifically the palatal rugae pattern (rugoscopy), are new markers for forensic identification. The overall stability of both lip prints and palatal rugae, make them useful for forensic identification. But the vast majority of identifications performed by forensic dentists are done on the basis of teeth, particularly clinical data though not only (for example photosuperimposition of dental profiles is a well known method of identification).

The existence of antemortem dental records is still very diverse around the world: whereas in some countries there is prevention of dental pathologies with free consultations until adulthood, in others, a vast majority of the individuals have never been to a dentist. In other words, the utility of odontology to provide a PI varies a lot between very developed countries and 3rd world countries. This asymmetry is also reflected in the lack of uniformity in both the codification and completeness of dental features, despite the great efforts of Interpol in that sense.

The multitude of dental treatments have turned forensic odontology into a very specific discipline implying the presence of specific experts in the DVI teams and Legal medicine Institutes. Likewise, ID can only be achieved by a comparative analysis, which can be performed either with clinical ante mortem data or dental X rays [13].

Yet, in cases where the teeth / or mandible are missing postmortem, or dental am data is not available, forensic dentistry is useless. No matter what, a dental record is of no value until a possible identity has been achieved. On the other hand, in the cases of suspicious identity, or in close mass disasters, odontograms are of paramount importance to identification.

25.2.4 Forensic Anthropology

When the physiognomic traits are no more available to enable recognition or identification, a forensic anthropologist is usually called to identify the remains. That is, the longer the PMI (postmortem interval), the more probable it is that forensic anthropology is involved in the expertise. In these bodies the skin is no longer preserved, precluding the application of dactiloscopic techniques. Furthermore, in many instances there is no identity suspicion. Thus, the only solution is to commence, through the generic identity factors, the so-called biological profile. An osteological profile is just a generalized descriptor of an individual, but is an essential step of the reconstructive identification procedure. With the biological profile- sex, age, ancestry and stature (with some caution)- exclusions can be accomplished and thus the possible matches are considerably narrowed down. In this regard, the increasing incorporation of statistical methods which allow the quantification of each of these parameters has provided more clear-cut results [14]. The second step of the anthropological reconstructive process of identification is to search for identity factors, the ones that make the remains unique.

25.2.4.1 The Uniqueness of Bones

In what is the skeleton unique? Fortunately, bones might be unique in several parameters/aspects which will be summarized here.

Firstly, the pattern of the trabecular bone is unique, visible on radiographs and suitable for unique individualization [15, 16]. As such, every bone can be used to prove an identity with the advantage that even fragmentary bone remains can allow a PI.

Then, the spectrum of anatomical variants is quite wide. The frequencies of these morphological variants are helpful for the purpose of ID, since the rarer a feature the more it can contribute to ID. The knowledge of their inheritance can also help to narrow down the possible matches. The features on the skull and chest will have more probability of contributing to ID, since these are the most radiographed regions, allowing ante and post mortem comparison. Examples can be given by sternal perforation, wormian bones, metopic suture and variations of the xiphoid process, among others. One should bear in mind that each of them could lead to ID if an antemortem record exists.

Within ante mortem variations, besides the morphological ones, we have the unique medical features which have a great potential to provide ID. Despite the fact that the skeleton is normally one of the last organic systems to react to aggression, it can be affected by various types of aggressions: bacterial, viruses, physiological and physical are some examples. Even if pathognomonic lesions are rare, the type and pattern of lesions can enable the diagnosis of some diseases. This is the case of some infectious (tuberculosis, syphilis, leprosy), metabolic (osteoporosis, paget), neoplasic, rheumatic (spondyloarthropathies, dish), degenerative (osteoarthrosis), circulatory (osteocondritis), congenital and genetic disorders (limb deficiencies, craniosynostosis), and, of course, traumatic injuries [17]. The most useful pathologies for forensic anthropology identification purposes can be found at Cunha and Pinheiro, 2013 [18].

Sometimes, more important than arriving at the diagnosis of the disease is the interpretation of the effects of the lesions on the daily life of the individual. Whether he or she was active or already suffering from severe limitations of movement; whether gait or posture were affected or unusual are key elements to reach the right identification. Spondyloarthropathy is a good example of this: although the family might not know that his relative suffered from that disease, they may recognize the effects of this disorder, such as the limited mobility of the individual [18].

Occupational stress markers justify a special reference, because of their potential to give insight into physical activity. Lesions on the entheses are almost always due to hyper-use of the muscles and or tendons, which are consistently used to perform the same actions throughout life. These repetitive movements can then be linked to some type of activities which may narrow down the possible matches.

Another special mention has to be made to trauma, which can be a very valuable aid to identification. With a misaligned fracture a large bone callus will work as an identity marker. At the other extreme, when such a fracture is treated with surgical intervention, the chances of ID are increased [18]. Thus implants, orthopedic devices and bone plates are excellent elements for obtaining ID. Whenever a logo or serial number is present, the task of associating the device with an identity is facilitated [19]. Plus, certain countries have national registries of implanted products [20]. A consultation of that database can permit a direct link to an individual. Furthermore, a logo (there are logos for various orthopedic companies) can lead to its manufacturer who, in turn, can give indications concerning the hospital to which he/she sold it. In that hospital, the consultation of medical files can lead to identity. In all, signs of surgical intervention can be very valuable for identification.

Still with respect to antemortem trauma, bone changes occurring after the traumatic event may lead to understanding of how long the individuals survived that trauma. This is a very helpful element for identification as well. In particular, bone remodeling after surgery may provide a means of estimating the length of time that has passed since a surgical procedure or injury occurred. Yet, the wide individual variability does not permit results of the desired accuracy [20].

25.2.4.2 Frontal Sinus

Within anthropological approaches to ID, it is worth mentioning the frontal sinus. The uniqueness of the frontal sinus makes it a sinus print, parallel to fingerprints and DNA. Their unique pattern confers them an immense value for forensic purposes. Even monozygotic twins present different sinus patterns [21]. One of its benefits is that the antemortem radiograph does not need to be recent. This is because after 20 years old the sinuses generally remain stable. Trauma, pathology and surgery [21] may alter the sinus but, as when a person undergoes an x-ray, these alterations might be recorded. A drawback is that just a minority of the individuals has an imagiological record of his skull. The first case of identification using the frontal sinuses was published in 1927 [21]. Basically, a superimposition match of antemortem and postmortem X-rays is the main technique applied to achieve a PI through the frontal sinus. Nowadays, this technique is widely accepted by the scientific community, since it has been tested using the scientific method and has been giving very accurate results. In all, frontal sinuses are accepted as unique to each individual and meet the Daubert criteria.

When none of the above procedures are enough to reach an absolute identification, accessory techniques of identification can still be tried. Craniofacial superimposition is an example, although its potential as an identification technique is still dubious. Whereas facial approximation has nothing to do with identification, as it is only a means by which to convey a biological profile: the reconstructed face indeed may not resemble the decedent closely.

25.2.5 Forensic Imagiology

Radiographs and other imagiological sources (CT scans, multislice computed tomography (MSCT) and magnetic resonance imaging (MRI), among others) are excellent sources of data for comparison of anatomical features, both for anthropology, odontology and pathological identification [22–24]. That is, this technique is transversal to several methods and there is an increasing trend to replace classical Xrays by modern forms of imagiology.

As early as 1896, Bordas suggested that x-rays be used "… for identification through the visualization of old fractures, bullets, or other peculiarities" [25]. Later, in 1927, radiological identification was reported for the first time using frontal sinus [15, 21].

Images can be superimposed digitally to prove positive identification. Superimposition pattern matching should be the standard methodology to prove an ID. But obtaining a postmortem radiograph at the same angle and distance to the antemortem cannot be the luck of the draw [21]. Only in 1987, Harris and colleagues [26] published guidelines to adequately do so when using the frontal sinus. Nowadays, the variety of sources of imagiology has been very helpful for identification purposes. Most recently, virtual anthropology, the introduction of modern slice imaging to anthropology [24], has gained popularity. As already pointed out, the pattern of trabecular bone is used in human identification contexts, since this pattern is unique and visible on radiographs and suitable for unique individualization [27].

In the complex task of identifying the burnt, generally a task for forensic anthropology, Campobasso et al. [28] point out the usefulness of radiology, particularly as applied to the dentition and sinus frontalis. Forensic anthropology has been playing a major role in the identification of burnt remains, which is critical in mass disasters and/or terrorist attacks [29].

25.2.6 Clothes and Personal Belongings

Although these are always circumstantial means of ID, in many cases, these items, once shown to the victim's family and/or friends, signify a very good start for the identification process. Usually they are a very good guide in the process of identification and can both create a suspicion of identity or corroborate an identity. They are invariably secondary identifiers and include jewelry, articles of clothing and personal identification documents.

25.3 The Need of Implementation of Best Practices to ID and the Comparative Phase of Identification

This brief exposition shows that identification is a multidisciplinary task to which a variety of different disciplines merge to create a picture of an individual. Yet there are four main disciplines involved in the identification of human remains: genetics, dactiloscopy, odontology and anthropology [30], which are the most used scientific approaches. Yet, some others were not mentioned, such as earprints, which are known as good identification tool, although they can be encompassed within facial recognition [31], or even the tattoos, which in some particular instances can be enough as a proof of identity. Tattoos are indeed a good proof of the centrality of the skin for both human identity and identification [32].

To face the multiple requests of modern societies, where the number of John and Jane Does is unknown [5, 33, 34], it is of paramount importance that all the countries adopt a same policy to collect ante and post mortem data, which has been claimed by all international agencies dealing with this subject.

One of the major pitfalls for ante and post mortem comparisons, with respect to anthropological and pathological features, implying a radiological comparison, is the lack of consensus on the number of matching features necessary to reach a positive identification [35] and on how to compare these features. Therefore standards are needed for establishing identity from radiographs. Ross and collaborators gave a very good contribution to this problem [35]. Another aspect to be detached is that the rapid replacement of traditional antemortem Xrays by multislice computed tomography (MSCT) and other new technologies requires the presence of a forensic radiologist in the interdisciplinary identification teams. That is, this discipline is becoming more important where ID is concerned, even if few forensic centres have access to sophisticated X-ray technologies [36]. International, more complete and accessible databases on the missing are also a major requirement [1]. Above all, when a person is missing, he or she should be reported as such. It is not easy find a person that was never reported missing.

The achievement of more identifications is not only dependent on the identification techniques, but also on the existence of complete and reliable missing persons lists. Besides, to each of these persons a complete enquiry about ante mortem features should be appropriately filled. Only in this way can a good match be attained. Therefore, the comparative phase of identification has to be done mandatorily after the reconstructive phase has been accomplished. Whereas ante and post mortem confrontation can be done, manually or by means of software, with a more uniform and standard way of filling the same ante and post mortem questions about particular features, the confrontation process could be facilitated.

Scientific rigor and objectiveness are essential while reporting the results of identification in the final forensic report. The results have to be categorized within one of the possible classifications provided above or should be strongly justified similarly to other classification systems or basic medico legal criteriology. Since the report is to be sent to the judicial authorities, we also argue that a better complicity between science and law is needed. In many cases, the forensic experts go to court to answer questions on how the identification was accomplished. There is still some asymmetry between proof of identity accepted by the court from one country to another. Moreover, whereas the admissibility criteria for expert testimony is well legislated in the US after Daubert versus Merrel Down [37], this is not the case in Europe. Maintaining professional and scientific standards will always be a challenge to which the professional certification of experts can give a key contribution [38]. Finally, we argue that in the near future anthropological methods, considered as secondary identifiers, can become primary ones. Once the quantification of the results or an appropriate statistical strategy can be adopted, some unique skeletal features can indeed work as such. The number of John and Jane Does can thus be decreased.

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Chapter 26
DNA-Based Methods for Age Estimation

Matteo Cassina and Maurizio Clementi

Abstract The age estimation of unidentified cadavers and human remains is a challenging field of forensic medicine and several methodologies have been proposed, including morphological and biochemical analyses. Since the identification of a number of age-related DNA modifications, several new molecular approaches have been proposed. The first DNA-based method that has been extensively studied for the application in forensic age estimation was the analysis of telomere repeats of human chromosomes; subsequently, other techniques have been proposed, including the analysis of the mitochondrial DNA variants and the more recent approaches based on the evaluation of sjTREC rearrangements in T-cells and the methylation status of the human genome. Many studies have been conducted to standardize the sampling methods, the accuracy and the reliability of age determination using molecular techniques; the most promising results have been obtained with the analyses of sjTREC rearrangements in blood samples and the methylation profile of tissues. Conversely, most studies have shown that the accuracy of both the analyses of mitochondrial DNA and telomere length are not sufficiently high to be used in forensic practice. In fact, age-related DNA modifications are susceptible to a number of variables that can alter their measure and limit the precision and reproducibility of the assays; important factors include the type of tissue used for the analysis, the characteristics and life style of the subject, the level of degradation of DNA due to the effects of post-mortem environmental agents. The real challenge is to create a model that can provide the most accurate estimation in consideration of this large number of variables.

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26.1 Introduction

The age estimation in unidentified cadavers, human remains and living individuals without proof of date of birth is essential in public health and forensic medicine. Therefore, several approaches based on different methodologies have been developed by researchers.

The discovery of a cadaver or body with no identity is an event associated with possible significant administrative, legal, social, and ethical consequences; therefore, it is necessary to implement a series of investigations, sometimes long and complex, for an accurate identification of the corpse. In particular, the estimation of the age at death is one of the preliminary procedures performed, since it allows a rapid selection of a subgroup of possible candidates among many missing individuals.

In recent years, age identification in living individuals without valid documents has also become an essential administrative procedure in many countries that are facing an increasing number of immigrants and people requesting asylum; in fact, defining the age of refugees is a fundamental step. This aspect is particularly relevant for minors (aged less than 18 years), who are protected by the European law, especially if unaccompanied by relatives or caregivers (Directive 2011/95/EU of the European Parliament and of the Council of 13 December 2011). Finally, but not less relevant, there is an increasing request of pension claims of older immigrants and defining their age is becoming a new emergency.

Recent sociopolitical events have made this issue particularly relevant. In fact, in addition to the well-known problem of unidentified cadavers and missing individuals, terroristic attacks and the movements of millions of individuals escaping war and poverty have made this topic of primary importance in European countries.

Several methods have been developed in the past based on the analysis of the anthropological and dental status. These allow trained personnel to quickly obtain an approximate age of bodies/individuals.

However, the amount of biological material is not always sufficient for accurate age estimation, especially when body remains have to be analyzed. This problem, together with the need to obtain even more accurate age estimations, has motivated researchers to develop and test innovative methodologies.

The recent development of technologies for DNA analysis, the results of the human genome project, and the development of the "omic" sciences, has certainly led to the development of new approaches to this challenging aspect of forensic medicine. In particular, the first DNA-based method that was extensively studied for application in forensic age estimation was the analysis of telomere repeats of human chromosomes; subsequently, other techniques have been proposed, including the analysis of the mitochondrial DNA variants and the more recent approaches based on the evaluation of sjTREC rearrangements in T-cells and the methylation status of the human genome.

In this chapter, the main studies evaluating each of these methodologies will be presented and discussed.

26.2 DNA-Based Methods for Human Age Estimation

26.2.1 Analysis of Telomere Repeats

Telomeres are the terminal regions of chromosomes and consist of highly repetitive DNA sequences (TTAGGG in humans) [1, 2]; they stabilize and protect chromosomal ends and regulate the cellular replication capacity.

The DNA polymerase, the enzyme required for DNA replication before cell division, is not able to replicate the ends of the lagging strand at the end of each chromosome; as a consequence, in somatic cells, DNA replication results in shortening of the telomeres, which leads to cell senescence, a condition characterized by the incapacity of the cell to further replicate. Conversely, germ cells, stem cells and cancer cells maintain their replication capacity indefinitely, since they are protected from the progressive shortening of telomeres by the activity of telomerase; this enzyme acts as a reverse transcriptase and contains a RNA template, which is used to extend the 3′ strand of chromosomes.

Several methods have been used to measure telomere length, including Southern blotting [3, 4], slot-blot hybridization technique [5], PCR-based techniques [6, 7], quantitative fluorescence in situ hybridization (Q-FISH) [8, 9], and flow-fluorescence hybridization techniques (Flow-FISH) [10, 11].

The observation that in somatic cells telomeres shorten during a lifetime suggested the possibility of a correlation between their length, the biological age and the chronological age of a donor. The first published studies testing the measurement of the length of telomeres for the estimation of age in humans used the Southern blot technique [12].

Tsuji et al. [13] evaluated the correlation between telomere length and age in 60 Japanese individuals between 0 and 85 years. Genomic DNA was extracted from the peripheral blood and digested with restriction enzymes; then, the length of terminal restriction fragments (TRF) was analyzed by Southern blot analysis, using a Digoxigenin (DIG)-labeled probe specific for the TTAGGG telomeric repeat. The experiment was also performed with DNA extracted from bloodstains stored at room temperature for 5 months. The Authors observed that TRF length tended to shorten with aging, allowing an estimation of age with a standard error of approximately 7 years. In addition, terminal restriction fragments in stored bloodstains were shorter by about 500 bp compared with those in fresh samples.

An inverse correlation between age and TRF length was also confirmed using DNA extracted from the dental pulp of 100 Japanese subjects [14]. These results permitted the creation of a formula for the estimation of chronological age with a standard error (approximately 7.5 years) comparable with that observed using blood. The experiment was also performed using DNA extracted from 4 teeth stored at room temperature for 1 year; the authors observed that TRF length was 10–952 bp shorter compared with that of teeth evaluated immediately after the extraction. In addition, the validity and applicability of the formula was tested calculating the age of 15 forensic autopsies: the chronological age of 12 cases was inside the range of standard error of the estimated age using the protocol the authors described; conversely, the formula failed to detect the chronological age for three subjects whose cause of death was drowning. Therefore, the authors suggested that the immersion of corpses for several days led to an alteration of telomere length.

The same method was used by Ren et al. [15] for the analysis of DNA extracted from the blood cells of 105 individuals aged between 0 and 81 years. Their results confirmed a correlation between age and TRF length, but the results permitted estimation with a standard error of 9.8 years.

More recently, Srettabunjong et al. [16] performed the terminal restriction fragment (TRF) assay using blood samples obtained from healthy fresh Thai cadavers. The Authors found an inverse correlation between the mean TRF length and age; however, the correlation was too low to be used for forensic age estimation.

The Southern blot method has several limitations: it does not analyze the real length of telomeres but evaluates terminal fragments of chromosomes that include various subtelomeric sequences; large amounts of DNA are required; the DNA used needs to be as intact as possible, limiting the analysis of samples with degraded and unpurified DNA; the estimation of the mean TRF length may be inaccurate and a significant variability has been observed in all datasets; finally, Southern blot analysis is time consuming.

For these reasons, faster, more reliable and more efficient techniques were chosen to analyze the telomere length in recent studies.

In particular, Cawthon [6] developed a singleplex quantitative polymerase chain reaction (QPCR) assay; this method requires less DNA and less time than the standard analyses using Southern blot. A few years later, Cawthon [7] developed a multiplex version of the assay, in order to improve the accuracy, raise the throughput and lower the costs of the analysis.

Karlsson et al. [17] used the quantitative PCR method to evaluate the number of telomere repeats in healthy blood donors of age 0–86 years; the aim of the study was to create a standard curve to be used for age estimation of individuals with unknown age. The variations of telomere repeats among different tissue (blood samples vs. buccal swab samples) and within individuals (blood samples collected in 2007 vs. blood samples taken approximately 20 years before) were also studied. The authors found a lower correlation between telomere length and age compared with the previous studies using the Southern blot method; this resulted in a high standard error of age prediction (22 years). In addition, the degree of telomere shortening was found to vary significantly among different individuals and different tissues. Therefore, the authors concluded that this method was too imprecise and was not appropriate for forensic applications.

The non-applicability of the above-mentioned quantitative PCR method for forensic age prediction was confirmed also by Hewakapuge et al. [18] in a study evaluating telomere length in buccal cells.

In conclusion, all these studies confirmed that telomere shortening is inversely correlated with age; however, telomere length is affected by both genetic and environmental factors, including diseases, lifestyle, level of oxidative stress and the efficiency of the antioxidant systems. Therefore, the correlation is not sufficiently high to be used in forensic practice and the standard error has been found to be particularly high with PCR-based techniques compared with the older and time-consuming Southern blot techniques. In addition, a significant variability between different tissues of the same subject has been observed and the variations of telomere length in non-fresh tissues have not been exhaustively studied.

26.2.2 Mitochondrial DNA Mutations

Mitochondria are intracellular organelles with their own independent genome, a circular double-stranded molecule. Mitochondrial DNA (mtDNA) is more vulnerable to mutations than nuclear DNA due to the mitochondrial production of reactive oxygen species during the oxidative phosphorylation reactions, the high replication rate of DNA, and the lack of efficient DNA repair mechanisms.

Several heteroplasmic large-scale deletions have been reported at very low levels in several tissues of healthy subjects and have been observed to increase in an age-dependent manner [19]. In addition to deletions, also three tandem duplications (150, 190, 260 bp) have been reported to accumulate both in bone and muscle tissue [20].

Among mtDNA variations, a common 4977 bp deletion has been detected in brain, skeletal muscles, heart muscle, and other tissues of older individuals [21–24]; the proportion of mtDNA carrying the deletion varied largely in the different examined tissues, but was higher in muscle [25].

In particular, Meissner et al. [26, 27] studied the correlation between aging and the amount of the deletion in iliopsoas muscle tissue taken at autopsy from 50 individuals aged 24–97 years. Subjects with chronic and degenerative disorders that could have affected the levels of the deletion were excluded; cases with death-to-sampling postmortem intervals exceeding 48 h were also excluded. The deletion was quantified using a kinetic polymerase chain reaction (PCR) followed by Polyacrylamide gel electrophoresis. The Authors observed a positive correlation between the amount of the deletion and the chronological age of the individuals. In addition, their results were used to produce a formula for the calculation of age at the time of death; however, because of the broad confidence intervals, this method allowed only rough estimates discriminating between young and elderly subjects. The quantification of the deletion by real-time PCR allowed only a slight improvement in the age estimation [28].

In a subsequent study with a larger cohort of 92 individuals, this positive correlation was confirmed not only in iliopsoas muscle tissue, but also in other post-mitotic tissues (substantia nigra, caudate nucleus, putamen, frontal cortex, cerebellum, anterior and posterior wall of the left ventricle of the heart, right ventricle); however, a significant variability was observed among different tissues and individuals of the same age decade, limiting the accuracy of forensic age estimation [29]. In general, in iliopsoas muscle the deletion started to appear in the early thirties and the highest abundance (0.14%) was detected in a 102-year-old woman. On the other hand, the best linear logarithmic correlations between the amount of the deletion and the chronological age were observed in substantia nigra and anterior wall of the left ventricle. However, this study confirmed that the methodology is imprecise, only allowing differentiation between young and old subjects. In addition, it is important to note that cerebral tissues may not be suitable for the analysis, since they undergo putrefaction relatively early after death.

In addition to deletion/duplication mutations, high heteroplasmic levels of single nucleotide variants localized in the control region of mtDNA replication have been detected in aged healthy individuals.

Michikawa et al. [30] observed that more than 50% of the individuals older than 65 years had a high proportion (up to 50%) of mtDNA molecules carrying the T414G transversion in skin fibroblasts; conversely, this variant was not found in any of the younger subjects. A subsequent study failed to detect the T414G transversion in a significant proportion of mtDNA molecules from skeletal muscle, but reported the aging-dependent accumulation of other two different variants (A189G and T408A) in the same control region [31]. Del Bo et al. [32] confirmed that both mutations significantly accumulate with age in muscle biopsies.

In addition, Theves et al. [33] evaluated the presence of the A189G transition in muscle samples and buccal cells using three different methods: automated DNA sequencing, Southern blot analysis, and peptide nucleic acid (PNA)/real-time PCR combined method. The latter technique was particularly sensitive for heteroplasmy detection and permitted the demonstration of the absence of the A189G transition in buccal cells in young individuals and its presence in older individuals from the same maternal lineage, suggesting that it is a somatic mutation. Finally, Lacan et al. [34] reported an age-dependent accumulation of the A189G mutation also in bone tissue.

26.2.3 sjTREC Rearrangements

The thymus is the primary lymphoid organ for the development of naive T-cells; it reaches its maximum activity during childhood/adolescence and then undergoes a physiological, progressive involution [35]; however, despite the significant thymic atrophy, the production of naive T-cells does not stop completely even in advanced age.

T-cell mediated immune response is triggered by binding a foreign antigen to the variable amino-terminal region of a T-cell receptor (TCR) and an efficient immune system needs a large pool of T-cells with a wide repertoire of different TCR molecules.

TCR consists of a variable (V) amino-terminal region and a constant (C) region. In order to create a wide repertoire of TCR molecules, each immature T-cell undergoes unique somatic rearrangements in its TCR loci during intra-thymic development to form complete V-domain exons; these somatic modifications lead to the production of episomal DNA molecules, called signal joint TCR excision circles (sjTRECs). Since these molecules do not replicate, they are found in high concentration in the most recent thymic emigrants and are progressively lost during the subsequent cell divisions.

Several studies observed a positive correlation between thymic involution and the reduction of the number of sjTRECs in the peripheral T-cell pool [36, 37]. In addition, Pido-Lopez et al. [38] found that this age-dependent decline is gender-linked, with females having a statistically significant higher number of sjTRECs compared with males. The overall age-dependent reduction of sjTRECs displayed the classic bi-phasic trend of the decline of naive T-cell production, with greater decline prior to 40 years of age and slower decline thereafter.

Zubakov et al. [39] confirmed in whole-blood samples of 195 healthy Dutch individuals aged from few weeks to 80 years a correlation between sjTRECs levels and chronological age (standard error = 8.9 years), with a small but statistically significant gender effect.

Ou et al. [40] measured sjTREC levels in peripheral blood samples from 248, unrelated, healthy Chinese subjects ranging from 0 to 78 years old and confirmed the correlation reported in the previous papers (standard error = 10.47 years), without detecting significant gender effects. Similar results were observed in a subsequent study analyzing sjTREC levels in bloodstains from 264 individuals aged 0–86 years (standard error = 9.42 years) [41]. In addition, the authors tested fresh and stored bloodstains from the same individuals in order to detect any effects of storage time; while no statistically significant difference in sjTRECs contents was observed between the fresh and old DNA samples over a 4-week of storage time, a significant loss in sjTRECs contents was detected after 1.5 years of storage in 31 samples [41].

Similar results were also obtained with a study including 172 Korean individuals aged from 16 to 65 years (standard error = 8.49 years) [42].

The analysis of sjTRECs in blood samples/bloodstains is a promising method for forensic age estimation. However, the genetic and environmental factors that may regulate and modulate the age-dependent decline of sjTRECs are largely unknown and additional studies evaluating possible confounding factors are needed to confirm the applicability and validity of the technique. Further studies on cadavers are warranted to confirm these preliminary results.

26.2.4 Methylation Studies

DNA methylation is one of the mechanisms involved in regulation of gene expression without changes in the DNA sequence; it involves the addition of a methyl group at the 5′ position of cytosine residues in CpG dinucleotides, a reaction catalyzed by a number of enzymes called DNA methyltransferases.

DNA sequences containing a high frequency of CpG sites called “CpG islands” are located close to the regulatory region of many human genes and their methylation results in the silencing of gene expression.

The methylome, i.e. the DNA methylation profile, varies substantially between different tissues resulting in specific patterns of gene expression and cell differentiation. In addition, there is increasing evidence that the methylome changes in response to environmental stress during the embryo-fetal developmental and over the lifetime, suggesting that epigenetic modifications are an important mechanism of genome adaptation. This hypothesis is supported by the observation that identical twins progressively differ in their DNA methylation profile during their lifetime [43]. In addition, many diseases in adulthood have been associated with modifications in the methylome.

A number of studies demonstrated age-dependent methylome variations but the underlying mechanisms are still largely unknown [44]; however, there is an increasing number of studies evaluating these epigenetic regulations and their possible application in forensic age estimation.

Several methods for DNA methylation analysis have been described, including: (a) qualitative and quantitative analysis of CpG sites after sodium bisulfite treatment; (b) precipitation of DNA using proteins that interact with 5-methyl cytosine (for instance, specific monoclonal antibodies); (c) analysis of CpG sites using methylation sensitive restriction enzymes.

Methods based on bisulfite treatment seem to be particularly appropriate for forensic analyses, since DNA integrity is maintained and a limited amount of DNA is required.

Rakyan et al. [45] performed genome-scale DNA methylation profiling of whole blood from 93 different healthy females (31 twin pairs and 31 singletons) ranging from 49 to 75 years of age; the methylation status of 27,578 CpG loci covering more than 14,000 promotors in the human genome were analyzed. The authors identified 213 CpG sites that become more methylated with age (hyper-aDMRs). These results were validated by analyzing the DNA methylation profile in sorted $CD14^+$ monocytes and $CD4^+$ T-cells from an independent cohort of 25 singletons; for 131 of 213 CpG sites an increase of methylation with age was confirmed.

Bocklandt et al. [46] performed a similar analysis in saliva samples of 34 pairs of identical twins, aged from 21 to 55 years. Eighty-eight loci were identified to be highly correlated with age and in most cases (83%) were located within CpG islands upstream of transcription start sites of genes. Among the 88 loci, 10 were found to be correlated with age also in the study previously performed by Rakyan et al. [45] on blood samples. These results were replicated and validated by the analysis of CpG sites in the promoters of three genes in a general population sample of 60 individuals between 18 and 70 years of age. In addition, a regression model predicting the age of a subject with an average accuracy of 5.2 years was built.

The authors were not able to replicate the results obtained by Fraga et al. [43]: in fact, significant age-dependent differences between identical twins were not detected. However, these results may not be contradictory; Bocklandt et al. [46] focused their analysis on CpG sites close to genes, while Fraga et al. [43] studied random sites, most of which were located in nonfunctional repeated sequences. Therefore, the Authors concluded that an age-dependent drift might occur in nonfunctional DNA regions, while important regulatory sequences remain under strict epigenetic control throughout life.

Koch and Wagner [47] analyzed the DNA-methylation profiles of several cell types (dermis, epidermis, cervical smear, T-cells and monocytes) and selected 5 CpG sites to build a model for the prediction of the age of the donor with an average precision of ±9.3 years.

Hannum et al. [48] analyzed the methylome profiles of a cohort of 482 individuals spanning a wide age range; the methylation status of 485,577 CpG loci in blood samples was evaluated and then a predictive model of aging, including both methylomic and clinical parameters such as gender and body mass index, was built. The accuracy of the model was high, with an error of 3.9 years. Seventy-one methylation markers were highly predictive of age and most of them were located within or near genes with functions in aging-related conditions (including Alzheimer's disease, cancer, tissue degradation, DNA damage, and oxidative stress). The model was also validated in another independent cohort of 174 individuals; predictions were accurate with an error of 4.9 years.

Weidner et al. [49] combined 575 DNA methylation profiles of blood samples from four studies including individuals aged from 0 to 78 years. Methylation profiles were obtained using the same platform, which analyzed 27,578 CpG sites. A multivariate linear model, based on 102 age-related CpG sites selected by Pearson correlation, was built to predict donor age. The results correlated with chronological age with a mean absolute deviation from chronological age of 3.34 years. This model was also validated using 3 other available datasets derived from blood samples; mean absolute deviations from chronological age ranged from 4.02 to 5.79 years. In addition, the model was also applied to the dataset published by Hannum et al. [48]; in this case, an adjustment was necessary because the methylation analysis was performed with a different platform. However, after the adjustment, the mean absolute deviation from chronological age was 4.12 years.

Studies on genome-wide methylation analyses detected that the CpG site with the strongest positive correlation of methylation with age mapped to the *ELOVL2* gene. In particular, Zbieć-Piekarska et al. [50] analyzed 7 CpG sites located in the *ELOVL2* gene in cohort of 303 individuals aged 2–75 years; the examination was firstly performed on blood samples and the final linear regression model, including 2 of the 7 CpG sites analyzed, enabled age prediction with an error of 6.85 years and a mean absolute deviation from chronological age of 5.03 years. The developed prediction model was evaluated in an additional set of 124 samples, resulting in an only slightly higher mean absolute deviation (5.75 years). The Authors also observed that the methylation status of *ELOVL2* did not change significantly in bloodstains after 4 weeks of storage in room temperature conditions. In addition, the analysis of 45 bloodstains deposited on tissue paper after 5, 10 and 15 years of storage in room temperature conditions indicated that the success rate for age prediction remained similar; however, a gradual decrease of positive PCR results was observed.

In recent years, other age prediction models based on the analysis of specific CpG sites in blood and bloodstains have been published [51–54]. Overall, these results suggest that the analysis of the methylation status of specific CpG sites in blood cells is a relatively precise method for forensic age estimation; however, further studies are necessary to evaluate the accuracy of the method by analyzing a greater number of different tissues, both fresh or stored at room temperature for a long time, from individuals of different ethnicities.

26.3 Conclusions

The estimation of the age of unidentified cadavers/remains or living individuals is important for several legal and ethical issues and a number of methods have so far been proposed.

The evaluation of morphological features, especially of the dental and skeletal system, is very accurate for age estimation in childhood, but is not accurate after the end of skeletal growth and development. Therefore, other methodologies have been tested, including the analysis of aspartic acid racemization in permanent proteins of various tissues, which has been considered one of the methods of choice in the past years, and the more recent molecular analysis of specific DNA modifications.

In fact, several different biological mechanisms have been shown to modify the structure or the functionality of DNA; in particular, a number of age-related modifications have been described, including the length of telomeres, the DNA methylation, the accumulation of spontaneous mutation in both nuclear DNA and mitochondrial DNA, and the production of episomal DNA molecules in immature T-cells.

Many different groups have tried to standardize the sampling methods, the accuracy and the reliability of age determination using molecular techniques. Most studies have shown that the accuracy of both the analyses of mitochondrial DNA and telomere length are not sufficiently high to be used in forensic practice. Both methodologies allow differentiation between young and old individuals, but give estimations of too high a margin of error to be proposed as standard reliable methods.

More promising results have been obtained with the analyses of sjTREC rearrangements in blood samples and the methylation profile of tissues. However, the results have been obtained in relatively small samples, in a limited number of tissues (in most cases blood samples) and under specific conditions (i.e. fresh samples of living individuals, bloodstains stored at room temperature, tissues of fresh cadavers).

The real challenge is to understand how all genetic and environmental factors affect the biological mechanisms used for age estimation and create a model that can provide the most accurate estimation in consideration of a number of variables. This will be achieved only by the accumulation of a large amount of data regarding both the physiologic DNA modifications during the lifetime in different tissues and the post-mortem pattern of DNA degradation according to different post-mortem intervals and a wide variety of environmental conditions.

The advent of next generation sequencing (NGS) methods which facilitate and accelerate DNA analysis will allow a further step in the understanding of age-dependent DNA modifications. The applications of these high-throughput technologies in molecular biology and medicine include the analysis of the entire genome sequence, the characterization of RNA transcripts in specific tissues, and the profiling of DNA methylation patterns. As previously discussed, the analysis of the epigenome from samples collected by cadavers has already been used in forensic studies with regard to age estimation; furthermore, an interesting field of research is the analysis of the accumulation of somatic mutations in human tissues related with age.

However, DNA methylation and other age-related DNA modifications discussed in this paper are susceptible to a number of variables that can alter their measure and limit the precision and reproducibility of the assays. Important factors include the type of tissue that has been used for the analysis, the characteristics and lifestyle of the subject (including gender, race, smoking, diseases, and diet), in addition to the level of degradation of DNA due to the effects of *post-mortem* environmental agents.

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Chapter 27
Radiodiagnostic and Molecular Innovation in Personal Identification

Silke Grabherr, Tanya Uldin and Fabrice Dedouit

Abstract The forensic radiological examination permits identity to be attributed to individual bodies in the context of molecular or odontological data that is unavailable or not readily accessible. Among the proposed techniques, post-mortem computed tomography (PMCT) allows the rapid and non-invasive acquisition of illimitably consultable multiple data, even in the identification of multiple bodies (e.g., mass disasters). Radiological assessments are mostly centered on the comparison of ante and post-mortem aspects, these latter representing the main factor determining the choice of the examination methodology, applicable to the determination of the biological profile, in particular age and gender. The absence of a consensual qualitative-quantitative criterion in the definition of identity and the inferential problems of virtual anthropological studies raise questions of reliability and scientific validity. In light of the promising role of forensic radiological techniques in intra-vitam and post mortem personal identification, the limits and applicative perspectives of which are presented and discussed in this paper, a multi-disciplinary collaboration, also aimed at training experts in the field, is advisable.

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27.1 Introduction

Identification of bodies or body parts is an important task in death investigation. Several methods exist that are either used by the police or by forensic experts. In order to decide which method is the most adequate for identifying a human body, many different aspects have to be evaluated: the condition of the body, the availability of ante-mortem data and technical equipment, the degree of emergency as well as the financial aspects.

During the last decades, the importance of radiological post-mortem exams has increased impressively, especially concerning modern cross-sectional imaging techniques, such as MDCT (Multi-Detector Computed Tomography). In fact, the combination of radiological exams and conventional autopsy methods is today proposed with the aim of increasing the quality of the post-mortem exam. A post-mortem Computed Tomography (PMCT) scan allows a rapid exam and a digitalization of the body. Within some minutes data can be obtained that contains a huge quantity of information, especially concerning the presence of foreign bodies, and the skeletal system, but also concerning major traumatic lesions or preexisting illnesses or malformations. Such information can be used to get an idea of the circumstances and cause of death as well as about possible identification methods. Therefore, if an unknown body is scanned, the obtained images should be searched for particularities that could lead to identification.

A radiological identification can be performed in different ways [1–3]. Images can be obtained by using conventional X-ray units, MDCT-units or even Magnetic Resonance Imaging (MRI). Again, the most important feature is not the type of post-mortem imaging that is chosen, but the availability of ante-mortem data. In single unknown corpses, there are often already one or few presumed identities. In most cases, the role of the forensic pathologist is to confirm an identity, which is proposed by police investigations. Therefore, the medico-legal exam has often to be adapted to the presence of such ante-mortem data. Depending on the case, it is easier to obtain radiological images than to obtain a dental report or a DNA-profile from family members. If this is not the case, even the best radiological images will not help with identification.

In mass fatalities the situation is different. The most important point is the rapid examination and identification of the victims. Cases of natural catastrophe or mass disaster of other origins always present a situation of acute crisis in which the rapid intervention of first aiders, security and police are demanded. Once there is no longer any possibility of saving lives and the emergency units withdraw, there is often need of forensic pathologists. Their role is to identify victims, to diagnose their causes of death and to help investigate the mechanism that led to the catastrophe. In order to be able to intervene rapidly, the forensic pathologists have to be organized and need to have a detailed plan of intervention that is ready at any time. Therefore, organizations like DVI (Disaster Victim Identification) units exist. The aim of such teams is the coordination of the intervention of specialized persons and their formation. Their role is also to increase the quality of the intervention and to propose different and new approaches that could be useful for investigating such catastrophes. More and more DVI-Teams propose the use of post-mortem imaging, especially PMCT. Its great advantage is the rapid digital documentation of a corpse that allow unlimited investigation of the images later on. The obtained radiological data enable the visualization of foreign bodies such as medical implants, projectiles and many more. It also gives a detailed overview about the skeletal system including its pathological changes due to existing diseases or due to the acute event that lead to death. Although the visualization of soft tissue such as muscles and organ parenchyma is limited by using PMCT alone, it still permits the identification of major traumatic lesions and preexisting modifications of the anatomy due to malformation or pathologies. For these reasons, the implementation of PMCT in the investigation of victims of mass disasters is extremely useful. Multiple studies have already proven this approach [4, 5], which is of utmost importance if the body presents advanced alteration due to putrefaction or traumatic lesions. Once the first acute intervention including the identification of the victims is done, the PMCT data remain available for a detailed investigation including the definition of the cause of death. Many studies have shown the utility of PMCT for investigating especially traumatic death [6, 7], such as it is mostly the case in mass disaster. More complex reconstructions are also possible based on PMCT data, such as the reconstruction of bullet trajectories in cases of ballistic trauma [8]. This can be of interest in cases of terrorist outrages for example. The digital PMCT data can be explored even a long time after the body has been restituted to the family, allowing a good compromise between a rapid identification and restitution and the possibility to perform medico-legal investigations. As all the described advantages are obvious, the use of PMCT for investigating mass disasters has already been proposed [3–5], and several DVI-teams include the performance of a PMCT-scan in their protocol. In order to avoid transporting the bodies to centers that have a CT-unit, even mobile CT-units are proposed that can be rapidly brought to the scene of the disaster as these are installed inside of trucks.

Medical implants are the most promising features and are very easy to detect on the PMCT images. The presence of osteo-synthesis material such as screws, metallic plates or nails, for example, allows a comparison with ante-mortem radiological images, leading in most cases to a positive identification of the victim. But also other implants such as vascular stents, surgical clips or drains are regularly associated with ante-mortem images that have been utilized to verify their correct position and are therefore suitable for a comparative identification. Most surgical interventions leave traces that can be seen in PMCT. As in such cases, radiological images are regularly performed in the peri-operative period, and they can be used for an ante-and post-mortem comparison.

Beside such modifications of the normal anatomy by medical interventions, there can be preexisting modifications which are rare and may lead to possible identifications. Examples are anatomical malformations or variants, such as vascular variations (vena azygos, double renal artery etc.) malformations of inner organs (e.g. polycystic kidneys, situs inversus etc.). Once such variations are identified on post-mortem radiological images, corresponding information can be searched, either in medical reports or in ante-mortem images that may even be performed for other reasons and on which they may have been reported as additional findings.

Also pathological changes can be used as markers of identification, if ante-mortem images exist. A typical example is the comparison of metastasis in cases of advanced cancer, if radiological exams were made shortly before death. By a detailed comparison of the localization and size of the metastasis, as well as their morphology (osteolytic, necrotic etc.), a radiological identification can be obtained.

The simple study of the individual anatomy can also help to identify an unknown body. In fact, every individual has a typical anatomy within the range of "normal", non-pathological morphology. Thus, the size, number and localization of sesamoid bones and the shape of sinuses are particular for each individual. Therefore, they can be used for ante-and post-mortem comparison. Examples are the comparison of sesamoid bones of the hand or feet or the shape of the frontal sinuses [9, 10].

Also for dental identification, radiological images are used. The methods for obtaining such pictures are variable. Either conventional X-ray images can be made by using different types of mobile or static machines, or the data can be reconstructed from PMCT images [11] by using special CT-protocols or post-acquisition treatments with specific software [12, 13].

If no identity is presumed at all, the presence of such abnormalities, foreign bodies and pathological changes can help to focus the search of the victim's identity. In fact, the more information about the body is known, the faster and easier investigations can be focused that lead to the identification.

If such a radiological identification is not possible, molecular identification methods can help. By analyzing samples of the body, the genetic profile can be obtained. This profile can either be compared with the profile of the presumed identity or with relatives, mostly the parents or siblings. For the first case, ante-mortem samples can be collected. Most convenient samples for such direct profile comparisons between a presumed victim and a body are medical samples of a person, such as biopsies or blood samples.

27.2 Main Part

Today, radiological methods are well established in forensic medicine and also techniques for allowing forensic anthropology exist [14–16]. They can be used to directly identify the deceased by comparison of individual characteristics in ante and post mortem radiological images [1, 2, 17]. The simplest way to obtain such post-mortem radiological data is plain radiography, which is relatively simple to handle, as well as inexpensive. An alternative, that is more and more used today, is PMCT. In fact, many forensic centers use PMCT scans and this method has been proposed as a tool for investigating victims of mass disasters [4, 5]. Methods for such radiological identifications are multiple. For example, Quatrehomme et al. [18] used trabecular bone morphology for identifying a person. Stephan and co-workers [19, 20] used optical surface scans and radiographs for which they have developed a geometric-morphometric method that allowed comparing the shape of clavicles for identification. Even specific software can be used for radiological identification. In this sense, Derrick et al. [21] have modified software that was initially used for spinal injury diagnosis to identify victims based on the comparison of the vertebrae in ante and post mortem radiographs. Concerning the comparison of sinuses, most work has been done based on ante and post mortem radiographs of frontal sinuses [22, 23]. The same procedure has been adapted to PMCT by Reichs et al. [9, 10] and by Uthman et al. [24]. Ruder et al. [25] have integrated the comparison of paranasal sinuses in such an approach. Knowledge of normal bone variations may also be very useful for comparative identification. The reference textbook for radiologists on this subject is Keats and Anderson's "Atlas of normal roentgen variants that may simulate disease" [26].

However, comparative identification in radiology must always be performed cautiously. Indeed, there is no official scientific consensus on the specific number of concordant traits required to positively establish an identity. The lack of a standard ruling on which and how many features and matching points are needed for positive identification makes the process difficult. Some authors consider that one to four concordant features and no discrepancies usually establish positive identification [1, 2, 27]. However, others state that, in some cases, the combined presence of numerous common and non-specific features are required [28].

The expert who bases identification on a feature that he considers to be rare is making a subjective judgment. Caution is always needed because some authors have demonstrated that some morphological characteristics of bone, such as fractures, pathological conditions and surgical material, supposed to be rather rare, may in fact be quite common [29]. When performing those kind of evaluations, the Daubert standard or Daubert challenge has to be known by the radiologist, forensic pathologist or anthropologist [30]. Indeed, even if applied in the USA, those principles must always be kept in mind, because they concern the scientific expert testimony, which is of course crucial for a judiciary expert. The guidelines of the Daubert standard underline many questions concerning science and the expert and, of course, his testimony: What is the basic theory and has it been tested? Are there standards controlling the technique? Has the theory or technique been subjected to peer review and publication? What is the known or potential error rate? Is there general acceptance of the theory? Has the expert adequately accounted for alternative explanations? Has the expert unjustifiably extrapolated from an accepted premise to an unfounded conclusion? These are indeed a lot of questions, which should necessarily be answered by the expert who is performing an anthropological assessment or a work on comparative identification.

It is clear that, for reconstructive identification, the methods applied are derived from a population sample which is usually different compared to the geographical origin or the socio-economic status of the individual examined. The problem is always the same: adaptation of different population data to one single individual. In this case, statistics and particularly Bayesian statistics are helpful. It permits to answer different questions, particularly concerning the age assessment with probability. This kind of answer seems to be the most "scientifically honest" and the one which respects the Daubert rules. Prior to 1993, federal and most state courts adhered to the 1923 Frye standard of “general acceptance” in the relevant scientific field. Some examples of judgments or appeals decisions of the District’s Court were clearly based on the fact that the proposed testimony simply did not meet the Daubert standard of being scientific knowledge, citing untested methodology, lack of peer review and publication, the potentially high error rate, and lack of general acceptance in the scientific community. Consequently they rejected the conclusions of the experts.

Beside radiological comparative identification, **forensic anthropology** can help to provide information about age, sex, size and ethnic of the victim(s) [31]. In fact, several studies have shown that such anthropological estimations can be made on “virtual skeletons” that can be reconstructed from PMCT data [32]. In terms of innovations in this topic, we will focus on two different points: Firstly, the geometric morphometric methods (GM) and, secondly, MRI.

GM permit the quantitative representation and analysis of morphological shape using geometric coordinates instead of measurements [33–36]. The major goal of GM is measuring morphological similarities and differences. Results can be presented visually as a “shape”. Data can be collected from digital photographs or MDCT images. For anthropological purpose, the great interest is the possible differentiation between size and shape. This is of utmost interest when sexual dimorphism is studied. Such dimorphism permits the characterization of differences between males and females, in terms of size but also shape. In GM the size is mathematically removed from the analysis to focus on pure shape. The anthropological use of GM is not new and has already been used based on photographs, and two dimensional (2D) documents. With the use of MDCT and the three dimensional (3D) information contained within the images, new possibilities are offered for GM. It is possible to obtain population data using clinical MDCT explorations. Of course, depending on national laws, the approval of ethic commissions is sometimes necessary. The great advantage is the possibility to obtain recent and modern data, which is not possible when working on dry bones. The possibility of obtaining “consensus shape” is very useful, because it allows a representation of “hypermale” and “hyperfemale” subjects. Of course, the representation for male, female and indeterminate subjects is also possible. The visualisation of deformations on a so-called Thin Plate Spline (TPS) is also useful as it permits to localize anatomical differences in terms of shape. Those can, for example, be located on the cranium or the pelvic bones. GM can be also used in cases of mummies which cannot be autopsied, or for bodies or parts of bodies, for which bones are surrounded by tissue or muscles.

MRI is an X-ray free imaging method. The most important difficulty relates to the performance of MRI, mainly due to limited access to installed machines. However, in the recent literature many articles dealing with age assessment use this technique, since it is particularly suited for examination of the growth plate, and especially the metaphyseal–epiphyseal junction, which appears as a more or less complete gap between the metaphysis and epiphysis, depending on the state of union.

Most MRI studies focusing on bone age assessment using T1-weighted sequences, which provide a good indirect imaging of the bone as well as anatomical information. Many anatomical regions have been already explored: teeth, clavicle, wrist, iliac crests, knee and ankle. New population data are now published [37] with new classifications or staging methods, as they already exist for radiographs and MDCT. One problem of such MRI methods is the fact that they cannot be compared to other imaging methods, as they are mostly based on the exploration of cartilage, which is only clearly visible in MRI. Another difficulty with the MRI is the need for specific sequences that have to performed at the imagine acquisition. Indeed, the diagnosis and analysis possibilities are different if the acquisition is performed on T1-weighted, on proton density-weighted or on T2-weighted sequences, in a coronal, axial or sagittal plane. In addition, the choice of the radiofrequency coils, which can be volume or surface coils, plays a role. Another parameter that can modify the quality of the images is the magnetic field strengths. For example, higher magnetic field strengths may contribute to improve the signal-to-noise ratio, and potentially improve the quality of the images. Consequently, the comparison of results of different population studies for the same anatomic region implicates a standardization of the technical parameters. Results of some studies seem interesting for experts that have to determine the age of an individual around the legal age of eighteen. Krämer et al. [38] showed that all males with complete union of the distal femur on a T1-weighted sequence were aged at least 18 years.

However, even with modern radiological machines, the anthropological assessment will be limited by classic anthropological problems. The “attraction of the middle” is one of these limits the consequence of which is an overestimation of the age at death in younger individuals and an underestimation in older individuals. Of course, other parameters like intra-, inter observers variabilities, standard deviations etc. have to be taken into account for the evaluation of the quality of publications dealing with MRI and age assessment [37].

There are also projects trying to combine molecular biological methods with anthropological approaches, especially to perform cranio-facial approximation. Claes et al. [39, 40]. Incorporate genetic information into the determination of facial shape. They investigated DNA-based facial composites of 592 adult individuals from an admixed population in the US, Brazil, and Cape Verde. In order to verify the feasibility of their method, predicted faces were compared to the real ones. Preliminary results are promising but further studies are needed.

27.3 Conclusion

The increasing implementation of modern radiological imaging methods into forensic and medico-legal investigations leads to a more and more widespread accessibility of radiological techniques in post-mortem exams. Also, forensic pathologists are nowadays beginning to train in radiological data-reading and co-operations with radiologists. This development means that forensic radiology is gaining more and more importance. Centers all over the world have started to perform PMCT or even MRI on the deceased before the conventional exam of the body is done. Therefore, more and more radiological data is available that can also be used for identification procedures. Methods for such radiological identification are various. However, today, studies are often missing that are investigating their validity.

Although the technical equipment is often available, the most important problem today is to have trained experts in forensic imaging. Particularly in relation to the performance of radiological identifications, knowledge about medico-legal procedures is important. For this reason, clinical radiologists are often overstrained with the question of performing a radiological identification on their own. They need the help of the forensic pathologists, which is the reason why a common approach has to be pursued.

Once such collaboration is obtained, the technique of radiological identification is a fast and easy approach that allows a non-invasive identification of a victim. Of course, all success depends on the presence of ante-mortem data. Especially in cases of mass disaster, where a rapid investigation and identification of all victims is of utmost importance, PMCT will naturally play an important role. Therefore, mobile CT-units are proposed that can be brought easily onsite and that can facilitate the task of DVI teams, firstly for identification and then for reconstructive issues.

The possibility to investigate the skeletal system by using the radiological images allows the performance of "virtual anthropology". As multiple studies have shown, the radiological data can be explored in order to perform anthropological measurements, age and sex estimations and to find out information about habits, geographical origin and pathology of the investigated body. Therefore, even if no presumed identity is given, anthropological examination of the radiological data helps to give a good idea of the identity of the victim. As the radiological images often provide more details and information than the examination of a dry bone, new methods for such virtual anthropological estimations are proposed nowadays. Depending on the imaging tool used, either the bone structures (MDCT) or cartilage (MRI) can be examined in detail. The scope of non-invasive anthropological identification of a body is today a field of very large and ongoing research, which is booming with new results and possibilities that are discovered nearly every day.

The possibility to use MRI, which is functioning without any radiation, also permits the investigation of living persons, where age estimation has to be done for juridical reasons. As such, radiological exams do not have a medical, but a juridical, indication, and are the object of heated debates in many countries. MRI could be a possible solution for these cases, as it permits an imaging investigation without any harm to the examined individual.

The actual tendency, with increasing use of radiological imaging, promises that radiological identification will gain even more importance in the near future.

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Part VIII
Innovation, Unitariness and Evidence. Forensic Genetics and Genomics

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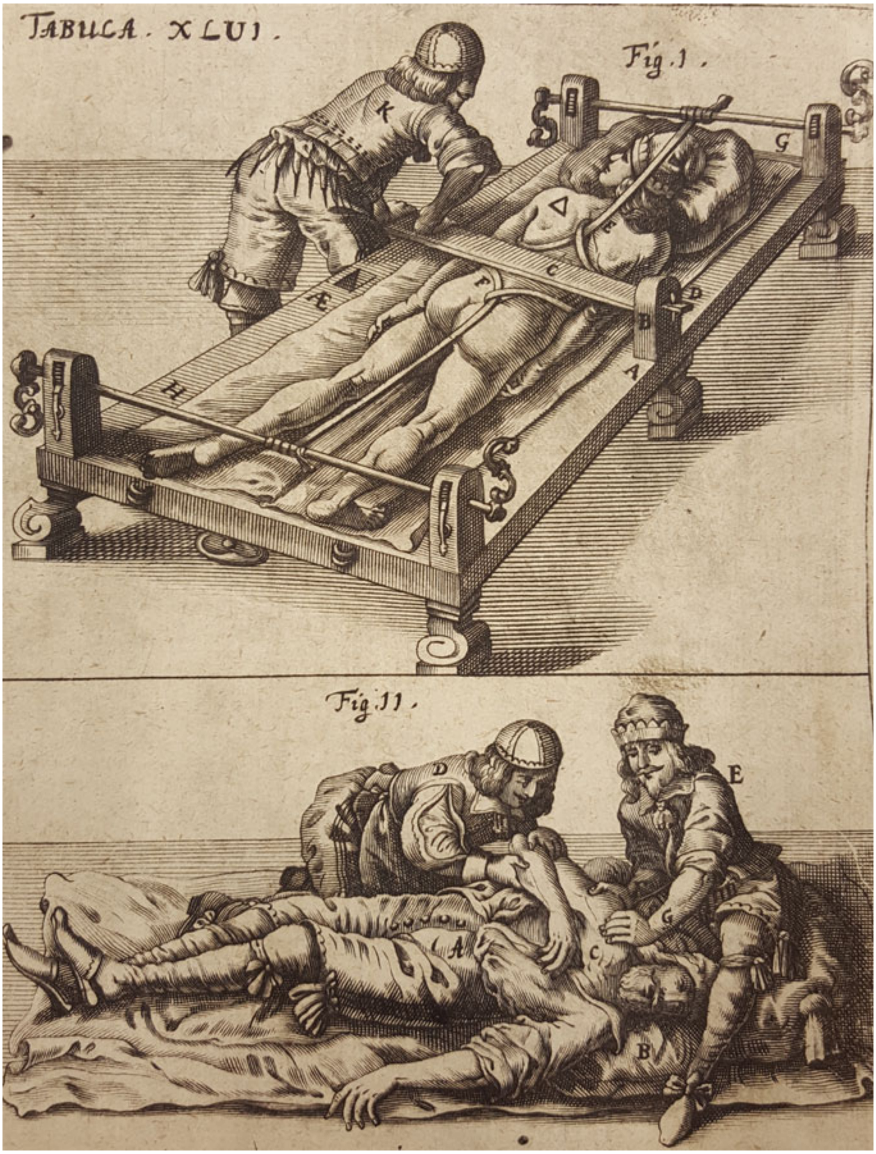

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Chapter 28
From Hemogenetics to Forensic Genomics

Angel Carracedo

Abstract The chapter traces the milestones of the historical evolution of forensic genetics, citing the fundamental contributions that have allowed this specialist discipline to resolve judicial cases, through the analysis of human and non-human biological samples. The process of refinement of analytical methods has ecountered the following key moments: the discovery of autosomal SLP and STR polymorphisms of DNA, for the determination of individual genetic profiles; the standardization of analytical processes and guidelines for interpretation by the DNA Commission of the International Society for Forensic Genetics (ISFG); the discovery of mitochondrial DNA polymorphisms and of heterochromosomes; the construction of forensic data banks and the identification of particular SNPs for the determination of the individual phenotypic characteristics. Forensic genetics currently aspires to establish itself as a forensic genomics thanks to the introduction of next generation sequencing techniques, whose massively parallel sequencing represent the second generation and the successive step in the endless revolution in this field.

28.1 Introduction

Forensic Genetics is a speciality of forensic science and genetics. A medical speciality or a scientific discipline is commonly defined when academic chairs, scientific journals and scientific societies are specifically devoted to this topic and named as such. This field satisfies these three requirements in order to be considered as a discipline. I had the opportunity to follow the history of forensic genetics from

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the very beginning to the present, since I had been working in this field for 40 years when the field was known as forensic biology (which is still the case in some European countries). The denomination was then changed to forensic haemogenetics, then to forensic genetics, and it is quite likely that in the near future it will be referred to as forensic genomics.

Forensic genetics is the application of genetic tools and knowledge to the resolution of judicial conflicts. Paternity testing cases, criminal casework (i.e. biological stains, contact samples, hairs), identification of human remains, criminal DNA databases, and a variety of applications of non-human DNA typing are the kinds of expertise usually requested by the judges regarding forensic experts. In the last few years forensic genetics has also been applied in other fields of legal medicine, namely in forensic pathology through the genetic diagnosis of sudden cardiac death and also toxicogenomics.

28.2 The Evolution of Forensic Genetics

The evolution of forensic genetics has been driven by the analysis of human genetic variation, which began over a century ago with Karl Landsteiner's discovery of the human ABO blood group variants (termed polymorphisms) and his early realization that this variation was applicable to solving paternity testing cases as well as crimes.

During the first half of the 20th century different polymorphic human red cell antigens were discovered, although the application of forensic genetics to criminal casework was rather limited due to the difficulties of analyzing minute bloodstains and body fluids other than blood. In addition to the ABO system, there were about 15 other well-established blood-groups with potential for forensic serology, including the MNSs system, and the Kell, Duffy, Kidd and Lutheran systems amongst the most widely used in forensics.

The characterization of human variation in the immunoglobulins, but especially the discovery of polymorphic proteins and enzymes in serum and red blood cells, represented a significant advance in this field, particularly for paternity testing.

Since the discovery of the first serum protein polymorphism in 1956, the number of polymorphic proteins that can be analyzed by electrophoretic methods continuously increased up to the late seventies. The same occurred with red cell and leukocyte polymorphic enzymes, most of them discovered in the 60's and 70's.

MHC Class I loci were introduced in 1962 and Class II some years later and were of major importance in the field. HLAs were more polymorphic than any other genetic markers used up to that moment and represented an important improvement in paternity testing despite the fact that the methodology used (based on a microlymphocytotoxicity method) brought with it some difficulties of interpretation due, in part, to the cross-reactivity of some antigens and the complexity of statistical interpretation due to linkage disequilibrium within the main HLA loci.

The use of these classical genetic markers was nevertheless limited when it was necessary to analyze minimal or degraded material, which is commonly encountered in forensic cases. In addition, it was, difficult to analyze biological material other than blood, and therefore the information obtained from hair, saliva and even semen in rape cases was rather limited.

In spite of the discovery of all these polymorphisms, the information that forensic geneticists were able to report in many cases was clearly insufficient and therefore the discovery of hypervariable loci in minisatellites by Jeffreys et al. in 1985 [1] represented a milestone in the field and one of the most important discoveries in the history of forensic science.

Minisatellites were initially detected by hybridisation of probes to Southern blots of restriction-enzyme-digested genomic DNA, and shared 'core sequences' between different minisatellite loci permitted the application of probes for the detection of many independent minisatellites simultaneously, yielding the hypervariable multi-band patterns known as DNA fingerprints.

Originally, multilocus probes were proposed for forensic genetic analysis. However, these types of probe were not very successful in the forensic field, because, although highly informative, statistical problems of evaluation of the evidence in cases of band matching and standardization problems arose. For these reasons, multilocus probes were substituted in the forensic field by the use of specific cloned minisatellites, 'single locus probes' (SLPs), where each revealed only a single, highly polymorphic, restriction fragment length polymorphism, thus simplifying interpretation. It was with SLPs that the first DNA-based criminal investigation was carried out; this case culminated in the conviction of Colin Pitchfork for a double rape and homicide in Leicestershire in 1986. Very soon DNA analysis became the standard method in forensic genetics as it was used by the majority of laboratories in the full range of applications, but especially in criminal forensic casework (stain analysis and hairs) and identification.

Until the introduction of short tandem repeat (STR) analysis by polymerase chain reaction (PCR) amplification, the analysis of minisatellites with single locus probes was very popular in forensic laboratories.

The main advantage of SLP analysis is the enormous variability of some of the minisatellite loci and well-documented knowledge of the mutation rate in some of them. The main disadvantages are the time needed for the analysis and the need for relatively large amounts of non-degraded DNA required for successful SLP typing. Since DNA extracted from forensic specimens is often degraded due to environmental conditions, SLP techniques have often failed to produce reliable results. The polymerase chain reaction (PCR) has overcome these difficulties and it has strongly enhanced the usefulness of DNA profiling techniques in forensic science.

Most PCR-based DNA typing systems allow alleles to be identified as discrete entities, therefore making standardization easier by avoiding most of the statistical issues that arise in matching and binning SLP bands that occupy a continuum. Additionally, apart from the increased sensitivity inherent in any PCR technique, it is more likely to be successful in analyzing old or very degraded material mainly because of the smaller size of many of the DNA polymorphisms (SNPs and STRs), making them more amenable to analysis by PCR.

STRs were discovered in 1989 and were applied to forensic cases at the beginning of the 90's and are now the markers of choice for DNA-based forensic identification.

The advantages of using tetra and pentanucelotide repeat STRs (4 and 5 base repeat units) over di and trinucleotides (2 and 3 base repeats) soon became apparent and a systematic search for the most convenient STRs started. Another important step was the possibility of amplifying multiple STR loci in a single combined (multiplex) PCR reaction. When this PCR approach was coupled with direct detection of amplified products in polyacrylamide gels, STR DNA profiling becomes amenable to automation. Commercial STR multiplexes for manual electrophoretic systems were available from 1993. Denaturing polyacrylamide gels were used for the separation of DNA fragments until the introduction of capillary electrophoresis that revolutionized the field together with the introduction of fluorescent-based dye-labelled primer technology and the use of DNA sequencers, which allowed the typing of large STR multiplexes. Several commercial dye-labelled multiplexes have since become available and all include a range of STRs plus amelogenin for sex determination. The multiplexes used nowadays combine more than 15 STRs. The combined discrimination power of STRs is very high and the probabilities of two unrelated individuals matching by chance (random match probability: RMP) are lower than 10^{-15} for most of the larger STR multiplexes.

Although there are thousands of STRs in the human genome, forensic scientists all over the world have agreed to use almost the same ones and there is a common nomenclature for all of them. (Indeed, there are some popular forensic kits as the GlobalFiler, a first 6-dye, 24-locus STR kit that combines maximum compatibility with global databasing loci standards).

28.3 The Process of Standardization

If DNA analysis is nowadays accepted in countries all over the world, it is in part due to the progress made in standardization.

The standardization of forensic DNA analysis has made enormous progress in the last few years and this innovation in standardization is comparable to the introduction of DNA technology itself.

Standards are crucial for forensic geneticists. This is due to the fact that only with an agreement about standards is it possible to develop quality control programs and quality assurance programs. In other words, standards are the only way to guarantee the judges, juries and the public that the tests performed and laboratory efficiency are reliable in any specific case.

In addition, standards are necessary to allow for second opinions for the exchange of data between labs and for the creation of uniform searching procedures in cross border crime.

Two types of standards need to be addressed: technical and procedural. Technical standards include matters such as the genetic systems to be used (including type, nomenclature and methodology), the statistical methods for evaluating the evidence and the communication of the final report. Procedural standards encompass matters of operation such as laboratory accreditation, laboratory performance, accreditation and licensing of personnel, record keeping and proficiency testing.

Proficiency testing programs for DNA analysis are established in some countries, and external and internal controls have been set up by most of the labs in western countries. Progress in accreditation has been effective in many countries in the last few years.

Even more advances have been made in attaining common technical standards. Agreement on genetic systems, types and nomenclature is widespread.

Establishing common standards in Forensic DNA analysis is not easy due to the fact that very different legal systems exist as well as a variety of laboratories performing forensic genetic analysis. The success of the forensic geneticist in achieving common standards (at least compared with other aspects of forensic science and genetics) has been greatly facilitated because of the fact that a great number of them are members of the International Society of Forensic Genetics (ISFG) (www.isfg.org), which has many national and international working groups actively involved in establishing common standards. The DNA commission of the ISFG has an important role in resolving scientific conflicts between standardization groups and producing recommendations for the use of DNA polymorphisms in forensic casework.

However, efforts in standardization should continue. Progress in common procedural standards and particularly progress in similar requirements between countries for accreditation are necessary. Concerning technical standards, other priorities include the harmonization of criminal databases, the coordination and compilation of population databases (especially for mtDNA and Y STRs) and a continuation of the progress initiated over the last few years on statistical evaluation and communication of the value of evidence provided by DNA analysis.

This latter is a priority field of standardization. The question is that in most cases, but not all, the value of the evidence provided by DNA analysis is enormous. However, uncertainty always exists. As scientists, we must measure this uncertainty and for this we use a standard: that of probability. Likelihood ratios are nowadays used for weighing the value of the evidence and for communicating this value to the courtroom and the Bayesian approach to inference provides a coherent framework for interpretation.

28.4 The Refinement of Current Analytical Methods: STR and SNPs

Since the mid-1990s, computer databases containing STR profiles from crime scene samples, convicted offenders, and in some cases persons arrested but subsequently cleared of a crime, have provided law enforcement agencies with the ability to link offenders to crime scene STR profiles.

Application of this technology has enabled thousands of crimes to be solved around the world.

Y-chromosome specific polymorphisms have proved to be especially useful in forensic analysis and have been used since 1995. Applications of Y-chromosome polymorphisms include the analysis of deficiency paternity testing of a male off-spring (e.g. mother unavailable for testing) and different applications in criminal casework. Y polymorphisms are particularly interesting for the analysis of the male DNA fraction in stains involving male/female mixtures, the most common biological material available from the analysis of sexual crimes.

In the same way as autosomal Y-STRs have risen in number, Y-STR marker sets have expanded and commercial multiplexes now offer a minimum number of Y-STRs to create the minimum Y STR haplotype. As uniparental loci, passed on in the parental lineage from male to male, statistical interpretation in cases of a Y-STR match are more complicated and appropriate corrections must be made by taking into account population substructure and sampling errors. Population surveys are therefore very important and quality-controlled population databases have been compiled by developing a global database organised as the Y-chromosome haplotype reference database (YHRD). At the same time as Y-STRs, STRs in the X-chromosome were also introduced and these markers are of interest for some deficiency paternity testing cases.

During the period in which STR typing was beginning to utilize fluorescent labeling in the early to mid-1990s, mitochondrial DNA (mtDNA) was introduced for forensic applications. Analysis of the mtDNA control region, a segement of the whole mitochondrial genome, is an efficient method for the study and comparison of bones, old and degraded DNA and, especially, the analysis of telogenic hairs. In these cases, samples of mtDNA variation can be analysed using a variety of strategies. The combination of PCR amplification with direct DNA sequencing is usually the optimum approach for identification and it has proved to be a reliable and reproducible method in forensic casework, used in forensic laboratories since the mid 90’s.

The last years have been very exciting in the field of forensic genetics [2]. Firstly, a new type of marker has been introduced: single nucleotide polymorphisms (SNPs). SNPs have a number of characteristics that make them very appropriate for forensic studies. Firstly, they have lower mutation rates than STRs and this is valuable for paternity testing. Secondly, they can be analyzed in short amplicons and, in general, short sizes are desirable since the size of the amplified product is critical for the successful amplification of degraded samples. Finally, they are very suitable for analysis using high throughput technologies and the use of high density SNP microarrays has recently been successfully implemented for the identification of very distant relationship investigations in incomplete pedigrees. A variety of SNP panels for different forensic applications have been proposed and validated. Similarly, insertion-deletion polymorphisms or indels have proved to be especially valuable since they are simple, robust and easy to analyze and interpret.

Perhaps the most important advance and progress in the last few years were the use of SNPs for forensic DNA phenotyping (FDP), which in its narrow definition comprises the prediction of externally visible characteristics (EVCs) (i.e., appearance) of a stain donor as the phenotypes of interest from trace DNA directly. Because some EVCs are age-dependent, and age itself characterizes a person and is visible, age is included in the wider definition of FDP. In addition, bio-geographic ancestry i.e., the geographic region of origin of a person's biological ancestors, which further characterizes a person and is linked to some EVCs, is included in the wider definition of FDP.

Progress in the field of human appearance genetics made over the last 10 years has provided promising expectations for the construction of composite sketches from DNA for investigative intelligence purposes [3, 4]. Various studies, searching for the genetic basis of EVCs, have been carried out for pigmentation traits, body height, hair morphology, ear morphology, facial shape, and for some age-related appearance traits such as hair loss, pigmented age spots.

Based on research that identified the key genes involved in human eye and hair color variation, predictive DNA markers (mostly single nucleotide polymorphisms, SNPs) were developed and validated, and statistical models were built and validated to obtain eye and hair color phenotype probabilities from the DNA marker, providing provide prediction accuracies for eye and hair color that are sufficiently high to be useful in practical FDP.

A second approach for constructing a composite sketch from DNA is the use of age information stored in the DNA. Inferring the age of an unknown crime scene sample donor from DNA found at a crime scene allows the characterization of the unknown person and their differentiation from other persons, which on its own provides intelligence information in the context of FDP, since age is noted in registers. Moreover, age progression influences appearance and there are various EVCs that strongly depend on the age of the person. From all the methods proposed so far, methylation analysis is the most accurate. DNA methylation regulates gene function and about 20% of variation in DNA methylation in the human genome is correlated with age. DNA methylation-based age prediction models for blood developed so far have provided age prediction with a mean absolute error of even less than 3 years [5].

A third approach that contributes to a composite sketch from DNA is the use of biogeographic ancestry information stored in the DNA. Bio-geographic ancestry describes the geographic region in the world where the person's genetic ancestors originate from (also referred to as genetic ancestry). Inferring the bio-geographic ancestry of an unknown trace donor from his/her trace DNA allows the characterization of that person and the differentiation from others, which in itself provides investigative information in the context of FDP. The genetic-geographic structure of worldwide human populations shows a minor proportion of genetic differences between people from different places, but exploitable in order to derive ancestry-informative DNA markers and for developing DNA tests for ancestry prediction, which are useful in forensic applications. Ancestry informative marker SNPs with marked allele frequency differences in populations have been found to be particularly useful in predicting the geographic origin of individuals from biological material and have been successfully used in important forensic cases, such as the analysis of unmatched STR profiles in the investigation of the 11-M Madrid terrorist attack [6].

Non-human species in forensic genetics is an emerging field with a promising future. Forensic analysis of animal DNA has been used both when animal material (usually pet hairs) is found at crime scenes, and in investigations of the illegal trade in endangered species. As with animal material, plant material can be associated with a crime scene and provide vital evidence. The same is true for analysis of bacterial strains in soil through new metagenomic approaches.

One of the most difficult problems facing the forensic biologist is the identification of body fluids. Molecular biological approaches to the identification of blood, semen and saliva stains through the analysis of specific mRNAs (which are surprisingly stable) have been described, and are likely to increase in use and importance.

28.5 Conclusion and Future Perspectives

New generation sequencing technologies are also going to have an impact on many of these new applications. At the present time, the most suitable technology to analytically combine most of the new applications of forensic DNA testing is targeted massive parallel sequencing (MPS) [7]. Almost 10 years ago, technology emerged termed "Next Generation Sequencing" that took advantage of DNA molecules being bound to a substrate which could then be independently analyzed in a highly parallelized fashion. For this reason, the first two generations of NGS are also referred to as Massively Parallel Sequencing. With MPS, DNA template preparation does not necessarily require an amplification step. Additional advantages over the conventional Sanger method are the direct determination of the nucleotide and the ability to pool multiple DNA samples into one sequencing run differentiated by individual adaptors. The second generation of MPS instruments and reagents (also termed bench top sequencers) brought the main breakthrough in providing detailed, reliable, and high throughput sequencing for the field of molecular genetics, including forensic DNA analysis, and represent the next step in the never-ending revolution in this field.

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Part IX
Innovation, Unitariness and Evidence. Forensic Toxicology

Casseri, Giulio 1552–1616, Tabulae anatomicae 78 cum supplemento 20 tabularum Danielis Bucretii (Adriani Spigelii Bruxellensis ... Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden ... Amsterdami: apud Iohannem Blaeu, 1645)

Schultes, Johann 1595–1645, Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666

Chapter 29
Post-modern Medicolegal and Forensic Toxicology

Hans H. Maurer

Abstract The ongoing progress in analytical and life sciences is opening up new perspectives in post-modern medico-legal and forensic toxicology. In times of personalized medicine, the interpretation of analytical results in clinical and forensic cases should also be based on genetic aspects, pharmacogenomics in particular. Pharmacologic and toxic effects of drugs or poisons may be influenced by the genotype and phenotype of an individual, but also by the isoenzymes involved in their metabolism and membrane transport. Further individual factors such as body mass, age, sex, kidney and liver function, and drug-drug (food-drug) interactions may have an impact. Detailed knowledge of all these factors is a prerequisite for evidence-based case interpretation. In this chapter, the current knowledge of possible risks in variations of the effects of relevant therapeutic drugs, herbal drugs, and drugs of abuse will be presented. A critical discussion of the impact on the interpretation of analytical results in clinical and forensic toxicology will follow.

29.1 Introduction

In times of ante-modern forensic toxicology (FT), drug concentrations were determined, for example, by spectrophotometry after thin-layer chromatographic separation and elution of the scraped spots following internal approaches. In modern FT, the blood concentrations are determined using the latest high-end mass spectrometry equipment in high resolution [1, 2] following all international

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guidelines for method validation and internal and external quality control [3] in an accredited laboratory. However, this analytically determined "true blood level" was/is often interpreted toxicologically by simply comparing it with a published reference list, e.g. by Schulz et al. [4]. Such lists are mainly based on data coming from controlled clinical trials of pharmaceutical companies or single case reports. Of course, this procedure is too simple for evidence-based case interpretation in times of P5 medicine and justice. While the pharmacokinetic variability within such trials is up to ten times, the variability among the real population may range up to 100 times [5]. Case reports are often well documented only for the medical or for the analytical part [6].

In (post-)modern toxicology, various effects influencing the individual drug/poison response have to be considered, such as age, gender, body-mass index, drug-drug or drug-food interactions, lifestyle and nutrition, the microbiome, and particularly the genome and epigenome [7, 8]. All effects may, for example, increase or decrease the bioavailability and plasma elimination half-life of a drug. Pharmacogenomics (PGx) plays a major role in the relationship of P5 medicine and justice [9]. In contrast to pharmacogenetics describing only the genetic variations, PGx describes multifactorial variations of drug responses caused by variable gene expression (polymorphisms), influence of drugs on genes (epigenetics), and time-dependent changes of gene expression [10]. These genetic and post-translationally acquired variations then describe the phenotype.

PGx is nowadays well established in P5 medicine, such as in oncology, transplantation, and psychiatry with the goal to find the right dose of the right drug for the right indication for the right patient at the right time [7]. Sim and Ingelman-Sundberg [11] discussed important pharmacogenomic biomarkers influencing treatment response and/or incidence for adverse drug reactions. However, the prerequisite is that the drugs are characterized during drug development concerning the influence of polymorphically expressed target proteins (receptors, ion channels, or enzymes) and proteins involved in drug transport or metabolism.

As the new psychoactive substances (NPS) are neither tested for preclinical nor clinical pharmacology and toxicology before distribution and consumption, such data have to be elucidated by, for example, academic institutions. The author's group, for example, has studied metabolism intensively, including the kinetics of the involved (iso)enzymes [12–20], the cytochrome P450 (CYP) inhibition potential of NPS [21, 22], and the possible interaction with drug transporters [23]. In pharmacokinetics, various drug transporters are involved in absorption, distribution, and elimination [24, 25]. For elucidating possible impact on drug response, PGx variations or interactions have to be tested for. Meyer et al. [23] described that the investigated NPS were no substrates of the major efflux transporter P-glycoprotein (P-gp), but some were potent inhibitors. Thus, PGx variations or interactions will have no impact on the effect of these NPS, but they can produce interaction with substrates of P-gp. For example, loperamide and domperidone, both effluxed by P-gp at the blood-brain barrier, are discussed to act centrally by coadministration with P-gp inhibitors such as verapamil or quinidine.

For personalized case interpretation, Wong et al. [9] discussed various advantages and disadvantages of PGx as an adjunct biomarker in personalized justice. It is advantageous that the DNA is stable in postmortem settings and may provide a personalized approach for assessing the relation of drug response with the postmortem drug concentrations. However, there are only limited postmortem reference data available in contrast to clinical medicine. As already discussed, the legal interpretation is challenging as many posttranslational modifications have to be considered as well as interactions with inhibitors or inducers of drug metabolizing or transporting proteins.

In the following, published examples for the impact of PGx and/or interactions on real case interpretation will be discussed.

29.2 Evidence-Based Case Interpretation

29.2.1 Missing Drug Effect Caused by PGx Variations and/or Interactions

29.2.1.1 Tramadol in Personalized Pain Management

Tramadol is an enantioselectively metabolized by the polymorphically expressed CYP 2D6 to the more potent opioid receptor agonist *O*-demethyl tramadol. In the context of personalized therapy in pain management, Stamer et al. [26] could show that a patient with a CYP 2D6 poor metabolizer genotype formed the lowest blood concentrations of the active metabolite, while the ultra-rapid metabolizers formed the highest. They could confirm the clinical response correspondingly. In a further study, they gave a CYP 2D6 inhibitor to the ultra-rapid metabolizers and, as expected, they could be transferred to functional poor metabolizers with low blood concentration of the acting metabolite. Again, the blood levels correlated with the clinical outcome. This example demonstrates that the case interpretation would not be correct when the blood levels of the parent drug would have been correlated with published data.

29.2.1.2 Missing Pain Treatment Under Oxycodone

Lee et al. [27] described the case of a patient under pain treatment with oxycodone, but without analgesic effects. The anesthetist wanted to monitor his adherence by urine drug testing for oxycodone. The test, performed with an assay focused on the parent drug only, was negative. After intake under supervision and still no response, they thought of possible PGx variations or interactions. Oxycodone is also a prodrug bioactivated by CYP 2D6. The patient was genotyped as CYP 2D6 poor metabolizer explaining the missing analgesic effects, but what was the reason for the negative urine test? They found that the patient was under treatment of the tuberculostatic rifampicin for years. Thus, the patient showed a significant induction of CYP 3A4, the enzyme responsible for the major metabolizing step leading to the pharmacologically inactive *N*-dealkyl metabolite. This example shows the above-mentioned complexity of evidence-based case interpretation. Incomplete drug testing with missing targets may lead to misinterpretation of the adherence test, drug-drug interaction as well as genetic variations in forming the acting metabolite, making a simple case interpretation impossible.

29.2.2 Poisoning Caused by PGx Variations and/or Interactions

29.2.2.1 Narcotic Syndrome After Codeine Administration

Gasche et al. [28] described a narcotic syndrome of a patient under therapeutic doses of codeine. Genotyping revealed that the patient was a CYP 2D6 ultra-rapid metabolizer forming a higher rate of the acting *O*-demethyl metabolite morphine. He was additionally under co-administration of the potent CYP 3A4 inhibitors clarithromycin and voriconazole. Thus, the main metabolizing step, namely the formation of the inactive *N*-demethyl metabolite, was blocked, resulting in even more morphine production. Finally, the patient suffered from an acute renal failure resulting in a limited elimination of the morphine-6-glucuronide, which can pass the blood-brain barrier and act as potent opioid. Again, this case shows that all aspects of PGx, interactions, and the body functions have to be considered when interpreting analytical results.

29.2.2.2 Fatal Poisoning After Breast Feeding of the Mother Under Codeine Treatment

Madadi et al. described a fatal morphine poisoning of a newborn after breast feeding of the mother under codeine treatment. The mother was a CYP2D6 ultra-rapid metabolizer forming high concentrations of morphine. This example shows that PGx and interactions must also be considered in such cases when the mother may be accused of having killed the newborn by application of morphine.

29.3 Conclusions

In post-modern toxicology, it is a must—at least in any unclear cases—to consider pharmacogenomic variations and interactions in interpretation of analytical results. However, it is essential that the above-mentioned limitations are considered of genotyping detecting only the genetic risks. Antemortem, phenotyping [29] is preferred to detect genetic and acquired posttranscriptional variations. The future will show whether in postmortem cases, direct determination of the modified proteins (metabolizing enzymes, transporters etc.) can be determined by high-resolution mass spectrometry. On the other hand, over-interpretation of all above-mentioned aspects by untrained experts must be excluded.

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Chapter 30
From Drug Identification to Systems Toxicology

Donata Favretto

Abstract Biomedical sciences are at the edge of an extraordinary transformation in the conduct of toxicological evaluations using modern biomolecular analysis techniques to elucidate mechanisms of toxicity. To this transformation have contributed the increasing power and availability of molecular measurement tools, the possibility of probing biological networks inside organisms, organs, tissues, and cells, the affordability of high-throughput characterization tools, and the availability of potent bioinformatic tools. The classical toxicant-by-toxicant approach, that has been applied to solve clinical and forensic toxicology challenges for decades, has now turned to a multidisciplinary approach. The application of the newest biomolecular measurements to the field of toxicology led to the emergence of new sub-disciplines, such as toxicogenetics, toxicoproteomics, and systems toxicology. The leading approaches are briefly reviewed, with a special focus on technological advances, the omics era, systems toxicology and the toxome.

30.1 Introduction

The knowledge of the natural, chemical and biochemical properties of plants, animals, insects and minerals has accompanied the development of civilization over the centuries, and the study of poisons, drugs, chemicals or toxins, *toxicology*, is ancient, as witnessed by documents dating as far back as 1500 B.C.E. such as the Smith and Ebers Papyrus. In the early development of toxicology many important milestones were reached, based upon experimentation and discoveries, but it is only from the beginning of the nineteenth century that the science of toxicology evolved

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into a scientific discipline. As a discipline, modern toxicology comprehends both clinical and forensic toxicology, running parallel and complementing each other, though with peculiar areas of interest. Whereas forensic toxicology needs to identify and quantify poisons in a medico-legal setting, establishing relationships between toxicant levels in biological fluids and impairment or a probable cause of death, clinical toxicology deals with emergencies in hospital settings managing overdoses, poisonings, and attempted suicides. In both sub-disciplines, however, the toxicologists need efficient tools to identify and quantify poisons/drugs/toxicants, to understand the mechanisms of intoxication and to face the challenges often posed by emerging new drugs or new uses of drugs. The history of modern toxicology has largely been determined by the development of technological devices, and the availability of laboratory diagnostic methods represented a turning point of the toxicology evolution, enabling evidence based decisions either in the forensic or the clinical settings.

More recently, the new "global" methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites have been termed "-omic" technologies, based on their ability to characterize all, or most, members of a family of molecules in a single analysis. After the achievement of the human genome project, technology developments and biostatistics elaborations allowed enormous amounts of information to be gathered on the genotypes and the phenotypes of species. The impact of omics onto toxicology made new sub-disciplines emerge: toxicogenomics, toxicoproteomics and metabolomics.

Toxicogenomics is the study of the relationship between the structure and activity of the genome (the cellular complement of genes) and the adverse biological effects of exogenous agents. In its broadest sense, it combines transcript, protein and metabolite profiling with conventional toxicology to investigate the interaction between genes and environmental stress in disease causation. Toxicogenomics has three principal goals [1–3]: to understand the relationship between environmental stress and human disease susceptibility; identify useful biomarkers of disease and exposure to toxic substances; elucidate the molecular mechanisms of toxicity.

Toxicoproteomics can be defined as the application of proteomic approaches analysis to address toxicological demands, from investigating the molecular targets of toxicants to understanding the molecular responses of cells and tissues to toxicants. The choice of the correct proteomic platforms and the characterization of specificity, validation, and hierarchization are the main issues that need to be addressed in toxicoproteomics.

Metabolomics is the study of the cellular metabolites formed and degraded under genetic control. The non-invasive nature of metabolomics and its close link to the phenotype make it an ideal tool for biomarker discovery and drug safety screens. In the future, with the availability of personalised metabolomics, we will potentially be able to track the trends of our own metabolome for personalised drugs and improved treatment strategies. Personalised treatment is likely to be more effective than our current medical population-based approaches.

The integration of the aforementioned bioanalytical approaches with classical toxicology is **Systems Toxicology**, when quantitative analysis of large networks of molecular and functional changes, occurring on multiple levels of biological organization, are gathered and integrated. The potential health risks associated with exposure to xenobiotics largely present in our environment lead to an increasing demand for new, more accurate and predictive risk-assessment approaches. A detailed mechanistic understanding of the ways in which chemicals perturb biological systems and lead to adverse sequels is needed; the combination of computational with advanced analytical tools in Systems Toxicology should allow such mechanistic knowledge, as well as identification and application of biomarkers for improved safety assessments. In Systems Toxicology, systems-wide molecular changes deriving from an exposure are quantitatively measured, and a causal chain of molecular events linking exposure with adverse outcomes is built. These processes are further described in a quantitative manner by mathematical models predictive of toxicological processes.

A complementary "omics" approach is also used to map and annotate pathways of toxicity (PoT) in the **Human Toxome Project**, a long-term vision to modernize toxicity testing for the twenty-first century. The project will comprehensively map pathways of endocrine disruption (ED) representing a proof of concept of mapping PoT by systems toxicology and a first step towards mapping the human toxome. The project will develop a common, community-accessible framework and databases that will enable the toxicology community at large to comprehensively and cooperatively map the human toxome using integrated testing strategies that combine "omics" data with computational models. Specific aims of the project are the development of software and visualization tools to enable the integration, analysis and visualization of data across multiple omics hardware platforms, identification of PoT, development of a consensus-driven process for pathway annotation, validation, sharing; establishment of a public database on PoT, validation of PoT and extensions of the PoT concept to additional toxicants.

30.1.1 Technological Advances for Drug Identification in Clinical and Forensic Toxicology

With origins in basic research, mass spectrometry (MS) emerged as a toxicology research tool when it was first applied to fingerprint molecules for drug screening in the fight against drugs of abuse. Since then, the technique has evolved, and in combination with separation technologies such as gas chromatography (GC), liquid chromatography (LC), and the advent of tandem mass spectrometry (MS/MS) technology to support targeted experimentals, smaller and smaller concentrations of active principles and metabolites could be identified and quantified. Due to its extremely high specificity in the identification of analytes, mass spectrometry rapidly became the gold standard of forensic toxicology. Successively, macromolecule ionization methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), enabled the study of proteins and large biomolecules, and with the adoption of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for small molecule targeting and translational clinical applications, MS technology is now routinely applied in clinical laboratories to improve the sensitivity and specificity of clinical tests, screen for diseases, monitor drug therapy, analyze peptides and proteins for diagnostic testing, and identify causes of infections for targeted therapies.

Matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) now supports direct tissue analysis with diagnostic potential and reduced analysis time [4]. Pathologists are now able to examine biological tissue directly, even in real time during an operation using MS with smart electroknives. Mass spectrometry imaging (MSI) enables histologists to define tissue types by chemical composition rather than structure [5] and all these recent advances promise even more sophisticated extensions to forensic and clinical applications. To the purpose of general unknown screening, clinical research and forensic toxicology laboratories already use high-resolution, high accurate-mass spectrometry (HRMS) or time-of-flight (TOF) MS, as well as for multi-analyte drug screens. Whereas triple quad-based techniques can quantify targeted analytes, newer HRMS systems with multiple mass spectrometry capabilities can simultaneously detect and deliver the full product ion spectrum, and retain high selectivity, high resolution and high mass accuracy.

Analyte multiplexing and automation deliver superior and highly reproducible data to simplify increasingly sophisticated methods and support test validation. HRMS using triple quadrupole MS/MS is now used to screen and quantify toxic drugs, offering novel metabolomic methods to distinguish between and eliminate candidate isomers. Electrospray MS/MS and a computer-assisted metabolic profiling algorithm can automatically flag abnormal profiles in newborns from a single spot of blood [6].

Automated MALDI-TOF MS enables clinical microbiology laboratories to identify and classify bacteria and other microorganisms [7, 8].

Exponential growth in MS methods in clinical and forensic laboratories is expected in high-throughput and quantitative clinical and translational workflows, bacterial identification, imaging of tissue sections, diagnostic testing, and functional assays. Improvements in automation will support validation and seamless communication with laboratory information management systems to bring mass spectrometry-based detection into larger and more complex clinical laboratories. The development of handheld mass spectrometers will enable measurements to be performed on-site or in remote environments.

As technology advances and technological challenges such as sample preparation, online extraction, throughput, automation, and system interfacing are overcome, we can expect the impact of MS in clinical laboratories to mature. MS will increasingly be relied upon for sensitive, highly reproducible, accurate results. As for forensic toxicology, the current demands that are likely to drive the future technology trends are protein analysis, peptide determinations, and large molecule bioanalysis.

30.2 The Omics Era

30.2.1 Toxicogenomics

Through integrating genomic technology with bioinformatics, toxicogenomic emerged as a new scientific sub-discipline soon after the introduction in the mid-1990s of microarray technology [9]. Toxicogenomics has enjoyed widespread attention as an alternative means to study the underlying molecular mechanisms of toxicity and address challenges that are difficult to overcome by conventional toxicology methods [10]. It represents a new paradigm in drug development and risk assessment, which promises to generate information and understanding of the molecular mechanisms that lead to drug toxicity and efficacy, and of DNA polymorphisms responsible for individual susceptibility to toxicity. Gene expression profiling will aid in establishing links between expression profiles, mode of action and traditional toxic endpoints. Such patterns of gene expression, or 'molecular fingerprints' could be used as diagnostic or predictive markers of exposure characteristic of a specific mechanism of induction of that toxic or efficacious effect. It is anticipated that toxicogenomics will be increasingly integrated into all phases of the drug development process particularly in mechanistic and predictive toxicology, and biomarker discovery. Transcriptomics or gene-expression profiling is perhaps the most widely used measurement technology and is used to study the changes in expression of all mRNAs in a cell population, organ, or organism. Transcriptomic analysis is also a well established approach for identifying perturbed biological networks and thereby gaining mechanistic insight into the system's response to an exposure [11]. With microarray-based toxicogenomics, the expression levels of thousands of genes can be simultaneously monitored, permitting the assessment of alterations in gene expression induced by different compounds or associated with different physiological conditions. Importantly, the large number of genes tested together provides opportunities to identify gene patterns and signatures that provide unique insight into a drug's toxicity that are difficult to recognize by conventional technologies.

As an example of toxicogenetic application in the medico-legal context, studies on the cytochrome P450 enzyme system were published in relation to drugs of forensic interest. With respect to drug metabolism, the most important cytochrome P450 fractions are CYP 2C9, 2C19, 2D6, 3A4, and 3A5, the polymorphisms of which can be relevant in the metabolism, for example, of codeine and tramadol for CYP 2D6, valproic acid for CYP 2C9, and diazepam and amitriptyline for CYP 2C19. Polymorphisms in these genes can change the enzyme activity, from a complete deficiency to an ultrafast metabolism and these differences could lead to severe toxicity or therapeutic failure by altering the relationship between the dose and the blood concentration of the pharmacologically active parent drug or metabolite. An opioid-related respiratory depression in a patient receiving tramadol was reported by Stamer et al. [12]. Analysis of the patient's genotype revealed a CYP2D6 gene duplication resulting in ultra-rapid metabolism of tramadol to its active metabolite. A concomitant renal impairment resulting in decreased metabolite clearance further enhanced opioid toxicity. In 33 autopsy cases, Levo et al. [13] analysed both the CYP2D6 genotype and the concentrations of tramadol and its metabolites O- and N-demethyltramadol, finding a correlation between the number of functional CYP2D6 alleles and the ratios of tramadol to its metabolites. The two important findings of this study were: genetic variation in drug metabolizing enzymes can be analyzed in post-mortem blood, and genetic variation correlates well with the parent drug to metabolite ratios.

Comparable to tramadol, O-demethylation of the prodrug codeine to its metabolite morphine is essential for its opioid activity and, thus, the CYP2D6 genotype specifically influences the efficacy and side effects. The analgesic effect of codeine has been reported to be substantially reduced in subjects found to be poor metbolizers. Some case reports concerning codeine are presented. A breast-fed neonate whose mother received codeine 30 mg/day died on day 13 of morphine poisoning. The mother was an ultrarapid metabolizer and high amounts of morphine were formed from codeine, which were then transferred to the baby [14]. A 29-month-old previously healthy child experienced apnea resulting in brain injury following a dose of acetaminophen and codeine two days after an uneventful anaesthetic for tonsillectomy. A genetic polymorphism leading to ultra-rapid metabolism of codeine into morphine resulted in narcosis and apnea. Genotyping of CYP2D6 is not only performed to prove the responsibility of genetic polymorphism for side effects, but also to prove an (accidental) dosage error when an ultrarapid genotype could be seen as responsible for a fatality [15].

More recently, with the invention of high-density array printing, next-generation sequencing (NGS) technologies are emerging as the measurement methods that may supersede microarray technologies on the basis of greater accuracy, providing exact transcript counts and results that closely approach quantitative PCR. Furthermore, NGS methods are more flexible as they enable gene-expression studies in organisms for which microarrays are not available, such as model systems used in environmental toxicology. Finally, they are likely to offer a higher throughput than microarrays as new developments will likely allow for the analysis of thousands of transcriptome samples in a single sequencing run.

Therefore, toxicogenomics is expected to revolutionize the traditional approaches for assessing toxicity and has been considered as a paradigm shift in toxicology. However, the lack of advanced data mining tools can significantly hamper progress in this new vanguard of the toxicological sciences. Recently, large toxicogenomics databases were made freely available to the public. Among those, the Comparative Toxicogenomic Database (CTD) is a robust, publicly available database that aims to advance understanding about how environmental exposures affect human health. The program is supported by funds from the U.S. National Institute of Environmental Health Sciences (NIEHS). The CTD provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships. These data are integrated with functional and pathway data to aid in the development of hypotheses about the mechanisms underlying environmentally influenced diseases. These wide-ranging studies are expected to stimulate knowledge discovery and development of novel data mining tools, which are essential to make advances.

30.2.2 Toxicoproteomics

The proteome represents the full complement of the proteins in a cell, organ, or organism, and proteomics is a systematic approach to characterizing, all or an enriched subset of, proteins therein. Measuring changes in levels and modifications of proteins is applied to diagnostics, drug discovery, and investigating toxic events. The emerging field of toxicoproteomics is focused on the proteomic studies of toxicity, caused in response to toxic chemicals and environmental exposures, both in episodes of acute exposure to toxicants along with the long-term development of disease. Toxicoproteomics uses the discovery potential of proteomics in toxicology research by applying global protein measurement technologies to biofluids and tissues after host exposure, with the aim of identifying predictive biomarkers of toxicants exposure. Toxicoproteomic studies will provide critical tools in the evaluation of the safety of chemicals and design of appropriate measures to minimize adverse effects. Proteomic data are of particular value in Systems Toxicology, because the proteome is an important mediator of altered biological responses as a consequence of exposure to active substances. Increases or decreases in protein levels may be a direct consequence of corresponding mRNA-expression changes, but increases or decreases in protein function may also be influenced by post-translational modifications. For example, protein phosphorylation, which can be further addressed by high-throughput phosphoproteomics, enables the characterization of molecular events proximal to disease-related signaling mechanisms [16].

MS is widely considered to be the fundamental technology for modern proteomics, largely because of its unrivalled sensitivity and highthroughput. Thanks to the high accuracy of MS, peptides in the sub-femtomolar range can be detected in biological samples with a mass accuracy of less than 10 ppm. This level of accuracy is necessary to compare proteins between samples derived from exposed and control systems. In comparative proteomics for Systems Toxicology, Isotope tagging for relative and absolute quantification (iTRAQ) is used because it enables the relative quantification of protein species between samples in a non-targeted manner. This method can be further complemented with a targeted method based on selected reaction monitoring (SRM) for the precise quantification of predefined proteins after controlled enzymatic digestion of the proteome. Because it is a targeted approach, any proteomics analysis by SRM requires the a priori selection of the proteins to quantify, using the results of previous experiments using non-targeted approaches such as iTRAQ combined with a review of the scientific literature. The list of selected proteins is then processed with bioinformatics tools to identify at least two proteolytic peptides that optimally represent the protein and distinguish it from all others, after several optimization and validation steps to ensure unique identification and accurate quantification. By this method, a multiplexed approach is obtained by which hundreds of proteins can be quantified in a single MS run. An emerging example is the development of surface-capture probes to interrogate the surface-reactive subproteome, a critical response gateway for active substances, as recently demonstrated for hepatocytes.

These data can lead to the refinement of mechanistic details of toxicology pathways that were mainly based on transcriptomic data [17–20].

30.2.3 Metabolomics

Being an emerging field of "omics" research, metabolomics has been increasingly used in toxicological studies, mostly because this technology has the ability to provide more detailed information to elucidate mechanisms of toxicity. As an interdisciplinary field of science, metabonomics combines analytical chemistry, bioinformatics, statistics, and biochemistry. When applied to toxicology, metabonomics also includes aspects of patho-biochemistry, systems biology, and molecular diagnostics. During a toxicological study, the metabolic changes over time and dose after chemical treatment can be monitored. Therefore, the most important use of this emerging technology is the identification of signatures of toxicity—patterns of metabolic changes predictive of a hazard manifestation. This chapter summarizes the current state of metabonomics technology and its applications in various areas of toxicological studies.

Metabolomics involves a comprehensive and quantitative analysis of all metabolites or organic or inorganic chemicals of low molecular weight that are products or substrates of enzyme-mediated processes [21, 22]. In the context of Systems Toxicology, metabolomics is unique because it can be used to define the amounts of internalized xenobiotic chemicals and their biotransformation products [23, 24] as well as the perturbed endogenous metabolome, which represents the ultimate change in the levels of chemical species resulting from molecular perturbations at the genomic and proteomic levels [25–27]. In the first case, an understanding of the kinetic behavior of xenobiotic toxicants and their metabolites (as well as related biomolecular adducts) [28] is necessary to identify candidate biomarkers of exposure for human and environmental monitoring. The second is a more conventional Systems Biology perspective of metabolomics, which involves both identification of metabolites and quantification of changes in their abundance and rates of production caused by an exposure. From a technical perspective, metabolomics most commonly involves NMR spectroscopy and/or MS analysis techniques in untargeted profiling or targeted analysis strategies [29, 30]. Profiling of a metabolome may entail global detection and relative quantification of a large number of metabolites without a priori knowledge. However, targeted experiments involve the absolute quantification of a small number of metabolites (around 20 or less) to test a defined hypothesis. In Systems Toxicology, metabolomics may be accompanied by concomitant transcriptomic and proteomic measurements to provide the full context of the exposure. Integrated analysis of these diverse data types is necessary to enable a full understanding of the past decades of advancements in NMR, which have made it a very powerful tool for metabolic research. Despite its limitations in sensitivity relative to mass spectrometric techniques, NMR has a number of unparalleled advantages for metabolic studies, most notably the rigor and versatility in structure elucidation, isotope-filtered selection of molecules, and analysis of positional isotopomer distributions in complex mixtures afforded by multinuclear and multidimensional experiments. In addition, NMR has the capacity for spatially selective in vivo imaging and dynamical analysis of metabolism in the tissues of living organisms. In conjunction with the use of stable isotope tracers, NMR is a method of choice for exploring the dynamics and compartmentation of metabolic pathways and networks. Various direct and isotope-edited 1D and 2D NMR methods can be employed to profile metabolites and their isotopomer distributions by stable isotope-resolved metabolomic (SIRM) analysis. It is to highlight the importance of sample preparation methods including rapid cryoquenching, efficient extraction, and chemoselective derivatization to facilitate robust and reproducible NMR-based metabolomic analysis. NMR has been applied in vitro, ex vivo, or in vivo in various stable isotope tracer-based metabolic studies, to gain systematic and novel metabolic insights into different biological systems, including human subjects. The pathway and network knowledge generated from NMR- and MS-based tracing of isotopically enriched substrates will be invaluable for directing functional analysis of other omics data to achieve an understanding of the regulation of biochemical systems [31–33].

30.3 Systems Toxicology

Systems Toxicology is aimed at decoding the toxicological organization of active substances that interact with living systems. It resides at the intersection of Systems Biology with Toxicology and Chemistry. It integrates classic toxicology approaches with network models and quantitative measurements of molecular and functional changes occurring across multiple levels of biological organization. The multidisciplinary Systems Toxicology approach combines principles of chemistry, computer science, engineering, mathematics, and physics with high content experimental data obtained at the molecular, cellular, organ, organism, and population levels to characterize and evaluate interactions between potential hazards and the components of a biological system. It is aimed at developing a detailed mechanistic as well as quantitative and dynamic understanding of toxicological processes, permitting prediction and accurate simulation of complex (emergent) adverse outcomes. Thereby, the approach provides a basis for translation between model systems (in vivo and in vitro) and study systems (e.g., human, ecosystem). Systems Toxicology, therefore, has an ultimate potential for extrapolating from early and highly sensitive quantifiable molecular and cellular events to medium- and long-term outcomes at the organism level, and its application could be part of a new paradigm for risk assessment. The development of dynamic adverse outcome pathway (AOP) models enabling the simulation of the population-level effects of an exposure is the ultimate goal of Systems Toxicology. This development follows three broad steps of maturity. The first level consists of the development of causal computable biological network models that link the system's interaction of a toxicant with the organ-level responses. Such models can be used to quantify the biological impact of an exposure in the context of quantifiable end points such as histology or physiological measurements. In a second step, as more mechanistic knowledge derived from quantitative measurements accumulates, dynamic models linking the exposure with the organ-level responses can be developed. Ultimately, the third level of maturity is reached when the link between the exposure and the population outcome can be represented by mathematical models that enable the simulation of population-level effects of an exposure. As an example, the liver is known for its outstanding capacity to regenerate after toxic damage [34]. Within a relatively short period of time millions of cells find their new position to restore functional tissue architecture. Until recently, little was known about which mechanisms orchestrate this process [35, 36]. In principle, cytokines released from dead cells may be responsible. However, numerous further possibilities, e.g. oxygen gradients, cytokine release from stellate cells or Kupffer cells, etc., may alternatively play a role. However, Systems Toxicology based simulations demonstrated that an unknown mechanism, named "hepatocyte sinusoid alignment" (HAS) is crucial [37]. During HAS hepatocytes align in the direction of the endothelial cells of the sinusoids. Therefore, the endothelial cells control the architecture of the liver's sheets of hepatocytes and also provide the critical stimuli to proliferate. This Systems Toxicology driven prediction of the key role of endothelial cells was later confirmed by knockout experiments [38, 39]. The practical relevance for toxicology: as soon as sinusoidal endothelial cells are destroyed by chemicals the risk of fibrosis strongly increases.

The principal question thus arises: What is a toxicological mechanism? The answer is complex because we are looking into highly dynamic networked systems. It is difficult to distinguish where normal response, defense, and adversity start. Most substances are promiscuous in the sense that they have not only one target for interaction with the biological system (molecular initiating event) but they will perturb more and more downstream pathways with increasing dose or duration of exposure. It is difficult to identify which is the pace-making (causal) pathway of a hazard manifestation. Further complications arise from the fact that an organism is a moving target, i.e., developmental processes, adaptive processes, cyclical processes, aging and degeneration all interfere with the perturbation under study. This is especially problematic if we look for the more subtle effects of low-dose chronic exposures. Next, it is not clear how much variance we face: Are these pathways sufficiently conserved between cells, species, or for a given group of toxicants employing the same mechanism? Last but not least, will we still see the mechanisms at work when dealing with the real-life exposures to mixtures? All these questions can only be answered by simply doing it [39, 40].

30.4 The Human Toxome

The Human Toxome Project [41, 42], funded by National Institute of Health as an NIH Transformative Research grant is focused on developing the concepts and the means for deducing, validating and sharing molecular pathways of toxicity (PoT). Using the test case of estrogenic endocrine disruption, the responses of MCF-7 human breast cancer cells are being phenotyped by transcriptomics and mass-spectrometry based metabolomics. Endocrine disruption was chosen as the pilot for the human toxome, because of the urgency to complement current risk assessment approaches [43] and the fact that many endocrine system molecular pathways are known. This allows comparison of the PoT (deduced in an untargeted way) with established toxicity pathways. The overall strategy is to use omics (initially, transcriptomics and metabolomics) to map and annotate PoT, to develop software and visualization tools for integration and analysis of the multi-omics data streams, and to identify, annotate and validate PoT. The project will develop a consensus framework and a community database enabling toxicologists to map the human toxome. The bioinformatics tools for PoT deduction represent a core deliverable. A number of challenges for quality and standardization of cell systems, omics technologies and bioinformatics are being addressed. In parallel, concepts for annotation, validation and sharing of PoT information, as well as their link to adverse outcomes, are being developed. The Human Toxome Knowledge-base, a reasonably comprehensive public database of PoT, could become a point of reference for toxicological research and regulatory test strategies. The project is unusual in that it is developing many of the concepts to address these challenges while exploring and further developing, in parallel, the necessary technologies. Human exposure to environmental estrogenic chemicals (xenoestrogens) is widespread and testing for their effects is a high priority for regulatory agencies. The possible effects include altered development in utero through puberty and beyond, as well as effects on reproductive tissues and the development and progression of cancer, especially breast cancer. Environmental chemicals capable of estrogenic endocrine disruption include various organic pollutants such as polychlorinated biphenyls (PCBs), pesticides, dioxins, aromatic hydrocarbons, and various natural chemicals (such as genistein). In particular, there is great public concern about bisphenol A (BPA). Some studies in animal models have shown effects of low dose in utero exposure to xenoestrogens (such as BPA) to be associated with abnormal fetal reproductive tract development in male and female offspring [44] and mammary tumor development in rats [45]. It remains controversial, however, whether the low dose exposures to xenoestrogens in humans are associated with adverse health effects. The estrogenic activities of many compounds have been examined in in vitro systems using fluorescent reporters [46, 47] and cell proliferation assays [44]. These assays, however, only provide information on a single endpoint and not the underlying pathways. More recently, microarrays have been used to determine gene expression induced by estrogens [48] and metabolomic patterns of metabolite changes [49]. However, these endpoints have not been systematically integrated to elucidate classical nuclear and non-classical cytoplasmic/membrane estrogen receptor-mediated (or other) pathways. Thus, it becomes important to develop an approach combining transcriptomic and metabolomic analysis—and later expand to further platform technologies—of the response to estradiol and xenoestrogens to discover PoT using relevant human cell lines such as MCF-7 and T47D [50]. The establishment of the quality-controlled cellular test system and definition of toxic treatment was done in the first two years of the project. Two independent labs were responsible for establishing the test model system and providing the biological material. In parallel, and continuing in year 3, the standard operating procedures for omics and their performance assessment took place. In order to generate the PoT, transcriptomics and metabolomics experiments are conducted in parallel in two additional labs. Throughout the project, software tools development and data analysis are supported. The definition of the concept of PoT, their identification and validation, started in year 2. A series of workshops developing the concepts is a key component of the project—for example, a workshop developed the following working definition of a PoT: A Pathway of Toxicity is a molecular definition of the cellular processes shown to mediate adverse outcomes of toxicants. All data and metadata (i.e., experimental descriptors) are made accessible to the consortium via a centralized cloud server. The last two years of the project include the establishment of the Human Toxome Knowledge-base [51, 52].

30.5 Conclusions

Toxicology is gradually evolving into a systems toxicology that will eventually allow us to describe all the toxicological interactions that occur within a living system under stress and use our knowledge of toxicogenomic responses in one species to predict the modes-of-action of similar agents in other species. We stand at the edge of an unprecedented transformation in the conduct of toxicological evaluations. A central tenant of the new toxicology involves applying modern molecular analysis techniques to elucidate mechanisms of toxicity and is being enabled by several factors. The first is the increasing power and availability of molecular measurement tools able to probe the functioning of biological networks inside organisms, organs, tissues, and cells. The second is the increasing affordability of high-throughput and high-content characterization approaches that can be applied to thousands of chemicals in short time periods rather than the chemical-by-chemical approach of the past four decades that involves thousands of animals and perpetual high costs and years of duration. The third enabler is the increasing computational power, data-storage capacity, and information-management tools now available to the scientific community that has facilitated the ability to employ complicated Systems Biology models. The fourth enabler is the acceleration in the development of adequate in vitro test systems to complement and gradually replace animal models. The fifth enabler is that of significant resource investment by governments throughout the world in funding efforts to develop the scientific foundation of Systems Toxicology. As an example, the Defense Advanced Research Projects Agency (DARPA, USA) has planned to develop up to ten "organs on chips" within the next 5 years. Likewise, the EU has invested on Safety Assessment Ultimately Replacing Animal Testing with prospects to continue the investment in the European funding framework, Horizon 2020. Eventually, these international efforts need to be combined to produce a real shift in the risk assessment paradigm that not only assures the highest levels of protection of public health and the environment, but also enables economic growth and global trade [53].

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Chapter 31
Omics in Forensic Toxicology a Bridge Towards Forensic Medicine

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Abstract Cutting-edge omic strategies are bringing new opportunities to many fields of investigation from the biomedical to the biomedicolegal. Comprehension of molecular mechanisms associated with pathologies such as acute or chronic toxicity or sudden cardiac death and the discovery of associated biomarkers represent key elements in the development of novel routes for a better understanding of complex phenomena. Omics and mining of complex generated data have reached a state of maturity. Recent innovations, notably for mass spectrometry with a remarkable gain in sensitivity and selectivity, and the development of next-generation sequencing technologies, have considerably increased the power of these approaches. In spite of ongoing progress in the omic methodologies, their application can already provide informative results leading to more accurate interpretations and evidences. Beyond the remarkable potential in forensic toxicology such as the research of new biomarkers, we predict that these technologies will reinforce translational research between forensic and clinical disciplines.

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31.1 Introduction

The development of omic sciences and their recent integration are bringing new insights to many areas of research. Multi-omic investigations aim at studying the overall changes and associated mechanisms of complex traits occurring in response to system-level perturbations [1]. With the development of cutting-edge technologies and computational tools, a state of maturity has been reached, offering the possibility of overcoming the complexity of the life sciences. Beyond the clinical applications, such tremendous opportunities are expected to have a considerable impact in the biomedicolegal field. Interestingly, forensic sciences are built on the subtle association of researchers with multiple skills and knowledges, integrating heterogeneous and synergic backgrounds. Many forensic institutes already integrate physicians, geneticists, toxicologists, chemists, pharmacists, biologists, and biostatisticians, i.e. combining all the competences required in system-level investigations.

In a modern laboratory of forensic toxicology, mass spectrometry (MS) plays a central role for detection, identification and quantification of pharmacologically active and/or toxic substances in various complex biomatrices which may be involved in death cases (direct or indirect) or may affect the behavior or deteriorate the physical and mental capacities. Among these procedures, toxicological screening is usually one of the major steps throughout the systematic toxicological analyses. By definition, screening procedures should ideally enable the detection and identification of thousands of molecules and/or related metabolites potentially present in complex biological samples [2]. The use of peak detection algorithms, MS/MS fragmentation and library searching tools are different steps of the process allowing the detection and identification of molecules of interest [3]. Although the purpose is totally different, the concept behind toxicological screening overlaps in certain points the definition and the methodology of metabolomics in certain points, at least from a technological point of view. Metabolomics is the science aimed at characterizing and monitoring the broadest range of metabolites in biological matrices [4]. The recent advancements and innovations in mass spectrometry (MS) in terms of sensitivity and resolution have driven the development of metabolomics by overcoming technical limitations associated with the complexity of this field [5].

The integration of metabolomic strategies in the bioanalytical research pipeline has created new opportunities to better understand the molecular mechanisms involved in physiological and pathophysiological states [6]. The methodology has been successfully applied in numerous areas, non-exhaustively including cancer, cardiovascular diseases, obesity, diabetes, neurology, and plant biology. In the population-based cooperative health research of Augsburg (KORA), 140 metabolites have been quantified for 4297 participants and the level of several metabolites were meaningfully altered in pre-diabetic individuals [7]. Using metabolite-protein network and targeted approaches on serum samples, seven T2DM-related genes associated with these metabolites were identified by multiple interactions with four enzymes [7]. Among them, glycine and lysophosphatidylcholine (18:2) metabolites have shown to be strong predictors of glucose tolerance, even 7 years before disease onset. Other serum metabolites have been identified as predictors of diabetes in the European Prospective Investigation into Cancer and nutrition (EPIC-cohort) [8]. More recently, Wang et al., using longitudinal data on 201 type 2 diabetes mellitus converters, identified a signature of five metabolites for which individuals in the top quartile exhibited a five-fold higher risk of becoming diabetes converters [9].

Because of its capability to detect slight changes in large datasets, metabolomics is also impacting the field of toxicology [10]. In the last couple of years, clinical and forensic toxicologists have indeed shown increased interest in applying this approach to determine signatures of exposition, susceptibility, or toxicity (for instance early signature of hepatotoxicity), and to investigate, in relation to the observed molecular patterns, the cellular mechanisms leading to the adverse effects of toxic agents and to the development of associated diseases [11]. Interestingly, the comprehensive nature of metabolomics can also be used to simultaneously identify products of drug metabolism, such as reactive metabolites involved in toxicity mechanisms [10].

In a pioneer study, Nielsen et al. performed untargeted metabolomics of retrospective forensic cases of humans exposed to 3,4-methylenedioxymethamphetamine (MDMA) to determine associated metabolites as well as endogenous changes related to drug response and toxicology [12]. From whole blood samples collected over a period of two years, their retrospective study revealed significant relative changes of several metabolites, including known metabolites of MDMA and different endogenous metabolites such as acylcarnitines, amino acids, and tryptophan. In addition to the possibility of identifying new direct or indirect biomarkers of drug abuse from human data usually not available, this work confirms the interest of the approach for providing new insights into drug effect and toxicology.

The changes in metabolite levels that can be measured by metabolomics will reflect the end response of biological systems to genetic or environmental modifications. The ultimate goal is therefore to link these changes within biochemical pathways to the enzymes involved and then to the underlying genetic alterations. Development of multi-omic approaches is the next step to better characterize and understand a complex pathophysiological state as a system organization. For instance, integration of genome-wide associations study (GWAS) and metabolomic profiling revealed how genetic polymorphism influence the human metabolome, providing strong association between metabolic traits and loci for biomedical and pharmaceutical interests [13, 14]. The potential of omic integration is still untapped and will expand in the near future to cover a deeper picture of the dynamics of molecular systems [15]. Many areas will be positively impacted by the promise of the post-genomic area. Not only the identification of more accurate interpretation, we can expect that these tools will enhance collaborations by potentially joining efforts between the different experts of a forensic institute. In this chapter, we will discuss the promising impacts that omics could have on forensic sciences, notably by using metabolomics. We also aim to introduce imaging mass spectrometry

(IMS), an emergent approach in MS for the molecular (proteins, metabolites, drugs) imaging of tissue sections, providing new opportunities in forensic toxicology and pathology.

31.2 Omic Study of Alcohol Consumption

Alcohol is a social drug whose harmful use is estimated to 3.3 million of deaths each year (www.who.int/substance_abuse/publications/global_alcohol_report/en/). Chronic alcohol abuse is a major cause of liver-induced toxicity and leads to the development of many other diseases including cardiovascular diseases, cancers and neuropsychiatric disorders [16]. Direct and indirect biological markers are used to determine alcohol consumption. Indirect markers, including gamma-glutamyl-transpeptidase (GGT) and carbohydrate deficient transferring (CDT), are biological parameters for which a modification in the concentration may be caused by high and regular alcohol consumption [17]. Direct markers, including ethanol (EtOH), ethyl glucuronide (EtG), phosphatidyl ethanol (PEth), ethyl sulfate (EtS), and fatty acid ethyl esters (FAEEs), demonstrates a great sensitivity and specificity with respect to a recent consumption and present the advantage of being detectable in the organism only after alcohol consumption. Some of them, like EtG, are easily incorporated into hair allowing the extension of the windows of detection in order to assess chronic alcohol consumption [17]. With respect to the burden of alcohol abuse and the number of related diseases, identification of novel biomarkers is of major importance to develop novel routes for better assessment of alcohol intake, prevention and therapeutic strategies but also to underly mechanisms associated to its toxic effects on associated disease onset and development [18].

Recently, GWAS have identified common single nucleotide polymorphisms (SNPs) associated with alcohol consumption, increasing the catalogue of susceptibility loci and the understanding of the genetic etiology of alcohol use disorders [19, 20]. Kutalik et al. performed a GWAS study on three population-based cohorts (each based on more than 5000 volunteers) to assess whether genetic factors affect CDT concentration in serum [21]. Their study revealed three single-nucleotide polymorphisms (SNP) significantly explaining some of the variation in CDT% measured in parallel within the different cohorts.

In addition to genomic studies, transcriptomics thought of “what appears to happen” refers to the identification and measurement of all transcripts (mRNAs, noncoding (nc) RNAs and small RNAs) expressed in a specific cell, tissue or fluid to capture its specific and complete gene expression set [22]. Transcriptome profiling has generally been achieved by hybridization-based cDNA microarrays, but is being progressively replaced by the recently developed next-generation sequencing (NGS) technology [22]. NGS extends the conventional process of capillary electrophoresis-based sequencing across millions of fragments in a massively parallel fashion by means of a generation cluster from a library prepared by random fragmentation of cDNA sample [23]. NGS is a powerful and cost-efficient tool for ultra-high-throughput transcriptome analysis, thus providing a discovery tool untapped in forensic sciences for deciphering the regulation and networks of gene expression [24]. As an example, Farris et al. performed a RNA-sequencing study on postmortem human prefrontal cortex to investigate the perturbations in gene expression profiles and their network organization in alcohol-dependent individuals [25]. Their work revealed associations of multiple ion channels, including a human-specific isoform of the voltage-gated sodium channel subunit SCN4B, underlying lifetime alcohol dependence.

Other omic strategies have been applied to assess biological processes in the development of drug abuse and the research of potential biomarkers [26, 27]. Among these approaches, proteomics has been widely applied and Wang et al. listed 237 proteins meaningfully associated with alcohol exposure from 17 proteomics studies [27]. In a population based cohort study, targeted metabolomics was also performed to investigate potential correlation between alcohol intake and changes in serum metabolite levels and to identify potential biomarkers that could predict high levels of alcohol consumption [16]. This study allows the identification of a metabolic signature, including lysophosphatidylcholines, ether lipids and sphingolipids species, that differentiates between moderate-to-heavy and light drinkers and which was further replicated in other cohorts. Although the study was based on a metabolomic strategy targeting 131 endogenous metabolites, the data suggested new insights into the impact of alcohol consumption on human metabolism and demonstrated the possibility of revealing new biomarkers.

Analysis of gene polymorphisms, gene expressions, proteins, metabolites or their potential combination is necessary to find key players with the largest detectable effect on a pathophysiological state such as alcohol abuse. This argument underlies the importance of integrating network information from multi-omic approaches. Taken together, these examples should encourage new study in different areas of forensic sciences to substantially improve our capacity of investigation at an unprecedented scale.

31.3 Proteomics and Metabolomics Imaging by Mass Spectrometry: A Link Between Forensic Toxicology, Pathology, and Radiology?

In the last years, radiological imaging techniques have been remarkably developed and implemented in forensic institutes, especially in Switzerland [28, 29]. Including multi-detector computed tomography (MDCT) and magnetic resonance imaging (MRI), the advantages of these approaches are undeniable by providing digitalized data to the forensic scientists that can be used in court or post-processed over time according to expert requirements [30].

The development of imaging procedures will continue to grow in forensic sciences by taking on increasing importance in case studies. This phenomena is due to the basic principle that complex set of data can be more easily understood from a single image, worded by the adage “A picture is worth a thousand words” [31].

In the last decade, an innovative MS-based approach has emerged for direct molecular imaging of tissue sections [32]. In addition to the MS efficiency for monitoring and identifying molecules, imaging mass spectrometry (IMS) leads to information on the spatial location of analytes in thin tissue sections [33]. For the first time, IMS enables the simultaneous mapping of the untargeted profiles of hundreds of molecules including proteins, peptides, lipids, drugs and metabolites while maintaining a high correlation between the resulting molecular images and the histology of the sections [34–36]. Although the principle has been comprehensively explained elsewhere, the concept lies on the use of an ion or laser beam to raster a thin tissue section mounted on a microscopic plate according to a grid array of fixed resolution defined by the user [37]. At each coordinate (usually between 10 and 200 μm), a mass spectrum will be acquired allowing the generation of ion images of the tissue section for each detected peak [38, 39]. The possibility of carrying out proteomic and metabolomic imaging from thin tissue sections is bringing new insights into the understanding and diagnosis of diseases. During the last decade, IMS has been applied in various fields such as cancer research, cardiovascular diseases, neurosciences, drug metabolism and distribution, and plant sciences among others [35, 40]. In addition, the technology is also gaining in popularity in the field of forensic sciences [41, 42]. As an example, chemical imaging of latent fingerprinting were recently demonstrated, providing an associated chemical information for the physical identification of individuals [43, 44].

Although IMS has been extensively applied to the analysis of endogenous molecules, IMS of drugs and metabolites is being more deeply investigated in recent years, as for instance in preclinical studies for monitoring the distribution of drugs and metabolites in organs [45]. As a remarkable example, IMS has been applied for mapping drug incorporation from a single hair analysis [46]. In a recent study, the use of IMS provided crucial information by visually demonstrating that methoxyphenamine is incorporated at two different sites, i.e. the region of hair bulbs and sebum glands [47].

The implementation of IMS will also bring research opportunities in forensic pathology. A recent publication has demonstrated the interest of IMS for the profiling of protein in post-mortem human spinal cord samples obtained from amyotrophic lateral sclerosis [48]. In our institute, we are applying proteomics and metabolomics IMS in order to determine molecular changes occurring in the context of early myocardial ischemia/infarction (EMI—ischemia during the initial 4 h) which includes lack of specific symptoms, markers, and tissue morphology as well as the timing of markers’ appearance [49].

Another emerging area in the field is the development of 3-D IMS to provide spatial molecular information of tissues or organisms [50]. Indeed, 3-D reconstruction of multiple 2-D IMS datasets presents an opportunity for deeper study of many biological phenomena such as cardiovascular diseases and neurological disorders [51, 52]. Despite numerous technical challenges still to be overcome, the method is highly promising and has already been successfully applied [53]. In a recent study, we demonstrated the potential of the approach in both mouse model and human carotid atherosclerosis tissues to describe 3-D biological system and mitigate concerns about the representativeness of the sample [54]. Interestingly, 3-D IMS has also been integrated to MRI enabling to correlate post-mortem proteomic imaging with corresponding in vivo anatomical data provided by radiological imaging [55, 56]. Looking at the growing importance of post-mortem imaging notably for the investigation of cardiovascular pathologies, integration of IMS to radiological methods into multi-modal strategies will extend the potential of investigation by allowing the molecular information to be associated with the anatomical features [30, 57]. From a general point-of-view, IMS is offering exciting new possibilities in the forensic and toxicological field to obtain completely new information.

31.4 Conclusions

Implementation of omic strategies in forensic sciences is opening exciting novel routes of investigations. The power of omic methods and the possibility of integrating multi-omic approaches will bring new opportunities in the future to better understand complex phenomena and extend our capacity of investigation. A plethora of technologies have reached a mature state of development now allowing the in-depth analysis of genome, epigenome, transcriptome, proteome, and metabolome. Although the future is promising, efforts are still required to push omic technologies forward, especially from the bioinformatic point of view, where the computational resources still have to be improved so as to fully take advantage of the tremendous quantity of data generated per analysis. This is among the most important aspects for which the community has to unite in their efforts.

Although it will never replace conventional forensic investigations, omic research has to be promoted to bring complementary tools. A way to move in this direction is also to accept that such an implementation represents an investment and may need time to deliver concrete applications. Recent success stories concerning omic applications in biological or clinical studies should encourage the use of these strategies in legal medicine. Elucidation of forensic cases is often a complex task overcome by the convergence of multidisciplinary experts working closely together, including physicians, geneticists, toxicologists, chemists, pharmacists, biologists, and biostatisticians. All the elements are combined to foster the development of omic approaches in the field. These approaches have been already successfully applied to forensic toxicology with, for instance, the use of metabolomics to determine the signature of drug exposition and toxicity, bringing new opportunities to determine novel biomarkers of drug exposition, susceptibility, and toxicity. Enriched by a decade of remarkable developments, IMS has also witnessed a phenomenal expansion, bringing remarkable opportunities to develop new fields of investigation at the frontier between forensic toxicology, pathology and radiology investigations.

The continuous innovations of omic strategies have considerably increased the power of these approaches. This gives the possibility of tackling very difficult issues in a "new" era of forensic biology. Among the potential future applications, we can note the understanding of various molecular events involved in pathology (such as acute or chronic toxicity or cardiovascular disease) and the research of associated biomarkers. Recent literature indicates that application of omic approaches in forensic research are only in the early stages despite their remarkable potential. It seems obvious that their growing interest in the legal medicine community will provide meaningful output, enhancing our knowledge and our ability to provide novel evidence in forensic sciences.

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Chapter 32
Medicines and Driving Personalized Medicine and Medical Liability

Rossella Snenghi and Alessandro Amagliani

Abstract Physicians establish which medicines should be administered for the benefit of patients. The system of categorization for medicines and driving developed by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) and research groups within the European project DRUID (Driving Under the Influence of Drugs, alcohol and medicines) would allow the prescribing doctor to look for safer alternatives within a specific therapeutic class. A prime and exemplificative medico-legal case of driving under the influence of psychotropic medications is presented, analyzing the responsibilities of each participant: the patient, the general and specialized physician, and the driving licensing authorities. It is necessary to establish guidelines relating to the prescription of psychoactive medications, to be shared and adopted by all of the parties involved. The point of reference is that of *Personalized Medicine*, a paradigm which also entails the personalization of therapy, including a careful evaluation of the social sphere and impact on the community.

32.1 Introduction

Driving a motor vehicle is a relatively complex activity, entailing a number of abilities, comprised of cognitive, psychological and motor skills. The large number of medicinal drugs currently being marketed and prescribed, combined with the issues of polypathology and polypharmacy, as well as the potential off-label use of

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said drugs, raises concerns in terms of the potential insurgence of side effects and adverse reactions causing driving impairment, which prescribing physicians might be unaware of [1].

Medicinal drugs can alter all of the functions required to operate vehicles, namely visual, cognitive, and/or motor abilities needed for safe driving. Therefore, such abilities influence the smoothness and correct functioning of the driver information processing model (DIP), switching between its various stages of perception, decision and reaction. Psychoactive medications, as well as other types of medication, can determine blurred/double vision, dizziness, impaired coordination and tremor, or affect the central nervous system (CNS), producing sedation, confusion or dizziness, and therefore imply a driving hazard. According to published data, up to 5–10% of medicinal drugs produce impairment of driving performance as a result of their pharmacological action and/or side effects [2].

Although traffic accidents and injuries are a common cause of death in many countries, the effect of medicinal drugs, among other numerous risk factors, has not been investigated and analyzed thoroughly [3]. The evidence of the effects of said drugs on driving fitness obtained through laboratory testing provide only a partial insight into the true impact, given that in real conditions a number of other variables are involved, such as the driver's behavior, health and interaction with the given environment.[1, 2, 3]

However, that a number of psychoactive substances, among which can be included alcohol, illicit substances, sedatives, antidepressants, mood stabilizers and other medicines acting on the CNS, negatively affect driving ability, has long been common knowledge [4, 5]. Indeed, the combined use of the aforementioned substances significantly increases the likelihood of a driver being involved in a traffic accident [6, 7], as can be inferred from the data published by the European Commission's Directorate-General for Mobility and Transport, Road Safety Unit. According to said body, accidents involving the consumption of alcohol, medicines and/or illicit substances account for up to 25% of the total, which roughly translates into 6500 fatalities on a yearly basis in Europe.[4]

Given the emphasis on the significant role played by medicinal drugs in traffic accidents, as stressed in recent Reports on drugs and driving,[5] the necessity to provide adequate guidance and information to patients and drivers is crucial. Recent European-based research has demonstrated the demand, on the part of patients, for

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[1]European Monitoring Centre for Drugs and Drug Addiction. Literature review on the relation between drug use, impaired driving and traffic accidents. Lisbon: EMCDDA; Feb, 1999.

[2]European Monitoring Centre for Drugs and Drug Addiction. Driving Under the Influence of Drugs, Alcohol and Medicines in Europe—findings from the DRUID project. Lisbon: EMCDDA; 2012.

[3]Jones R, Shinar D, Walsh JM. State of the knowledge of drug impaired driving. National Highway Traffic Safety Administration; Sep, 2003.

[4]European Commission–Road Safety–Fitness to drive. (http://ec.europa.eu/transport/road_safety/topics/behaviour/fitness_to_drive/index_en.htm).

[5]See foot note 2.

reliable information on prescribed medicinal drugs and the related potential adverse effects, also in relation to driving fitness [8, 9]. In this scenario, the provision of such information and knowledge is the responsibility of healthcare workers, family practitioners, prescribing doctors and pharmacists, who should effectively communicate with the patient. Nevertheless, the patient's responsiveness is also influenced by such factors as age and educational level [10].

Physicians establish, with complete autonomy and freedom, which medicines should be administered for the benefit of patients, and a therapeutic prescription is appropriate when it satisfies criteria of efficacy and safety. Accordingly, the physician must have a thorough knowledge concerning a given medication, such as indications, contraindications and interactions. However, health professionals prescribing *dangerous medicines* rarely make responsible and informed choices in relation to the prevention of traffic accidents.

In 2001 the ICADTS (International Council on Alcohol, Drugs and Traffic Safety) established a working group to evaluate "*Prescribing and Dispensing Guidelines for Medicinal Drugs Affecting Psychomotor Performance*". The aim was the development of criteria for better warning systems and guidelines for safe administration of psychotropic drugs. The ICADTS Guidelines highlight the role of standard practice for treating various medical conditions. "*In cases where medication has been selected as the preferred treatment option, side effects of medication that could harm the patient or diminish the drug's action should be avoided*". In accordance with the need for a best standard practice, the ICADTS claimed that the drug categorization system should be used to adjust the existing guidelines for all major illnesses entailing the prescription of psychotropic drugs. Another relevant point is the Patient's Education, which has to be a substantial part of the prescribing and dispensing guidelines. Furthermore, Driving Licensing Authorities should pay specific attention to medication history of applicants when they are evaluated for issuing or renewing driving licenses.

In 2006, the ICADTS published a list of 389 driving impairing medications, based on the Belgian, French and Spanish classification systems. The ICADTS three-tier categorization system was developed by comparing the effects within the three categories with the effect of different blood alcohol concentrations (BAC): categories I, II and III as respectively equivalent to BACs <0.5 g/l (<0.05%), 0.5–0.8 g/l (0.05–0.08%), >0.8 g/l (>0.08%). The use of 0.5 g/l is the most significant, since it is the legal limit in the vast majority of EU countries. According to the three-tier categorization system medicinal drugs are classified into: (1) Presumed to be safe or unlikely to produce an effect; (2) Likely to produce minor or moderate adverse effects; (3) Likely to produce severe effects or presumed to be potentially dangerous.

In 2011 the ICADTS and research groups within the European project DRUID (Driving Under the Influence of Drugs, Alcohol and Medicines) established the system of categorization for medicines and driving, developed to allow the prescribing doctor to look for safer alternatives within a specific therapeutic class.

The main aim of the DRUID Work Package (WP) 4 was to provide the bases and the methodology for the development of a European classification system for medications with respect to their impact on the ability to drive.

The DRUID WP4 expert group established and agreed that, according to its influence on the ability to drive, a medicine could be categorized as follows regarding driving: (a) category 0 (no or negligible influence on fitness to drive); (b) category I (minor influence on fitness to drive); (c) category II (moderate influence on fitness to drive); (d) category III (severe influence on fitness to drive).

This was in line with the SmPC (Summary of Product Characteristics) guidelines adopted in September 2009 by the EMA, proposing that in the Sect. 4.7, entitled "Effects on ability to drive and use machines", it should be specified whether the medicinal product has (a) no or negligible influence (b) minor influence; (c) moderate influence or d) major influence on these abilities.

32.2 Medico-Legal and Toxicological Ascertainment Applied to a Paradigmatic Case

The medico-legal literature does not report on judiciary cases relating to driving under the influence of psychoactive medicines. Indeed, its focus tends, for the most part, to be on cases of driving under the influence of alcohol and/or illicit drugs, whereas the issue of driving under the influence of medicinal drugs with potentially impairing effects, also in relation to individuals undergoing psychiatric therapy, has been, from a historical perspective, confined to a niche of interest to pharmacologists and specialists in pharmacovigilance. In light of such a premise, it is therefore interesting to carry out a medico-legal and toxicological study of a judiciary case of involuntary manslaughter caused by a patient undergoing treatment for a psychiatric illness.
The medico-legal and toxicological protocol entailed the following steps.

1. *Circumstantial history*, including the collection of data regarding family, school and employment conditions, the time and circumstances of the violation of the Italian Highway Code.
2. *Clinical history*, including the collection of previous medical reports, as well as any laboratory and/or instrumental findings of potential interest.
3. *Toxicological history*, focused on the type and quantity of medicinal drugs assumed, their manner and timing of consumption, as well as the possible assumption of alcohol and illicit drugs (based on the overall score obtained by the evaluation of the ten items of the Alcohol Use Disorders Identification Test—AUDIT, and the number of criteria fulfilled relatively to the substance use disorder in the Diagnostic and Statistical Manual of Mental Disorders).
4. *Clinical-Objective evaluation,* specific to internal medicine, neurology, psychiatry, orthopedics and toxicology.

5. *Toxicological analysis* of both blood and urine samples taken during the medical assessment for analysis of ethanol and the main classes of psychoactive substances and medicinal drugs, by means of screening immunochemical procedures, followed by Liquid Chromatography Mass Spectrometry *(*LC/MS*)* and Gas Chromatography-Mass Spectrometry (GC/MS) confirmatory analysis.
6. *Medico-Legal and Toxicological epicrisis* with a final evaluation of driving fitness.

32.2.1 Circumstantial Synopsis

A 62-year-old woman driving an SUV hit and subsequently dragged the body of a young woman for 3600 meters, which resulted in the latter's death. The environmental conditions were of excellent visibility and good weather. The accident took place on a straight country road where the speed limit was of 70 km/h. The ground was dry and no brake trails were found at the site of the accident.

32.2.2 Clinical Synopsis

The evaluation of the medical records demonstrated that the woman suffered from a mood disorder evaluated with different diagnoses during her long clinical history: atypical psychosis, anxious depressive disorder, major depression (MD), recurrent depressive episodes, bipolar disorder (BD). Three cycles were observed, in the third of which, from 54 to 62 years old, she maintained the psychopharmacological polytherapy. The medicines were prescribed by a General Practitioner according to the indications of different Psychiatrists. Other relevant clinical aspects aggravating the subject's psychiatric condition were the presence of stressful events, such as chemotherapy for breast cancer at 61 years old and, more recently, hospitalization for recurrent depressive disorder at 62 years old.

From the analysis of the hospital records, it resulted that the woman was hospitalized for acute stress disorder (ASD) two hours after the accident. In relation to the anamnesis, she declared «I am under antidepressant therapy and I don't have any symptoms». The psychiatric evaluation showed: normal space-time orientation; congruent ideation; unstable gestures; low mood; heightened anxiety; normal cerebral CT.

32.2.3 Toxicological Synopsis

On discharge from the hospital the polypharmacotherapy included: two antidepressants (amitriptyline + perfenazine—Mutabon Forte®; venlafaxine—Efexor®);

a mood stabiliser (lamotrigine—Lamictal®); a neuroleptic (Quetiapine—Seroquel®) at low dose; a hypnotic benzodiazepine (Flurazepam®). The treatment combination was briefly changed before the accident due to complaints of excessive medication on the part of the woman's husband. However, due to a recurrence of depressive symptomatology, it was restored.[6] Subsequent to the accident, the psychoactive therapy combination came to light and the Public Prosecutor ordered a medical-legal and toxicological evaluation of driving fitness.

The woman had no history of alcohol and/or drug abuse/dependence.

32.2.4 Clinical-Objective Evaluation Synopsis

The clinical-objective evaluation highlighted: absence of medical and surgical pathologies, normal space-time orientation, congruent ideation, unstable gestures, low mood, heightened anxiety and a normal cerebral CT. The woman admitted that she misrepresented her status in the "*self-anamnestic statement*"[7] 10 months before the accident, so she wrote NO for Mental disorder and NO for legal and illegal drugs.

32.2.5 Toxicological Analysis Results

The blood and urine samples were taken within 5 h 20 min of the accident. Both matrices showed concentrations within the therapeutic range for all of the prescribed medicinal drugs, with the exception of perphenazine, which was present only in the urine sample, given that the patient skipped the second daily dose of Mutabon as a result of the accident.

32.2.6 Medico-Legal and Toxicological Epicrisis

The medico-legal evaluation demonstrated that the patient suffered from a recurrent depressive disorder. The general rationale of recurrent depressive disorder treatment is the prevention of new episodes, treatment of residual symptomatology and control of the chronicity of the disease [11, 12]. Nevertheless, the main problem is

[6]The paradox is that the Generic Recommendation written by the prescribing physician was «Prudence in driving».

[7]This document is a self-declaration written in the presence of a physician authorized by the State. It is filled out every ten years for drivers. In the past it was an anamnestic certificate written by Italian general practitioners, but they asked for its abrogation due to conflict of interest with professional secrecy.

Table 32.1 Antidepressant drugs

SSRI (Selective Serotonine Reuptake Inhibitors)
paroxetine, sertraline, citalopram, escitalopram, fluoxetine, fluvoxamine
NARI (Selective Noradrenalinie Reuptake Inhibitors)
reboxetine
SNRI (Serotonine-Norepinephrine Reuptake Inhibitors)
venlafaxine, duloxetine
NaSSA (Noradrenergic And Specific Serotonergic Antidepressant)
mirtazapine
TCA (Tricyclic Antidepressant)
clomipramine, amitriptyline, imipramine, etc.
Others
Trazodone, bupropion, «physiological» composites (hypericum eXtract)

that each patient responds differently to a specific medicine or polytherapy. Generally, antidepressant drugs are classified in six categories (Table 32.1).

In this case of recurrent depressive disorder the polytherapy diversification was necessary, with the prescription of antidepressants (TCA + SSRI), together with neuroleptics (quetiapine), mood stabilizers (lamotrigine) and benzodiazepines. Such an antidepressant therapy diversification demonstrates a pharmaco-resistant disorder [13]. In relation to the medicines taken, the Scientific Literature [14–19] and the relevant product information sheets,[8, 9, 10, 11, 12, 13] highlight the following common side effects with a potential to cause driving impairment (Table 32.2).

The medico-legal analysis also studied the information relating to driving fitness reported on the *packages* of the medicines assumed, as well as considering the presence of warning labels (*pictograms*). The principle is that they are the most accessible source of knowledge for patients, their families and the health professionals involved. The 2011 DRUID WP4 guidelines foresee the presence of warning information and pictograms based on the category of medicines (Fig. 32.1) [20].

[8]Amitriptyline hydrochloride product information: Mylan Pharmaceuticals Inc., Morgantown, WV, (PI revised 03/2016).

[9]Trilafon perphenazine product information: Schering Corporation 505 Kenilworth, NJ, (PI revised 04/2002).

[10]Lamictal lamotrigine product information: GlaxoWellcome Inc., Research Triangle Park, NC, (PI revised 05/2015).

[11]Flurazepam hydrochloride product information: Mylan Pharmaceuticals Inc., Morgantown, WV, (PI revised 01/2015).

[12]Venlafaxine hydrochloride product information: Wyeth Pharmaceuticals Inc. Philadelphia, PA, (PI revised 02/2008).

[13]Quetiapine Fumarate product information: Sandoz Canada Inc., QC, (PI revised 09/2015).

Table 32.2 Common side effects correlated with driving impairment

Medicinal drug	Side effects
Amitriptyline	*Hallucinations, Confusional states, Disorientation, Incoordination*
Perphenazine	*Blurred vision, Lethargy, Drowsiness*
Lamotrigine	*Diplopia, Blurred vision, Dizziness*
Flurazepam	*Reduced alertness, Confusion, Dizziness, Double vision*
Venlafaxine	*Somnolence, Dizziness, Blurred vision*
Quetiapine	*Somnolence, Dizziness*

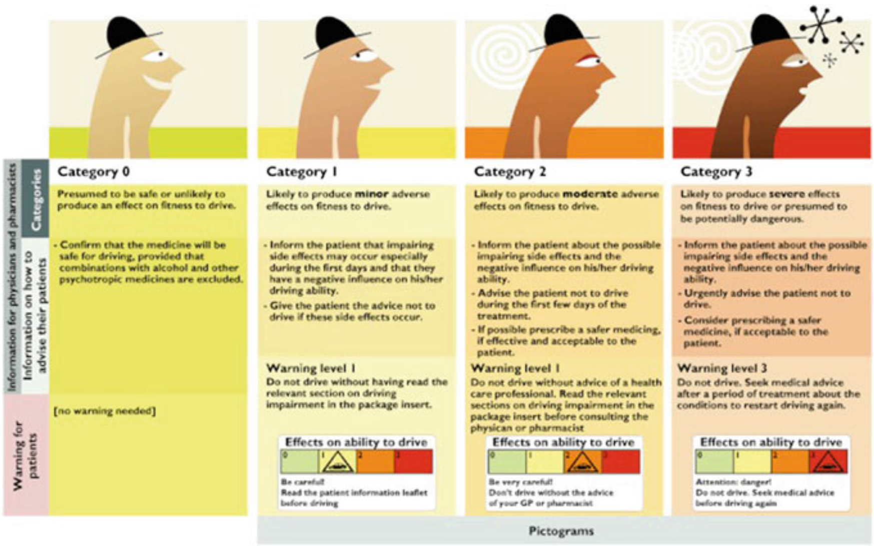

Fig. 32.1 DRUID categorization system for medicines and driving [20]

For the association of *Amitriptyline* and *Perphenazine* the side effects reported on the package are sleepiness, hallucinations, disorientation and anxiety. The driving warning is specified. DRUID categorization labelling classifies Amitriptyline in the third category, Perphenazine in the second category, whereas the association of Amitriptyline with psycholeptics is not evaluated.

For *Lamotrigine* the side effects are vertigo and double vision. Driving fitness is not evaluated, but prudence is advised. DRUID classifies lamotrigine in the second category.

For *Flurazepam* the side effects are sedation, amnesia and attention deficit. The driving warning is specified. DRUID classifies it in the third category.

For *Venlafaxine* the only side effect is akathisia. Moreover, in this case driving fitness is not evaluated and prudence is advised. DRUID classifies it as not evaluated.

Quetiapine's side effects are sedation and sleepiness. The driving warning is specified and it is classified in the second category by DRUID.

None of the packages had pictograms printed on them. The presence of such pictograms is the most effective warning for the patient and family.

The Toxicological Analysis demonstrated that the woman properly followed the prescribed therapy, as revealed by the results showing therapeutic concentrations. However, considering the polytherapy and the unwanted effects of medicinal drugs reported by DRUID, it is likely she experienced an impairment of her ability to drive due to episodic somnolence, disorientation, hallucination, diplopia and vertigo.

The thesis of the defense was comprised of the presence of brain damage (based on PET and CT scans), and a pharmacoresistant disorder (based on the genetic analysis demonstrating a homozygous variation of enzyme MAOA-3, an indicator of relapse vulnerability).

The medico-legal conclusion was a diagnosis of driving under the influence of medicines. Accordingly, the penal sentence was of 6 years for involuntary homicide, driving under the influence of psychotropic substances and making a false declaration.

32.3 Conclusions

The role played by medicinal drugs in traffic accidents is currently underestimated and this entails a significant social risk. Unfortunately, the inefficiency of the system is particularly damaging to the patient, who is individually responsible in the event of a traffic accident causing death or injury to others.
The main critical areas concern the following.

- *Compliance* of the *patient*, often clearly uninformed of legal responsibility and any related insurance implications that may arise.
- *Insufficient involvement* of the *prescribing physician*, with regard to the possible impairment arising from the prescribed medications, including *lack of communication* with the patient and discussion of potential therapeutic alternatives.
- *Inadequate medico-legal procedures* of *ascertainment* by the Authorities involved in the assignment, renewal and management of driving licenses.

In light of the aforementioned, there is a clear need to establish Guidelines dedicated to the prescription of *psychoactive medications*, to be shared and adopted by all of the parties involved, so as to reinforce the awareness and conscientiousness of the various health professionals.

Within such a framework, the ***patient*** would have to respect the posology and avoid misuse. Furthermore, the patient's health condition would have to be disclosed to the driving authority in charge of evaluating driving fitness by means of a specific procedure.

At the same time, the ***prescribing physician*** would be in charge of providing adequate medical information, through an effective dialogical consultation and the utilization of "information forms", clearly disclosing side effects, possible driving impairment correlated to the drug/disease, and recommending adherence to the principle of "*don't drive if you think you are experiencing side effects*". Of equal importance is the provision of advice not to combine the prescribed therapy with other psychoactive medicines and/or alcohol, as well as clear information concerning the possible legal and insurance implications.

The ***Patient Evalutation*** would include the assessment of those side effects related to his therapy/pathology, his awareness of the disease and any correlated impairments arising from it. Moreover, a ***scheduled follow-up*** of the patient, possibly involving his family, with his consent, and accurate archiving of the ***patient's records,*** would be essential to prevent the emergence of legal consequences for the physician.

From the perspective of the ***driving authority*** the Guidelines would have to focus on the identification of a set of *minimal requirements for driving fitness*, based on the *DRUID categorization system* and the *Driver Evalutation*. Such an evaluation would have to include a *standardized Medico-Legal and Toxicological protocol of ascertainment* focused on the consumption of alcohol, illicit drugs and medicinal drugs, as set out above in the aforementioned example in the form of an *ex-post* evaluation of a criminal DUI case, as well as specialized "tests of skills and behavior" (to be entrusted to qualified psychologists and/or psychiatrists).

In many countries, in response to violations of the Highway Code for Driving Under the Influence (DUI) of alcohol (and/or licit and illicit drugs), legislation provides for a mandatory evaluation of the subject prior to the reissuing of the driving license. Such an evaluation can lead to a medico-legal conclusion of fitness/unfitness to drive. However, the same laws do not provide or suggest any methodological standards and the whole reissuing procedure is entrusted to local health authorities.

A valuable initiative was carried out in 1993 by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) through the establishment of a working group that outlined 2 levels of assessment (medical screening and medical–psychological assessment) for drinking drivers, in relation to individual risk parameters, such as blood alcohol concentration (BAC), category of license, and recidivism [21].

In the Italian framework the methodological and evaluating guidelines, published in 2006 by the Italian Society of Legal Medicine and Insurance (SIMLA) [22], represented the first step towards a much needed standardization, as well as a launch pad for the further development of Medico-Legal and Toxicological protocols of ascertainment.

The application of such guidelines allowed for the identification of underlying polydrug use in drunk drivers with a significant increase in the rate of unfitness to drive [23]. Such evidence demonstrates that there is a need for a revision of the relevant European law that should cover the entire system of Traffic Safety.[14]

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[14]In Italy the exponential increase in fatal road accidents in the face of the perceived inadequacy of the repressive system has led the Italian Parliament to introduce Law n. 41 of March 23rd, 2016, making vehicular homicide, together with road traffic injuries, a criminal offense, both of which to be punished as a result of gross negligence. The penalties of the new law are doubled compared to

In conclusion, the practitioner cannot ignore the collective risk by merely privileging the interests of the patient and/or emphasizing the treatment of the illness.

The medical practitioner must consider that the prescription of "*dangerous medicines*" entails types of responsibility inherent to the various healthcare procedures: diagnosis and classification of the psychopathological illness, choice of treatment, clinical and compliance monitoring, as well as education and provision of information to the patient. The paradigm for medical conduct would hence become more closely aligned with that of Personalized Medicine, namely, personalization of therapy with patient-centered decisions truly benefiting from a rational balance between efficacy and safety, including a careful evaluation of the social sphere (especially in relation to driving and working) and impact on the community.

In light of the aforementioned, a significant effort shall also be made by the Driving Authorities together with those Practitioners of Legal Medicine involved in the issuing and reissuing procedures, with a view to the promotion of a virtuous system in which all of the parties involved are no longer acting in a compartmentalized fashion, but within a framework regulated by positive and negative feedbacks where, at its center, is situated the patient.
In this perspective, the role of the practitioner of Legal Medicine shall hopefully play a pivotal role in the near future, especially by promoting the following.

- Multidisciplinary research projects concerning human—machine interactions, with the involvement of clinical pharmacologists and bio-engineers, so as to investigate the potential for driving impairment of the various medicinal drugs under realistic testing conditions.
- Medico-Legal and Forensic Toxicology Protocols for the effectuation of roadside testing programs, with emphasis on medicinal drugs, which, up until now, have not been adequately taken into account and reported.
- Standardized Medico-Legal and Phamaco-Toxicological procedures to be implemented by the Driving Auhorities in the granting/regranting of the driving licenses.
- Diffusion of Medico-Legal guidelines of conduct aimed at all of those practitioners involved in the prescription of *"dangerous medicines"*, so as to reinforce the conscious awareness of all of the potential legal and insurance implications of their therapeutic choices.
- Promulgation of a set of minimum requirements to be shared at a European level for determination of driving fitness, thereby establishing the basis for a harmonization of those criteria and procedures involved in the decision-making processes concerning driving fitness. Up until now, there exists a striking

(Footnote 14 continued)

"general" involuntary homicide: driving a motor vehicle in a state of psycho-physical alteration resulting from the consumption of narcotic drugs or psychotropic substances is punished with imprisonment from 8 to 12 years.

paradox, that of the so-called “International Driving License”, with no corresponding “International Driving Fitness Standards”.

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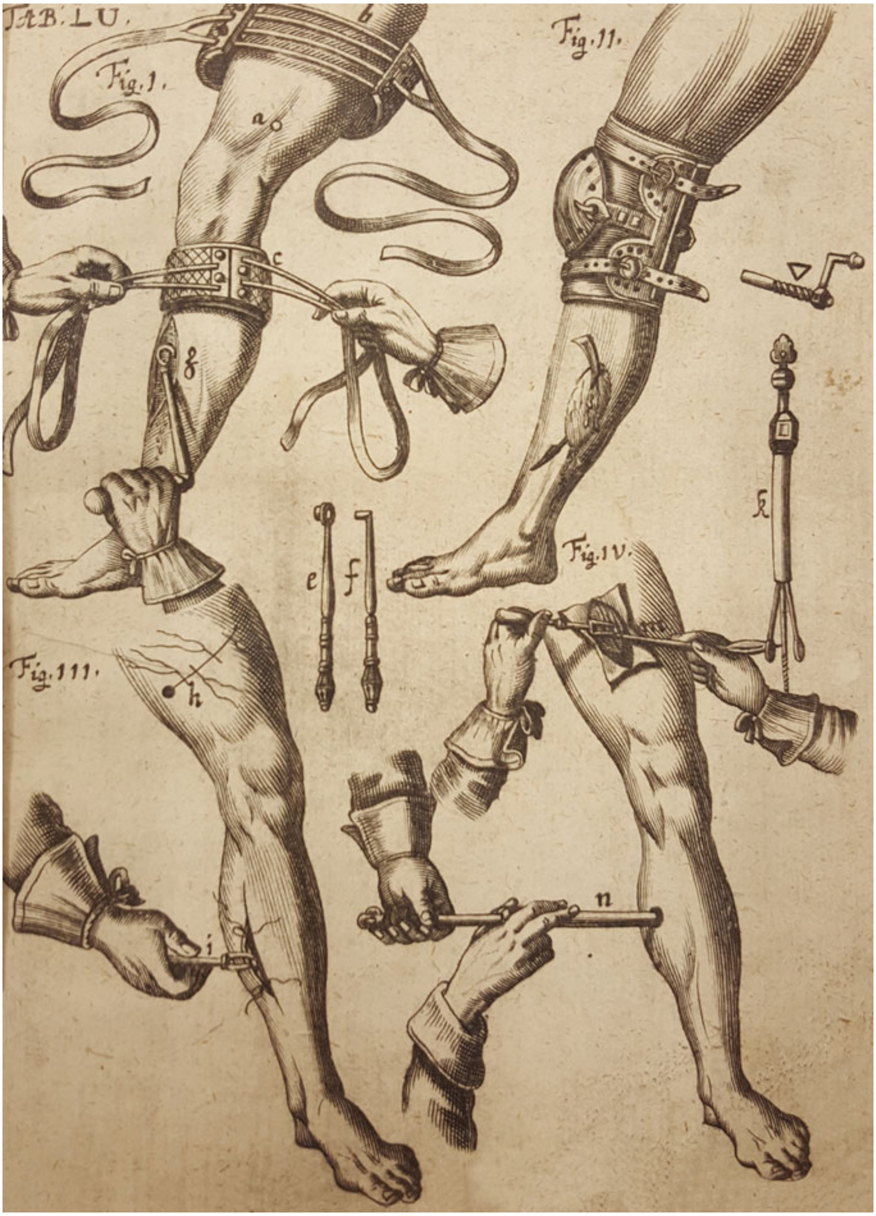

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Chapter 33
Single Hair Analysis Monitoring Concept for P4 Implementation

Markus R. Baumgartner

Abstract P4 medicine is a holistic concept describing man as a complex biological system. With a viewpoint of general healthcare management this concept focuses mainly on prevention and not curative treatment. On the other hand, forensic toxicology investigates (adverse) effects caused by drugs and chemicals in cases with medicolegal consequences. Thus, on a first glance, these two concepts seem to have nothing in common. Hair testing is a young technique among all the analytical tools applied in forensic toxicology. The main advantage of this method is the prolonged time frame represented by a segment of the keratinized part of the hair. This allows long term monitoring of exposure, not only to drugs, but also to environmental influences or situations pertaining to health such as, for example, an on-going stressful situation. Hair metabolomics hast just started; however, the development of new markers will—together with standardized toxicological hair analysis—enable a predictive and preventive monitoring over a controlled time frame. The highly sophisticated techniques currently developed for single hair analysis allow an even deeper insight into hair, incorporation pathways, but also degradation mechanisms. Even today, first experiments have shown that metabolic ratios observed in the hair matrix might provide some evidence for the phenotype of the individual regarding metabolism. A single hair does not only consist of a dead, keratinized part containing mitochondrial DNA (mtDNA). The hair follicle at the bottom end of a plucked hair contains enough nuclear DNA (nuDNA) for complete sequencing. In addition, microscopic examination of the hair follicle is used to determine the state of the hair growth cycle the hair is in. In summary, single hair analysis allows individual characterization and therefore supports personalized assessment and facilitates participatory medicine.

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33.1 Introduction

Modern concepts of general healthcare management focus on prevention and not just on curative treatment. Included in these concepts is consideration of individualized patterns of behavior, optimized dietary habits, and self-responsibility. These concepts are associated with visions of personalized medicine, where medical devices are developed or enhanced with a view to efficiency, safety, and treatment optimization in clinical use. There is a main focus on tailoring medical treatment to each individual patient, towards maximizing effective results, while minimizing side effects and maintaining safety. Expanding this system by the substantial inclusion of tools of systems biology led to a major concept established by Leroy Hood [1–6]. His concept of systems medicine encompasses genetics and genomics to identify genetic variants associated with health and diseases, the discovery of biomarkers by use of targeted proteomics, to the stratification of diseases into subtypes in order to achieve more effective treatment, and also the identification of new types of targets to accelerate the discovery process of new drugs. A last developmental step includes the involvement of subjects in such visionary systems and leads to the pioneering new paradigm of P4 medicine: predictive, preventive, personalized and participatory. This holistic concept, describing man as a complex biological system, includes much more than just personalized medicine. Thus, P4 medicine is the convergence of systems medicine (the application of systems biology to the study of disease), big data and patient (consumer) driven healthcare and social networks with the simplified goal of optimizing wellness and minimizing disease for each individual, embedded in a health care management system [7]. The main goal of such a proactive system can be subsumed in a comprehensive concept of maintaining or improving health via diagnosis, treatment, prevention and the demystification of disease, illness, injury, and other physical and mental impairments in human beings. Healthcare is delivered by health professionals and includes work or activities done in different care service structures, as well as in public health.

Forensic toxicology is a sub-domain of legal medicine, which is a discipline of medical services that is less a part of healthcare than of legal services. Therefore, the main concern of legal medicine and forensic toxicology follows its own principles. The Forensic Toxicology Council (FTC) defines forensic toxicology as a discipline that investigates adverse effects caused by drugs and chemicals on cases or issues with medico-legal consequences [8]. Accordingly, the results of forensic toxicological investigations and their interpretation must withstand critical scrutiny in front of the court. A non-final list of such incidents includes death caused by intoxication, driving while impaired or under the influence of a drug or a medication, diminished responsibility for an action, for example a homicide, assassination, rape etc., or limited ability to act due to impairment, to name just a few. Thus, at first glance, P4 medicine and forensic toxicology have only a limited overlap.

However, a fundamental principle of forensic toxicological investigations is individualized expertise. This demand for single-case-oriented or personalized expert input is an interesting first point from which to reflect on the applicability of the concept of P4 medicine to forensic toxicology. The basis of any forensic toxicological expertise is the quantification of the different drugs, medications or other chemicals, as well as of metabolites and endogenous compounds. However, this expertise must always take into account not only the concentration levels of any *noxa* quantified in biological samples taken after the incident, but also the incident-related circumstances or observations, and finally a retrospective interpretation of all case-relevant aspects with knowledge of metabolic behavior, potential of interactions, etc. Pharmacogenomics data are therefore of great relevance to characterize the subject and to improve the personalized interpretation of concentration values. For an individualized interpretation of analytical findings, it may be important to include additional information about the genotype or—even better—the phenotype of the individual. However, in forensics, it is rarely or sometimes not possible (post-mortem cases) to determine the phenotype with a simple standard experiment. Notably, post-mortem cases pose a particular, exceptional challenge. Therefore, it is important to establish new concepts for deriving the activity of enzymes as an example in post-mortem cases. Taking into account that both factors, genetics and expression, determine metabolic capacity, a group in Montreal, Canada, presented a new concept [9]. They established a novel proteomic approach for identifying the metabolizer type in post-mortem cases. Peptides, characteristic of normal CYPs and mutants, were detected, which enable the assessment of genetic metabolic capacity potential. Differences in CYP expression levels were then evaluated using proteotypic peptides common to normal and mutant CYPs. As a conclusion it was stated that this method yields a more precise and accurate estimate of the metabolic capacity than genotyping.

Individualization is an intrinsic aspect of the work of each forensic toxicologist. Promising new concepts are always investigated with a view to optimizing the personalized interpretation of analytical results, e.g., genomics and genetics data. Thus, the questions arise: What about the other three P-words, namely predictive, preventive, and participatory? And: which role might be assigned to forensic toxicology in the evolution of P4? Direct use of these terms with an inherently prospective perspective is not evident in the field of forensic toxicology, with its focus on the retrospective appraisal of findings mainly related to single incidents. However, changing the perspective allows the indirect application of this concept across a broad spectrum of topics. Extending the forensic examination in the direction of prediction of appropriateness of imprisonment, success of therapeutic treatment or addiction hazards—provided that toxicology can make significant contributions to the assessment of these aspects—additional preventive and participatory components can perhaps be added. The same is true if predictions of developments—an example of the local drug market—consider prevalence data from a long-term period of monitoring.

Sample matrixes collected for forensic toxicology cover a wide range. If the analyses are made from body fluids, the observation window is punctual or, ideally, a mirror of the last hours before the collection time point. Basically, one could also imagine enlarging the window of the retrospective observation. This results not in a snapshot but rather in a retrospective long-term monitoring of at least weeks or months, which is more suitable for comprehensive predictions. Keratinized material is the matrix of choice for long-term monitoring in the field of forensic toxicology. Hence, hair analysis should be considered as a futuristic new perspective with which to perform P4 applicable monitoring.

33.2 Hair Testing as a Window for P4 Medicine

Hair differs from other human materials used for toxicological analysis such as blood or urine because of its substantially longer detection window (months to years) enabling retrospective investigation of past consumption [10]. Because of the solid and durable nature of this keratinized material, hair analysis can be performed even centuries after growth. Compared to other keratinized materials, the most important advantage of hair analysis is the possibility for retrospective monitoring of an accurately determined time period [11]. This time frame is defined by the hair growth rate and the hair growth cycle. The growth rate ranges for scalp hair between 0.7 and 1.4 cm/month with an average of about 1.1 cm per month. As a rule of thumb, in forensic investigations an average value of 1 cm/month is commonly applied. Considering this feature, a time-selecting filter can be set with the investigation of a hair specimen of a certain length which defines the monitoring time frame. Thus, an average value over time—as for example a substance concentration—can be determined experimentally. The advantage of this strategy is considerable. For example, if one is interested in stress monitoring, cortisol level is an excellent marker to investigate [12]. However, cortisol in blood is subject to a marked circadian rhythm. The incorporation rate into the matrix is directly correlated with this up-and-down variation of the blood concentration. Together with hair growth, a one-dimensional concentration timeline is recorded along the hair shaft. Measuring cortisol in a hair segment of a certain length centers these daily variations over the corresponding time frame. Therefore, average values can be recorded that allow a comprehensive evaluation of health-related stress. This provides an opportunity to record stress load during a defined period. Comparing hair cortisol levels in adjacent hair segments opens the possibility to monitor changes in stress, such as, for example, relaxation during a therapeutic timeout after a heart attack or in the case of a posttraumatic stress syndrome.

Time-dependent concentration changes are an intrinsic peculiarity of a living system. Circadian changes as mentioned above are an example, as are diet-related changes in nutrient concentration or—in a more toxicology-related example—the metabolization of drugs, and therefore the concentration of the parent compound as well as of the active form or of any metabolites changes over time. Kinetics (concentration changes over time) is not only triggered by the genotype, which describes mainly genetic variations, but also by other factors. These multifactorial variations in drug response, which are caused by variable (polymorphism) and time-dependent changes of gene expression as well as the influences of drugs on genes (epigenetics), can be subsumed by the term phenotype and are investigated by pharmacogenomics approaches [13]. Hair testing offers, under certain circumstances, the opportunity to test for the genotype as well as the phenotype by investigating metabolic ratios. The following examples illustrate this option. A first example was the investigation of hair samples taken from a large group of children supervised by a nurse in a day care [14]. The hair samples of all these 23 children were positive for amitriptyline, nortriptyline and its 10-hydroxy metabolites. However, all samples could be divided into distinct groups defined by the metabolic ratios. Amitriptyline is metabolized by CPY2D6 and CYP2C19, respectively. Both isoenzymes belong to the large group of the Cytochrome P450 family (CYP) and are polymorphically expressed. An association of the metabolic phenotype with the genetic disposition was observed, whereby genotypes of CYP2C19 and CPY2D6 were examined by conventional polymerase chain reaction (PCR). A similar observation of high variability of metabolic ratios was obtained by investigating workplace exposition of different groups of employees of a tramadol manufacturer and comparing these findings with data from a large group of patients medicated with tramadol [15]. The metabolic ratio of O-desmethyl tramadol, which is metabolized from tramadol by CYP2D6, shows a widely scattered distribution, whereas for N-desmethyl tramadol (CYP3A4, CPY2B6) an asymmetrical distribution pattern of the ratio values was observed. Unfortunately, investigations on genotype were not possible due to ethical reasons. Heroin addicts often attend an opioid maintenance therapy to improve their medical and social situation. Hair testing is a common tool to check for side consumption. A systematic examination of the metabolic ratios of patients treated with methadone showed some markedly increased values [16]. The patients of the one group with significantly deviating concentration rates of EDDP vs methadone had all been co-medicated with the antiretroviral drug efavirenz used for the treatment of HIV. Efavirenz is known to promote overexpression of CYP3A4 leading to increased activity of this enzyme, which is, together with CYP2B6 and CYP2C19, relevant for the metabolic degradation of methadone. All of these examples demonstrate that hair testing enables the determination of a phenotype in cases with known regular medication or drug use over a longer time period; with extended knowledge of metabolic ratios in hair samples, this survey might be applicable for certain drugs even in post-mortem cases.

The second important issue in hair analysis is the fact that the incorporation of xenobiotics and endogenous substances into the hair matrix is constrained to various and partially very selective mechanisms [10]. Since the beginning of hair testing as an analytical tool of forensic investigations, the incorporation mechanism is a major topic of investigation. Based on the present state of knowledge one can note that the incorporation efficiency into the hair matrix is highly dependent on compound-specific physicochemical parameters with significant differences between various groups of substances [17]. An incomplete list of such parameters includes the pKa value and charge of the molecule with regard to the pH of the biological compartment involved in the incorporation pathway, respectively, the log P value (lipophilicity, log Kow), molecular geometry and chemical nature of functional groups, and molecular weight. E.g., the immunosuppressive drug cyclosporine with a molecular weight of 1.2 kDa is—according to present knowledge—the largest xenobiotic incorporated into the hair matrix [18].

The investigation of substances incorporated into the hair matrix offers even more potential advantages. Once incorporated, the molecules are trapped in a highly stable structure. In addition, the molecules are not only fixed within this matrix, but also seem to be sustainably protected from many degradation processes. Only exposure to very strong conditions, such as bleaching the hair sample with oxidizing agents like hydrogen peroxide, or enforced radiation by UV light, leads to a significant decrease in incorporated substances as well as a partial destruction of the hair matrix itself [19–21]. Together with the aforementioned option of time-resolved analysis, hair matrix offers a unique opportunity for the long-term archiving of low molecular weight substances. The incorporation of compounds and their fixation within the hair matrix during the keratinization process demonstrates that hair can be regarded as a matrix featuring a selectivity filter. This filter function promotes the amplification, but also the suppression/reduction, of the detectability of substances in hair extracts. Hair testing can therefore open a new door to metabolic profiling, a strategy for investigating the metabolome of cells, tissues or organisms. The metabolome of complex biological fluids such as blood is highly dynamic and varies with diet or immune status. In contrast, hair metabolomics offers several advantages [22]. The stable matrix retains low molecular weight substances and accumulates them over a longer period and therefore eliminates effects caused by singular short-term influences. This allows the discovery of robust biomarkers of long-term environmental exposure, chronic intoxication or ailments, or other multifactorial phenotype signatures of different etiology. In the future such markers might help to detect cancer at a very early stage, but they can also be used to check for stress resilience, longevity and additional aspects.

Hair testing is still a young discipline compared to other analytical tools applied in forensic toxicology. For a long time, forensic hair analysis was an investigation just for specialists. However, this analysis is nowadays widely accepted and applied in various fields not only in forensic toxicology, but also in workplace drug testing, the re-granting of driving licenses, and more recently in clinical applications. The main goal of these applications is the long-term monitoring of misuse of drugs, pharmaceuticals, and alcohol or the documentation of the subject's abstinence. Clinical requests mostly focus on compliance monitoring in cases of long-term medication or therapeutic drug monitoring. The orders for monitoring the consumption habit of a person by hair analysis are generally imposed by authorities or issued by a physician in charge. The results of these analyses retrospectively document consumption behavior over a longer period of time. They are used by the client either to check the compliance of a proband or patient or of a larger group of persons, e.g., if the security-related behavior regulations of a company are complied with (randomized monitoring of employees). The goal of this ordered forensic or clinical testing is to induce ongoing change in behavior, which may have a predictive, preventive, personalized and/or participatory impact for the individual or the group of people. Retrospective evaluation of anonymized consumption behavior data recorded during assessments for driver's license re-granting or data of side-consumption of subjects under opioid maintenance treatment is a common source of prevalence data. Based on hair testing, the positivity rate is often higher than in urine testing due to the prolonged term of continuous observation. Prevalence data of club drugs, often misused pharmaceuticals, or detection of dangerous co-medication, are of great importance for prevention [23].

In summary, it can be stated that the features of hair as a unique matrix in forensic toxicology are determined by two filter functions, namely long-term monitoring combined with the possibility of time-resolved investigations by segmental analysis on the one hand, and the incorporation selectivity of molecules into the matrix, together with an extended stability towards degradation processes on the other. These features recommend hair testing as a unique tool not only in forensic toxicology, but also in clinical applications. The results of hair analysis investigations yield reliable information from personalized data with predictive and preventive impact, together with participatory behavior.

Routine hair testing for the detection of drugs, pharmaceuticals, alcohol markers or substances due to exposure to environmental toxins is performed with the classical methods of forensic analysis. These methods usually need a lock of hair with a weight for the segment of interest of 10–50 mg. This sample amount typically ranges from 50 to 100 single hairs. Locks of hair are cut with scissors as close as possible to the skin. Body hair, which allows only a rough estimation of the corresponding time period, can be collected with a disposable razor. To monitor the temporal profile of a consumption habit, the total tuft of hair must be divided into shorter sections. Therefore, the necessary mop of hair must be thick enough, because each segment of hair must weigh the amount described above. The development of more sensitive techniques enables a significant reduction of this quantity of hair matrix. However, the segmented investigation of a single hair is currently only possible for selected analytes using standard LC-MS/MS [24, 25]. As mentioned above, segmental hair analysis can be used to obtain a retrospective timeline of drug use. However, this is a laborious and time-consuming process. As an alternative, an untargeted analytical method was developed using DART-HRMS (direct analysis in real time—high-resolution mass spectrometry) [26]. This method scans longitudinally an intact lock of hair without extensive sample treatment or segmentation. The ionization technique DART uses a heated, metastable gas (e.g., helium) to desorb ions from the surface under ambient conditions. The spatial resolution resulting from investigations using the DART technique is approximately 5 mm and corresponds to a retrospective timeline accuracy of ± two weeks. This resolution is determined by the diameter of the standard exit cone. It cannot be foreseen whether this rapid technique has the potential for routine forensic application. Other recent analytical developments for hair testing methods are imaging technologies known for visualization of substance distribution patterns in solids. For example, scanning electron microscopy can be used for metals. However, any mass spectrometric technique suitable for forensic investigations requires a special ionization technique that enables the documentation of compound distribution along the hair shaft. This technique must detach the molecules from the hair matrix and allow a spotty generation of ions detectable by mass spectrometry. This leads to the need for the implementation of ionization techniques such as MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) and MetA-SIMS (metal-assisted-secondary ion mass spectrometry) for single hair analysis [27–32]. The result of the measurement is a substance-specific distribution pattern along a single hair recorded with a spatial resolution of less than one mm in the longitudinal direction of hair, which corresponds to a time frame of less than 3 days, or a depth profile of a transverse section of the hair, respectively. An even better temporal resolution is not necessary as the keratinization process itself takes some time. These techniques are currently only used for studies on incorporation mechanisms into the hair matrix or other foundational data. However, these combined techniques have the potential to be adopted in routine hair testing, so that a single hair would be sufficient for a qualitative screening or targeted analysis with high temporal resolution.

Today, tufts of hair tend to be examined and are collected by cutting close to the skin. Single hair analysis would permit the removal and analysis of just a few hairs. Besides the disadvantages of an invasive sampling, there would be some remarkable benefits. Microscopic examination of hair follicles can be used to determine the stage of the hair growth cycle the hair is in (anagen, catagen, and telogen), and, in addition, to examine the section of the hair that usually remains in the skin after cutting. This section corresponds to a time frame of approximately 10–14 days. Under certain circumstances it might be interesting to investigate this section as one can assume that this part of the hair is contaminated neither by sweat nor by exposure to dust, smoke or any other environmental sources. Eventually, one can also investigate DNA obtained from the hair bulb. This part of the hair is rarely investigated by hair analysis. However, the hair follicle at the bottom end of a plucked hair contains enough nuclear DNA (nuDNA) for successful profiling or next generation sequencing (NGS) [33, 34]. Apart from that, the dead, keratinized part of a hair contains mitochondrial DNA (mtDNA) suitable, for example, for excluding suspects.

33.3 Conclusions

Commonly, blood is used as a diagnostic window for viewing the health and disease state of the individual. It is the matrix of choice for toxicological screening and for investigations to update databases of individualized genomics or metabolomics data, etc. From a different viewpoint, forensic hair testing can be regarded as an alternative window for the implementation and evolution of P4 medicine. This is especially due to the unique features of the hair matrix, namely its selectivity based on the incorporation of xenobiotics and endogenous compounds into the hair shaft and the feasibility of long-term monitoring. This allows the discovery and analysis of specific biomarkers and the creation of a retrospective timeline of drug use or exposure to environmental effects. Combined with optimized single hair analysis, this analytical tool can provide significant contributions to the progress of proactive P4 medicine: predictive, personalized, preventive, and participatory.

In all of these reflections, only scientific, analytical, and, where needed, medical aspects were considered. However, the promise of P4 medicine goes much further. As a network of networks it is a virtual link not only between the database of the entire sequenced human genome and the results of systems biology investigations, but also to small hand-held devices recording relevant parameters of each individual online, and big data made available by patient-driven and/or social networks. The implementation of such a visionary, holistic system raises challenging questions. One of them is due to the fact that the P4 concept faces an uncertain regulatory environment [35]. This is of greatest importance in the context of legal medicine. Forensic toxicology is part of that discipline, which belongs to the juridical system and not to a healthcare concept. The responsible handling of this information has top priority, especially in a legal environment, and the responsibility for it is undividable.

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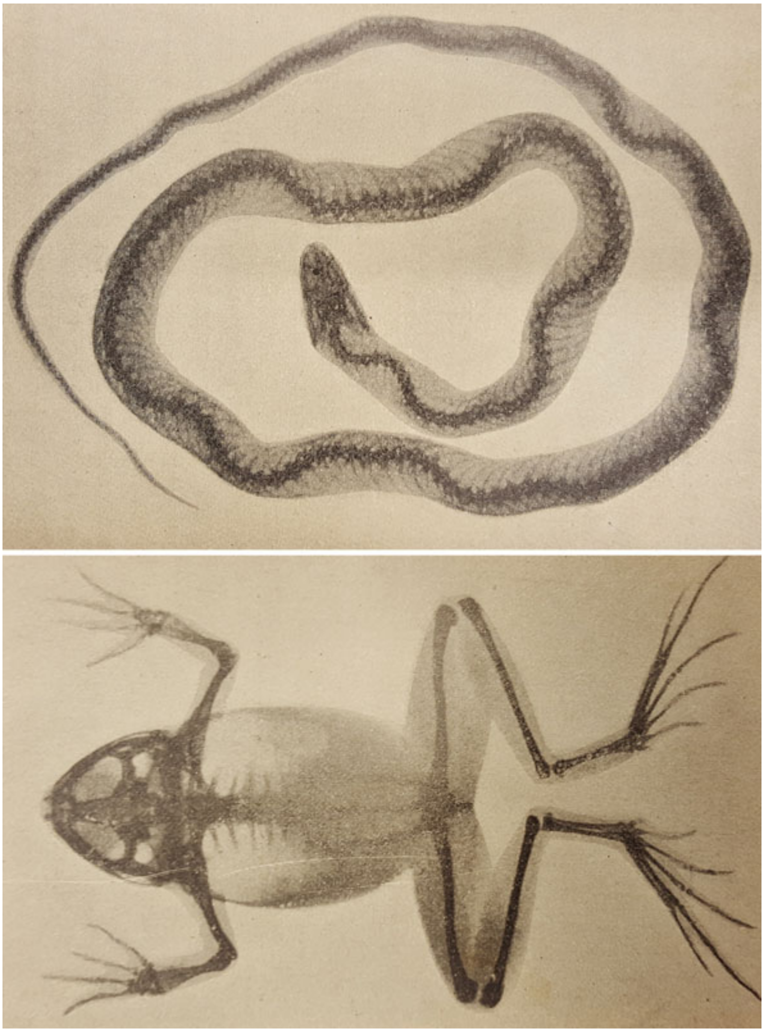
Part X
Innovation, Unitariness and Evidence. Forensic Imaging



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Chapter 34
History and Current State of Forensic Imaging

Hermann Vogel

Abstract When Wilhelm Conrad Röntgen discovered X-rays on the 8th of November, 1895, not only Clinical Diagnostic Radiology but also Forensic Radiology came into being. Röntgen himself created the first radiographs, which showed the potential of the new rays concerning forensics. In 1896, Röntgen's discovery induced fantasies—in 2016, some of these fantasies have materialised in forensics. Since 1896, X-rays have been utilised by forensic pathologists. As parts of new techniques and methods, X-rays help to answer old questions. In this chapter Milestones, Tools, and Techniques of post mortem and ante mortem forensic imaging are presented and discussed. The future will show whether and where Virtopsy shall be used in addition to forensic pathology—or instead of autopsy—and how far the applications of imaging methods without ionising radiation will increase the utilization and the diagnostic power in the clinical forensic medicine.

34.1 Introduction

Today, Forensic Radiology has become Forensic Imaging. This happened by including other imaging methods. MRI, nuclear medicine, endoscopy, and ultrasound are examples. Today, Forensic Imaging includes the whole spectrum of Clinical Diagnostic Imaging. The methods of Clinical Diagnostic Imaging have been adapted and modified to meet the questions posed in the Forensic Sciences. Forensic Imaging can be considered either a sub-discipline of the Forensic Sciences or of Clinical Diagnostic Imaging. In the future, the developments in Clinical Diagnostic Imaging will create opportunities for the forensic specialist, which are unknown today. Furthermore, the fight against terrorism has induced a development

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of imaging technologies for the control of countries' borders and airports with surprising solutions.

34.2 Röntgen's Discovery and First Images Showing the Discovery's Potential for Forensics

The eight of November 1895, Wilhelm Conrad Röntgen (1845–1923), Professor for physics in Würzburg, experimented with cathode rays [1].

In the room, not far from the tube with which Röntgen was experimenting, there was a screen covered in fluorescent material. This screen was illuminated when the tube was switched on. Röntgen observed that the screen illuminated in the distance larger than the theoretical range of the cathode rays. The illumination increased when he held a book between the tube and the screen. The illumination ceased when he replaced the book by plumb sheets or platinum sheets. Röntgen then held his hand in the path of the rays. He noticed that flesh and skin absorbed less rays than bone; the screen displayed a skeletal hand. Röntgen replaced the screen with a photographic plate and documented the displayed image [2]. Röntgen experimented with these phenomena for eight weeks, intensively. Thereafter, he concluded that when the cathode rays hit the glass surface of the tube radiation comes into existence similar to light. This radiation has the following properties.

- Blackening of the photographic plate.
- Inducing fluorescence.
- No deviation in a magnetic field.
- Linear propagation.
- Invisible to the human eye.
- Penetration of solid material.
- Absorption depending on the density of the penetrated material [3].

Röntgen reported his observations in "Über eine neue Art von Strahlen (Vorläufige Mitteilung)" [4], "On a new kind of ray (preliminary communication)". On December 28th, 1895, he submitted his manuscript to the Physikalisch-Medizinische Gesellschaft in Würzburg [3]. The printing of this "preliminary communication" took place during the first days of 1896, and on January 6th, 1896, the discovery became known worldwide after it was transmitted by cable [5], printed and commented upon by the international press.

During his investigation, Röntgen made radiographs of his shotgun (Fig. 34.1) and of a box containing weights (Figs. 34.2 and 34.3). Both radiographs indicate possibilities for the forensic sciences: the radiograph of the shotgun shows irregularities of the barrel and the ammunition; the radiograph of the box visualizes the contents (weights of metal), demonstrating the possibility of analysis with leaving the box intact. Today, radiographs from arms are common in the fight against terrorism; the analysis of luggage and transported goods is part of the prescribed security at airports [6–8].

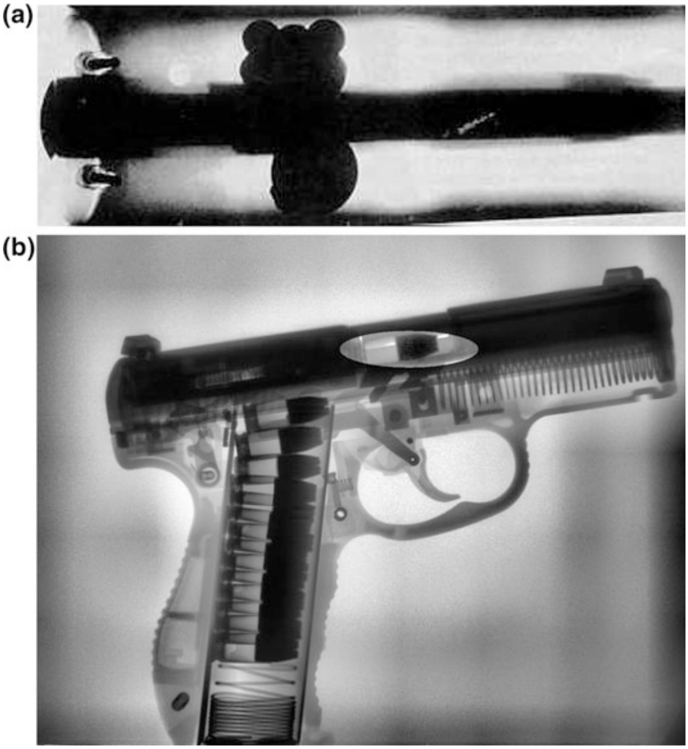

Fig. 34.1 Radiographs of guns 1895 and today. **a** Roentgens's shotgun, 1895. The barrel shows an irregularity. The ammunition is visible. **b** Modern radiograph of a pistol. Apparently, the pistol is charged and ready to fire

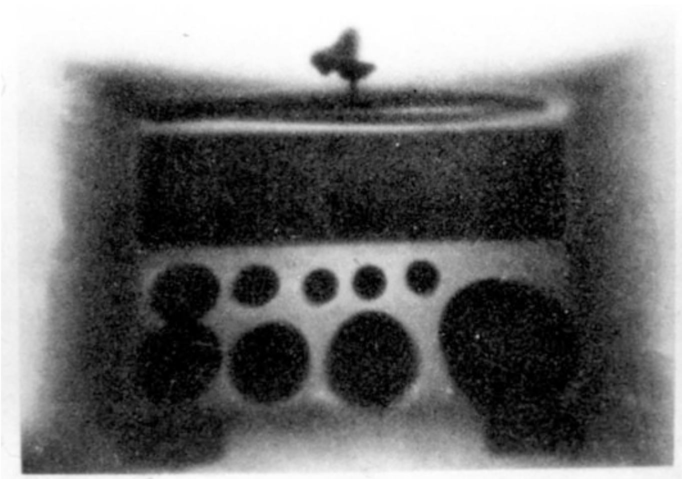

Fig. 34.2 Analysing the contents of boxes. Box with weights, one of Roentgens first radiographs, 1895

Fig. 34.3 Modern analysis of Improvised Explosive Devices with radiographs. Exhibition at the ECR, European Congress Radiology [6]

34.3 First Applications

On January 6th, 1896, the New York Sun reported that "The professor is already using his discovery to photograph broken limbs and bullets in human bodies" [9]. Although not correct, this statement expressed the perception of the new rays. During the same year, 1896, the first applications were made.

Professor AW Wright probably was the first American who produced a radiograph; he radiographed a rabbit, visualising its skeleton [10].

In the US, the surgeon Dr. RC Kirkpatrick was the first to localise the projectile with a radiograph. The projectile was removed and the radiograph was submitted to court. The shooter was convicted [11–13]. In 1897, in the criminal case of the Haynes murder trial in Watertown, New York, Dr. Gilbert provided his expertise in relation to two metal foreign bodies. He interpreted these foreign bodies as fragments of one bullet [13, 14].

In England, radiographs demanded by Dr. Frankish were used in civil action for the first time in 1896. The radiographs showed the injury of Mrs. Folliott's foot after a fall. There were radiographs of both feet for comparison. The difference was visible. It was obvious that a defect remained and Mrs. Folliotts could not work [15]. In a criminal case, the shooter had fired several times before committing suicide. Dr. William Little induced radiographs of the still living victim. The projectiles could be localised, but they were out of reach and the victim subsequently died [16, 17].

In France, Dr. T Bordas mentioned the potential of the new rays for identification by visualizing fractures, projectiles and other peculiarities [14]. Furthermore, he proposed the investigation of suspicious parcels with the new rays. Already in 1897, the Bureau de Douanes checked luggage, purses, and hats for contraband at the Gard du Nord in Paris.

34.4 Early Fantasies and Application in 2016

The X-rays were mysterious. The reports stimulated the public imagination. Fantasies resulted. However, some of these early fantasies correspond to techniques of today, used in forensics. Examples are search of persons, of letters, parcels and goods, brain imaging and Superman.

Search of persons, nakedness. Shortly after the discovery of the X-rays, the fact that the X-rays penetrated the clothing induced the advertising of X-ray opaque clothing for women. [18]. Today at airports, whole body scanners with backscatter imaging are used for searching persons. These scanners have the potential to show the scanned person naked (Fig. 34.4). Backscatter imaging helps to detect arms, explosives, and drugs hidden on the body's surface under the clothing. This technology has induced a discussion about intimacy and the protection of privacy. Whole body scanners with transmission imaging visualise objects hidden in the body (Fig. 34.5) and under the clothing. The backscatter imaging and transmission imaging both employ X-rays. The fact that X-rays belong to ionizing radiation, which produces biological effects, has induced the development of imaging techniques using non-ionizing radiation instead. Terahertz imaging is but one example [8]. Today, the development of imaging techniques used for checks progresses rapidly.

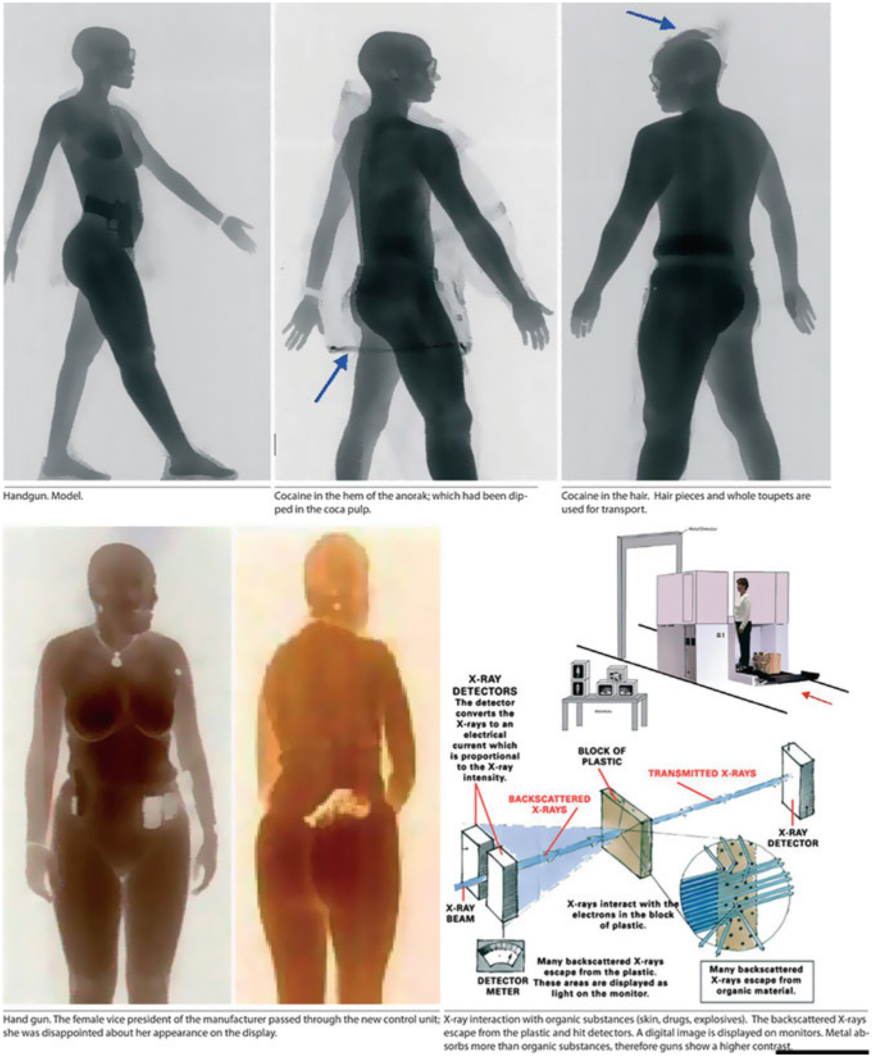

Fig. 34.4 Backscatter imaging and privacy. Air port control. Exhibition at the ECR, European Congress Radiology [6]

Letters. From 1896, a postcard shows a concierge reading a letter without opening it (Fig. 34.6). Another postcard shows the monitoring of a door through a wall (Fig. 34.7). Today, transmission imaging of letters and parcels with X-rays visualises explosives and ignition devices. After the fall of the wall in 1989, it became known that the Secret Service of the German Democratic Republic ("Stasi") had flagged manuscripts and letters with gamma rays emitting substances. This happened with the aim of identifying the persons transporting these

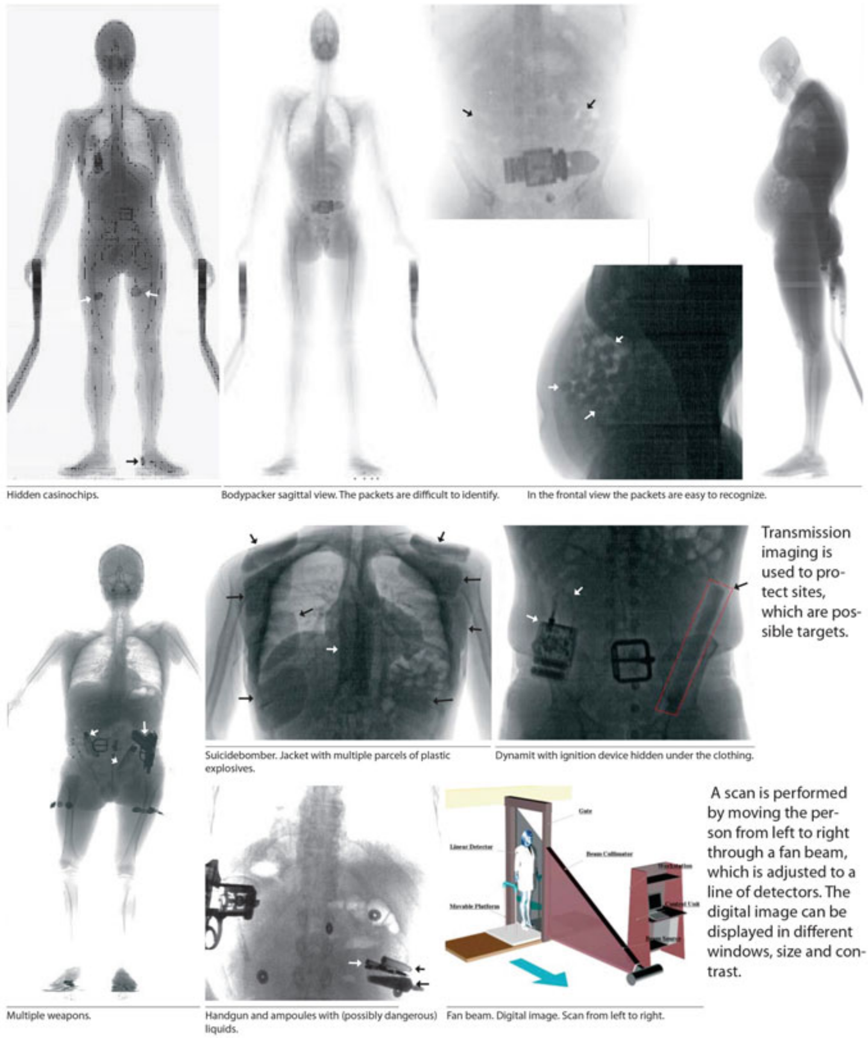

Fig. 34.5 Transmission imaging. Air port control. Exhibition at the ECR, European Congress Radiology [6]

manuscripts and letters. Furthermore, it became known that the Stasi disposed of equipment for radiographing walls and cars in search of hiding places, hidden objects, and hidden persons (Fig. 34.8) [6].

Brain and Thoughts. Early on, Thomas Alva Edison, the American inventor and businessman, tried to commercialise the X-rays. He constructed a fluoroscopy unit for everybody. For a cost everybody could use them. The advertising played with the idea that radiographing the head of the spouse or the neighbour could give access to their memories and thinking. Today, MRI visualizes neurons in the brain.

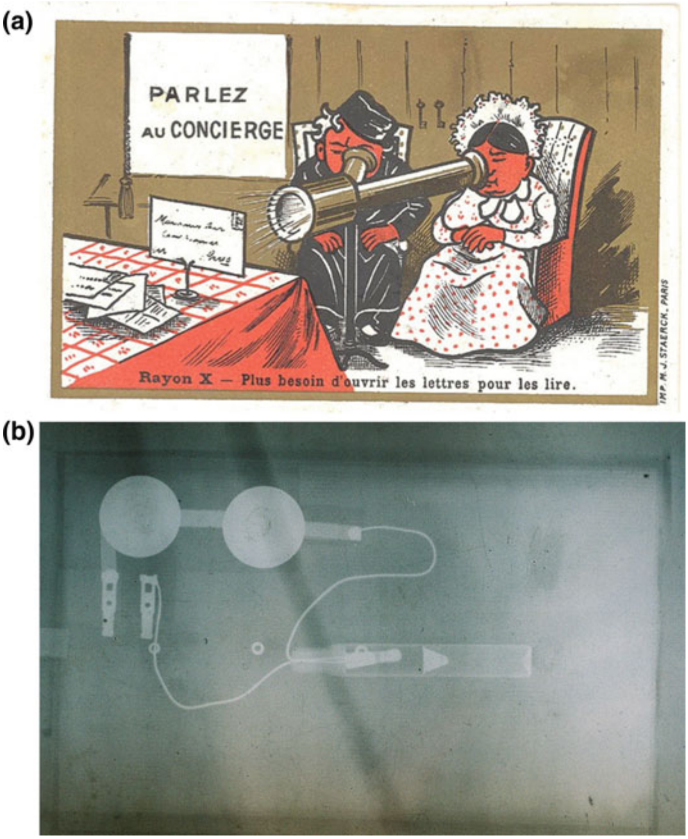

Fig. 34.6 Letter analysis 1896 and today. **a** 1896, fantasy: Concierge reading the letters of the residents with the help of X-rays. **b** Letter bomb, radiograph. Explosives and ignition device

Fig. 34.7 1896, imagination of future technical solutions for surveillance

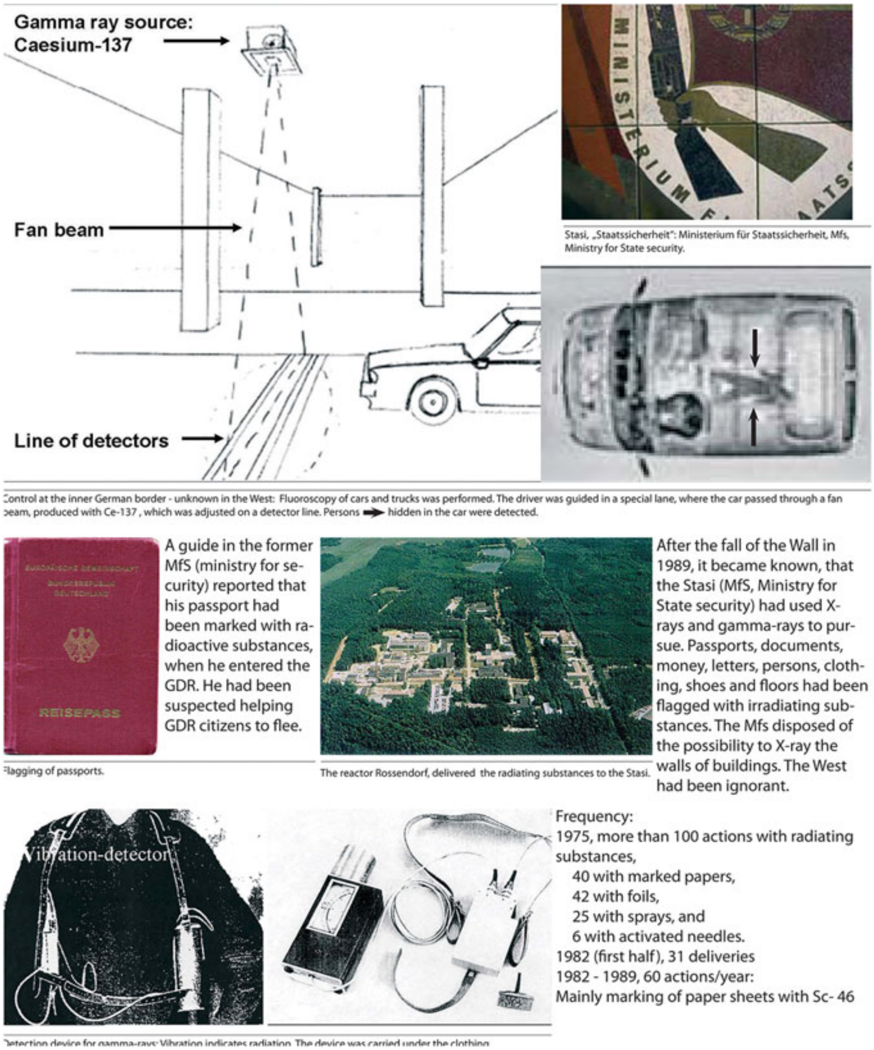

Fig. 34.8 X-rays and gamma radiation employed by secret service. Exhibition at the ECR, European Congress Radiology [6]

MRI localises active brain centres. Research concerns free will and sequels of torture and possible consequences are proposed which may one day have effects on forensics. One has to add that X-ray exposure impaired Edison's eyesight and killed his assistant Clarence Madison Dally [19].

Superman. Superman may serve as an example for how imagination and wishes have been projected onto X-rays and radioactivity. Superman has X-Ray Vision. He fights for good, which he has in common with the forensic specialist. With X-ray

Vision, Superman can look through walls and is even able to melt metal. These capacities remain a fantasy, a dream. One may speculate why. Perhaps, Superman's X-ray Vision has nothing in common with the X-rays discovered by Röntgen. The appellation X-ray Vision only suggests that there may be something in common.

34.5 Milestones, Tools, and Techniques

Identification, Teeth. In 1896, W. König visualised teeth with the first intraoral radiograph. However, not before the 1940s did identification with dental X-rays contribute to important cases [20, 21]. Famous also is the identification of Hitler's head by comparison of ante mortem radiographs with radiographs obtained from the corpse—the Russians kept the identification secret for two decades [22]. The identification of the corpse of Mengele, the Doctor of Death from Auschwitz, is nearly as famous. The comparison of ante and post mortem radiographs of the teeth was essential in this case, too [23].

Identification, superimposition of radiograph and photograph [24]. In 1935, the Ruxton case demonstrated the potential of combining radiography with photography. This proceeding is an example for the possibilities of combining different imaging methods. The court accepted as proof the superimposition of radiographs of the head of an unidentified person with photographs of a missing person. This identification was essential. The court convicted the accused, Dr. Buck Ruxton. The proceeding of the experts has similarities with identification by biometrics (Fig. 34.9) [24].

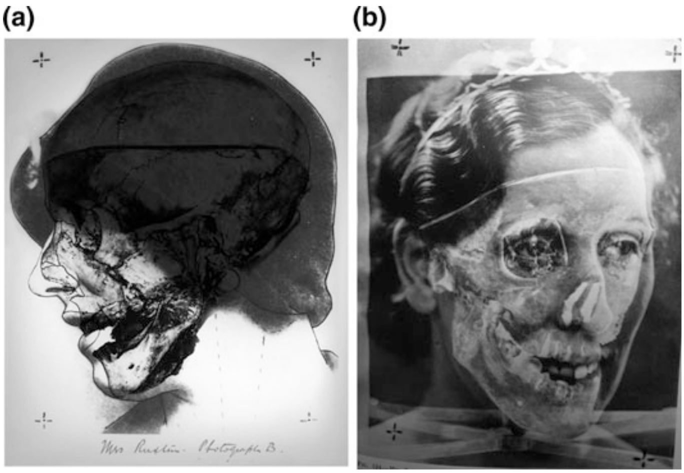

Fig. 34.9 Head, superimposed radiograph and photograph. Case Dr. Buck Ruxtong. **a** Lateral view with hat. **b** Frontal view with tiara

Identification, the Lost Head of Henry IV, King of France [8]. A recent case, concerns Henry IV, king of France (Fig. 34.10). The king's head was lost after revolutionaries desecrated the royal graves in St. Denis Paris, in 1993, during the French Revolution. The forensic pathologists used all available methods, because at first they could not obtain enough DNA for Identification. In the end, DNA could be obtained and the identification was confirmed [8].

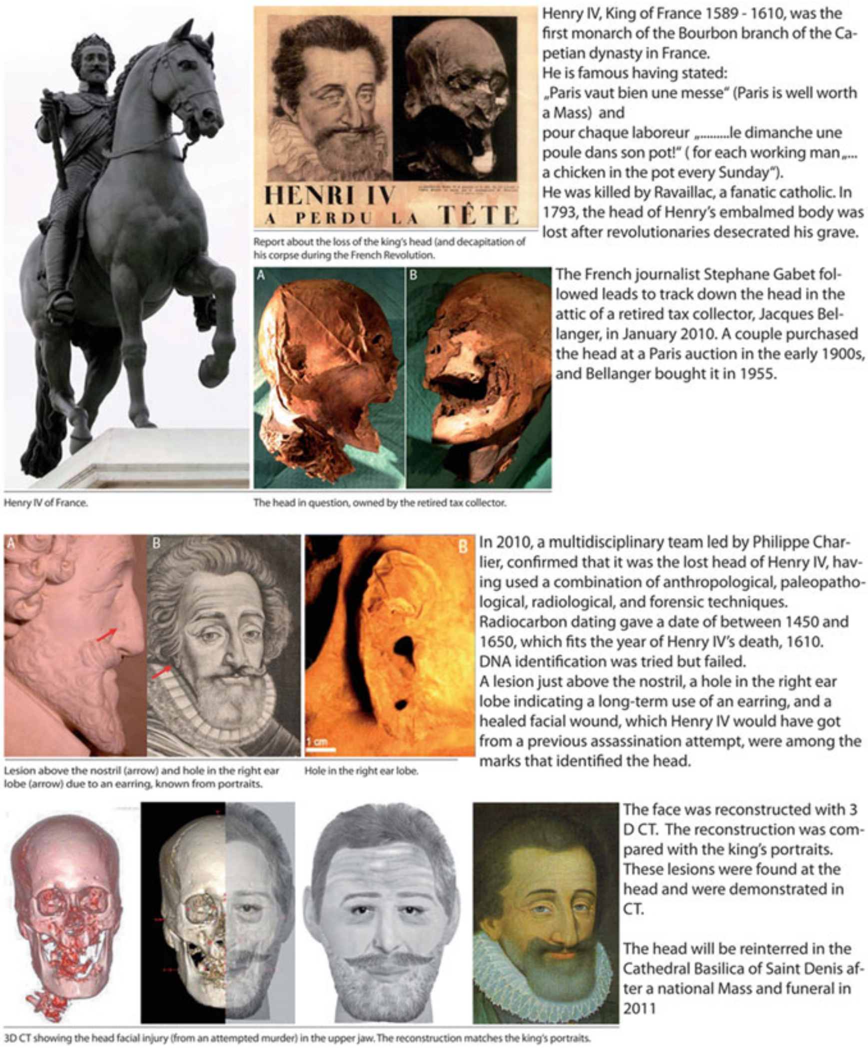

Fig. 34.10 Recovery and identification of the lost head of Henry IV, King of France. Exhibition at the ECR, European Congress Radiology. [6]

Fingerprints Documented with Radiographs. Many methods for identification were proposed or even described. Several methods were never realised. For example, Beclère in Paris proposed to visualise the papilla lines of the fingers with soft X-rays. Metal powder should enhance the lines. Furthermore, Béclère proposed radiographs of the distal digits for identification; he referred to the characteristic spongiosa pattern of the distal digits [25, 26].

Reconstruction of the Crime Scene. Repeatedly, Presidents of the USA have been the targets of attacks. Some were killed. Several times, forensic imaging was performed and showed the methods available [1]. The assassination of President Kennedy on the 22nd of November, 1963, demonstrates the existing limits. Up until today, there have been many theories put forward concerning what occurred, concerning the number of the shots and the number of the shooters. There were radiographs, but their interpretation differs [1, 27–30]. Today, Computed Tomography creates a data set of the deceased. This data can be displayed repeatedly in different fashions. 3D-Reconstruction can be performed under different angles. The crime scene can be scanned, too. Both data sets can be combined for reconstruction. Figure 34.11 shows an example [8].

Age determination with Radiographs, Asylum seekers, Criminals and Prostitutes. Individuals claim to be younger or older according to their interest. It is quite common for asylum seekers to claim to be younger than they are in order to avoid being sent back. In Paris, minor prostitutes claim to be older than 18—her customers would be punished if they had had sex with minors, and the woman would be out of business. Austria recently prepared a new law for the age determination of asylum seekers. Human right groups protested. Age estimation is done with radiographs of the left hand, the clavicle sternal junction, and the teeth; practically every other bone of the human body has been used, too. Employing Diagnostic Imaging, one has to be aware of its inaccuracy, which can reach up to two years. An expertise resulting in an age estimation that has not been consented to by the examinee has been considered an ethical problem for the physician. The left (non-dominating) hand offers an approach of age determination up to the age of 18. The clavicle permits an estimate thereafter. The teeth can be of help in children, adolescents, and young adults. The growing bone and especially the epiphysis is the point of analysis. Even the foot can be used to assess the age: The secondary centre in the calcaneus has appeared (>6 years), but has not united (<14 years). One can limit the gap to 8 years by examination of just this bone [8].

Age Determination with MRI, Sports. In soccer, the U-17 World Championship is a prestigious event. To prevent participation in the incorrect age group, and owing to the fact that in some Asian and African countries registration at birth is not compulsory, other methods of age determination need to be available. Standard radiographs of the left wrist have been used for assessment of skeletal age for many years. However, this has been criticised as unethical in the sporting environment. MRI of the wrist has been proposed as alternative, non-invasive method of age determination in 14–19-year-old male adolescents. FIFA organized a study. The open epiphysis of the radius has been found appropriate to prove an age of 17 years and less. After the age test, Nigeria dropped 15 out of 38 players [8].

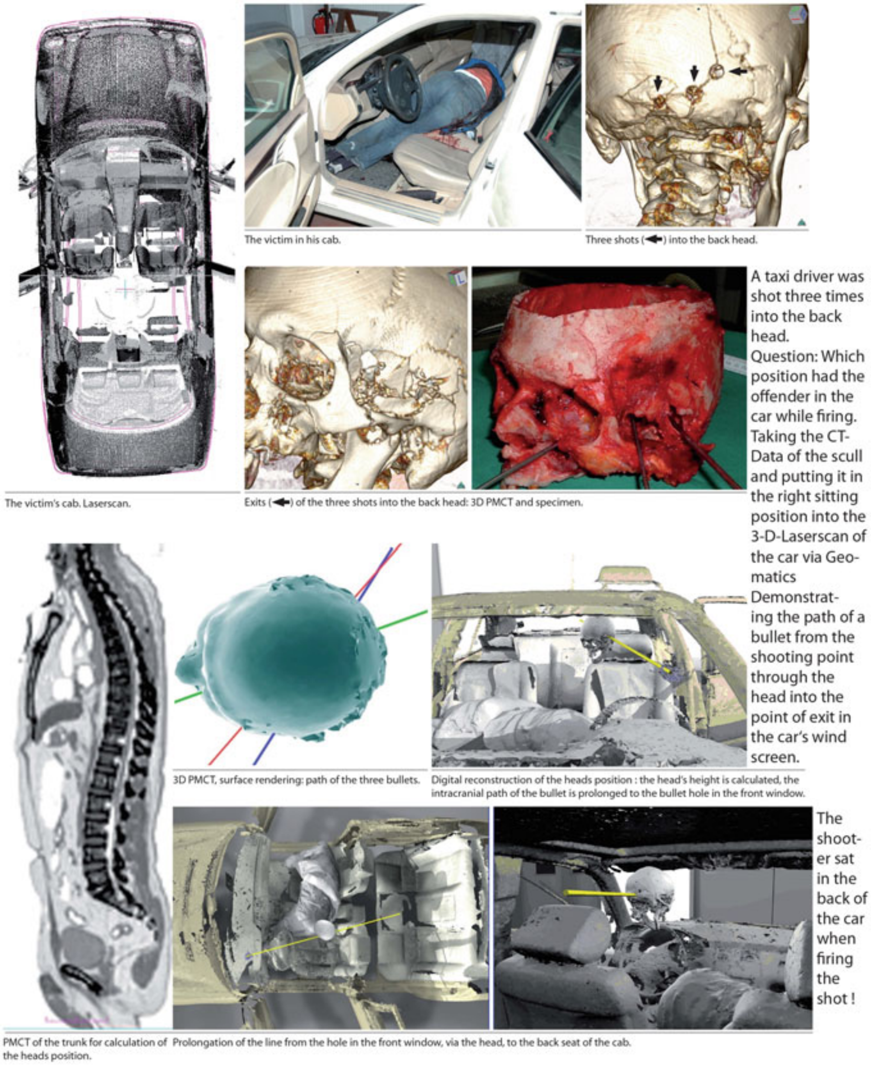

Fig. 34.11 Reconstruction of the crime scene. Fusion of data sets. Exhibition at the ECR, European Congress Radiology [6]

These cases, observations and methods are only examples. Forensic Imaging brought advances when the public was ready to accept that children could be battered and babies could be shaken to death [1, 6, 8]. Forensic Imaging plays an important part in archaeology. Well known are the results of the computed tomography of Tutankhamen, Egypt, and of "Ötzi", the Iceman. Diseases and injuries became apparent or were excluded [31, 32].

Several accidents with *mass-casualties* proved the value of Forensic Imaging for identification. In 1949, the fire aboard the Noronic in Canada killed 119 people. X-rays were obtained of 79. They were compared with 35 radiographs taken ante mortem. The comparison allowed the identification of 24 of the deceased. A new approach had been established [33, 34].

Paintings and other *works of art* can be imitated or forged. In Rembrandt's case, the value of an original is several million dollars or Euros. Radiographs and computed tomography help in the analysis. Sometimes the results of imaging analysis can be decisive [8, 35].

34.6 Outlook

Methods and Application. At present, conventional radiographs and Post Mortem CT are established as tools of the forensic pathologist. Works in progress analyse the potential of MRI, Endoscopy, Sonography and others. Angiography as Multislice Post Mortem CT Angiography, MPMCTA finds its place in forensics [36]. Hybrid Imaging, the combination of different Imaging methods are tested.

Using the established tools and employing Imaging methods, one notices that post mortem imaging is different from diagnostic imaging of the living. The questions to be answered are different. Clinical imaging concerns functions, while post mortem imaging concerns morphologies, which are modified by post mortem changes.

The questions in post mortem imaging concern the cause of death, age, sex, time since death, and identification. The questions in Clinical Diagnostic Imaging concern the injury, the treatment (surgery or conservative), and the prognosis. The imaging specialist uses radiographs obtained for medical reasons to answer questions in forensics. Law explains this: in Germany the Röntgenverordnung prescribes a medical cause to justify a potentially damaging X-ray exposure of the living. A health benefit must exist. The consequence is that radiographs of the living are hardly made. MRI, which uses non-ionizing electromagnetic waves, is permitted. However, MRI is expensive and MRI is only rarely available in the daily routine in forensics. Additionally, one has to have in mind that training in clinical radiology helps one to become familiar with post mortem imaging. However, without surveillance and training in forensic imaging there is the risk of mistakes, even for the expert in clinical radiology.

Developments such as virtual autopsy, "Virtopsy" [37], demonstrate the possibilities of imaging methods for forensics. The future will show whether, where and how far Virtopsy shall be used in addition to forensic pathology, as a tool of forensic pathology, or instead of autopsy. Legislation shall influence the development: in Germany the law prescribes the autopsy of the deceased child, when a battered child syndrome is suspected. Familiarity with the new technology, reliability and costs, are decisive.

The employment of imaging for dealing with mass casualty situations [38, 39] demonstrates the gap between possibilities and realities. The worth of imaging methods is established. New methods are known. However, their use is limited. Examples are the actual analysis of mass graves in Iraq, former Yugoslavia, and after the Tsunami catastrophe. If available at all, units for radiographs and imaging intensifiers have been used. Only in singular cases has more sophisticated imaging technology been employed.

Imaging of the Living. War, torture and crime force us to separate existing from alleged damages. If a damage/injury is found, it must be attributed. In the past, one rarely employed radiographs for this reason [40, 41]—a new approach is the use of scintigraphy to demonstrate preceding torture [42]. The reason was a possible damage due to the exposure of ionising radiation. It seems reasonable that in the future imaging methods without ionising radiation shall answer these questions. MRI and ultrasound are candidates. A limiting factor will be the availability of the specialist of forensic imaging.

At countries' borders and airports, imaging in the search for men, drugs and explosives shall employ non-ionizing radiation, as Terahertz rays. In the search for goods, the use of neutron rays and of spectroscopy has also produced promising results. The necessity to deal with possible terrorist attacks and the political situation shall influence the developments of these techniques.

34.7 Conclusion

Since the discovery of the X-rays by CW Röntgen, imaging has been part of the forensic sciences. The potential of forensic imaging has been established and is accepted. Developments in clinical diagnostic imaging have induced new approaches in forensics. Outside, medical imaging methods have shown their value in the control of borders and airports. Art, antiquities and archaeology represent another field of forensic imaging. The imaging of the living has the potential to prove preceding abuse and torture in singular cases at least. Post mortem imaging is different from ante mortem diagnostic imaging. Special training and experience prevent mistakes and errors.

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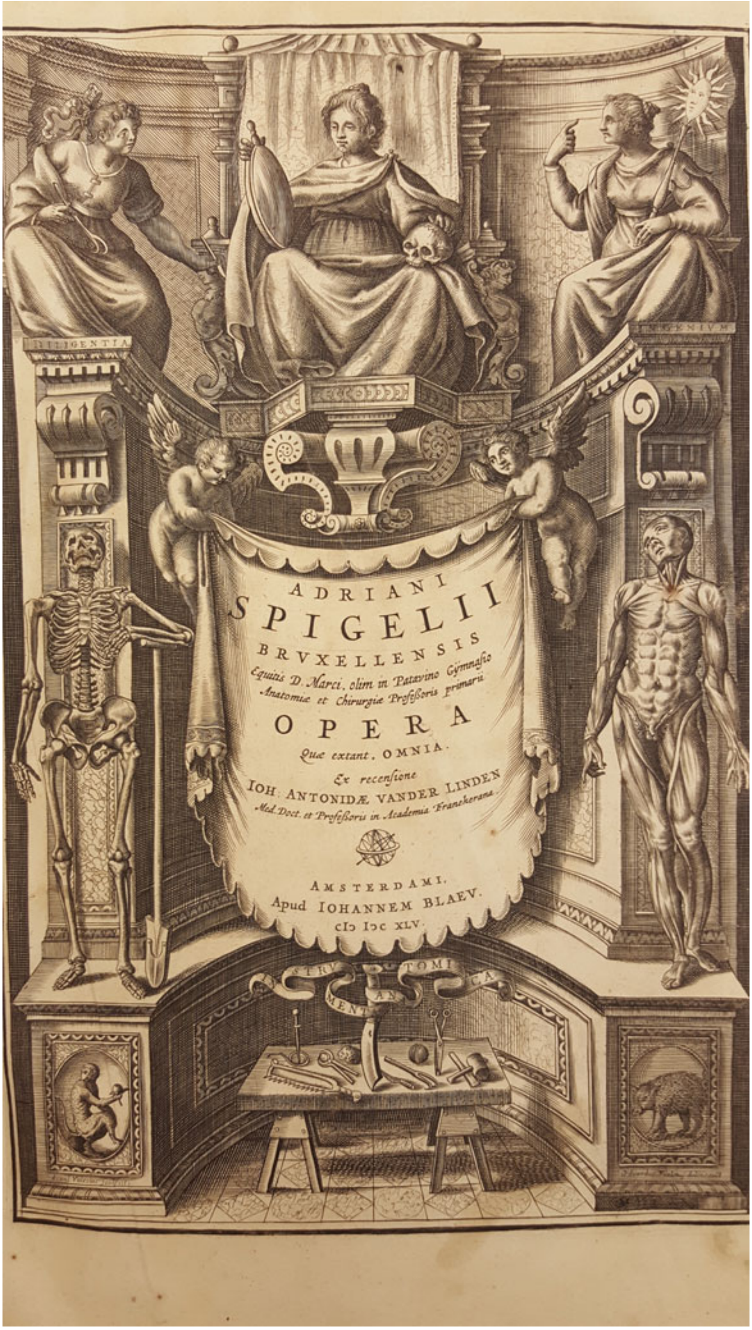

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Chapter 35
Clinical Forensic Imaging

Kathrin Yen and Astrid Krauskopf

Abstract Clinical Forensic Imaging (CFI), i.e. the forensic application of imaging methods in living persons following assault or other issues of potential legal interest, emerged a few years after post-mortem imaging had become one of the most belabored fields of research in forensic medicine. However, still rather few systematic studies on clinical forensic imaging exist, although the value of imaging methods for the detection of traumatic injuries and other findings in living persons has meanwhile been demonstrated clearly. Clinical forensic imaging, due to the absence of predominantly MRI radiation exposure, can add unique information regarding, for example, injuries in the body tissues and organs, about the age of an injury or the age of a person. In the following text, the current status of CFI shall be outlined with a specific focus on recent advances in CFI research.

35.1 Introduction

Clinical Forensic Imaging (CFI) is a relatively new diagnostic tool for the forensic assessment of living victims of violence, accident or self-inflicted injury. In clinical diagnostics, imaging methods became routine years ago and modern diagnostics and treatment seem unimaginable without them. In forensic medicine, the rise of Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) started around the year 2000 with the "Virtopsy"—and other research projects that focused on the postmortem non-invasive assessment of injuries and other case-related findings [1]. Before that, imaging was mainly limited to classical radiography that was applied in selected situations, e.g. for identification purposes, foreign body

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searching, or age estimation. Occasionally, imaging data from clinical examinations were used for forensic expertise. In such cases it was, however, mainly referred to the results from clinical radiologic assessment and usually no secondary forensic image reading with a specific focus on the forensically relevant findings and information took place. This is still a frequent praxis, although the lack of clinical image reading only in the context of forensic expertise is well recognized.

In the nineties, the focus of clinical research on CT and MRI that was related to forensic issues was mainly on head and neck traumatology, child abuse sequelae and their differentiation from accidental injury, identification and neurobiological or neuropsychiatric findings. In 2004, a forensic imaging study on the application of clinical cranial CT in forensic case assessment was published by Bauer et al. [2]. The first prospective clinical-forensic study using MRI was published by Yen et al. in 2007 [3]. This study on survived strangulation demonstrated the principal applicability of modern non-invasive imaging methods in such a context and showed their potential regarding the assessment of inner injuries that the expertise could be based on besides the results from an external examination of the body. From that time on, clinical forensic imaging began its rise, being increasingly recognized as a useful assessment tool that could support diagnostics by offering access to internal injuries and findings also in living persons [4, 5]. An important step in the evolution of CFI was the foundation of the Ludwig Boltzmann Institute of Clinical Forensic Imaging LBI-CFI (www.cfi.lbg.ac.at) at Graz in 2008, a research institute funded by the Austrian non-profit organization Ludwig Boltzmann Company. The LBI-CFI follows a focused research program and undergoes strict evaluation after fixed periods. The main advances in the field of clinical forensic imaging that will be discussed below are the result of LBI-CFI and associated institutions' research. There are still, however, a number of open questions; although the imaging of living patients is routine in the clinical process, its indications, application and specifications in forensic cases are to date rather unsystematic and need further development. The following article intends to outline the current status and actual developments in CFI and its applications.

35.2 Recent Advances in Clinical Forensic Imaging

A number of topics were at the center of CFI research in the past years, with some of them having proven to be of outstanding relevance for clinical forensic praxis. These are the followings.

35.2.1 Imaging Blood Including the Detection of Small Bleedings, Blood Layers and Microbleedings

Trauma sequelae are usually reflected by the presence of hemorrhage in body tissues and organs which can be assessed by CT and/or MRI depending on their size and localization. The larger the hemorrhagic alteration, the easier it will be found at imaging. Often hemorrhages accompany other findings such as fracture or laceration, thereby giving direct hints for the presence of such traumatic lesions. Imaging blood is of the utmost importance in clinical imaging and extensive experience exists from clinical trauma patients and studies. This experience also builds a perfect basis for forensic-radiologic examinations. However, some tissues are not in the clinical focus but important for forensic case assessment—e.g., bleedings in the subcutaneous tissue will be present, but are usually not described in clinical readings, as such findings are of no relevance in clinical trauma diagnostics in the vast majority of cases. The forensic knowledge about the detailed morphological appearance of subcutaneous hemorrhage in MRI or CT is still mainly restricted to a postmortem pilot study on fat tissue lesions by Yen et al. [6]. Later, a study was added on scalp lesions by Malli et al. [7] who examined persons following blunt injury to the head with subsequent clinical CT. Other studies demonstrated how forensic reconstruction could be improved by the use of imaging data [8–10], which is again mainly due to the ability of imaging methods to display hemorrhage in the soft tissues. In view of the importance of imaging blood in a forensic context it would, however, be highly desirable to add further prospectives and systematic studies regarding subcutaneous and soft tissue injuries, their detection and forensic interpretation.

Well recognized in postmortem forensic imaging is the fact that small layers of blood or microbleedings escape current imaging possibilities. Even when using 3T MRI, they will not be seen in normal clinical settings. Only if multiple small lesions are situated close together, or if relevant edema is present around the lesions, might they be detected. As known from the autopsy, small layers of blood and microbleedings may be present in many cases and might provide important clues for what happened to a person or how the injuries occurred. Therefore, imaging should offer possibilities that allow the detection of such discrete findings. Again, clinical forensic imaging learns from the dead—as recent post-mortem ultra-highfield MRI scans show, even hemorrhages in the micrometer range can be seen in the brain tissue when the imaging technique allows excellent resolution and is adapted to the specific forensic requirements. Yet unpublished data from this postmortem 7T neurotrauma study that is currently conducted at the Heidelberg Institute of Forensic and Traffic Medicine and the German Cancer Research Institute indicate that in future the current limitations of imaging regarding discrete hemorrhages might be overcome with new technical developments, even if it will still last some years until imaging in the living will be able to display these findings routinely.

35.2.2 Time-Dependent Changes of Blood

Another very important question is when an injury occurred. Until nowadays, age estimation of hemorrhages in living persons is still mainly based on color changes of externally visible bruises, although it is well known how difficult such diagnosis is and how variable color changes may be even in one single person. From the start of CFI, one of the main questions was if imaging, especially MRI, could add supportive data that offers an improved age estimation of hemorrhages based on objective tissue data. Besides clinical knowledge from neurotraumatology [11], the first forensic studies have shown that such an approach is highly promising, as the data from Neumeyer et al., Hassler et al., and Petrovic et al. [12–14] demonstrate. The authors were able to prove that bruises show a linear and characteristic time-dependent behavior when MRI was applied at certain defined times after the bleeding occurred. In two studies the hemorrhages were caused by injection of blood in the subcutaneous tissue of volunteers, one was an in vitro study using human blood samples that were repeatedly scanned over a period of 30 days. MRI showed a change of contrast over time, specifically in T2 and T1 sequences [14], proton density or IR sequences [12, 13]. In the studies from Hassler and Neumeyer, the Michelson contrast between blood and muscle was analyzed, with again typical changes of blood versus muscle from hyper- to hypointensity over time. On the whole, these studies gave promising results regarding the development of objective measurement methods for the estimation of the age of bruises. The potential of such a method in forensic casework is extremely high, as the question of the date of origin of injuries plays a major role, for example, in many child maltreatment and other interpersonal violence cases. Reflecting some limitations and questions that arose in the mentioned studies, such as the influence of metabolic factors or the shape of hemorrhage, further studies should be made to add reliable and validated data specifically from “real” cases.

35.2.3 Forensic Age Estimation by Means of MRI

Another field of CFI where modern imaging is currently going to change praxis is age estimation of living persons. The forensic methods for age estimation have been well defined in the past years and were continuously adapted to data from new studies. Imaging played a major role in this field of expertise since many years; first, the assessment was based on classical roentgenography and then increasingly on additional CT in defined cases. However, both methods are associated with radiation exposure for the examined persons. This was one reason for major discussion about the legitimacy of applying age estimation methods, for example, in asylum seekers, as there usually is no medical indication that would warrant roentgenography or CT in otherwise healthy persons. However, again, MRI could offer a solution. Since 2014 a number of publications showed that MRI has the potential not only to improve forensic age assessment, but also to replace radiation based imaging methods in the future [15–30]. Even the examination of teeth that is difficult at MRI could become possible; this would be required for a full replacement of the traditional methods [25]. Some studies tried an approach of automatic assessment of MRI data regarding the findings required for age estimation, again with promising results [17, 21, 26]. The current state of research indicates that MRI will not only help to overcome the limitation of radiation exposure in forensic age estimation, but also that it will become possible to develop (semi-) automated evaluation tools that could support the assessment.

35.2.4 Improvement of Forensic Reconstruction by New Visualization Methods and 3D Printing

Forensic reconstruction of the sequence of events is of central interest following violent acts or accidents. To find out what happened and how injuries occurred, the findings from clinical and/or forensic examination, additional examination results from e.g. DNA analyses, the "case circumstances" including reports of involved persons and witnesses, police reports and in some cases the results from on-scene evaluation are analyzed. The results from forensic reconstruction have to be "translated" to persons without medical training and demonstrated in court. For all these purposes modern visualization tools have turned out to be highly supportive [31–33]. For example, new visualization techniques help to demonstrate gunshot channels and stab wounds and in future could possibly even find them (semi-) automatically [34]. Tools such as a "magnifying glass" help to show inner injuries in context with the body surface [35], and 3D printing allows to demonstrate a bone fracture system in court even if the patient has survived [36, 37 and own unpublished data]. Furthermore, new developments provide possibilities to display inner findings assessed by imaging methods in relation to the body of the examined person [38]. Applications such as the aforementioned can be based on CT, MRI, or surface scan data, as all of these offer all well-known advantages of modern digital imaging methods.

Techniques for visualization are in continuous development not only in forensic research, and the coming years will show which of these are finally going to enter the courtroom and how they will be applied in routine cases. However, research will be necessary to further optimize the methods for a routine forensic use, for example, in view of getting a more realistic display of fracture lines on a 3D reconstruction without digital smoothing of small scissures, as is frequently observed when standard software is used.

Regarding surface scans, most forensic institutes currently do not have the infrastructure or collaborations that offer access to such examinations. This problem might be overcome, as a recent study by Campana et al. demonstrates [39].

In general, the best results in forensic reconstruction can be reached when specific forensic CT or MRI scans are performed, but also clinical data can generally be well used for 3D and other reconstructions. As in clinical radiology and patient care, such techniques still play a minor role, and it is necessary that a forensic evaluation is added and forensic expert reports do not only refer to the written reports from clinical diagnostics.

35.2.5 Age Estimation of Fractures

Similar to the dating of hemorrhages the estimation of the age of fractures is of high relevance, specifically in child maltreatment cases or following torture. Ongoing clinical and forensic research of fracture healing processes [40–47], meanwhile, set the basis for an increasingly detailed analysis of the stage of bone healing to become possible. The Ludwig Boltzmann Institute of CFI has recently shown how MRI could support such analysis in a forensic context, as quantitative parameters change with the duration of the fracture healing process [47]. Similar to the hemorrhage age studies, T1 and T2 values showed alterations that could be linked to the phase of fracture healing in adult persons. Besides data from clinical studies, these new results indicate that the dating of fractures might be far improved by MR-based methods in the nearer future.

35.2.6 Application of Contrast Agents and Angiography

It has been clearly demonstrated in the past few years how postmortem angiography can support case evaluation specifically after trauma or, for example, in medical malpractice cases. In clinical medicine, angiographic methods are daily praxis and belong to the frequently applied techniques for numerous indications. In clinical-forensic situations, angiography has not yet entered research or routine praxis. There would be a number of issues that could potentially benefit from such diagnostics, for example, vessel lesions following stabbings or medical malpractice cases with suspected lesions of blood vessels. Before forensic examinations using contrast agents and angiographic methods could start, it would have to be clarified if such applications are feasible from an ethical and juridical view when a clinical indication is not given.

35.2.7 Interpretation of Imaging Findings Following Strangulation, Specifically in View of Life-Threatening Forms of Injury

The first MR examination of a young woman following life-threatening strangulation in the early 2000s at the Forensic Institute of Bern impressively demonstrated what modern imaging methods could supply in clinical-forensic cases. From this time on, systematic studies that focused on strangulation followed [48–51]. The knowledge about findings to be expected after a strangulation incident is meanwhile high—however it is still rather unclear how these findings could be interpreted in view of the severity and life-threatening ability of such an event. An ongoing cooperation study led by the Ludwig Boltzmann Institute of Graz could help to further clarify this important question.

35.3 Strengths and Weaknesses

Clinical forensic imaging has meanwhile proven to provide helpful data about the condition, injuries and pathologic findings in numerous situations [4, 5, 52]. It allows information to be obtained in relation to living persons where inner findings are not accessible otherwise. Only if clinical imaging had been performed is imaging data also available for forensic purposes; however, these do not often optimally meet the forensic requirements. Besides traumatology diagnostics, one main strength of forensic CT and MRI in living persons is that these methods offer additional information and quantitative data that could allow further analyses, for example, regarding the healing status of soft tissue injury or the age of a fracture. Furthermore, clinical studies with some forensic relevance, e.g. regarding the diagnosis of metabolic changes or the sequelae of substance abuse, have some potential that their results can also be implemented in future forensic casework.

The main weakness of clinical forensic imaging is that there is still a relevant lack of knowledge regarding numerous issues. The existing studies have been performed by only a few institutions and often with a limited number of cases or some methodic restrictions. Such studies can be regarded as fundamental "pilot studies" where validation should follow, but has rarely taken place to date. Accordingly, there are still only few standard procedures that have been defined for performing and reading the scans, the indications are still not clearly defined, and the question of assumption of costs is open. This all demonstrates the urgent need for further research activities, and it underlines the need for highly specialized research activities such as at the LBI-CFI at Graz, where numerous clinical forensic imaging studies have been provided in the past years and specific expertise is present that can help to further spread the application of imaging methods in clinical forensic medicine.

35.4 Relevance of CFI in Court

Clinical data were used for forensic expertise for many years, but in the majority of cases the forensic examiner only referred to the written radiology report and no specific forensic follow-up reading took place. Through such an approach a lot of relevant information might get lost. Apart from performing interdisciplinary forensic-radiologic readings, it is important that the radiology examiner knows the forensic aspects and findings that the forensic expert needs from the CT or MRI data. As many institutes still have restricted access to clinical radiology and only few radiologists are trained for the forensic needs and diagnostics, this can be a major obstacle for bringing imaging into the courtroom. On the other hand, besides the unique possibility to get inner findings that escape the external examination, the new visualization techniques offer a great potential to demonstrate even complex medical and forensic contexts in a way that is understood by non-medical experts. However, the relevance of CFI in court is still limited in daily forensic praxis and its application is restricted to few centers. The potential to become a routinely applied method and to be an integral part of forensic expert reports seems high, however, in view of the general potential of forensic imaging methods.

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Chapter 36
Post-Mortem Forensic Imaging

Fabrice Dedouit, Lorenzo Campana, Tanya Uldin and Silke Grabherr

Abstract For autopsies, in addition to classical non-enhanced post-mortem computed tomographies, (PMCT), an innovative technique consists in post-mortem vascular opacification. It is called post-mortem computed tomography angiography (PMCTA) and the most widespread method is multiphase PMCTA (MPMCTA). It permits the diagnosis and characterization of vascular lesions. Another radiological technique is the post-mortem magnetic resonance imaging (PMMR). Its great advantage is the optimal spontaneous inter-tissular contrast. The development of PMMR angiography (PMMRA) seems to be promising for post-mortem cardio-vascular explorations. 3D surface scanning combined with photogrammetry permits the documentation of information such as texture and colour, which may be combined with PMCT reconstructions, the surface scanning of objects or the environment of a deceased person, to perform virtual reconstitutions of the events. In forensic anthropology, PMCT is also very useful for identification purposes (comparative, reconstructive and lesional identification).

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36.1 Introduction

The word autopsy is of ancient Greek origin, being the combination of «*autos*» (self) and «*opsomei*» (see) and can be translated as "a seeing for oneself" [1, 2]. In forensic imaging literature, nowadays most authors use the term Virtopsy®, a combination of virtual and autopsy [3, 4] or virtual autopsy. Medico-legal autopsy has many aims: reconstitution of the events and circumstances which preceded and led to death, including pre-mortem diseases, determination of cause of death, its judicial classification (homicide, suicide, natural death), precise time of death and identification of the deceased [2]. Autopsy techniques are aided and improved by new advances in pathology, molecular biology, toxicology and imaging. Indeed, autopsy may be limited regarding the demonstration of intra- or extravascular gas and the detection of some skeletal lesions [3]. Furthermore, there are diagnoses that can only be made if a specific technical protocol is applied. However, such protocols are only used if there is a suspicion that leads to a change of the standard protocol. This is the case, for example, for demonstration of intra- or extravascular gas (such as pneumothorax, pneumomediastinum, rupture of emphysematous bullae, gaseous embolism, pneumoperitoneum), various skeletal trauma (cervical spine fractures, such as transverse or spinous process fractures or pedicular fractures), complex and multiple fractures of the face or limbs, bone marrow injuries) and also ballistic wounds in putrefied or carbonized corpses [1, 3].

For a long time, in routine forensic medicine, radiology was limited to plain X-rays, which converted the original three-dimensional data into two-dimensional data: this has always been a problem [5]. Thanks to the advent of clinical MultiSlice Computed Tomography (MSCT) or MultiDetector Computed Tomography (MDCT) in 1988, it was possible for the first time to obtain isotropic voxels, which are the basis of high-quality multiplanar reconstructions [6]. Four main rendering techniques are used for the reconstruction of CT (computed tomography) data:

- two-dimensional reconstructions in different axes, so-called multiplanar reconstructions (MPR) are easily obtained and used regularly in clinical radiology. Not only standard axes (axial, frontal, and sagittal), but also oblique and curved slices can be reconstructed.
- three-dimensional reconstructions.
  - Maximum intensity projection (MIP): This reconstruction corresponds to the evaluation of a volume by drawing a virtual light ray from the observer's eye through the data volume and selecting the voxel with the highest attenuation. This voxel is then used as a displayed pixel of the rendered picture.
  - Minimum intensity projection (MinIP): This reconstruction is quite similar to MIP, but in this mode the voxel with the least attenuation along a virtual light ray is visualized.
  - Shaded surface display (SSD): This type of reconstruction derives the surface from a small percentage of the available data. This technique is not indicated for structures which do not have well-differentiated surfaces: subtle

bony fissures will not be visible because of the smoothing artefacts of the extracted surface.

– Volume-rendering technique (VRT): This reconstruction renders the whole volume and not only surfaces defined by threshold values. VRT takes the entire volume data and sums the contribution of each voxel along a line from the viewer's eye through the 3D dataset. The resulting composite of each voxel is then displayed on the screen. With a 3D workstation, it is possible to modify opacity, brightness and color. Special uses for VRT are virtual bronchioscopy and colonoscopy, permitting endoscopic views inside air-filled body cavities.

All these post-processing techniques entail the use of reconstruction workstations with advanced commodity graphics cards and a high memory capacity.

Although MSCT and magnetic resonance imaging (MRI) are widely used in clinical medicine, the routine diffusion of these techniques in forensic medicine seems, unfortunately, to be limited on the worldwide scale [5]. Post-mortem magnetic resonance imaging (PMMR) is a powerful diagnostic tool with a wide scope in forensic radiology. However, in the past 20 years, PMMR seems to be underused in forensic pathology. Its role in forensic death investigation largely depends on the rules and habits of local jurisdictions, availability of experts, financial resources and individual case circumstances. Limited access to MR scanners, time constraints and the complexity of MR technology are thought to be the principal reasons why PMMR is used less frequently than PMCT [7].

Overall, the virtual autopsy approach leads to increased quality and added value in the fields of forensic medicine and science. Basically, modern cross-slice imaging techniques have two applications in forensic pathology: in thanatology and anthropology [8, 9].

In thanatology, virtual autopsy contributes to the documentation of judicial evidence. The advantages of this technique are numerous: it enables objective data archiving, is non-destructive, yields actual-size documentation, provides three-dimensional information, is an additional examination tool, and can be transmitted by teleradiology and the internet [3]. The post-mortem images obtained are of real value as evidence. Of course, virtual autopsy also has drawbacks, namely cost, limited tissue resolution and lack of colour visualization. However, 3D surface scanner and photogrammetry can give information concerning the texture and the colour of the surface scanned.

Virtual anthropology consists of the introduction of modern slice imaging into biological and forensic anthropology [10, 11]. Thanks to this non-invasive scientific revolution, some classifications and staging systems, first based on dry bone analysis, can be applied to cadavers with no need for specific preparation, as well as to living persons. Estimation of bone and dental age is one of the possibilities offered by radiology. Virtual anthropology may also help the forensic pathologist to estimate a deceased person's age at death, which together with sex, geographical origin and stature, is one of the important features determining a biological profile used in reconstructive identification. For this forensic purpose, the radiological tools used are MDCT and, more recently, X-ray free imaging techniques such as MRI and ultrasound (US) investigations. Lesion identification combines virtual autopsy and virtual anthropology. MDCT can be useful in paleopathology, seeking different diagnoses such as arthropathy, infection, oral pathology, trauma, tumours, haematological disorders, or for stress indicators or occupational stress in bones and teeth. Just as when working with patients in clinical practice, one of the central issues is benefit/risk, or, in other terms: is the investigation useful? What does the investigation add in terms of anthropological study? What does the anthropologist, the forensic anthropologist or the forensic pathologist who request the radiological examination expect? This must be discussed between the physician requesting the examination and the radiologist who performs it. Concerns relating to imaging of the bones and teeth of deceased persons are not very different from the concerns of clinical practice: the radiologist must always be aware of the reasons behind the examinations in order to use an appropriate CT image acquisition protocol.

Globally, for all the post-mortem imaging possibilities, a multidisciplinary approach is crucial and involves communication and data exchange between radiologists, forensic pathologists, anthropologists and radiographers.

36.2 Advances in Post-Mortem Imaging

36.2.1 Post-Mortem Computed Tomography (PMCT) and Post-Mortem Computed Tomography Angiography (PMCTA)

36.2.1.1 PMCT

PMCT is increasingly accepted worldwide and performed for post-mortem explorations with medico-legal interests [12]. Advantages of this technique are important for the forensic purpose [13]. This exploration is classically described as non-invasive, objective (although it is, however, based on the radiologist's interpretation). It permits measures of densities through HU (Hounsfield Units) that permit the characterization of gas, water, bone, and fat. The images, when stored on a PACS (Picture Archiving and Communication System) or DVDs are transferrable by internet. Depending on the circumstances images may be interpreted, re-interpreted, and new reconstructions may be made. This conservative role is also important, since a medico-legal autopsy is correctly done only one time. Also the role of lesion illustration and documentation is important: it is didactic, but can also be useful during trials. With the multislice technology, it is possible to have an optimal infra millimetric spatial resolution. Some technical advantages of the PMCT compared with the clinical CT are the absence of kinetic or dynamic artefacts: heartbeat, respiratory movements, and no limitation of the x-ray dose delivery.

Interpretation must ideally be made by a forensic radiologist, or by a tandem composed by a radiologist and a forensic pathologist. It is also important for the radiologist who will interpret the post-mortem explorations to be aware of the specific semeiology of post-mortem images and also to know specific pitfalls [14]. The team of radiologists and forensic pathologists involved in the project of post-mortem imaging has to know that the visualisation and radiological diagnostics differs between radiological interpretation performed by a forensic pathologist and a radiologist. Radiologists focused more on bone lesions and forensic pathologists on soft tissues and visceral lesions [15]. Interpretations of the images implicate the knowledge of a normal aspect, and also post-mortem modifications after death (early and post-mortem changes). The radiologist must know the limits of the PMCT [16].

PMCT can have two main pitfalls: One is technical and one is human. From a technical point of view, the scanning protocol is crucial. In order to increase the resolution of the obtained images, CT-parameters have to be adapted. The resolution can be increased by choosing very fine slices and by overlapping the slices. The second pitfall is the interpretation of the obtained images. Here, the training and experience of the image reader is essential.

However, even with ideal or apparently optimal conditions, the PMCT misses 50% of the lesions visualized during autopsies [17]. Such missed findings are mostly visceral abdomino-pelvic lesions. On the other hand, the PMCT permits visualisation of 15% of non-viewed lesions macroscopically with autopsies. Such findings are typically the presence of air, which can be intra vascular (an argument for gazeous embolism) or extra vascular: pneumoencephalus, pneumothorax, pneumomediastinum, pneumopericardium, pneumoperitoneum, pneumorachis etc., Compared to autopsy, PMCT also has a higher sensitivity for detecting bone findings. In particular, lesions that are located in areas that are difficult to access with classic autopsy methods can be much more easily viewed using radiological techniques. Examples are lesions of the face or the upper cervical spine. It also has to be considered that the conventional autopsy will only demonstrate lesions that are searched for. If the pathologist is not looking for lesions, for example, of the upper and lower members, they will not be described.

Taking the example of traumatic situations, PMCT permits the documentation and description of lesions and the formulation of a diagnosis. Depending on the context, it also permits the identification of vital signs and localisation of foreign bodies.

Existing literature describes many such examples. Besides mass fatalities and motor vehicle accidents, PMCT can be used to document [17]:

- direct and indirect trauma,
- abuse (children, the elderly),
- gunshot wounds,
- drowning,
- charred, carbonized, putrefied corpses,
- unidentified corpses.

36.2.1.2 PMCTA

Introduction of contrast agent in forensic pathology is as revolutionary as it has already been in clinic radiology. In clinical practice, CT-angiography (CTA) is the most widely used method for detecting and localizing clinically active haemorrhages. It is also the method of choice for the assessment of coronary arteries. PMCTA permits localization of the haemorrhage and its origin (arterial, venous, mixt). It also allows evaluation of vascular lumen [18–20]. Different techniques using different material have been developed [19, 21]. Most importantly, post-mortem whole body explorations are represented by multiphase PMCTA (MPMCTA) and PMCTA using aqueous contrast agent and polyethylene glycol. PMCTA using cardiopulmonary resuscitation (CPR) has also been described. Techniques of targeted coronary angiography (TCA) have been developed that are focused on the study of the heart.

While Jackowski et al. used an aqueous solution as a contrast agent, the methods described by Grabherr et al. used an oily solution as contrast agent. The use of an oily liquid was chosen because of its ability to remain intravascular, which makes them suitable for performing a post-mortem perfusion without high loss of the perfusate into the surrounding tissue and without an oedematization of the latter. Every technique has some disadvantages. The major problem with techniques using oily injection liquids was the appearance of a discharge of the perfusate into the stomach and the intestine, which showed a clear locus minoris resistentiae. The aqueous contrast agent solution used by Jackowski et al. caused tissue oedema and artefacts in histological investigations, which rendered its application in medico-legal cases difficult. To overcome these problems, the two different approaches had been further developed later on. Jackowski et al. proposed to add a hydroscopic polyethylene glycol (PEG) as a contrast agent dissolver, and Grabherr et al. changed the viscosity of the oily perfusate and induced the development of a new contrast agent for PMCTA.

When focusing on whole body PMCTA explorations, MPMCTA appears as the most standardized and widespread method [19, 21]. MPMCTA is a particular type of PMCTA. It consists in the injection of a contrast agent mixture composed of 6% of Angiofil® and paraffin oil of specific viscosity, using standardized injection parameters (injection time, pressure, volume and flow rate) for each angiographic phase. The injection is performed using a modified heart–lung machine or, as proposed in later articles, a Virtangio® perfusion device (Fumedica AG). The standard protocol of MPMCTA consists in the performance of one native CT scan followed by three angiographical phases (arterial, venous, and dynamic). To interpret a finding as real, it has been proposed that the same finding should be visible in at least two of the three phases; otherwise, it should be interpreted as an artefact.

In a study comparing the performance of MPMCTA and autopsy, MPMCTA demonstrated a higher sensitivity for identifying skeletal and vascular lesions [19, 20]. Autopsy provided more information about organ morphology and pathology. By considering all findings regardless of tissue type and importance, MPMCTA and conventional autopsy demonstrated nearly the same sensitivity concerning the detection of overall findings. MPMCTA could increase the sensitivity of native CT scan from 65 to 80.9%, whereas autopsy detected 83.1% of all findings. Concerning findings classified as "essential" for solving the medico-legal case, autopsy reported 77.2% and MPMCTA reported 93.3% of all findings. For this reason, it was concluded that MPMCTA is an extremely powerful and useful tool for post-mortem investigations.

In the literature, MPMCT can be used to document:

- traumatic cases, in which a source of bleeding should be detected [19, 22];
- trajectories such as trajectories of projectiles in cases of lethal gunshots or stab wounds in cases of sharp trauma [19, 23];
- cases of death following surgical intervention [19, 22];
- cases of cardiovascular disease and sudden cardiac death [18, 19].

For the later, MPMCTA proved especially useful in cases due to coronary artery disease. In fact, the images obtained from MPMCTA facilitate the visualization of coronary arteries and permit the evaluation of stenosis and occlusions [18, 19]. It also helps in visualizing cardiac infarction, which is visible as a so-called "pathological enhancement".

36.2.2 Post-Mortem Magnetic Resonance Imaging (PMMR) and PMMR Angiography (PMMRA)

36.2.2.1 PMMR

Limited access to MR scanners, time constraints and the complexity of MR technology are thought to be the principal reasons why PMMR is used less frequently than PMCT. As for PMCT, some differences have to be known concerning clinical and post-mortem aspects for MRI. Besides absence of motion artefacts, position-dependant sedimentations, which are criteria also found on PMCT images, some are specific to MRI [7]. The influence of the temperature on MR image contrast occurs on T1 and T2 weighted images, with a modification of the inter tissular natural contrast [24]. Similarly to clinical experiences, the ideal protocol must consist in T1- and T2-weighted sequences, performed in 3 different axes (or with 3D acquisition and post-processing MPR or MIP reconstructions). Of course, even in forensic imaging, what can seem a surprising balance time/acquisition of images is necessary. Indeed, post-mortem imaging methods must not implicate a significant delay of the realisation of the autopsy.

T2-weighted MR images allow fluid accumulations to be highlighted. This makes them an ideal diagnostic tool for a wide range of pathologies, including subcutaneous haematoma, bone contusion, organ laceration, internal haemorrhage and fluid collections, ischaemic injury of the heart, brain oedema, pericardial or pleural effusion and pulmonary oedema [7]. Short Tau Inversion Recovery (STIR) sequences are suitable for screening purposes because they emphasize the signal from tissues with long T2 relaxation times and fluid accumulations. Such visible signals, which indicate soft-tissue lesions, are called the “forensic sentinel sign” by some authors [7]. Concerning the cerebral exam, the study by Yen et al. [25] revealed heterogeneous results regarding the radiological detection of a wide range of pathologies (including injuries to the scalp, skull fractures, intracranial haemorrhage, intracranial pressure and gas collections). Sensitivity of PMMR and PMCT ranged from 100% (for gas collections) to 0% (for mediobasal impression marks, a typical autopsy finding of elevated intracranial pressure). The radiological methods prevalently failed in the detection of lesions smaller than 3 mm in size, whereas they were generally satisfactory concerning the evaluation of intracranial haemorrhage. The authors offer two reasons for the heterogeneity of their results: insufficiently standardized autopsy protocols and inadequate training in forensic medicine for radiologists. In addition, PMMR has also proved useful to visualize lesions of the skin, the subcutaneous tissue and muscles of the neck from strangulation and hanging [26]. In our experience, PMMR of the brain provides detailed in situ information with tissue contrast that is superior to PMCT very early after death, as soon as the dedifferentiation between grey and white matter appears [13].

Another big interest in today’s PMMR research is cardio-vascular imaging. Acute, chronic and even subacute infarction are visible on PMMR [27]. The post-mortem imaging findings of acute myocardial infarction are comparable to those found in clinical cardiac MR and consist of focal necrosis surrounded by perifocal myocardial oedema with increased signal intensity on T2 weighted images. Focally decreased signal intensity within the myocardium on T2 weighted images without perifocal oedema was interpreted as a sign of early acute myocardial infarction (with a survival time ranging from minutes to hours) [27]. Due to the excellent inter-tissular contrast, some measures of thickness of the myocardium can be made, which permits the identification of left ventricular hypertrophy [7]. Concerning abdomen and pelvic organs, it has to be stated that non-contrast PMMR reveals better soft-tissue detail than non-contrast PMCT. Therefore, PMMR is considered to be more useful than PMCT to assess the abdominal organs. However, there is no data comparing the diagnostic value of PMMR to PMCTA. High soft-tissue contrast and the ability of MR to visualize soft-tissue pathology are also the principal reason why PMMR is the modality also of choice in post-mortem neonatal and paediatric imaging [28].

Concerning the skeletal system, MRI is not the method of choice. In fact, in clinical radiology, MDCT is considered to be the method of choice to visualize bone fractures. However, the ability of PMMR to highlight bone marrow oedema on STIR sequences is also an interesting sentinel sign, which could challenge the performance of MDCT for detecting bone fractures.

36.2.2.2 PMMRA

It should be noted that the absence of circulation in the post-mortem setting precludes the use of dynamic clinical MRA sequences such as time of flight imaging. In 2012, Ruder et al. reported whole-body PMMR angiography using clinical aqueous iodinated contrast medium (Optiray 300®) diluted in a solution of PEG. Good image contrast was obtained using fat-saturated T1-weighted images [29]. Fat-saturated T1 weighted images offered a good image contrast. However, because of the relatively long scanning times, PMMR angiography was described in this study as susceptible to position-dependent sedimentation of contrast medium, which lead to a degradation of the image quality. The authors stated that the loss of intravascular contrast volume on PMMRA compared with PMCTA was most prominent in nondependent vessels, which was probably related to gradual gravity dependent settling of fluids. As suggested by Ruder, and based on our personal experience, we suggested that both the time between contrast injection and imaging should be minimized [29]. Recently Bruguier et al. [30] developed a protocol using an oily contrast agent for PMMRA on hearts ex situ, permitting high quality images and visualization of the coronary arteries.

36.2.3 3D Surface Scanning and Photogrammetry

3D surface scanning is becoming increasingly important in the field of forensics [31–34]. At the same time, the technologies of 3D documentation are developing very fast, with different methods of 3D documentation that are getting faster and easier to handle.

During the last 10 years, expensive industrial equipment was used for 3D documentation in the field forensic medicine [31–34]. This may be the reason why only few institutes implied such scanners. One technique that is reported regularly in the literature is, for example, the structure light scanner “ATOS” from the company GOM© (Gesellschaft für Optische Messtechnik, Braunschweig). Combining this technique with a photogrammetric system such as “TRITOP” of the same company, ultra-high resolution 3D models can be obtained. This material is used to document, for example, injuries, injury-causing weapons or vehicles that are involved in accidents [33]. As a very recent development, hand-held 3D Scanners, such as the “Go! Scan 3D” from the company Creaform/Ametek© have appeared on the market in the last years. Today, such handy scanners do not have the high geometric resolution that can be obtained by heavy industrial material, but for most forensic questions the resolution is high enough.

Perhaps the greatest novelty in 3D-documentation is 3D-photography. In fact, the simple technique of photography can be used to create 3D-models if an automated photogrammetry approach is applied. The obtained models do not have a high 3D resolution, but they have an excellent texture resolution, which is in many cases sufficient for forensic comparison. In fact, the true colour of the object and its texture is often more important than a high resolution in 3D geometry [32]. Therefore, a 3D-model obtained by photogrammetry can be used to compare an injury to an injury- causing instrument. In order to calculate such 3D-models from photographs, special computed the software is necessary. An easy to handle software, suitable for the calculation of 3D-surfaces, is Agisoft Photoscan©.

The fast technological development of the last years has made 3D documentation ever cheaper and therefore more accessible for widespread use. However, for some cases, a high-resolution structure-light scanner is necessary. But these scanners are expensive, often too heavy, too big and too complicated to bring to crime scenes or into a hospital to scan the injury of a hospitalized person. Furthermore, the exam needs time and the treatment of the data is complicated and should be performed by an expert. In contrast to this, the hand-held scanner is easy to handle, mobile and the examination time is extremely short. Still, the easiest and cheapest way to get 3D models is the photogrammetry. A short period of training allowed every interested person to capture adequate images with a simple camera [31]. Of course, the result depends not only on how the images are taken, but also on the quality of the camera used. A high quality Single-Lens Reflex (SLR) Camera would thus improve the result.

36.2.4 Forensic Anthropology

Radiographic methods are well established in forensic anthropology. They serve either to identify an unknown deceased, by comparison of individual characteristics in ante and post-mortem radiographies, or to develop a biological profile by estimating age, sex or stature of the unknown individual.

Nowadays, forensic radiology has become a routine application in a number of medico-legal institutes and research in post-mortem MDCT is rapidly growing. As far as forensic anthropology is concerned, we can differentiate between the following topics: the first group includes papers describing the general utilization of MDCT for Disaster Victim Identification (DVI) purposes that covers medico-legal and anthropological procedures, or case reports presenting the application of identification methods to MDCT-images [8, 35–37].

The second group consists of more specific studies evaluating the main characteristics that are building the biological profile of unknown human remains such as age, sex, and stature, or measurements in general [10, 11].

Additionally, several studies are investigating the application of conventional radiographic methods to MDCT. For example, the comparison of ante- and post-mortem radiographies of frontal sinus patterns has been implemented to MDCT [38].

Furthermore, Wade et al. (2011) and de Froidmont et al. (2013) have conducted studies comparing conventional radiography and MDCT for investigating trabecular bone for age-at-death estimation [39, 40]. Both studies are indicating that MDCT is superior to conventional radiography in analyzing fine anatomical structures. Other studies have been testing the potential of MDCT in investigating trabecular bone for age-at-death estimation [41–43]. Nevertheless, further investigations are needed to develop methods appropriate for MDCT.

Beside the identification of unknown deceased, age estimation in the living is a medico-legal field of activity using also radiography and MDCT. Specialists of different disciplines such as forensic pathologists, odontologists, radiologists, and anthropologists have to take into consideration mainly physical, dental and osseous development to assess the age of minor or young adult individuals, such as the ossification of epiphysis of the wrist and medial clavicles [44]. In contrast to post-mortem images, radiological images obtained from the bones of living subjects have mostly a limited image quality, as acquisition protocols have to follow clinical guidelines in order to keep the radiation dose as low as possible. MDCT acquisition parameters such as tube potential, tube current, beam collimation and others have thus to be adequately balanced in order to obtain appropriate image quality [10]. Schmeling and co-workers, who mainly worked on the ossification of the medial clavicle epiphyses, have therefore tested different reconstructed slice thicknesses in order to define a protocol with high quality but low doses for the investigated subject. They found that this parameter considerably influences the results of ossification stages and recommended to work with the smallest slice thickness [45, 46], although the radiation dose is high. This example clearly shows the importance of CT scanning parameters. However, many questions still have to be answered to better understand the utilization and the potential of MDCT. With regard to MDCT application in forensic anthropological studies, only a few of them have published sufficient protocols for data acquisition and imaging processing until now. Hence, data comparability, which is essential for sound science, remains limited.

36.3 Conclusion

Post-mortem use of modern cross-sectional imaging is a relatively young discipline [5]. The potentials that it offers in many fields of research still have to be fully explored.

The wide range of possibilities offered by MDCT may seem bewildering to a non-radiologist. If an MDCT examination should give optimal results, many technical conditions must be adhered to in terms of image quality, spatial resolution and contrast [6]. If not, the final images may even be unusable, in particular for analysis of fine structures such as the trabecular bone. The initial MDCT must be performed with appropriate voltage, amperage, field of view and slice thickness [47]. After acquisition, reconstruction time is critical. Choice of thickness and interval and choice of filters influence voxel size and the possibility of radiological interpretation. Post-processing requires powerful computers. Choice of reconstruction technique must be appropriate for the initial objectives. Nowadays, 3D visualization of radiological MDCT data is possible for isotropic documentation, with no loss of spatial resolution.

It is clear that for a non-radiologist, PMMR seems to be more difficult in terms of radiological interpretation compared with PMCT or even PMCTA. The education of the forensic pathologist in radiology seems crucial for the development of post-mortem imaging, as well as education in forensic radiology. To overcome some PMMR limitations, several investigators are currently evaluating the potential of quantitative PMMR analysis. However, normal values have to be determined for each tissue and also for every MR scanner, depending on the value in Tesla of the machine. Another work in progress concerns the differentiation between post-mortem clot and true pulmonary embolism, which proves to be a difficult task.

MPMCTA can be combined with PMMRA, which would be especially interesting in cases of cardiovascular disease. PMMR seems to have the potential to detect cardiac infarction in a way that is even more sensitive than conventional autopsy and histology [27]. Although this research has to be further developed, a combination of MPMCTA, which allows the detection of coronary stenosis or occlusions, with local cardiac PMMR angiography, would likely open up new possibilities for investigating cardiac death. Further work must also be performed to evaluate the aspect of lesions explored by PMMR before and after injection of a contrast agent and potential consequences of the injection on the classical post-mortem aspect of cardiac infarction. A complete work concerning the post-mortem semeiology has to be evaluated and tested.

For current anthropological purposes, MDCT has many advantages over dry bone analysis. One of its major assets in forensic anthropology is the elimination of lengthy bone preparation, which may damage fragile bone. This can be particularly useful when bones are burned or charred. Documentation by radiological imaging is classically described as observer-independent, objective and non-invasive. Another advantage of MDCT is virtual access to bones where physical access is impossible. An example are bones embedded in sediment or concrete blocks [48]. A major interest is the conservation of the obtained images and reconstructions. This allows further studies to be carried out on the scanned object, independently of its state of preservation. This opens up a new approach to quality control and expert supervision, as well as image transmission and use in forensic telemedicine. Taphonomy and pseudo-pathology should not be forgotten. Forensic taphonomy means the interdisciplinary study and interpretation of the post-mortem processes of human remains in their depositional context [49]. Taphonomic details are important for estimating time since death and differentiating injuries from post-mortem changes. The radiologist who performs post-mortem MDCT must be familiar with late post-mortem changes, to avoid misinterpreting normal changes as traumatic injuries. Such confusion, especially when exhumed bodies are concerned, could potentially have significant judicial consequences. Many anatomical parts may be involved such as post-mortem changes of the ossicular chain of the middle ear oraxial and appendicular joint disarticulations [50]. Due to loss of soft tissues and costal cartilages, the ribs, sternum and clavicles may collapse into the chest cavity. The hyoid bone may fall near the spine. The mandible may be disarticulated and some teeth may be absent because they have been lost after death. All these changes must not be confused with post-traumatic lesions.

Radiology is of course a vast specialty and its progress depends on technological advances. Post-mortem imaging, like clinical imaging, involves the multidisciplinary participation of numerous actors, among them radiologists, forensic pathologists, anthropologists, pathologists and toxicologists. Together, the can open up a unique path toward future progress and toward new methodologies rendering possible a high-quality service for the jurisprudence throughout the world.

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Chapter 37
Micro-Imaging in Forensic Medicine

Giovanni Cecchetto

Abstract Micro-radiology has a spatial resolution of a few microns and may be considered the bridge that connects diagnostic imaging and histopathology. The main advantages of micro-radiology techniques, such as micro-computed tomography, micro-magnetic resonance imaging, and ultrasound microscopy are that there is no need for optical transparency in the specimen and imaging is non-destructive. Despite remaining an emerging technology, their use in forensic medicine is growing because they permit the detection of tiny fractures or dislocations of bones, teeth or calcified tissues, the virtual analysis of the micro-architecture of nervous, pulmonary or cardiac tissues, and the 3D reconstruction of the spatial distribution of foreign bodies with a density higher than 1000 HU on the surface or inside biological specimens. In this overview, the prominent areas of practice of micro-imaging methods in the fields of forensic pathology, anthropology and ballistics are illustrated.

37.1 Introduction

Medical history has witnessed the division of many fields, with one nascent discipline budding off another. In this way, micro-radiology was born from radiology, but may prove to be the bridge that connects histopa-thology and diagnostic imaging as a fusion subspe-cialty of its own [1].

The prominent tissue micro-imaging methods (micro-computed tomography, micro-magnetic resonance imaging, and ultrasound microscopy) are microscopic analogues of clinical radiological techniques and their common advantages are that there is no need for optical transparency in the specimen and imaging is

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non-destructive, and they therefore represent a major advance in our ability to study functional histology [2].

Despite micro-radiology techniques remaining an emerging technology, its use in the forensic sciences is growing.

The leading application of micro-imaging in forensics is the structural study of bones and teeth. In fact, these techniques permit the measurement and calculation of bone mineral density in the trabecular bone, bone volume, bone to tissue volume or bone volume density, bone surface to tissue volume or bone surface density, trabecular number, and trabecular separation, as well as trabecular pattern factor, structural mean index and connectivity density. Consequently, it is possible to identify lesions, fractures or dislocations of bones or calcified tissues [3].

Moreover, the availability of systems with almost microscopic resolution and sufficient soft tissue contrast, as well as the use of different contrast perfusion and staining techniques, has opened up entirely new applications for laboratory investigation of blood vessels and soft architecture of different organs, such as the brain, lungs, heart or kidneys [4].

In addition, non-biological material above certain minimum levels of detectability, such as metal particles or other non-metallic foreign objects with a density higher than 1000 HU can be localized, permitting the 3D reconstruction of the spatial distribution of the foreign bodies on the surface or inside the specimens.

In this overview, the prominent areas of practice in which the tissue micro-imaging methods can potentially be applied to enhance forensic investigations are illustrated.

37.2 Micro-Imaging Methods

37.2.1 Micro-Computed Tomography

The term micro-computed tomography (micro-CT) stands for high resolution CT [5], which allows the virtual reconstruction of objects with pixel size in the micrometer range. This has two main forms, attenuation contrast and phase contrast. In both, the imaging chambers are quite small. Attenuation contrast micro-CT is the exact analogue of clinical CT, but uses a very small X-ray source spot size (2 mm), thereby allowing resolutions of this magnitude. The major disadvantage is that soft tissue contrast is poor, so it is mainly used for bony structures in the absence of contrast media. Phase contrast micro-CT uses a split X-ray source. This provides exquisite soft tissue detail, down to single-cell resolution in unstained tissue without a contrast agent. Moreover, to differentiate between areas of similar density (e.g., muscle, organs) and to visualize the microstructures of soft tissues, exogenous contrast agents with a high-Z element probe, such as osmium, gold, silver, iodine, platinum, mercury, and lead, can be used [6].

The process of turning 2D projection data into 3D image slices is called reconstruction. In the process of image reconstruction, parameters are set, including image voxel dimension affecting spatial resolution and image grayscale scaling [7].

The aims of quantitative analysis are the geometric characterization of the sample, the analysis of the microstructure and the prediction of the chemical and physical properties [5].

37.2.2 Micro-Magnetic Resonance Imaging

Micro-magnetic resonance imaging (micro-MRI), also known as magnetic resonance microscopy (MRM) or magnetic resonance histology (MRH) is an extension of conventional clinical MRI whose voxel can be 10^6 times smaller than the voxel in clinical imaging [2].

Micro-MRI is perhaps one of the best imaging methodologies available for assessing soft tissues. Contrast in MRI can arise from endogenous differences in the spins in different regions of the specimen. The rate of T1 (spin-lattice relaxation along the longitudinal plane) and T2 (relaxation time along the transverse plane) recoveries varies with the local chemical and physical nature of the specimen; these differences can be exploited to give contrast to the image. MRI is not limited to endogenous contrast; applied substances (known as MRI contrast agents) can be used to change the relaxation times of sub-regions so that they appear either brighter or darker in regions in which the agent has accumulated [8].

37.2.3 Ultrasound Microscopy

Ultrasound microscopy, using highly penetrative ultrasound, offers extremely good resolution. Contrast is generated by variability in tissue sonic reflectivity and absorption, without the need for staining [2]. Ultrasound imaging at ultrafast frame provides an analogue to optical localization microscopy by capturing the transient signal decorrelation of contrast agents—inert gas microbubbles, allowing both non-invasive sub-wavelength structural imaging and haemodynamic quantification of microvessels.

37.3 Forensic Applications of Micro-Radiology

37.3.1 Forensic Pathology

37.3.1.1 Traumatology

Bones or soft tissue calcification (i.e. larynx) can be assessed by measuring tissue volume, calcification volume and the percentage of calcified tissue, to detect fractures or dislocations. Micro-CT has been shown to provide comparable results to histology for the examination of chronic osteomyelitis, syphilis, hyperostosis frontalis interna, hyperparathyroidism, osteomyelosclerosis and healing following trauma. Moreover, micro-MRI can provide detailed insight into the architectural consequences of disease progression and regression in response to treatment. Key forensic applications targeted involve fracture risk prediction and evaluation of the effect of blunt or sharp trauma [3].

Kettner et al. [9] have scanned the whole larynxes of three cases of suspected strangulation, where advanced decomposition precluded detection of hemorrhages adjacent to fractures using a microfocus-CT, observing thin fracture lines of the superior horn of the thyroid cartilage and of the hyoid bone (Fig. 37.1a) [9]. More detailed micro-radiology images of laryngeal fractures in a case of fatal manual strangulation has been described by Fais et al. using a Skyscan 1172 h micro-CT (Fig. 37.1b) [10].

The use of micro-MRI can generate a more accurate understanding of the volume, contours, and location of each component of the laryngeal framework, providing architectural information about the cartilages, joints, ligaments, intrinsic musclesnad vocal folds, not easily evaluated using CT or ultrasound [11].

Moreover, micro-CT has been already applied for the analysis of toolmarks on bones produced by hatchets and knives. This technique has been tested to determine the size and shape of the injury-causing knife by measuring distance and angles on the stabbed bones [12]. Pounder et al. verified the potential utility of micro-CT in the assessment of striation patterns of stab wounds in porcine cartilages, producing a 3D virtual casting of the stab track walls and matching it with a scan of the knife blade [13]. Capuani et al. observed that micro-CT is an appropriate tool to analyze bone marks such as hatchet wounds, but is not discriminating with knife lesions, since the 3D reconstruction leads to smoothing of bone surface, making the differentiation between lesions produced by serrated and non-serrated knives not feasible [14].

More recently, Pelletti et al., through the integration between 3D renders and Multiplanar Reconstructions (MPR) of micro-CT scans, allowed the identification of the morphologic profile of false start experimentally produced on human bone sections using different hand-saws (Fig. 37.2) [15].

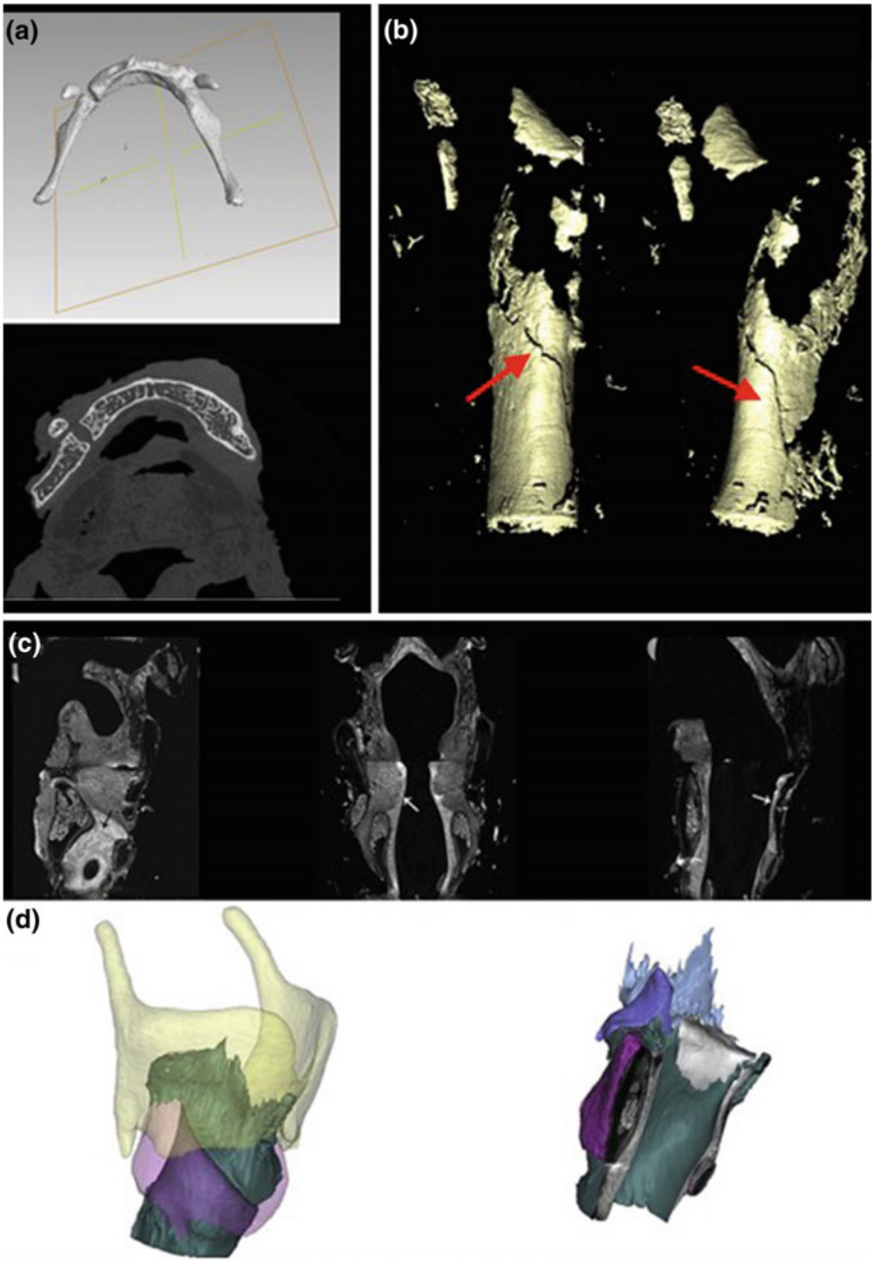

Fig. 37.1 Micro-CT and micro-MRI images of human larynx. **a** Micro-CT view of the hyoid bone, showing the lack of fusion between the body and left greater horn (reproduced from Kettner et al. [9]) **b** 3D reconstructions of the micro-CT images showing an oblique linear fracture of the left superior horn of the thyroid cartilage (reproduced from Fais et al. [10]). Micro-MRI images from the sagittal, coronal, and transverse planes, and 3D reconstruction of the thyroid, cricoid, and arytenoid cartilages (reproduced from Chen et al. [11])

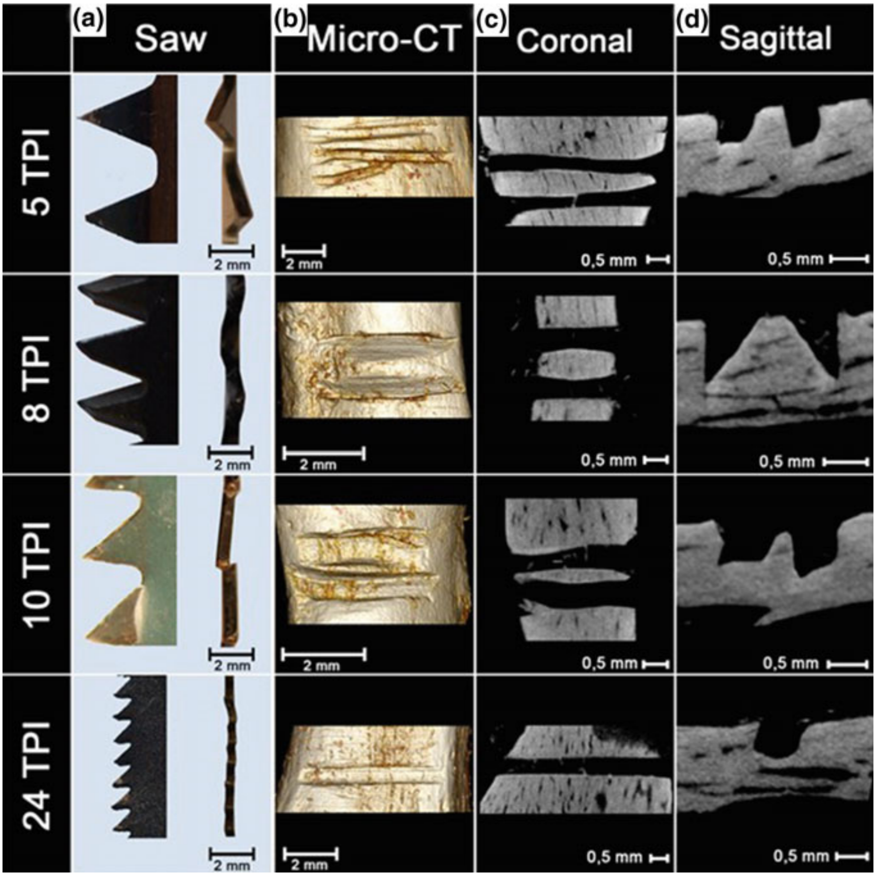

Fig. 37.2 Micro-CT images of false starts produced by different types of handsaws (reproduced from Pelletti et al. [15])

37.3.1.2 Asphyxia

Structural analyses of tomographic images based on model building have been reported for the study of pulmonary airway. Micro-CT provides the necessary spatial resolution of 3D images of the intact thoracic contents, as well as the basic micro-architectural structures, such as alveoli, can be individually visualized and quantitated.

Due to technical limitations such as detector size, micro-CT investigations of the human lung are restricted to the examination of small samples of excised tissue. By generating data sets consisting of isotropic voxels, information regarding spatial arrangement as well as quantitative dimensions of microstructures can be obtained from a 3D volume as well as from cross sections in any freely selectable spatial direction. Quantitative 3D measurements could apply to score the extent of emphysema in cases of asphyxia deaths [16].

37.3.1.3 Respiratory Work Related Diseases

Fusion of the micro-CT image data with other image data, such as micro-SPECT or histology, can enhance the information content beyond the mainly structural information provided by micro-CT [17].

Furthermore, 3D image editing techniques such as virtual endoscopy and shrink-wrap algorithms have been employed to describe lung anatomy and pathological changes of small airways and parenchyma in micro-CT data sets. Quantitative 3D measurements have been applied repeatedly for investigations of vascularization and for the quantification of tissue proliferation and rarefaction in lungs [18].

In cases of respiratory work related diseases, micro-CT could be useful to demonstrates the fine structure of the lung tissue and to assess quantitative information the fibrotic or emphysematous alterations (i.e. vessel density, volume and distribution of connective tissue) [16].

37.3.1.4 Neuropathology

Ex vivo micro-MRI imaging allows for 3D visualization of brain structures without slicing the tissue and provides a spatial resolution close to histology. This allows the estimation of the volume of small brain structures and of the neuronal loss, and the detection of amyloid plaques, microhemorrhages and age-associated rearrangement of hippocampal subfields (decrease of extended Ammon's horn relative volume and increase of dentate gyrus relative volume) related to age, cranial trauma or degenerative diseases [19].

Furthermore, a skeletonized model of human neurons can be built by tracing the 3D coefficient map obtained by X-ray micro-tomography, making it possible to analyse nervous circuits of any part of the brain [20].

37.3.1.5 Cardiovascular Pathology

A specific field of application of micro-imaging is cardiovascular pathology. Recent ex vivo studies have shown that contrast between muscular and connective tissue in micro-CT images can be enhanced by staining with iodine. X-ray microtomography can be used for exact volumetric measurements and the determination of mechanical properties of myocardium, as well as measuring fibre orientation [21]. This novel technique can be useful in non-destructive imaging of 3D cardiac architectures, due to the combination of relatively high speed and high voxel resolution provided.

A combination of micro-MRI, used for non-destructive reconstruction of fibre orientation, with subsequent histological sectioning, to obtain fine details of the tissue structure, has been successfully used to create a small-scale 3D computational model of the sinus node [22].

Microcomputed tomography is an important tool for vascular imaging, with micron-level resolution, allowing three-dimensional views of the entire microvascular structure, the collection of the volumetric data of blood vessels, and the morphometric analysis of human plaque [23]. Based on differences in grey-scale attenuations, micro-CT also correctly identifies the amount of mineralisation, and, consequently, the differential diagnosis atherosclerotic lesions that are histologically classified as fibrous plaques, calcified lesions, fibroatheroma, and lipid rich lesions.

Moreover, micro-CT is a promising method to visualize the architecture of the renal vasculature and, importantly, to separate cortex and medulla for the visualization of glomeruli and their afferent and efferent arterioles [4].

37.3.1.6 Perinatal Pathology

Several authors have published on micro-CT evaluation of foetal skeletal development. Those researchers pointed out the utility of micro-CT in terms of quantitative measures such as bone lengths, total bone volume, identification of small elements of ossification, as well as skeletal abnormalities [24].

A contrast-enhanced micro-CT protocol for post-mortem examination of whole-body specimens and isolated hearts from embryos or foetuses with gestational ages ranging from 7 to 22 weeks has been developed. This virtual autopsy yielded diagnoses in cases in which conventional autopsy or dissection was precluded due to size restrictions, furnishing additional details that improved diagnostic accuracy [25].

Whole-body high-field micro-MRI is a feasible option for post-mortem examination of human foetuses, and can provide good tissue characterisation even in small foetuses [26]. However, micro-MRI does not currently provide enough spatial resolution or sufficiently high contrast in tissues or cellular structures for a detailed analysis of early embryonic structures in the first Carnegie stages of the human development [27].

Compared with high-field micro-MRI imaging, micro-CT scanners are characterized by much lower purchase and operating costs, much shorter scan times and markedly higher spatial resolution.

Also the placenta has been examined by micro-CT after perfusion with a contrast agent [7]. Various parameters were analysed (e.g., total contrast agent, volume, vascular surface), finding that decrease in blood flow was associated with a decrease in foetal weight, while the enlargement of the arterial placental tree occurred primarily by increased arterial diameters with no changes in segment numbers. Combining of micro-CT data and total placental volume enables an estimation of the approximate surface of the placental vasculature [28].

37.3.2 Forensic Anthropology

37.3.2.1 Victim Identification and Age Determination

Micro-CT methods of age estimation are being increasingly explored, because they open new avenues for the assessment of bones, such as the pubis or rib ends, where age-related changes are to be assessed [29]. Compared with PMCT, micro-CT provides higher resolution imaging equivalent to macroscopic examination, which does not require the complete cleaning of the specimen and permits remote examination.

The micro-CT examination of the pubis avoids destructive analysis of human remains while providing an opportunity to estimate age-at-death using continuous, objective, quantitative measures based on the remodelling of the cancellous bone that underlies the symphyseal surface. This technique produces accurate and precise results when the symphyseal face is damaged and other indicators are inaccessible [30].

In forensic odontology, micro-CT examination of the enamel, dentine, and pulp cavity can contribute to the victim identification and age determination [31]. Someda et al. concentrated on applying micro-CT to the mandibular central incisors to investigate the relationship between age and age-related changes in the pulp/tooth volume ratio to elucidate the way in which the accuracy of age estimation could be affected by gender and regional differences [32].

As described above, compared with traditional gross anatomical observation or histological slicing, micro-MRI provides more accurate spatial information that can be translated into a 3D model. Successful 3D reconstruction provides useful and unique features of laryngeal structures that could be related to age and sex [11].

37.3.2.2 Vitality and Wound Chronology

Describing lesions as having occurred either ante-mortem, peri-mortem or post-mortem has severe limitations as it gives no estimation of the length of the time period between the moment of injury and the time of death (the post-traumatic survival time).

Although micro-CT cannot be used as a substitute for light microscopy, it could be useful to identify early callus formation that is still separable from the periosteal surface in order to estimate the post-traumatic survival time of bone fractures [33].

Moreover, Appleby et al. examined the mortal remains of King Richard III of England with micro-CT, concluding that the identified bones injuries were peri-mortem, since they did not present evidence of healing and the breakage characteristics were typical of fresh bone [34].

37.3.2.3 Time Since Death Estimation

By utilizing a combination of micro-CT and micro-MRI, it is possible to plan a human model study on samples in order to investigate the post-mortem interval and post-mortem changes [31]. Richards et al. used micro-CT to investigate the internal anatomical changes during blowfly metamorphosis, describing how micro-CT can be applied in the consideration of the post-mortem interval. They again report that it has the advantage over traditional methods of being rapid, inexpensive and non-destructive, allowing for multiple samples to be examined at one time [35].

37.3.2.4 Estimation of Cremation Temperature

The use of micro-CT proved to have clear advantages for the analysis of for the estimation of cremation temperature of burned human remains (bones or teeth).

It is well known that with the enhancement of the temperature ($\leq$ 600–1000 °C), the number of the multiple small cracks in the apical dentine as well as the large longitudinal cracks increases.

In contrast to other previously used methods, micro-imaging approach allows a non-destructive analysis of entire samples to determine local alterations. The results of a preliminary study showed an increase in mean crystal thickness in burned dentine and enamel that can be used to determine a temperature range. Moreover, a decrease in the degree of alignment and change in crystalline shape, as well as greater crystal perfection with larger grain growth, could be documented [36].

37.3.3 Forensic Ballistics

37.3.3.1 Gunshot Wounds Identification

Micro-CT analysis has been tested in several experimental studies to compare intermediate gunshot wounds produced on human skin under different experimental conditions [15, 37–39].

The results showed that this high-resolution tomographic technique detects radiopaque particles in entrance gunshot wounds, also covered by textiles, as well as altered by putrefaction, fire or water, allowing the tri-dimensional reconstructions of the spatial distribution of gunshot residue particles and the performance of a differential diagnosis between entry and exit wounds.

Consequently, if used as a screening test for the analysis of suspected wounds, in combination with other autopsy and crime scene findings, it may play an important role in reconstructing the shooting incident.

Obviously, considering that micro-CT is an invasive, but non-destructive technique, and the radiological analysis is limited to the presumptive identification of the GSR particles, positive results should be confirmed with a "gold-standard" method, such as environmental scanning electron microscopy coupled with an X-ray fluorescence energy dispersive spectrometry, inductively coupled plasma mass spectrometry and/or neutron activation analysis.

37.3.3.2 Firing Distance Estimation

In a recent study, intermediate-range gunshot wounds produced on bare human calves were analysed by means of a micro-CT coupled to an image analysis software in order to quantify the powder particles and to determine the firing distance [38].

The results, obtained through the scanning of the entrance wound, showed that in intermediate-range gunshot wounds, the amount of GSR resulting from the discharge of the firearm decreases at increased firing distance and is dependent in a non-linear fashion on the distance from which the gun was fired. Indeed, using a curve fitting process, a Gaussian model was found to best describe the relationship between the firing distance and the GSR percentage. Based on the Gaussian function it was possible to estimate the firing distance given a known percentage of the GSR deposit (Fig. 37.3).

On the other hand, the high variability of the results obtained on wounds produced in experimental trials simulating post-mortem alterations (i.e. submersion, decomposition or fire exposure) and considering that in forensic practice it is generally very hard to reproduce the same conditions of exposure from death until the retrieval of the corpse, the analysis of GSR through micro-CT, as well as other quantitative techniques, should not be used for estimating the firing range.

37.4 Conclusions

As emerges from this mini-review, the role of micro-radiology in the field of forensic medicine is already advancing. These non-destructive, high-resolution and high-accuracy imaging techniques should already be considered as an additional tool for forensic investigations, because they offer several important advantages and provide significant additional findings with respect to conventional autopsy and traditional radiological and histological techniques. Moreover, high resolution 3D images can be acquired relatively quickly, allowing a very accurate depiction of the samples [8]. They provide stable, detailed digital documentation of the post-mortem

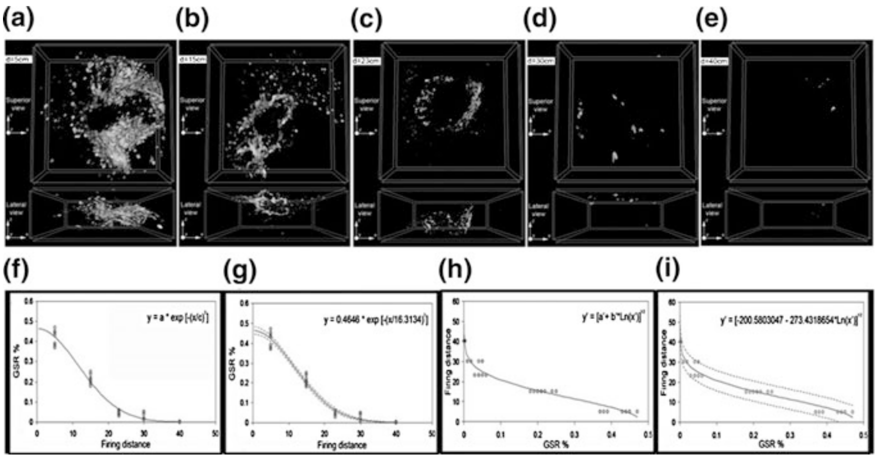

Fig. 37.3 Micro-CT reconstructions of the gunshot wounds at different firing distances (**a**–**e**) and graphs showing the Gaussian-like curve (**f**–**g**) and the regression function estimated through a nonlinear regression analysis (**h**–**i**) (reproduced from Cecchetto et al. [38])

findings that could be analysed and interpreted also retrospectively by an interdisciplinary team of specialists (i.e. radiologist, pathologist, cardiologist, neuropathologist), with potential gains in the accuracy of the diagnosis. The documented findings could be archived, re-evaluated and used in the court to show and illustrate to the judge and jury high-resolution images, which display all the advantages of digital evidence [25].

Micro-CT and micro-MRI, are currently limited by spatial resolution. These limits become particularly evident in fine structure imaging (e.g., in the visualization of terminal vessels and cellular lacunae in calcified had tissue). Moreover, the resolution of radiological measurements and reconstructions is influenced by several factors, which have to be taken into account while applying micro-radiology to forensic casework. Indeed, artefacts, such as beam hardening, scattering and metal scattering can hinder image analysis and interpretation. Finally, it is also important to underline that contrast solutions, generally used to enhance the visualization of soft tissues, can cause tissue shrinkage, alter the volume of the specimens, modify the relation between anatomical structures and tissues, and increase the volume of body cavities.

The future implementation of cross-sectional imaging is upon us, with the development of nano-radiology and molecular imaging. Actually, nano-computed tomography, a high resolution CT-technology for 3D imaging at sub-micrometer resolution, already exists and has been used for basic biomedical research. It has proved useful for the visualization and characterization of atherosclerotic plaques, cerebral vascularization, alveolar architecture of the lungs, and trabecular network of bones, furnishing anatomical information at a subcellular level [40] and revealing the structural basis of biological functions embedded into 3D images.

In the future, micro- and nano-technologies will probably be capable of analysing specimens not only from a morphological, but also from a chemical and molecular perspective, coupling structural information to biochemical and physiological data. In this view, cross-sectional high-resolution and high-accuracy imaging techniques could be tested and applied to many unresolved bio-medicolegal issues, such as the determination of the post-mortem interval, the identification of wound vitality, the reconstruction of the chronology of injuries, the timing of natural diseases, and the ascertainment/evaluation of personal injuries and damages that are problematic to objectify.

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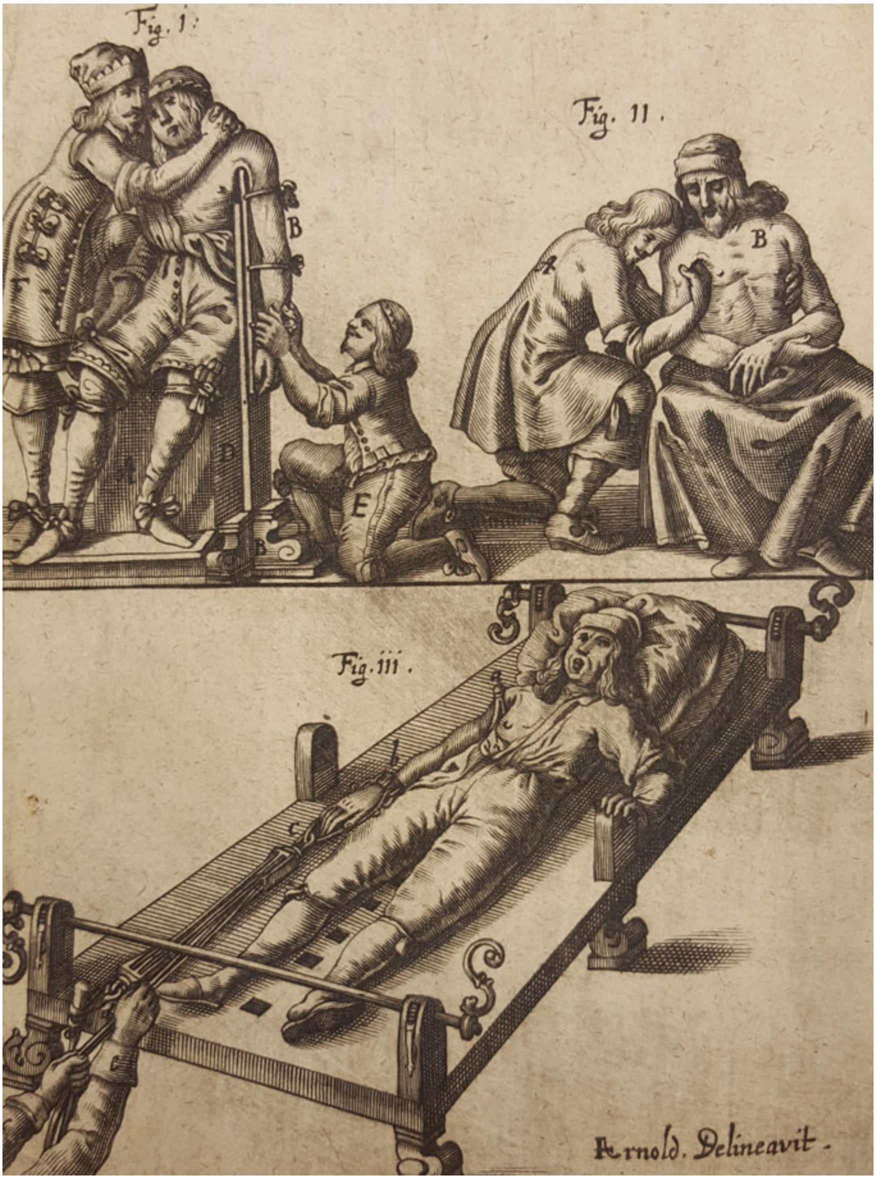

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Chapter 38
Future Evidence in Forensic Imaging

Guy N. Rutty and Bruno Morgan

Abstract The idea that the traditional autopsy can no longer be considered the "gold standard" and should be combined with or replaced, if necessary, by the radiological study (post-mortem cross sectional imaging—PMCSI) is slowly gaining ground, despite the presence of opposing currents thought. To encourage the application of PMCSI it is necessary to concentrate resources in three key areas: (1) Research, to improve its incisiveness, reducing its costs and keeping in mind possible applications in clinical settings; (2) formation of a "necroradiologist", with guaranteed specialist skills in the interpretation of radiological images of cadavers; (3) achievement of the standard, to ensure the quality of the results and give credibility to a still vulnerable field exposed to possible errors with catastrophic repercussions on its development and on the judiciary system. In the near future PMCSI will become the praxis in the study of the cadaver; however, for this purpose, it is necessary to promote research and a hyper-specialized training, to ensure the quality of results.

38.1 Introduction

Since 1983 when Kranz et al. first reported the use of computed tomography (CT) as an adjunct to autopsy investigation [1], the world has been undergoing slow but steady change in autopsy practice. This change has not been accepted easily, which is often the case in medicine when the traditional and longstanding "gold standard" of practice is challenged. To put it bluntly; medicine does not like change.

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Historical examples of resistance to change in medicine include the introduction of post-surgery antibiotic therapy or the introduction of keyhole surgery. More recently within the field of imaging itself, the use of CT for routine cancer staging was at first met with resistance and yet today is standard practice. When you add the police and the legal system into the mix then there is a significant mountain to climb!

The sequences of the change process is well recognised and in relation to post-mortem cross sectional imaging can be seen to have occurred over the last 35 years. The world is slowly coming to terms with the fact that the traditional invasive autopsy can no longer be viewed as the gold standard for death investigation [2, 3]. We are, as yet, not there yet in terms of the process of transformation, but we are nearing the top of the curve, somewhere between integration of the new "status quo". However, even when a new "status quo" is reached, what is certain is that there will still be invasive autopsies, but with routine post-mortem cross sectional imaging as an integral part of the autopsy practice.

Looking back over the last 35 years it is easy to identify why change has been so long in coming. After all the invasive autopsy has been around for a very long time; in essence why fix something that isn't broken? The most often quoted driver for change is the perception of the publics' dislike and religious objection to the invasive autopsy. Although this is in part correct, in one of the few studies to consider this matter Rutty and Rutty identified that it was not necessarily the public who were the main objectors to the invasive autopsy, but rather the medical profession's perception of the publics' attitude, or even the medical profession's own objection [4]. This explains in part the loss of the so-called "hospital" (non medico-legal) autopsy from modern medical practice. Thus, if both the public and medicine itself do not like the concept of the invasive autopsy, why has change been so slow in coming?

In fairness to autopsy pathologists, attempts have been made previously to try and seek alternatives to the full invasive autopsy [5]; needle biopsy autopsies, laparoscopic autopsies, limited and molecular autopsies have all been reported. In the case of deaths due to natural causes, if one undertakes an external examination and then, based on the past medical history and clinical presentation, suggest on a "balance of probabilities" that the death was due to ischaemic heart disease, the commonest cause of natural death in the western world, one will be correct in approximately 70% of case [6, 7]. So it is not for a want of trying that change has been slow in coming.

An alternative situation is where we do not wish to reduce the number of autopsies as such, but seek an alternative in order to actually increase the number of post-mortem investigations, for example in regions where autopsies are rarely performed. A good example of this is in Japan where they have probably the lowest invasive autopsy rate in the developed world. By seeking a culturally acceptable alternative, in this case cross sectional post mortem imaging, they have been able to increase and enhance their death investigation processes, without the need for invasive autopsies.

The main obstacles to the development of post-mortem cross sectional imaging are much simpler to identify. First there is the imaging technology. It has taken years for the scanners and computational software to develop to enable the living, let alone the dead to be imaged in the ways we know today. Then there is the access to scanners. Those who have been lucky enough to place scanners into their mortuaries will reap the benefits of 24-h access to imaging technology [8]. There is then the imaging enhancements such as post-mortem computed tomography angiography (PMCTA), including targeted coronary angiography [9], and ventilated post-mortem computed tomography (VPMCT) [10], all of which have had to be developed to enable diagnostic imaging of the dead to be able to provide a cause of death without the need for an invasive autopsy. However, the main hurdle currently is cost; cost of the equipment/facility and cost of the service. Post-mortem cross sectional imaging is perceived to be more expensive than the traditional invasive autopsy at a time of continuing global recession.

In 1994 Donchin et al. [11] proposed that some cases of trauma related death could be examined by sole use of post-mortem computed tomography (PMCT), as it later became known [12]. They were correct, although they lacked a research derived evidence base to support this pioneering suggestion. The world also lacked an autopsy imaging specific educated workforce to deliver this proposal to the medico-legal world. Finally, the processes of quality assurance, audit and standards of practice, which are so important within the criminal justice world, were all lacking. It is these three key areas i.e. research, education and standards, rather than the how and why, which remain to be addressed to allow forensic imaging to truly become integrated globally into medico-legal autopsy practice.

38.2 What Is the Question?

When looking to the future use of post-mortem cross sectional imaging (PMCSI) one first has to ask oneself "*what is the question we are trying to answer in investigating this death*"? To start with, for any medico-legal investigation there are usually 4 questions to be answered; *who* the person was, *where*, *when* and by *what* means, which is usually shortened to the simple terms '*how*' did the person come by their death? You need to know what the question is, before knowing if and how PMSCI can assist in the investigation. The question should not be asked in isolation but rather as a series of sequential questions (triage) so that when one has answered all the questions, to the burden of proof required for the relevant legal enquiry, the investigation stops. If these are all answered prior to PMSCI (view and grant system) then imaging is not required. If the question(s) are answered by PMSCI then there is no need to proceed to invasive autopsy. If PMSCI does not or cannot answer the question(s) then limited or full invasive autopsy is required.

If the question is solely or partly '*who*', can PMCSI assist with or address this question on its own? Well, when combined with an external examination of the body, and personal possessions, the answer is yes, to a certain extent. PMCT has been reported to be used for both odontology [13] and anthropology [14] identification, although further research is required to consider larger ethnic, age and gender data sets. As with the invasive autopsy PMCSI cannot tell where the person died. There are a number of publications considering the use of PMCSI for estimating a post-mortem interval but the field is too young for it to be considered more than a research interest currently [15]. As for '*how*', although there is a growing evidence base for its use in both natural and unnatural death, Morgan recently pointed out that there are large discrepancies currently between papers considering this question due to having different measures as to the required effect [16]. Due to changing context, different biases and inconsistent study comparators and end-points, it is very difficult to compare or apply published study findings to different areas of practice.

38.3 Research

When a new imaging technique is developed it needs to be tested to assess efficacy, effectiveness and efficiency. Efficacy and effectiveness are the extent to which the technique influences a favourable outcome, but are different. Efficacy is a technical term—does the test actually work, whereas effectiveness concerns the extent to which this 'efficacy' actually brings about the desired effect in the real world. For example an imaging test may accurately diagnose a condition, but this has no "effectiveness" if the diagnosis is untreatable or unimportant. So not only must the imaging test identify abnormalities and make the diagnosis, they must also displace or improve on other tests, contribute to service delivery and improve on outcomes for the whole population.

Not surprisingly, most diagnostic tests have good evidence for "efficacy" but often very little for their "effectiveness" [17]. Focusing purely on this technical performance can lead to failure to address the bigger picture and to incorrect assumptions about the new test. Therefore, when reviewing research or planning it, we must be a very clear about what effect we want. Put simply, if we ask the wrong question we will often get the wrong answer! [16].

These issues are particularly important in research into the relatively new discipline of post-mortem cross sectional imaging. Papers on technical performance abound with radically different conclusions. For example, we can argue that imaging is very poor at diagnosing the cause of natural death [18, 19] or actually very good [20].

These arguments cannot currently be satisfactorily resolved because the evidence suffers from a combination of changing context, different biases, and inconsistent study comparators and end-points. This makes it very difficult to compare and even apply study findings to work in different areas.

The key questions are wide-ranging but include:

- What type of death is it: natural or unnatural, single or multiple, witnessed or un-witnessed, paediatric?
- What is the main knowledge we want: Medical cause of death, mechanism of death or identification?
- Who wants to know it: The police, the local coroner, the family or the medical team?
- What is the tests place: To add information to traditional tests or replace them?

To answer these questions the evidence so far is nowhere near complete and all the author can do is read and critically evaluate the research.

Even when the question is similar the evidence can be unhelpful. Different study populations will have a different prevalence of different diseases. This pre-test probability will have a dramatic effect on the assessment of accuracy using the test. Many studies have different recruitment. For example, it is difficult to compare the results of excellent studies of hospital ITU deaths [21] compared with those from the community [22]. Likewise, large studies and systematic reviews may have a very heterogeneous population, including both natural and unnatural deaths [18, 23].

Another problem can be technical bias. The quality of CT scans available for Post-mortem imaging has improved over the last 20 years and this has almost certainly improved fracture detection and the development of angiography in the dead has changed the diagnosis of coronary artery and other vascular diseases [24, 25]. Old information may therefore not be good information and current research could easily become outmoded by technical advances.

Further research is therefore required to answer all these questions as technical performance/efficacy data needs to be more extensive and in different contexts. Only then can the more complex questions of effectiveness really be answered. Clearly at that point we can start to answer probably the most important question, efficiency or cost-effectiveness; is somebody prepared to pay for the test? Without the latter is is very unlikely that anybody is going to fund research at all.

There are also other potential research avenues. Instead of just looking at post-mortem imaging just to establish the "who, where, when and by what means", perhaps it is time to give something back. Medical research has all the same problems outlined above, but is compounded by the difficulty of research in living subjects due to the risk of harm or inconvenience to them. This is often resolved by clinical trials that are limited in how much investigative data is obtained per subject, or by moving into animal models. However, animal models are expensive and not universally accepted. They also often do not particularly resemble man, particularly the humble mouse. The post-mortem model therefore offers an opportunity to investigate clinical disease and investigate the tests we use in clinical disease in a proper human model, who has given their consent, either personally or via their next of kin. We have been pursuing this line of work in cardiology where there are many questions about grading the degree of coronary artery disease, not only in investigating cause of death but also in the living in order to evaluate the significance of coronary artery stenosis using new techniques such as optical coherence tomography and measuring pressure difference across stenosis. The cadaver potentially provides a good research model to investigate these techniques in the presence of real disease [26, 27].

38.4 Education

In 2004 Rutty and Swift, recognising the need for specialist training within an emerging field of practice, introduced a new practitioner to the autopsy world; the "necroradiologist" i.e. a specialist in the interpretation of radiological images of the dead [28]. But who is this person? Are they a radiologist with expertise in cadaveric imaging, trained in the external examination of the dead or a pathologist with focused training in post-mortem cross sectional imaging sufficient to report under the guidance and governance of a radiologist? In fact both exist depending upon where one practices in the world and one can argue that the most efficient system is to bring both skill mixes together and for both to work together.

What is clear is that for all branches of medicine, post-mortem imaging is not part of core medical training and in practice takes the practitioner outside what is considered conventional practice. For each profession to work within the criminal justice world training and accreditation will be required to allow a court to consider that the person undertaking the role is suitably medico-legally qualified. It is thus not unreasonable to predict that at some point in the not so distant future optional sub-speciality training in PMSCI will be introduced into radiology and medico-legal autopsy speciality training.

In the mean time we have to do with what is available. Although continuous professional development courses are run in several countries of the world, with the exception of the postgraduate certificate course of Zurich, Switzerland [29] and the postgraduate certificate, diploma and MSC courses of Leicester, United Kingdom [30] no others, to the authors knowledge, have attempted to develop postgraduate University accredited courses in PMSCI for medico-legal practitioners. Ideally a minimal global standard should be developed, possibly through an organisation such as the International Society for Forensic Radiology and Imaging (ISFRI) based upon the experiences of Zurich and Leicester. This will be a complicated task due to the differences both in medical practice and judicial regulations for all the different countries in the world. However, core curriculum activities should be able to be developed to inform and assist those developing similar courses within their respective countries. From experience in developing the Leicester courses, there is a considerable amount of work yet to be done within this key area for PMSCI to develop further.

38.5 Standards

It is all well and good gaining access to a CT scanner and scanning your next homicide victim, but do your scanning protocols, personnel, reports, audit, management, continuity of evidence, image database etc. all come up to the criminal justice standards related to your country of practice? Are your staff security cleared to work with restricted or higher level of material? Do you require your scanner to be licensed by a national body for sampling tissue from the dead? In terms of your management systems do they meet ISO 17025. The point is that practitioners new to this area of practice may not be used to the strict requirements of criminal justice systems.

To date this is an area of practice that has been completely overlooked mainly because the emphasis has been on research rather than service provision. As PMSCI becomes more globally accepted as an adjunct or replacement to the invasive autopsy practitioners within their respective country *must* address the issues of quality of standards and audit of practice. It only takes one very public error within the criminal justice system for the courts to lose confidence in the science and practitioners involved in any branch of forensic work. Currently PMSCI is extremely vulnerable to errors of practice which could have catastrophic repercussions on its development and future use.

38.6 Future Autopsy Practice

Rutty J et al. predicted that in the near future PMCT will become the ‘norm’ for death investigation, not the invasive autopsy and advised that pathologists amongst others must be prepared for the change that is already upon us [3]. However, to achieve this we, as a global imaging community, must embrace collaborative research undertaken in a similar manner to clinical medical trials, accept the need for postgraduate sub-speciality training and embrace the concept of quality assurance and audit of practice. The emergence of ISFRI and the dedicated Journal of Forensic Radiology and Imaging will hopefully draw the post-mortem imaging community closer together and drive forward the last stages of change to take us into a new era of global post-mortem imaging of the dead.

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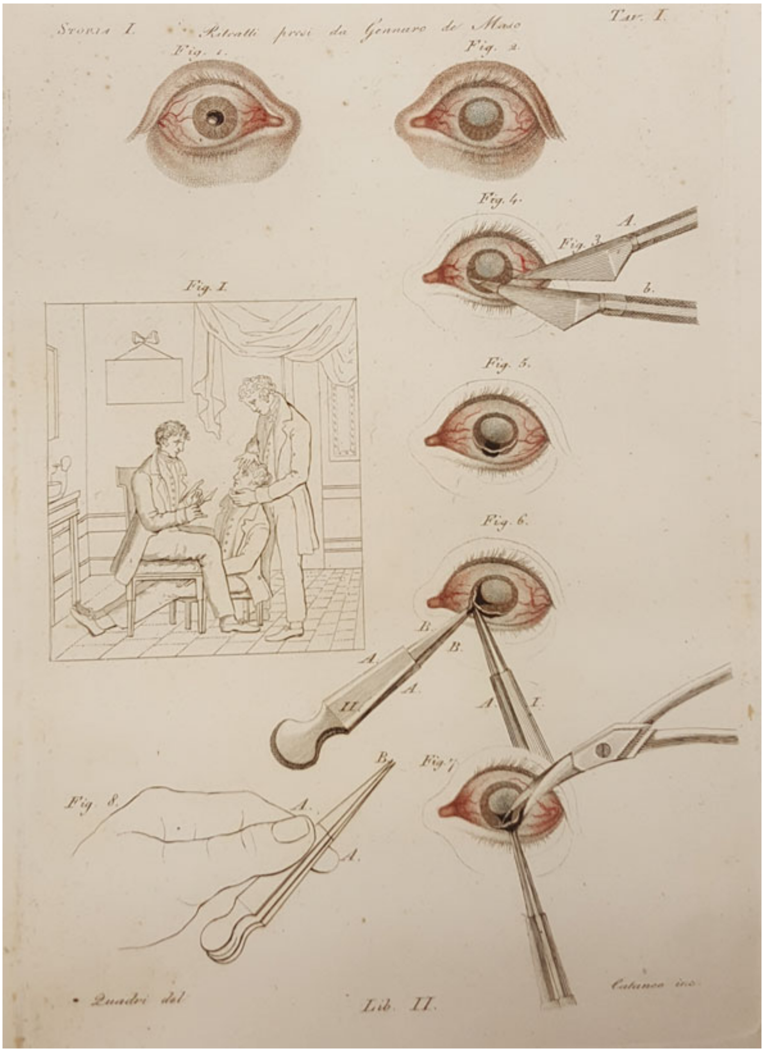
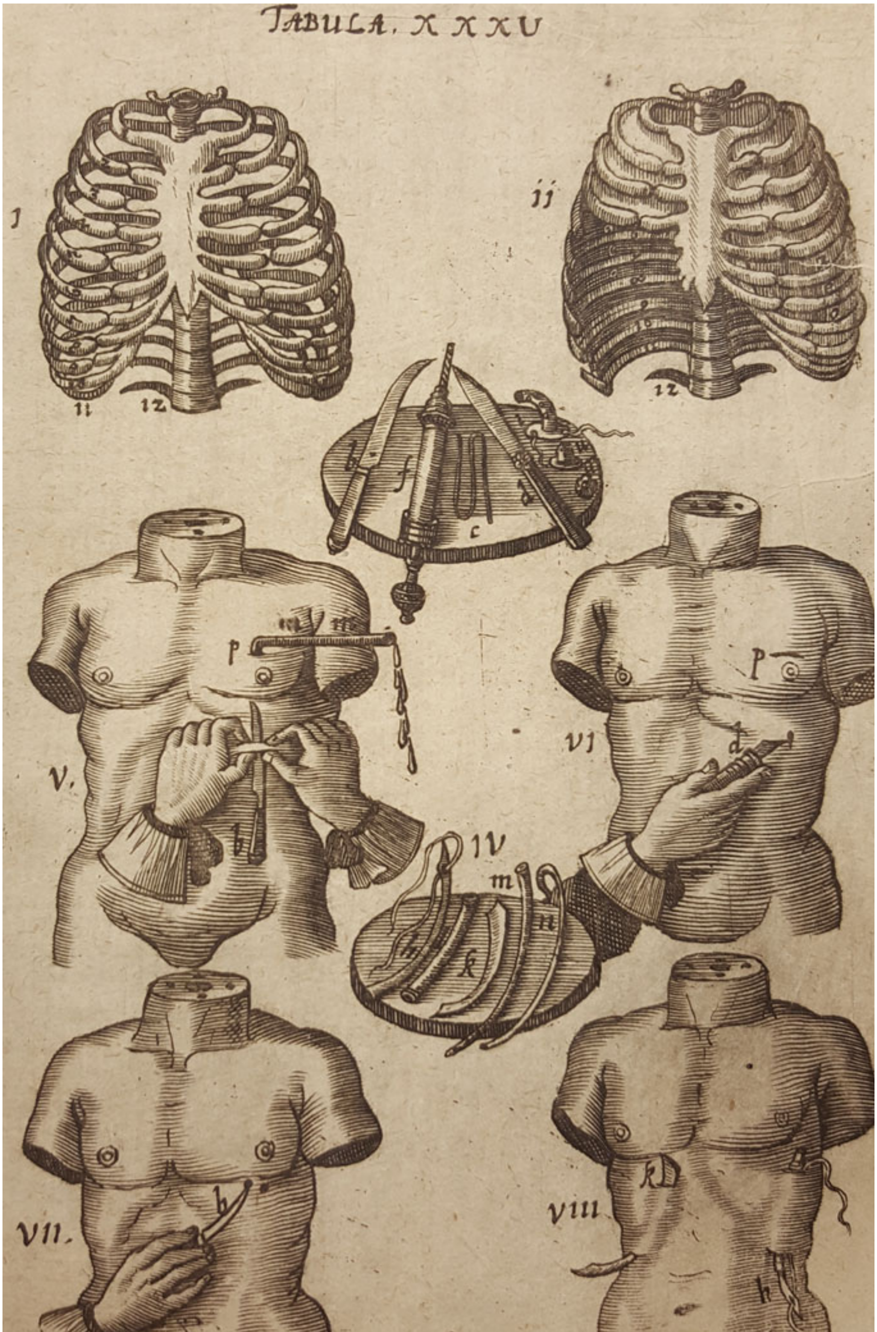
Part XI
Epicrisis, Conclusions & Future Perspectives



Ganz, Giuseppe, La medicina pittoresca, o Museo medico-chirurgico corredato di cento tavole d'anatomia generale, descrittiva, chirurgica e patologica, di patologia interna ed esterna, di medicina operatoria, d'ostetricia, di materia medica e terapeutica traduzione di Giuseppe Ganz Venezia: G. Antonelli, 1834–1839



Schultes, Johann 1595–1645, Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666

Chapter 39
Transdisciplinary Innovation and Future Evidence

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Abstract Future evidence in the bio-medicolegal sciences will emerge from transdisciplinary innovation, which, through the application of new technologies, involving the integration of imaging and bio-analysis, will be able to bridge knowledge gaps and reduce the black holes of knowledge, in an irreversible transition towards molecular evidence. The chapter depicts an overview of the contributions that every single discipline could bring to bio-medicolegal knowledge through its hyper-specialization, highlighting the role of transdisciplinary innovation, towards the realization of the Radiomics Project, the improvement of the level of Evidence and the diffusion of Educational Training, through an Interdisciplinary Masterplan, aimed at the scientific validation, certification and quality accreditation of the new technologies, with the ultimate goal of personalization, prediction and protection of human and personal rights, in the P5 Medicine and Justice perspective.

39.1 Introduction

In the future, evidence in the bio-medicolegal sciences will emerge from the entity of transdiscplinary innovation that each of them will be able to offer to the system of interdisciplinary culture, common and transversal to all disciplines. Evidence will emerge, that is, from the overriding ability to bridge knowledge gaps and respond to the numerous questions that still remain unanswered concerning etiopathogenesis and the ultrastructural and molecular physiopathology of pathologies of medicolegal interest.

The state of the art and the future perspectives of all fields of the bio-medicolegal sciences, accurately depicted in the previous chapters, raise, in particular, a series of unsolved questions, also referred to as *black holes of knowledge,* concerning:

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- *aetiology mechanisms, trait and state markers of diseases, damage and death;*
- *dating of injury and time since death;*
- *personal identification and age estimation*;
- *identification of trait-markers in criminogenesis*.

The reduction of *the black holes* should be accomplished through the application of new technologies, consisting in the integration of imaging and bio-analysis, as a vehicle for achieving a continuous and irreversible transition towards the attainment of the *molecular evidence*. This continuous evolution is undoubtedly laden with pitfalls, and the entire bio-medicolegal community should be aware of the contribution that every single discipline could bring to the future cause through its hyper-specialization, from Pathology to Criminology, through to Genetics, Toxicology, Anthropology and other more minor fields, geared towards the realization of P5 Medicine and Justice and the best Forensic Humanitarian Action, to which will be dedicated the brief profiles presented below.

39.1.1 Pathology

The achievement of evidence in forensic pathology has been evolving over the last decades. Some fundamentals remain valid (such as the principle of blunt and sharp trauma), while others have changed their frequency (illegal abortions and neonatocide) or their injury patterns (deaths due to poisons, drug toxicities, helium suffocation, plant toxins and vehicular accidents), with the continuous need to keep up with the daily casuistry. The consequence of all of this is the perennial impermanence of medicolegal knowledge, which remains valid only as long as it is not disproved by reality—which means by forensic casework—since only real cases can verify or deny new theories.

Although forensic pathology shows many features of an empirical science, traditional opinions must always be open to doubt. In fact, many problems have arisen from real daily challenges that remain unsolved and require more experimental research. This problem was also highlighted by Pollak in the related chapter of this monograph, where he reports the high number of homicides that go unrecognized in Germany. The solutions could and should be found in the research process, through the close relation between Legal Medicine and University Medicine [1], aimed at ensuring a high scientific standard and independence, in which concrete observations not yet fully understood could lead to hypothesis based studies and proper research activities, partaking in general scientific progress [2].

In the *post mortem ascertainment*, through Systems Pathology, a knowledge concerning the mechanism of death which takes place without morphological changes will in all probability be achieved (i.e. Sudden Unexpected Death and Sudden Unexpected Death in Epilepsy). The study of functional and metabolic status of the physiopathology of the death of cells and organs by bio-analytical platforms (mRNA microarrays, RT-PCR microRNA profiling, Genome-wide association studies, post-mortem biochemical profiles), will explain if and whether the integration between the genetic effects and the environment could be responsible for deaths that are nowadays defined as "sudden" and "unexplained".

A standardized and widespread application in forensic casework of specific biomarkers will facilitate the resolution of forensic questions, such as wound age determination, by means of the study of the changes in cytokine/growth factor profiles, the aging of thrombus, drowning in fresh/salt water and time of death, through changes in expression of "clock genes" [3–6]. Due to the unique dynamics of cytokines in the process of wound healing, mRNA analysis or DNA microarray have been used and have shown promising results for the study of the age estimation of wound aging, but need more application in daily casework [7, 8].

As for the advances in determination of time since death, new methods will be applied. Promising new approaches using classic technologies (i.e. Western Blotting) could be implemented and consequently improved, such as the study of degradation markers associated with temperature and Post-mortem Interval [9, 10]. Additionally, new technologies could be introduced in this field, such as Nuclear Magnetic Resonance and Mass Spectrometry based methods [11], which have given promising results in the experimental setting.

The evolution of the traditional autopsy will be influenced by new morphological techniques which can be further confirmed and validated with the so-called *advanced molecular autopsy,* a meeting point in which different skills come together and provide specific contributions. Technologies with encouraging prospects for their application in this field are Robotic Systems (so-called *robotic autopsy*), Laser Scanner Confocal Microscopy, Atomic Force Microscopy and the aforementioned Mass Spectrometry Imaging. Nevertheless, the high dimensionality of data generated from these new technologies requires the development of a mining approach, based on improved bioinformatics and computational biology tools [12, 13].

In *ante mortem ascertainment* on the living subject, combined proteomics and metabolomics approaches should provide a comprehensive understanding of the mechanism of diseases that are nowadays classified according to their symptoms. Through a molecular-systematic approach, new biomarker candidates will be identified and new insights about the underlying molecular mechanisms will be gained, in order to estimate brain injury grading [14], post traumatic disorders [15], depression and pain [16], and for a deep insight into the dynamic of human actions and behaviour, through a better understanding of the complex interplay and the relationship between nature, nurture, epistasis and epigenetics, which will be addressed in the section on Criminology.

39.1.2 Genetics and Genomics

The main achievements of Forensic Genetics and Genomics in the last few years have been the use of SNPs for forensic DNA phenotyping (FDP), which permits the prediction of externally visible characteristics (EVCs) from traces of DNA.

This progress is encouraging in relation to the construction of composite sketches from DNA for investigative intelligence purposes. Several studies have been performed for pigmentation traits, body height, hair morphology, ear morphology, facial shape and repeated appearance traits of age, such as hair loss or pigmented age spots. As a result, predictive DNA markers were developed and validated, such as statistical models to obtain eye and hair color phenotype probabilities from the DNA marker, as testified to by the developments of IrisPlex and HirisPlex systems for the prediction of eye and hair color [17, 18]. Other approaches consist in the use of age and bio-geographic ancestry information stored in DNA. Inferring EVCs, age and ancestry of an unknown trace donor from DNA collected from a crime scene, for example, in the near future forensic geneticists will be able to characterize an unknown person, with promising implications for forensic practice [19, 20].

The introduction of mRNA markers [21, 22] and DNA methylation [23] for body fluid identification will allow the use of Massive Parallel Sequencing (MPS) platforms [24, 25], with the potential to include many of the marker systems in one reaction, such as global STRs, SNPs, ancestry informative markers, EVC markers, as well as Y- and X- chromosomal loci. Consequently, the number of alleles per locus is likely to increase, leading to higher powers of discrimination.

The short-term goal is to achieve progress in common procedural standards and in similar requirements among countries, such as the harmonization of criminal databases, the coordination and compilation of population databases and the communication of the value of evidence provided by DNA analysis. The efforts in technical and procedural standardization should continue and improve, in order to obtain a shared international accreditation.

A reassuring and promising application of new technologies is the *innocence project* [26] a U.S. project which is trying to exonerate, through the use of DNA, people wrongly accused of having committed crimes. Up until now the project has been able to exonerate more than 200 people. It is interesting to note that most of the people were not guilty, and the main factor that led to such wrongful convictions was eye-witness misidentification. This is the reason why new technologies are important for the future.

Finally, forensic genetics related to non-human species is an emerging field with a promising future. Forensic analysis of animal DNA has been used both when animal material (usually hair) is found at crime scenes and in the investigation of the illegal trade in endangered species. As with animal material, plant material can be associated with the crime scene and provide vital evidence. The same is true for analysis of bacterial strains in soil through the new metagenomic approach [27].

39.1.3 Toxicology

The future of toxicology is influenced, even more than the other sub-disciplines, by the innovation introduced by the omics era. *Toxicogenomics* will open the door to a new paradigm in drug development and risk assessment, which promises to generate information and understanding of the molecular mechanisms that lead to drug toxicity and efficacy, and of DNA polymorphisms responsible for individual susceptibility to toxicity. The principal goals of toxicogenomics are to understand the relationship between environmental stress and human disease susceptibility, identify useful biomarkers of disease and exposure to toxic substances and elucidate the molecular mechanisms of toxicity [28]. *Proteomics* and *Metabolomics* will provide the full context of the exposure and the kinetic behaviour of xenobiotics, while *Toxicoproteomics* will permit the investigation of molecular targets of toxicants, the study of molecular responses of cells and tissues to toxicants and the identification of signatures of toxicity-patterns of metabolic changes [29, 30].

There are a number of aspects that differentiate metabolomics from the other omics, making it more affordable in forensic analysis. Apart from the lower costs and shorter time of analysis compared to other omics disciplines, it has the capacity to integrate data provided from different body compartments by different bio-analytical platforms that continuously improve their capacities (i.e. new mass spectrometers, hybrid quadrupole time-of-flight Q-TOF and Orbitrap systems, new chromatographic phases, multidimensional, ultra and nano chromatography), resulting in an integrated metabolomics approach [31].

An increasing number of techniques, such as Matrix Assisted Laser Desorption Ionization (MALDI), Nanostructure Initiator Mass Spectrometry (NIMS), Ion Mobility Mass Spectrometry (IMS) and Nuclear Magnetic Resonance (NMR) spectroscopy, will support and develop *Systems Toxicology,* derived from the integration of the Bio-analytical platforms with classical toxicology, and from the quantitative analysis of large networks of molecular and functional changes, aimed at decoding the toxicological organization of active substances that interact with living systems. This approach could provide a detailed mechanistic understanding of the ways in which chemicals perturb biological systems, measuring the molecular changes deriving from an exposure and building a causal chain of molecular events linking exposure, described in a quantitative manner by mathematical models [32]. A complementary omics approach is also used to map and annotate pathways of toxicity (PoT) in the Human Toxome Project [33], a long-term vision to modernize toxicity testing for the 21st century. The project will comprehensively map pathways of endocrine disruption (ED), as a proof of concept of mapping PoT by systems toxicology, representing the first step towards the mapping of the human toxome. In addition, all data and metadata (i.e., experimental descriptors) will be made accessible via a centralized cloud server. From a technical point of view, nano-robotization chemometry, by means of the LAB on a CHIP, Onsite Point of care and micro-NANO fluidics, will provide the realization of the adopted project of Human Toxome.

39.1.4 Imaging

In the near and more distant future, many imaging techniques and methods will be applied in the forensic fields, both in the dead and in the living subject, including

Functional MRI [34], Tractography MRI [35], Photoacoustic Tomography/Endoscopy [36] (in vivo histology and detection of genetic and molecular process and metabolism), Ptychographic X-ray CT [37] (nanoscale imaging), Optical Frequency Domain Imaging [38] (volumetric views of tissue microstructure at near-histological resolution), Spectral Domain Optical Coherence Tomography [39] (evaluation of microstructure and microvasculature) and the FMT-XCT [40] (in vivo detection of tissue anatomy and function). These promising techniques, whose level of accuracy remains undetermined, require the carrying out of additional research. As a consequence, the bio-medicolegal sciences should import these technologies from biomedicine, testing their usefulness and possible application in forensic casework.

For *post-mortem ascertainment*, at the present time the external and internal examinations could be performed with a multitude of techniques and methods, such as CT, micro-CT and MRI, in order to confirm the presence of soft tissue, bone or vascular trauma, with the application of a qualitative model. However, the modern system is vulnerable to errors of practice, with negative effects on the development of a shared protocol. Based on the current scientific literature, it is not possible to determine the diagnostic accuracy of post mortem imaging and its usefulness in conjunction with, or as an alternative to, autopsy. To correctly determine the usefulness of post-mortem imaging, future studies need improved planning, improved methodological quality and larger materials, preferentially obtained from multi-center studies.

In the near future, *micro-radiology*, whose use in forensic science is growing, may prove to be the bridge that connects histopathology and diagnostic imaging. Even if nowadays its application is limited (GSR, toolmarks, bone fractures) and it is not routine, due to the limited diffusion of the technologies, in the future micro-imaging will be the standard in post-mortem examination [41–47].

It is envisaged that Post Mortem Computed Tomography will become the "norm" for each investigation, in spite of invasive autopsy. Nevertheless, the best way to achieve this complex multi-factorial change is to continue with the traditional approach of constant transformation and evolution, so that it then becomes impossible to return to a fixed point in the past [48]. In this transition, a process of quality assurance, audit and standards and efficacy, as well as specific educational training programs are needed, in order to achieve consistent evidence.

Once qualitative imaging has become routine in forensic practice, a *quantitative model* and an *algorithm* will be applied and elaborated, as well as new technologies, such as Spectral CT, Synthetic and Fingerprint MRI, with the aim of providing quantitative multi-parametric maps with a high level of robustness and reproducibility.

The more distant future will be characterized by the creation of Big Data Imaging Platforms, universally accessible by cloud, with the progressive introduction of post-mortem Radiomics and Radiogenomics [49]. Imaging allows information to be obtained in relation to *ante mortem ascertainment* on living persons where inner findings are not otherwise accessible, offering additional information and quantitative data that could allow further analyses (i.e.) regarding some relevant forensic fields such as the healing status of soft tissue injury or the age of a fracture. The area of clinical forensic imaging is currently affected by a lack of knowledge and numerous issues, such as its indications, application and specifications, which are to date rather unsystematic and require further development. Research is still in progress and in many fields, such as the detection and the quantification of small bleedings and the dating of bone fractures and subcutaneous/ soft tissue lesions, increased and systematic research is needed. In fact, forensic knowledge concerning the detailed morphological appearance of some aspects in the living person is still mainly restricted to postmortem experimental studies [50].

Finally, in the field of age estimation, MRI will soon become the technique of choice, due to the absence of radiation exposure, since nowadays its application is strictly related to the elevated costs. Some recent studies have in fact reported promising results with an approach of automatic assessment of MRI [51, 52].

The main problem in clinical forensic imaging is that the existing studies have only been performed by a few institutions, without a systematic worldwide approach, and often with a limited number of cases or some methodic restrictions. Such studies should be regarded as fundamental “pilot validation” to achieve an adequate level of accuracy.

39.1.5 Anthropology

The identification of human remains is an interdisciplinary task, emerging from the cooperation of various disciplines, in which anthropological methods are nowadays considered secondary identifiers. The adoption of a quantitative approach and an appropriate statistical strategy could provide forensic anthropology with a role of primary importance, so that unique skeletal features can work as such and thus reduce the number of unidentified persons, whose number is still unknown [53, 54]. To face this problem, all the countries should adopt the same policy for the collection of ante and post mortem data, in order to obtain best practices in identification. The result of the identification process should be reported with scientific rigor and objectiveness, and categorized within one of the possible classifications provided by medico-legal criteriology, shared at international level. “Virtual anthropology”, also allowing the study of the bone structure, through MDCT, and cartilage, through MRI, should be implemented, and radiological data could be used even to perform anthropological measurements, age and sex estimation and to find out information concerning habits, geographical origin and pathology of the body [55].

39.1.6 Odontology

Forensic odontology is gradually improving its contribution to the field of bio-medicolegal sciences, especially in the field of age estimation and identification.

In sub-adults, age assessment procedures will be increasingly based upon the integration between dental and skeletal age estimation. The improved collaboration between forensic anthropologists, pathologists and experts in forensic odontology will improve the accuracy and reduce the occurrence of false positives [56]. New adult age estimation procedures will rely on new imaging technologies, focalized on the analysis of progressive physiological phenomena that reduce dental pulp through cone beam CT, an accurate, rapid and highly reproducible method [57]. Thanks to advanced technology such as laser scanning, scanning electron microscopy or cone beam computed tomography the forensic dentist may be able to identify more details in bite marks and in the individual teeth of the bite [58].

Future research should be carried out taking into consideration population-based studies regarding different ethnicities and ancestry, increasing the accuracy and the precision of each method in terms of identification and age estimation.

Under civil/tort law, conversely, forensic dentistry is aimed at the detection and the evaluation of injuries, disease or impairments to the jaws, oral tissues and teeth, and for further study of such topics it is necessary to consult the specialist material.

39.1.7 Entomology

Imaging could also provide useful innovations in the field of entomology. The *virtual forensic entomology,* through the application of 3D micro-imaging could describe internal and external modification, having the potential to estimate the age of blowflies and pupae with a higher degree of accuracy and precision than by using morphological characters alone. These techniques could aid in investigating the post mortem interval in cases of suspicious death [59].

Interesting innovations are also emerging in the developing field of *entomotoxicology*, with deep insights in the determination of the manner and cause of death through the identification of drugs in necrophagous insects, in cases where toxicological analyses are on decomposed tissues (high decay stage of decomposition or skeletonized remains) were generally less sensitive and yielded almost erroneous results [60, 61].

39.1.8 Criminology

In the last decades, the development of cognitive neuroscience has permitted the investigation of the neurobiological correlates of mental functions and the most intimate aspects of the human mind [62]. Most recent findings indicate that combinations of specific gene variants identified in serotoninergic and dopaminergic pathways have a greater frequency in individuals with antisocial and criminal behaviour than in noncriminal control subjects [63]. A great contribution is coming from the "omic sciences", to find a few novel genes that might play a role in shaping human social behaviour [64]. Also the environment itself exerts some influences on genes by modulating their expression, since environmental stimuli produce epigenetic modifications on our genome, adding some chemical markers, without changing the genetic sequence. Epigenetic may be considered as the missing link between nature and nurture, having a role in shaping behavioural responses or environmental factors [65].

As for the application of emerging technologies, the in vivo *Diffusion Tensor Magnetic Resonance Imaging* (DT-MRI) *Tractography* was used to analyse the microstructural integrity of the uncinate fasciculus in psychopaths, finding a correlation between antisocial behaviours and anatomical differences [66]. Functional Imaging, and in particular *Functional MRI* is an actual and promising tool for predicting recidivism in adults and young people. This kind of approach could lead to early intervention strategies for preventing recidivism, based on positive reinforcement strategies. Even if these methods are actually used in the research and a thorough validation is needed for forensic and judicial application, the near future will bring a better understanding of the interaction between brain function, genetic and environmental factors, ultimately informing improved interventions and prevention strategies and promoting better mental health as whole [67, 68].

These recent findings will have an important impact from an ethical and legal perspective, giving rise to the question of to what extent an abnormal behavioural control can be due to an anatomical abnormality or to a functional failure within those neural systems, or the lively debate concerning the use of neuroscientific evidence in court.

39.1.9 Humanitarian Forensic Action

The gradual growth of international attention towards humanitarian action and the emerging role of the forensic sciences in field have led to the rise of Humanitarian Forensic Action (HFA). All future innovations and applications of clinical legal medicine will be channelled into the HFA, since it is the core purpose of the United Nation's objectives. The real innovation lies not in the methods, but in the aim, since it honours the BML sciences with the purpose of providing the best aid and assistance to protect lives, health, human dignity and to improve the preparedness for the occurrence of man-made crises and natural disasters, which represent the new fundamental aims of the bio-medicolegal sciences. Most of the forensic disciplines have already been applied in this field, and in particular anthropology, odontology, genetics and pathology. The first goal is to share a medico-legal international protocol which also relies on new diagnostic technologies of Imaging and Bio-Analyses. Effective capacity building within local facilities represents a key element and the future success of global humanitarian efforts is augmented by the growing recognition and assistance of international societies [69].

In this era of mass migration, there is an increasing need for inexpensive, non-invasive and reliable methods of ascertainment and the synergy of all the information obtained, so as to decide on the “right combination”, in terms of the technologies that are going to serve us best in identification and age estimation.

Furthermore, a uniform methodology for the assessment of psychiatric impairment should be adopted, based on a rating instrument that truly assesses impairment rather than disability. It will represent an important step in eliminating the haphazard ways in which entitlement to damages are currently assessed. In the more distant future, it will be feasible to “label” patients, thereby providing a certain diagnosis, detecting malingering or the authenticity of the symptoms declared by the patients. This aim should be achieved through the use and the development of new methods, grounded on automatized psychometric index recording, such as reaction-times and kinematic aspects of mouse trajectories, which have shown promising results [70, 71].

39.2 Transdisciplinary Innovation

Transdisciplinary innovation will be the impetus of the future bio-medicolegal sciences, aimed at the realization of the Radiomics Project, the improvement of Evidence and related educational training founded on epistemic principles of scientific and humanistic culture.

39.2.1 The Radiomics Project

The future of the bio-medicolegal sciences is mainly entrusted to the aformentioned integration of imaging techniques and bio-analytical platforms, namely the *Radiomics Project*, including *Radiogenomics*, begun in oncology and currently being extended in other pathological nosographic classifications. The mineable omics data [72] conversion readily applies to quantitative tomographic imaging on multiple levels, each of which may contain millions of voxels and measurable features describing size, shape, and texture of organ-tissue pathologies [73], also characterized through new applications of improved methods of phenotyping. A complex of techniques and methods which add relevant challenges to the data management with the ultimate goal of increasing diagnostic, prognostic and *predictive* power and *accuracy* and therefore, from a forensic point of view, the scientific evidence to bring to trial.

The introduction of new technologies should be based on the principles of a collective and interdisciplinary work, in which all stakeholders operate together to promote the use of new technologies, methods and imaging procedures integrated with bio-analytical platforms and validate these new tools in terms of reliability, accuracy, precision and error rate, so that the judge can use them as real scientific evidence. This objective will be achieved through the unitariness of methodology and aims, which implies, in the near future, the constant application in forensic casework, leading to an improvement in the achievement of molecular evidence.

The *Unitariness* of the bio-medicolegal sciences has been guaranteed, up until now, by methodology. However, in the current post-genomic era such historical unity is no longer sufficient and the bio-medicolegal sciences must unite around the concept of *molecular evidence*. The bio-medicolegal sciences should utilize the new technologies as a stepping-stone for the integration of the scientific innovative contributions of each sub-discipline, and the knowledge should be enriched not through highly specialized sophistication, but through *interdisciplinarity* [74]. A remarkable example of the *interdisciplinary product* is Imaging Mass Spectrometry (IMS) which, in terms of molecular imaging and mapping in soft tissues [75], provides specific issues in forensic pathology and toxicology, allowing imaging distribution, the quantitation of drugs [76], the detection of latent fingerprints and a correlation of molecular information with traditional histology, applied in the living and in the dead subject [77–79].

In a dynamic and cyclic process of Hegelian and Vichian evolution, the historical identity of the "methodology for ascertainment", "criteria for evaluation" and for the solution of issues of "causality", occurring in daily routine work, will potentially and, in conclusion, restore the original unitariness by means of interdisciplinary research and transdiscplinary innovations.

39.2.2 Evidence

In order to survive the ongoing scientific progress of each specialty, research in legal medicine has to focus on the preservation of its unitarian knowledge and the development of *evidence* and *quality*. In fact, the central problem of the discipline will remain the achievement and measurement of the *level of evidence*. In the post-genomic era, the multidisciplinary approach, using innovative tools, will become the real key to obtaining objective evidence, undebatable in court, and the main sources are by now those produced by bio-medical science for clinical purposes, mainly in the fields of "imaging" and "bio-analysis" or "omics". Omic sciences, in particular, having reached a level of maturity and increased their application and power of approach, are ready to provide informative results that can improve evidence in all the bio-medicolegal fields. To increase and quantify the new level of evidence, new algorithms based on mathematical models should be developed. Furthermore, *systematic* and internationally shared guidelines and protocols on *methods of ascertainment* and *criteria of evaluation* will have the essential function of attaining "objectivity", capable of generating "proof" and "scientific evidence".

39.2.3 Educational Training

A quality system, focused on the achievement of "accuracy, efficacy, timeliness and efficiency" and founded on "continuous disciplinary education", must strive for "*certification*" and "*accreditation*" of "institutions" and individual "professionals". Continuous education, systems of control and proficiency, certification and accreditation hinge upon guidelines and protocols shared and used in the scientific and professional community of reference [74].

Educational training will be essential, because education leads to expertise, knowledge and know-how, and expertise strengthens evidence. The vision that law has been imposing on science, made up of dogmatic opinion, should be rejected, in a future perspective of *learning to doubt*, the typical emblem of the academic culture context, where the students could be trained and educated to become *error hunters*. This confirms the Hippocratic oath of the third millennium, which founded the ethical role of the physician on his professionalism, or rather, on the capacity to play the role of researcher, in the constant search for errors, the discovery of which reduces uncertainty in science, enhances professional formation and improves the quality of the system.

39.3 The Masterplan

An interdisciplinary synergy and a supranational perspective are adequate resources in the future preservation, development, innovation and unitariness of bio-medical learning, in step with post-modern times [74, 80–82].

In the so-called "iPOP era", it will be possible to combine in the single individual genomic, transcriptomic, proteomic, metabolomic, autoantibody profiles and dynamic changes in molecular components and pathways. The iPOP and other multiple high-throughput methods, derived from interdisciplinary knowledge and the integration of bio-analytical and imaging platforms, will be an expression of the main benefits provided in the future context of personalized medicine [83].

In a short time, a new value of the *protection*, *dignity* and *freedom* of the person will be added in the realization of so-called "P5 Medicine and Justice". *Protective Medicine* will be achieved by means of the "P5 network of networks", which is the result of the virtual link between the genome database, big data driven by patient and social networks, systems biology investigation and hand-held devices. But, first of all, the scientific community and in particular the bio-medicolegal community, should implement an *Interdisciplinary Masterplan* aimed at the scientific validation, certification and quality accreditation of the new technologies. To perform this transition, all the stakeholders should operate together to promote and validate the new tools, in terms of reliability, accuracy, precision and error rate, so that the judge can benefit from real scientific evidence. A joint effort that, it is hoped, will focus on the ultimate goal of personalization, prediction and protection of human and personal rights. In a perspective of a personalized justice, the main obstacle of this process of evolution lies in the Aristotelian principle of equality before the law. The transition should be slight, and should pass through a historical reversal of the criminal law, from a punitive system towards a restorative system, taking into account personal differences and peculiarities.

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Part XII
Iconography

Casseri, Giulio 1552–1616, Tabulae anatomicae 78 cum supplemento 20 tabularum Danielis Bucretii (Adriani Spigelii Bruxellensis ... Opera quæ extant omnia. Ex recensione Ioh. Antonidæ Vander Linden ... Amsterdami: apud Iohannem Blaeu, 1645

Schultes, Johann 1595–1645, Armamentarium Chirurgicum. Francofurti: sumptibus viduae Joan. Gerlini, Bibliop. Ulm. typis Joannis Gerlini, 1666

Chapter 40
The Fine Line Between Medicine and Torture. Historical Iconography of Instruments and Practices

Guido Pelletti, Sindi Visentin and Maurizio Rippa Bonati

Abstract Numerous instruments of torture, even on a superficial observation, resemble surgical tools and therapeutic aids. A more careful analysis of their respective uses and operations reveals, in effect, that when they do not simply involve the distorted and aberrant use of surgical effects, they represent an exaggerated application of forces and movements that, to attain a medical-therapeutic effect, should be limited and controlled.

There are situations in which the positivity of an action, in itself neutral, does not only depend on the quality of the intervention, but also on its quantity. In the medical-therapeutic field it is enough to consider pharmaceuticals, which, if administered beyond measure, lose their effectiveness as healing agents and become poisonous or even deadly.

Less immediately obvious examples, but no less disturbing for this reason, can be found by comparing surgical tools and therapeutic aids with instruments of torture. The devestating effects caused by an excessive application of force or by an exaggerated division of the tissues: in such cases the "bed of Hippocrates", instead of alleviating a dislocation, can result in the displacement of joints. A speculum can lacerate when its purpose was only to permit a brief inspection of the body cavities. A particularly emblematic example is that of Girolamo Fabrici d'Acquapendente

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so-called "machina" (Figs. 40.1, 40.2 and 40.3) [1], described and illustrated in such detailed images, in the numerous editions of his *Opera chirugica*, that they constitute veritable executive projects. And so, each of the orthoses—not prostheses—that constitute the complex apparatus, was designed and implemented to correct flawed positions through the forced movement of limbs, albeit slow and painless; it is evident that the same actions carried out quickly and excessively can cause pain, sprains and fractures.

Obviously, tortures have also involved veritable interventions, of which those involving the Christian martyrs comprise the most varied and tragic repertoire. Besides the flaying of St. Bartholomew, it is enough to consider the ablation of

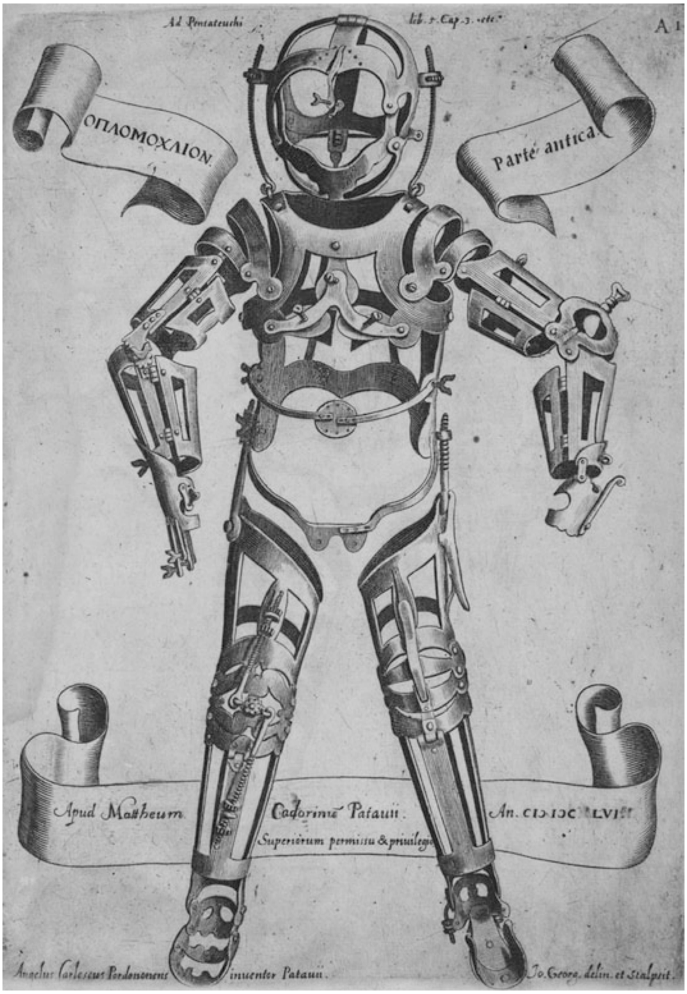

Fig. 40.1 Hieronymi Fabricii ab Aquapendente, Opera Chirurgica. 1723

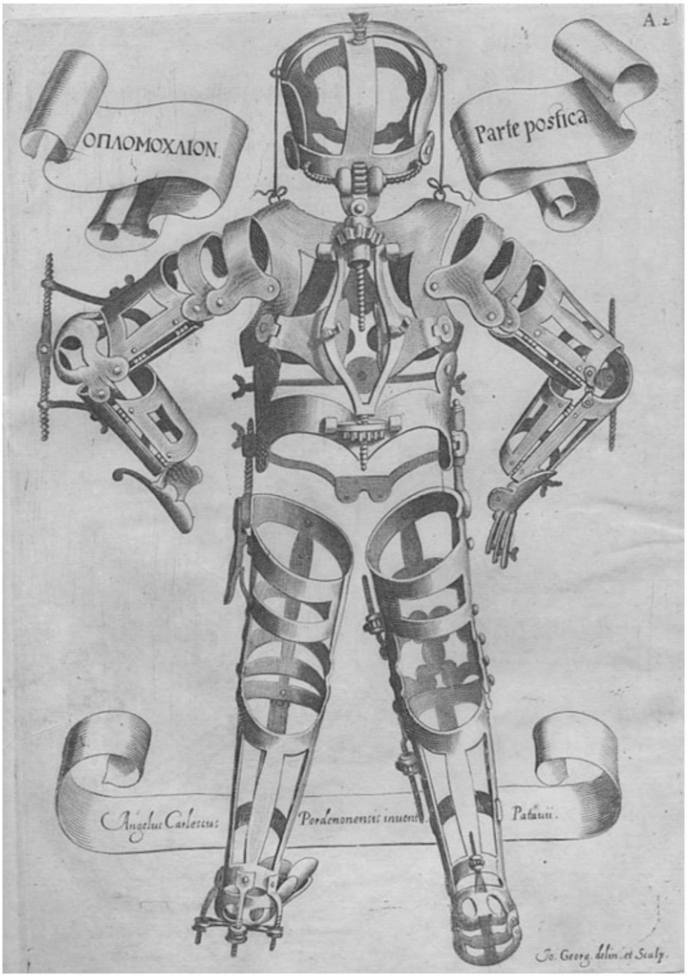

Fig. 40.2 Hieronymi Fabricii ab Aquapendente, Opera Chirurgica. 1723

St. Agatha's breasts, the enucleation of St. Lucy's eyeballs and the extraction of St. Apollonius' teeth.

Alongside countless beneficial operations and useful inventions, man's ingenuity has been able to devise an equal number of negative ones, of contrary effect. Indeed, words cannot do them justice: it is necessary to visualise them in order to grasp their ingenious cruelty. Some of the most important examples are displayed below [2, 3].

Figure 40.4 shows a breast removal, as well as a surgical practice for cancer treatment, was also used as a method of torture that become popular at the end of the XV Century. For this purpose, an iron instrument, usually heated, called a

Fig. 40.3 A picture of the "Coffin torture", a cruel medieval device where the victim was locked for hours

"*breast ripper*", was used to rip the breast as a form of punishment for women who were accused of adultery, heresy, blasphemy or self-performed abortion. An example is given in the Christian religion by St. Agatha, who is also the patron saint of breast cancer patients. Prior to her imprisonment and subsequent death, her breasts were cut off with pincers as a form of torture.

Figure 40.5 depicts a rectal and vaginal specula used in the XVII Century for medical purposes, composed of two (a) or three (b) spoon-like segments that could be operated by turning a screw. They worked on the same principles of the so-called "*pear of anguish*", a torture instrument used from the XVI Century. Despite the similarities between the two instruments, the pear of anguish differs from the medical one because of the absence of a safety limit that prevents the laceration of tissue.

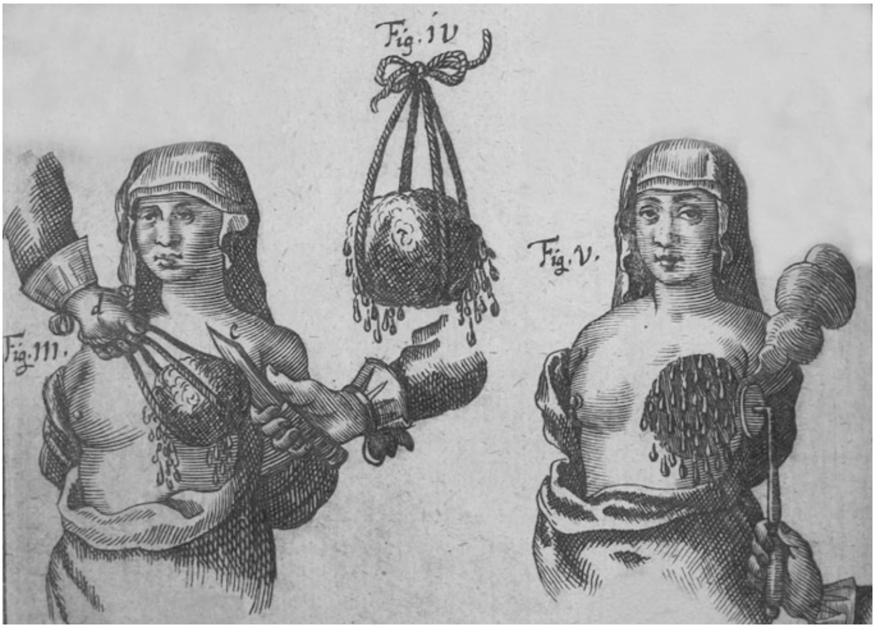

Fig. 40.4 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XXXVIII Fig III–V

Figures 40.6 and 40.7 represent instruments (Fig. 40.6) and procedures (Fig. 40.7) applied for the treatment of tooth disease and, particularly, for tooth extractions. Perhaps the most famous example of the extraction of teeth as a form of torture is the martyrdom of St. Apollonia. According to the legend, she had all of her teeth violently pulled out or crushed and, for this reason, became the patroness of dentistry and of those suffering from dental problems.

Figure 40.8 shows the surgical treatment of a pathological eye condition, called pterygium. Surgical or chemical treatments to the eyes, however, were also performed as a form of physical punishment. Early Christians, in fact, were often blinded as a penalty for their beliefs and, to give a representative example, St. Lucy was reported to have been tortured by eye-gouging prior to execution, thus becoming the patron of the blind and those with eye-troubles. In the Middle Ages, blinding was also used as a punishment for treachery or to neutralise a political opponent.

Figures 40.9 and 40.10 represent the Hippocratic bench, or scamnum (Fig. 40.9), which is the device that, using tension to aid in setting bones (Fig. 40.10), was used to reduce fractures and dislocations in different part of the body. It is the forerunner of both the traction devices used in modern orthopaedics, and of the instrument of torture known as "*the rack*". Both in torture and in the medical use of the instrument, the victim or patient lies on a bench, at an adjustable angle, and ropes are tied around their arms, waist, legs or feet. Winches are then

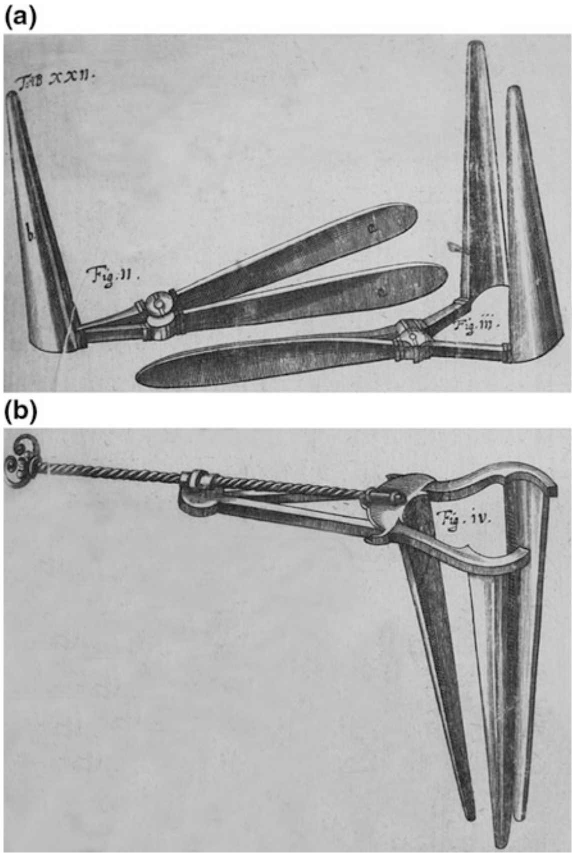

Fig. 40.5 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XXII Fig. II–IV

used to pull the ropes apart, but the rack is used to stretch the victim's body until their joints are dislocated and eventually separated.

Figures 40.11 and 40.12 represent other instruments (Fig. 40.11) and procedures (Fig. 40.12) used to apply tension to the legs in order to set bone segments, as with the Hippocratic bench, but in this case with the use of weights tied to the feet of the patient. The attachment of various weights to the victims feet was also used in torture, as it was in the "*Spanish donkey*" (also known as the "*wooden horse*"), but in this case the victim was put astride a triangular device with a sharp angle pointing upward, mounted on a "horse-leg" support. According to historical sources, the victim, who was put naked on the top of the device, could be sliced entirely in two as a result of the weight attached to his or her feet.

Figures 40.13, 40.14, 40.15, 40.16, 40.17 and 40.18 illustrate medical procedures such as clysters (Fig. 40.13), cauterization (Figs. 40.14 and 40.15), amputation (Fig. 40.16) and compression bandaging (Figs. 40.17 and 40.18).

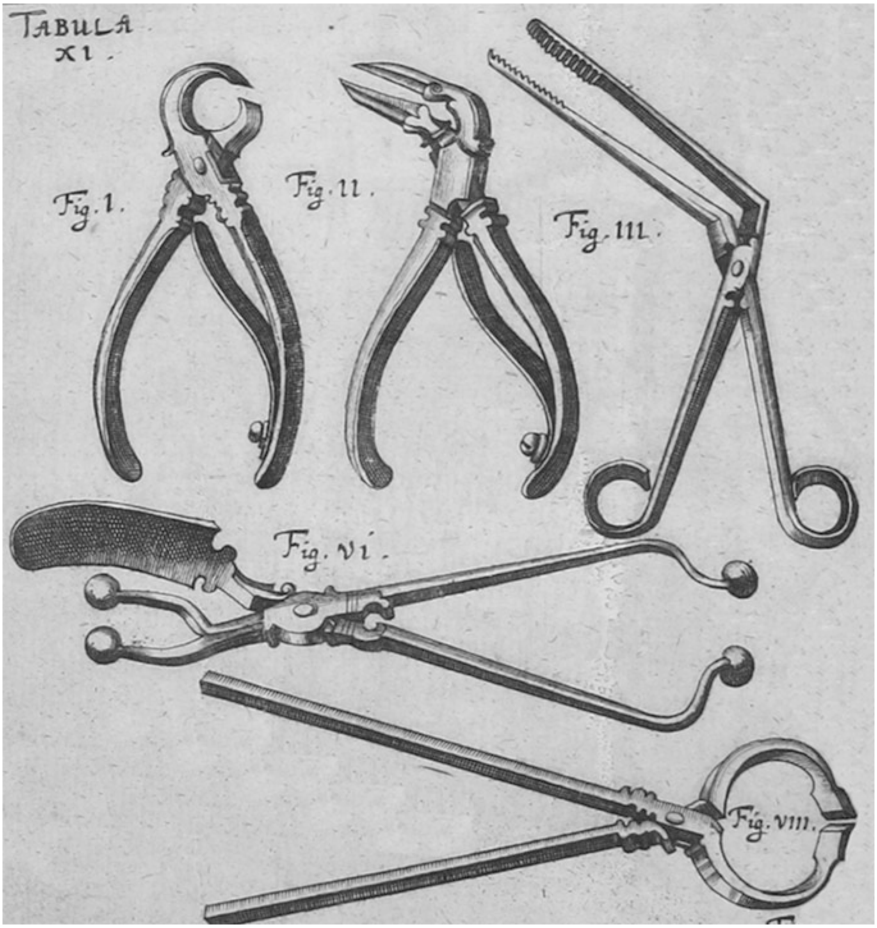

Fig. 40.6 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XI Fig I–VIII

Fig. 40.7 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XX Fig V, XIII

Fig. 40.8 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XXXV, Fig. VIII

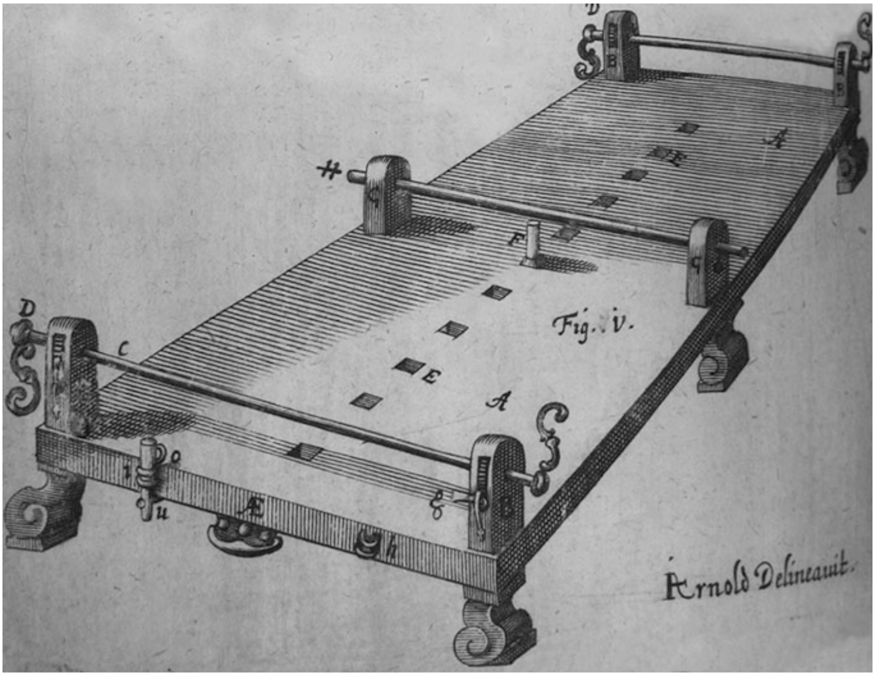

Fig. 40.9 Scultetus' Armamentarium Chirurgicum, 1655. Tabula XIII Fig. V

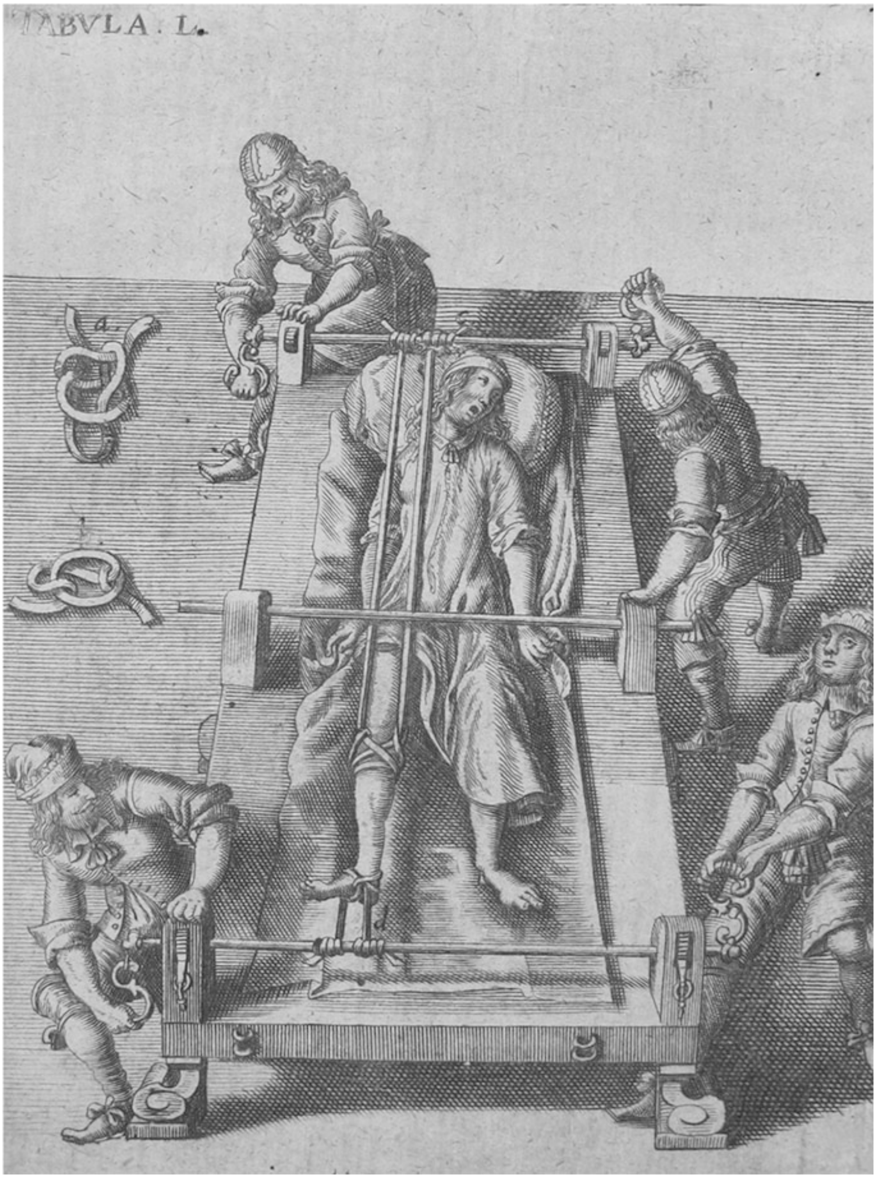

Fig. 40.10 Scultetus' Armamentarium Chirurgicum, 1655. Tabula L

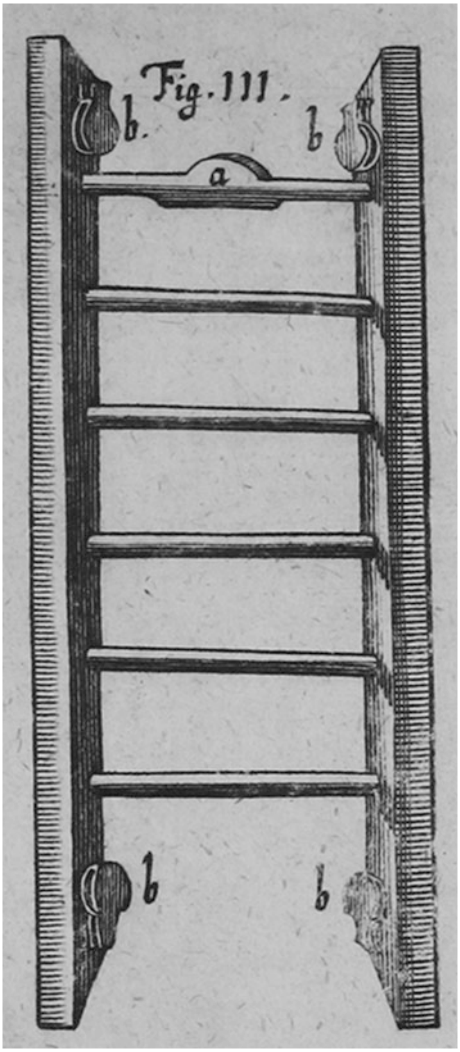

Fig. 40.11 Scultetus' Armamentarium Chirurgicum, 1655 Tabula XIV Fig III

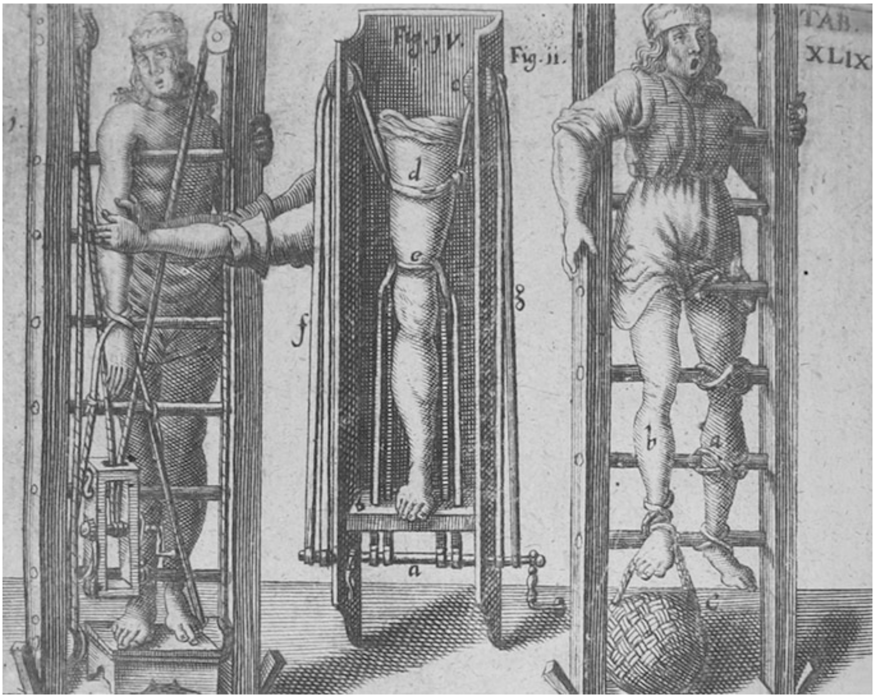

Fig. 40.12 Scultetus' Armamentarium Chirurgicum, 1655 Tabula XLIX Fig I–III

Fig. 40.13 Scultetus' Armamentarium Chirurgicum, 1655 Tabula XLIV Fig. I

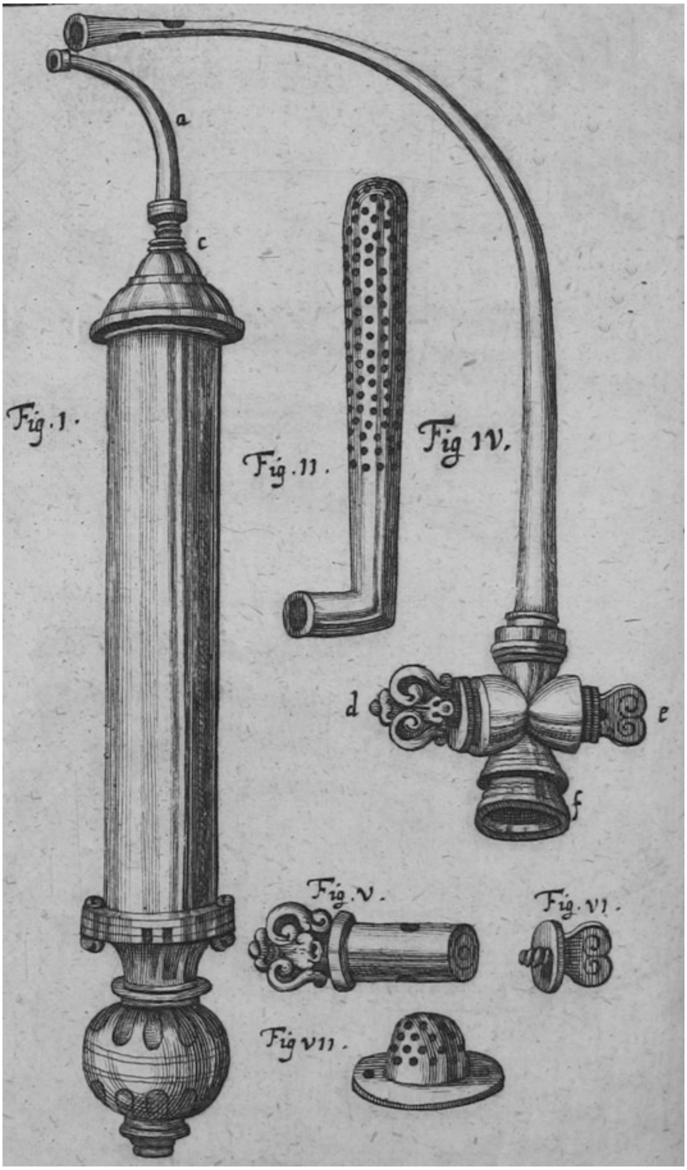

Fig. 40.14 Scultetus' Armamentarium Chirurgicum, 1655 Tabula XIII

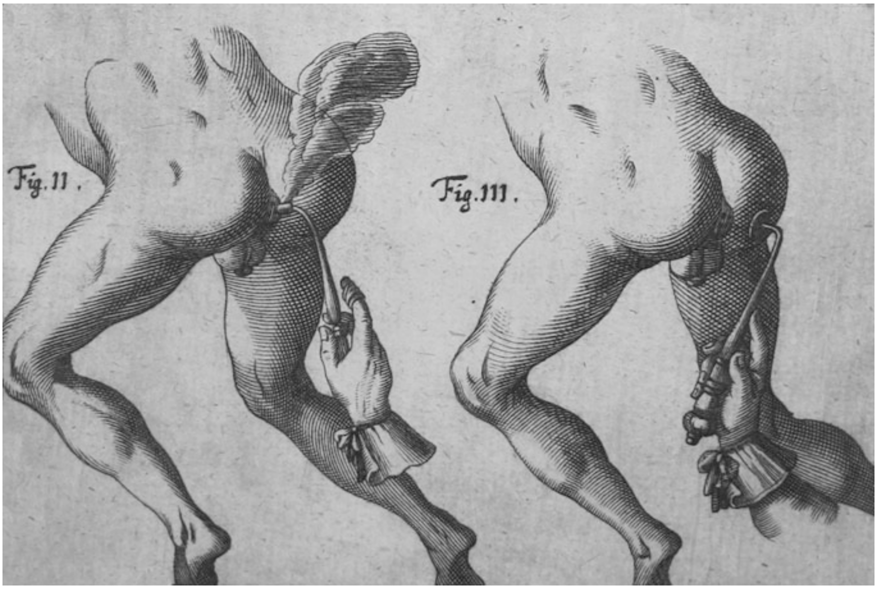

Fig. 40.15 Scultetus' Armamentarium Chirurgicum, 1655 Tabula XLIV Fig. II–III

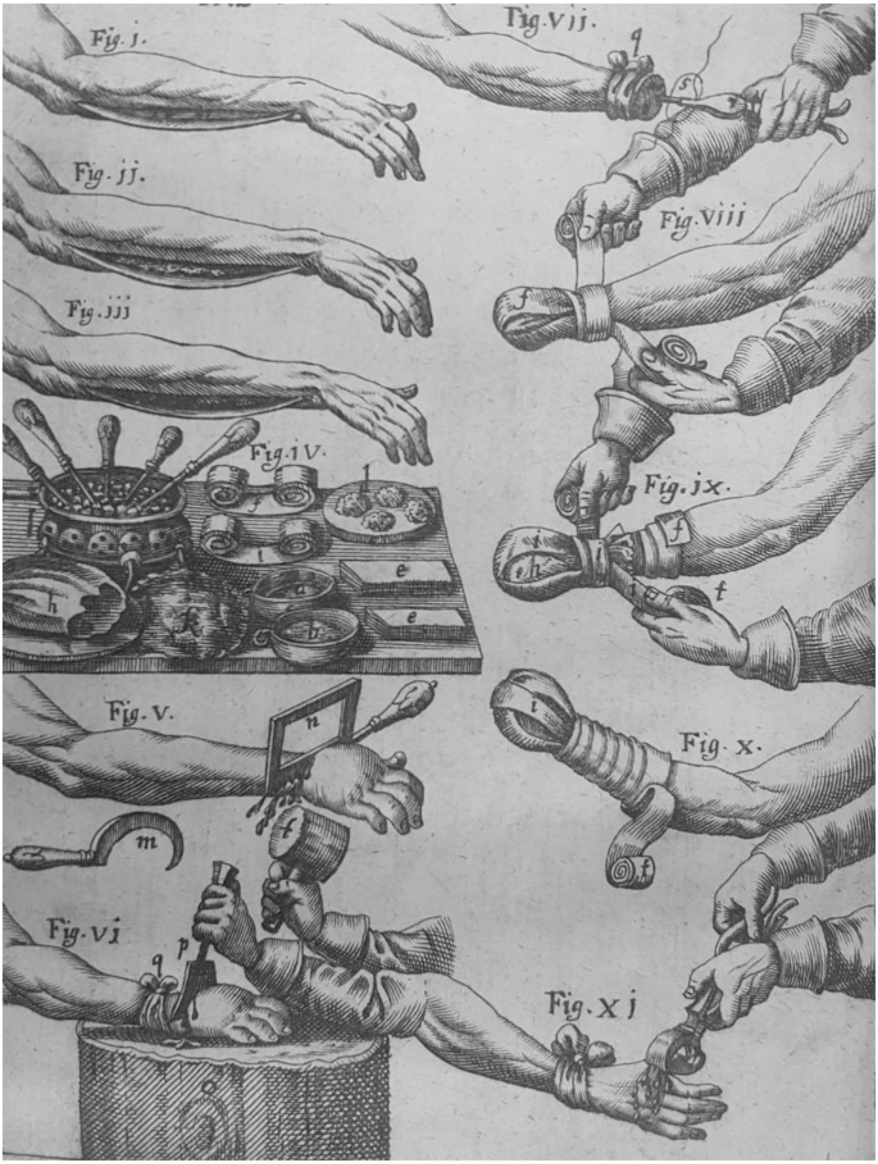

Fig. 40.16 Scultetus' Armamentarium Chirurgicum, 1655 Tabula LIII

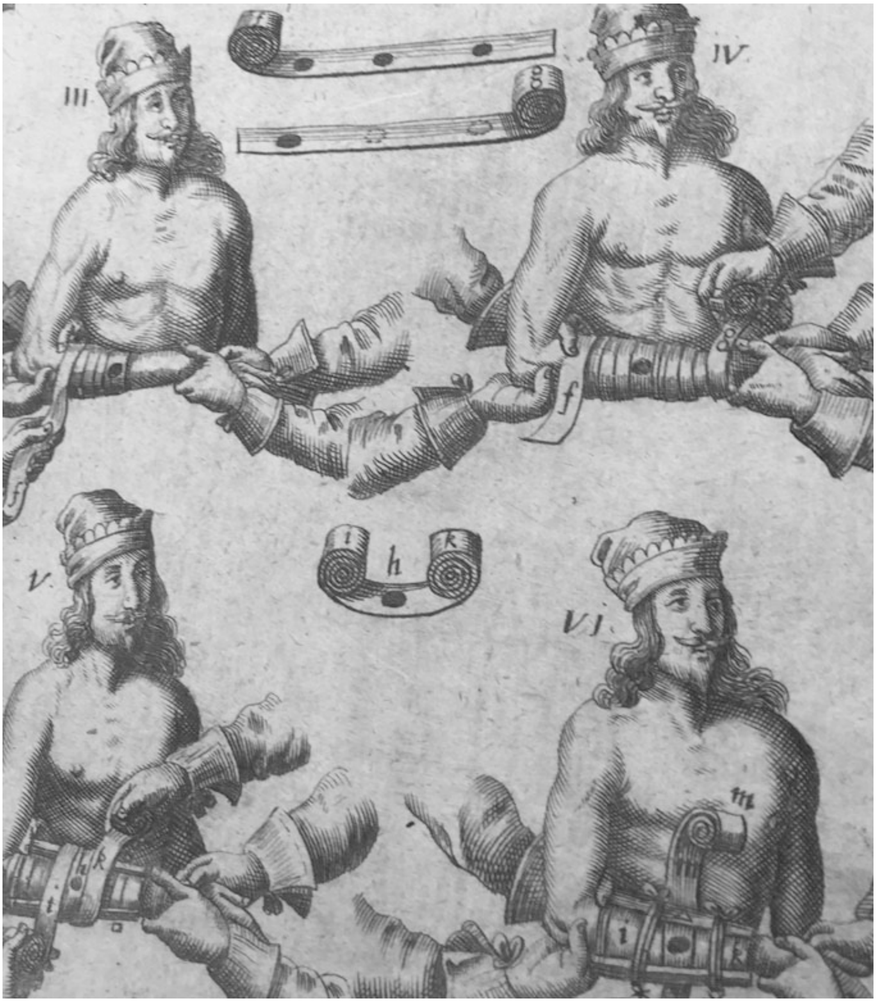

Fig. 40.17 Scultetus' Armamentarium Chirurgicum, 1655 Tabula LII

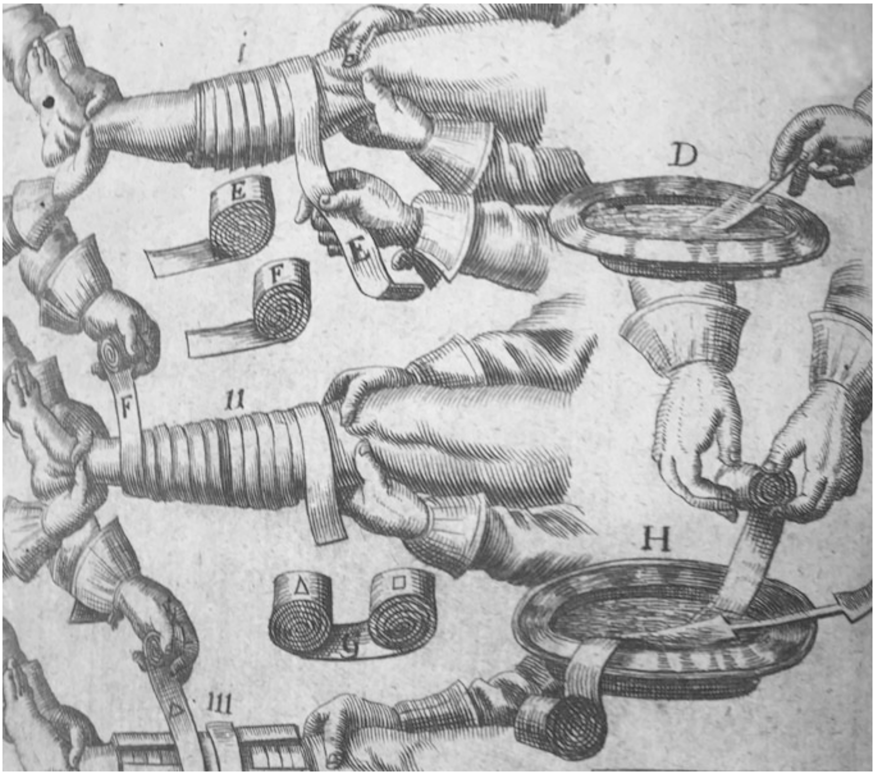

Fig. 40.18 Scultetus' Armamentarium Chirurgicum, 1655 Tabula LI

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Note on Bibliographical References

The editor has proposed to the authors the adoption of bibliographical references in accordance with their citation needs in relation to the specific chapter.

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