Essential Techniques in Certain Decedent Populations

5

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Introduction

The autopsy has been used in science and medicine since 300 BCE [1]. However, over the last few decades, the value of the autopsy has increased due to advances in and utilization of ancillary techniques [2]. Not every case will use every or even a majority of these techniques, but based on the decedent, certain ancillary studies should be undertaken to allow accurate certification of the cause and manner of death, as well as to identify any underlying congenital, hereditary, or contributory conditions or diseases. As science and medicine advance, epidemiologists have studied trends in worldwide populations to identify and prevent diseases. The autopsy is the greatest tool for public health. No matter how extensive and "cutting edge" clinical diagnostic modalities become, the autopsy ("to see for oneself") gives true final pathologic diagnoses and accurate causes of death. It expands and enhances data on every disease, inherent and acquired, in all people. It also identifies populations at risk so that diseases can be prevented. The autopsy identifies new diseases and different subsets of society particularly vulnerable to these new diseases. Answers to questions from all branches of medicine can be found in the autopsy.

As societies around the world change and develop, social norms, fads, dangers, and behavioral trends emerge. The autopsy often reflects these changes via pathologic diagnoses, causes of death, incidental findings, and manners of death. A routine autopsy is not actually routine. The autopsy pathologist must always strive to correlate the history with the autopsy findings and, if no known correlation exists, find it. New findings and new diagnoses will be overlooked if each decedent is examined in exactly the same way and by the same methods as in the preceding

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hundreds of years. This chapter will discuss postmortem techniques and studies that have proven useful in autopsy pathology. It will also discuss special patient populations that present unique challenges to the autopsy pathologist. In order to see for oneself, we need to know what we are looking at and what we should be looking for.

Basic Science

Postmortem Chemistry

Biochemical analyses of postmortem specimens and proper interpretation of the results can be extremely beneficial to the pathologist. Often when gross and microscopic autopsy findings are "negative," chemistry can provide answers. A brief overview of postmortem chemistry is needed to better approach certain cases [3–14].

Vitreous humor is the best specimen for analyzing concentrations of electrolytes, glucose, and ketones [2–4, 14]. Other low-molecular-weight, nonprotein-bound solutes such as ethanol can also be measured. Vitreous should be collected from the globe using a sterile syringe (usually 10 mL) and needle (18–20 gauge) with the needle inserted at the lateral canthus and directed to the center of the globe [2]. The vitreous should be withdrawn slowly and placed into a small, sterile, red-top vacutainer. Vitreous is a viscous, clear, colorless fluid. If the specimen is contaminated with retinal cells, the results will not be reliable. When interpreting vitreous chemistry results, it is important to understand that some postmortem vitreous values do not reflect premortem blood values of a given analyte. For instance, after death, potassium quickly leaks from the retina resulting in elevated levels in the vitreous. On the other hand, vitreous glucose concentrations fall rapidly after death. The College of American Pathologists published a useful reference table for vitreous chemistry results and their correlation with some entities likely to be encountered in hospitalized patients [2–14]. A basic metabolic panel to include these tests can be performed on postmortem vitreous by a hospital's chemistry laboratory.

Toxicology

Toxicology can be useful in non-forensic deaths as well as forensic cases, and hospital autopsists may be called upon to assist local medical examiner offices with collecting toxicology specimens, especially considering the growing opioid epidemic (see Chaps. 4 and 7 in this book for more details). Clinicians may be concerned about the blood concentration of a drug in the setting of liver or kidney disease or in neonates with seizures with possible transplacental drug overdose, as examples. The best specimen for a toxicology screen is urine

(approximately 30–50 mL) collected in a sterile container. For confirmation and quantitation, the best specimen is femoral venous blood placed in a gray-top vacutainer which contains the preservative sodium fluoride [2]. The preservative must be thoroughly mixed with the blood by gently rolling and inverting the vacutainer. Gastric contents should be examined for pills or pill fragments, quantitated, and a portion (50 mL) either analyzed or saved frozen in a sterile container. The liver, brain, and skeletal muscle are also good specimens to save (35–50 g of each), frozen if needed. Meconium (the first feces in a newborn) is an excellent specimen for toxicology. The thick, viscous meconium can be placed in a sterile red-top tube. The aforementioned specimens can be stored for many years at 4–6 °C.

Table 5.1 Dictionary of basic terms for special decedent populations

Term	Definition
Infectious diseases	
Bacteremia	The presence of bacteria in the bloodstream
Hypotension	Low blood pressure, usually less than 90/60 mmHg
Sepsis	Systemic response to bacterial, viral, fungal, or parasitic infection
Septic shock	Clinical syndrome of sepsis with critical reduction in tissue perfusion
SIRS	Response to infection, inflammation, and/or insult to the body
Toxic shock syndrome	Special type of shock caused by bacterial toxins
DIC	Systemic activation of blood coagulation resulting in excessive bleeding
Perinatal and infant decedents	
Embryo	Product of conception to the end of 8 weeks gestation
Fetus	Product of conception from 9 weeks gestation to birth
Neonate	A child from the time of birth to 1 month of age
Infant	A child between 1 and 12 months of age
Congenital	A genetic or non-genetic condition present in an individual at birth
Elder decedents	
Elder	Someone who is at least 60 years of age
Biologic aging	Changes of organs and tissues with advancing age
Atrophy	Wasting due to degeneration of cells and tissues
Decubitus ulcer	Damage to skin and underlying tissue due to prolonged pressure
Eating disorders	
Adiposity	The amount of body fat can be expressed as a percentage of body mass
Obesity	Excessive accumulation and storage of fat in the body (BMI at least 30)
BMI	Body mass index, mass in kilograms divided by length in meters squared
Anorexia nervosa	Eating disorder characterized by distorted body image and intentional weight loss
Bulimia nervosa	Eating disorder characterized by periods of binging followed by purging
Anabolic steroid	Steroidal androgen that promotes growth of skeletal muscle and male sexual traits

Infectious Diseases

Table 5.1 provides a dictionary of basic terms to be used in this section as well as several following sections of this chapter. Please also refer to Table 5.1 for definitions of key terms later in the text.

Infectious diseases result in many deaths in all age groups, and autopsy findings can be subtle. Some diseases such as bacterial pneumonia may be obvious upon gross examination, whereas others such as influenza provide no anatomic clues of their presence. Some basic definitions relating to postmortem evaluation of clinically suspected infections are important to bear in mind when reviewing clinical records and correlating pathology with clinical history in cases of suspected infectious deaths.

Sepsis and Sepsis Syndromes

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to an infection [15–23]. Septic shock is a critical reduction in tissue perfusion and abnormalities of cellular metabolism due to the infection, and it involves persistent hypotension and an elevated serum lactate level [19, 20]. The systemic inflammatory response syndrome (SIRS) is a specifically defined response and has four criteria [19, 20, 22]:

- Fever of more than 38 °C (100.4 °F) or hypothermia less than 36 °C (96.8 °F)
- Tachycardia (heart rate of more than 90 beats per minute)
- Tachypnea (respiratory rate of more than 20 breaths per minute) or hyperventilation with arterial carbon dioxide tension (PaCO2) of less than 32 mmHg
- Abnormal white blood cell count (>12,000/μL or < 4000/μL) or presence of immature neutrophils (>10% immature [band] forms)

SIRS has been used to identify early sepsis, but the criteria lack sensitivity and specificity for identifying increased mortality risk [19, 20, 22]. The sequential organ failure assessment (SOFA) scoring system is another method of predicting mortality in such situations and involves assessment of three criteria: respiratory rate $\geq$ 22 breaths per minute, change in mental status, and systolic blood pressure $\leq$ 100 mmHg [18].

Another dire consequence of systemic infection is disseminated intravascular coagulation (DIC), also known as consumptive coagulopathy. Eventually, the activation of coagulation depletes the coagulation proteins and platelets resulting in excessive bleeding. At autopsy, hemorrhage can be seen in the skin, mucocutaneous tissues, serosal membranes, and various organs. Platelet and fibrin thrombi can be histologically identified, especially in the renal glomeruli and pulmonary alveolar capillaries.

Two purported laboratory markers consistent with sepsis include C-reactive protein (CRP), a protein produced in the liver in response to inflammation, and procalcitonin, the precursor, or prohormone, to calcitonin which is secreted in response to inflammatory stimulation.

Utility of Postmortem Cultures

When bacteremia or sepsis is suspected, it is important to perform the autopsy as soon as possible (ideally within 18–24 h of death) and be prepared to take various cultures and specimens for microbiology/virology testing [24–28]. If medical records are available, a thorough review is strongly encouraged before beginning the autopsy, noting relevant vital signs and laboratory results, including antemortem microbiology findings.

The postmortem evaluation of a patient assumed to be infected is twofold. First, the presence and location of an infection must be determined, and second, the likelihood of morbidity and mortality from sepsis must be assessed. Clinicians have attempted to develop various criteria and scores to help them evaluate the severity of a patient's status [17–19, 21, 23]. The listed terminologies often overlap in individual cases but are also not absolute in every case. For example, not all infections result in SIRS, and not all cases of SIRS are secondary to infection. However, an infection plus SIRS is associated with organ dysfunction and substantial mortality in 5–16% of cases [22]. An infection in one location may stay localized, but toxins (such as in TSS) and/or an activated immune system can cause damage at distant sites. The SOFA scoring system is used to assess the severity of organ dysfunction and thus morbidity [18]. The predictive validity of the SOFA score for mortality has been reported to be superior to that of the SIRS criteria. Unfortunately, the applications of these criteria and scores vary between hospitals and between clinicians.

For the autopsy pathologist, it is important to know the clinical status of the patient. The clinical history, including microbiology culture results or presence of shock or organ dysfunction, can be correlated to the autopsy findings [2, 24–30]. Infections are among the 15 leading causes of death in the United States. Therefore, a thorough understanding of the different clinical pictures of sepsis is important in the interpretation of gross and microscopic autopsy findings as well as interpreting postmortem ancillary studies.

Performance of Postmortem Cultures

Controversy exists over the performance of postmortem microbiology cultures because there is confusion over their interpretation [2, 25–28]. Many studies have cited the lack of agreement between premortem and postmortem blood cultures [25]. There are several possible reasons for this which can be divided into two main categories: postmortem bacterial transmigration and iatrogenic contamination. Indigenous visceral microbial flora can transmigrate after death and spread throughout the body. To lessen this postmortem effect, the body should be cooled promptly after death, and body mobilization should be limited, as well. The autopsy should be performed as soon as reasonably possible, best within 15 h postmortem. To limit iatrogenic contamination, attention must be paid to using sterile technique when procuring blood, body fluid, exudate, or tissue for microbiology culture.

Growth of a single commonly recognized pathogen can usually be considered a true indicator of antemortem infection, whereas growth of multiple organisms and/or growth of a typical contaminant can usually be considered postmortem artifact due to transmigration and/or iatrogenic contamination (Table 5.2). Correlation of postmortem microbiology cultures with microscopic autopsy findings is of great value, as is corroborating histologic inflammation in a site from which cultures were taken.

So why perform postmortem blood or tissue cultures? Several good reasons exist despite the aforementioned controversy. First, a positive culture can determine the cause of death. Secondly, the autopsy pathologist can identify the agent as the cause of a previously undiagnosed infection even if that infection is not the proximate cause of death. Thirdly, postmortem cultures can confirm the premortem diagnosis and be of value in evaluating clinical assessment and response to antimicrobial treatment. Finally, public health benefits when epidemics and evolving antimicrobial resistance cannot be over emphasized.

Culture Techniques for Microbiology and Virology

When taking blood for culture, sterile technique must be used. If it can be performed safely, one preferred method is to sear the right cardiac atrium with a heated spatula or wide blade before inserting the sterile needle with syringe [2, 14, 24–28]. Another method is to flood the area with iodine and then insert the needle with syringe. Using a sterile needle and sterile syringe, withdraw 20–30 ml of blood for

Table 5.2 Common blood pathogens and blood contaminants

Common blood pathogens	Common blood contaminants
Staphylococcus aureus	Coagulase-negative staphylococci
Streptococcus pneumoniae *Streptococcus pyogenes* *Streptococcus agalactiae*	*Streptococcus viridans*
No common parallel pathogen	*Propionibacterium acnes*
No common parallel pathogen	Corynebacterium
Enterobacteriaceae	Mixed intestinal flora
Bacillus anthracis	Bacillus (other species)
Neisseria gonorrhoeae, meningitidis	*Neisseria subflava,* other species
Listeria monocytogenes	No parallel contaminant
Bacteroides fragilis	Mixed intestinal flora
Pseudomonas aeruginosa	No common parallel gram-negative contaminants
Haemophilus influenzae	
Escherichia coli	
Candida albicans	No common contaminants, though fungi often present in autopsy environment
Cryptococcus neoformans	

the aerobic and anaerobic culture/broth bottles. The rubber top of the bottle (where the needle is to be inserted) should be sterilized by iodine followed by isopropyl alcohol or sterilized by isopropyl alcohol alone. The inoculated bottle should be promptly transported to the microbiology laboratory.

Any organ or tissue can be cultured by the use of a culturette or a sterile biopsy [2, 14, 24]. The culturette can detect aerobic organisms and fungi. For organ/tissue biopsy, the surface of the organ is seared or flooded with iodine, as above, and a sterile blade is used to cut out a section of the tissue. Sterile aerobic and anaerobic transport media should be used. To maintain the anaerobic environment, make sure that the tissue is pressed into and covered by the solid media. Tissues can also be analyzed for viruses.

Mycobacteria can be detected from various specimens including blood, tissue, and feces. Mycobacterium can grow out of conventional blood culture media, but Middlebrook broth is recommended. The laboratory must be informed that mycobacterium is a consideration because laboratory smears, special stains, and special growth media will need to be used.

Rapid tests, usually utilizing molecular techniques such as polymerase chain reaction (PCR), are available for several viruses including respiratory and gastrointestinal viruses [31]. Blood procured and placed into a serum separator vacutainer can be analyzed for viral antibodies. Tissues for culture can be procured using sterile technique as described above, placed into viral media such as Hanks solution, and immediately transported on ice to the laboratory. Paraffin-embedded tissues can also be analyzed for viruses using immunohistochemistry and molecular diagnostic assays [30, 32–34]. Sometimes tissues will need to be sent to a reference laboratory for analysis. These can be paraffin-embedded formalin-fixed or fresh tissues [2, 24, 30, 34].

Fresh tissue samples for respiratory viruses can be procured by sterile technique, as above, and placed in a sterile container with a small amount of sterile saline. If they are to be shipped to a reference laboratory, the samples may need to be frozen. The following samples should be taken: bilateral lung hila, epiglottis, larynx, bilateral proximal and distal bronchi, and representative sections of bilateral lung parenchyma. Other organs and tissues may likewise be procured by sterile technique and frozen. Fecal specimens (10–20 ml) for viruses can also be collected in a sterile container and frozen for shipment to a reference laboratory. Some hospital laboratories have rapid viral enteritis tests and can microscopically examine for other agents of gastrointestinal infectious disease, such as parasites [31].

Perinatal and Infant Decedents

Important Procedures for Perinatal and Infant Decedents

Records Medical records of both the mother and the deceased child are extremely important to review before starting the autopsy. These include prenatal records, delivery records, and pediatric records as well as any required state genetic testing results.

Growth Measurements External measurements should be taken of all decedents 5 years of age and under. These include head circumference (occipital-frontal circumference), chest circumference (at the nipple line), abdominal circumference (at the umbilicus), crown-rump length (top of head to bottom of buttocks), crown-heel length (top of head to bottom of heel), and foot length (back of heel to end of great toe). These measurements should be compared to expected values for the age of the child. Charts for prematurely born children are available. See the following section on body mass index (BMI).

The best way to obtain accurate circumference measurements is to wrap a string around the body part (e.g., head) and then place the string along a ruler to obtain the circumference length.

Radiographs Full-body radiographs should be performed, and a skeletal survey including AP views of the skull, thorax, abdomen, individual limbs, hands, and feet and lateral views of the skull and thorax is highly recommended beyond the fetal age. Review by a pediatric radiologist can be very instrumental in assessing genetic defects, nutritional status, and birth or subsequent trauma. Previous radiographs should be obtained and reviewed for comparison, when possible.

Placenta In cases of perinatal or neonatal death, the placenta provides "the answer" in over one-third of the cases. The placental disc should be weighed without the umbilical cord and membranes. The membranes should be thoroughly examined for color and transparency. The umbilical cord length and diameter should be recorded and any unusual coiling noted. The site of cord insertion should be recorded. There is disagreement as to whether the placenta should be refrigerated (not frozen), unfixed, or fixed in 10% formalin. If refrigerated, the placenta can be stored at 4 °C for 3–7 days without loss of histologic integrity. The placenta ideally should be placed in a large, flat container so as not to distort its shape. The placenta should not be frozen because freezing results in lysis of red blood cells and marked distortion of histology. Keeping the placenta fresh (unfixed) allows for tissue procurement and the use of certain ancillary studies, such as bacterial and viral cultures, DNA and cytogenetic studies, metabolic studies, electron microscopy, and infusion studies. Tissue for bacterial and viral cultures may be taken in a sterile fashion from the subamniotic chorionic plate. The membranes also may be swabbed with a culturette for bacterial cultures. Sections of the fetal aspect of the placental disc can also be obtained for cytogenetic studies. If the placenta has already been discarded, or if it has been examined at another institution, the placental slides and accompanying pathology report should be reviewed by the autopsist.

For microscopic examination of placentas, the following sections should be submitted: (1) at least two full-thickness sections of the placental disc, (2) umbilical cord cross-sections from the fetal and placental ends, and (3) two sections of the placental membrane roll.

Microbiology Cultures If sufficient blood is not available due to the age (size) of the child, the spleen can be cultured. Lung cultures can be very valuable in this age group and should be taken. Sections of tissue, such as the liver and lung, or serum for viruses can be taken if a virus is suspected or for future analysis. Culture results can be correlated with histological findings as well as immunohistochemistry. See discussion of cultures.

Evisceration Technique In older children and adults, many pathologists use the Virchow method (organ by organ removal) of evisceration. However, in the perinatal and young pediatric age groups, when abnormal anatomical arrangements and/or vascular connections may not be immediately apparent, the en bloc or Letulle (also more recently referred to as Rokitansky) approach is advised so that anatomic relationships remain intact for evaluation. Remove the neck, thorax, abdominal, and pelvic block and begin the examination and dissection from the posterior aspect. The esophagus should be opened along its length to identify any tracheoesophageal fistula, before being taken down to the abdominal block. The heart-lung vascular connections should be carefully examined.

Fetal Brain Removal The fetal and often neonatal brain is very soft due to increased water content and can prove difficult to remove without damaging important landmarks and architecture. To avoid too many artifacts, cut open the fontanelles and sutures with scissors, cut the brain attachments, and gently remove the brain. In cases of very early gestation or maceration, removing the brain under water can best preserve its architecture.

Organ Weights In fetal, perinatal, and pediatric cases, especially 13 years of age and younger, it is important to compare the decedent's organ weights to expected weights for similar decedents. The expected weight is best based on the child's body (or crown-heel for fetuses) length. Expected body measurement and organ weight charts for prematurely born infants are available and are especially important for meaningful evaluation of premature babies and young children.

Genetic/Chromosomal Abnormalities and Inborn Errors of Metabolism Blood in an EDTA tube or blood as a spot on filter paper can be analyzed for numerous inborn errors of metabolism. Sections of the skin, fascia lata, and Achilles' tendon may be taken for fibroblast culture and cytogenetics and enzyme deficiencies. Skin sections (1 cm^3), and one or two entire Achilles' tendons, and fascia lata, taken using sterile technique, should be placed in transport media such as Hank's solution, and immediately transported on ice to the laboratory. Molecular pathology can be performed on paraffin-embedded sections using fluorescent in situ hybridization (FISH) or polymerase chain reaction (PCR) methodologies.

Many cardiac channelopathies can be detected in postmortem specimens. Blood, 5–10 mL, collected in an EDTA purple-top vacutainer is the specimen of choice. Other possible specimens include at least 5 g of fresh heart, liver, or spleen. The fresh tissues should then be frozen. Contact the reference laboratory for more specific instructions.

Postmortem Chemistry Vitreous should be procured for chemical analysis as described above. In children under 5 years of age, procurement of vitreous is usually performed at the end of the autopsy. This is because if any head pathology is identified, the eyes may need to be examined without any artifact of vitreous withdrawal.

Examination of the Elder Patient

In 2012, 809 million people, or 11% of the world's population, were over age 60. By 2050, about two billion people are expected to be over age 60, which will represent nearly 22% of the world's population at that time [35]. Life expectancy for the US population in 2016 was 78.6 years (males = 76.1 years, females = 81.1 years) [35]. Almost 40% of elders as defined above are obese, putting them at risk for diseases such as systemic hypertension, diabetes mellitus, coronary atherosclerosis, cerebral infarction, renal disease, respiratory diseases, decreased mobility, osteoarthritis, and cancer [35–39].

It is therefore not surprising that the top ten causes of death in this age group are [35]:

1. Heart disease
2. Malignant neoplasm
3. Chronic lower respiratory disease
4. Cerebrovascular disease
5. Alzheimer disease
6. Diabetes mellitus
7. Unintentional injury
8. Pneumonia and influenza
9. Nephritis, nephrotic syndrome, and nephrosis
10. Septicemia

As with children, specific approaches need to be taken when examining the elder decedent. With age, changes in the body make a person more prone to or at risk for various diseases and trauma.

Pathophysiology of Aging

A description by organ system of changes related to biologic aging, as well as to underlying pathologies that tend to be more prevalent with aging, follows and is summarized in Table 5.3 [36, 41, 42].

Table 5.3 The pathophysiology of aging and associated autopsy findings

Body systems	Effects of aging	Autopsy finding
Integumentary system	Thinning skin, fragile blood vessels, immobility, poor blood flow	Ecchymoses, decubitus ulcers, stigmata of diabetes
Musculoskeletal system	Decreased bone mass, Less vitamin D production, muscle mass decreased	Osteopenia, osteoporosis, fractures, muscle atrophy
Cardiovascular system	High cholesterol, high blood pressure	CAD, myocardial infarction, thrombosis, myocardial interstitial fibrosis
Respiratory system	Difficulty breathing, less ability to expand lungs and chest	Pneumonia, emphysema
Gastrointestinal system	Dry mouth, decreased gag reflex, decreased gastric secretions, slow digestion	Periodontal disease, aspiration, fecal impaction
Genitourinary system	Decreased blood flow to kidneys (vascular disease)	Kidney atrophy, glomerulosclerosis, arteriosclerosis
Hepatobiliary system	Decreased blood flow to liver	Liver atrophy, Glycogenated hepatocyte nuclei
Central nervous system	Trouble with walking and balance, greater fall risk, loss of memory	Brain atrophy, atherosclerosis, increased neurofibrillary tangles, subdural hemorrhage

Integumentary System The skin becomes thin with decreased elasticity, and often effects of long-term sun exposure are present, as well. The epidermis can be easily separated from the underlying dermis with minimal trauma due to flattened dermal-epidermal junctions and decreased interdigitations. The skeletal muscle is atrophied, and subcutaneous adipose tissue is overall decreased. The blood vessels can become fragile and decreased in number. Resultant senile purpura and senile ecchymosis, especially over the extensor surfaces and over bony prominences, are seen. Also, anticoagulant medications and drug-induced thrombocytopenias can result in easy bruisability. Underlying peripheral vascular disease and/or diabetes mellitus puts the elder at risk for poor wound healing, pressure ulcers, and infections. Decreased hair over the lower legs and other cutaneous signs of vascular stasis is also indicative of peripheral vascular disease.

A decubitus ulcer, also termed a pressure ulcer, is usually due to immobility, and the tissue breakdown is enhanced by urinary and/or fecal incontinence, malnutrition, a decrease in overall body fat including subcutaneous adipose tissue, muscular atrophy due to aging, decreased immunity, aforementioned skin fragility, vascular insufficiency due to atherosclerosis and/or diabetes mellitus, and generally delayed wound healing. Pressure sores can be divided into four stages [42]:

- Stage 1 Non-blanchable erythema of intact skin
- Changes in sensation, temperature, or firmness may precede visual changes.
- Stage 2 Partial-thickness skin loss with exposed dermis

- Partial-thickness loss of the skin with exposed dermis. The wound bed is viable, pink or red, and moist and may also present as an intact or ruptured serum-filled blister. These injuries commonly result from adverse microclimate and shearing forces, particularly over the pelvis and heels. This stage should not be used to describe moisture-associated skin damage (MASD) including incontinence-associated dermatitis (IAD), intertriginous dermatitis (ITD), medical adhesive-related skin injury (MARSI), or traumatic wounds (skin tears, burns, abrasions).
- Stage 3 Full-thickness skin loss
- Subcutaneous adipose tissue may be visible in the bottom of the wound, and the ulcer may include granulation tissue and epibole (rolled wound edges), but the underlying fibrous tissue, muscle, or bone are not visible. Sloughing and/or eschar may be present and undermining and tunneling may occur. If slough or eschar obscures the extent of tissue loss this is an unstageable pressure injury.
- Stage 4 Full-thickness skin and tissue loss
- Exposed or directly palpable fascia, muscle, tendon, ligament, cartilage, or bone in the ulcer. Slough and/or eschar may be visible. Epibole, undermining, and/or tunneling often also occur [42].

Musculoskeletal System With age, there is often decreased bone mass and less vitamin D production, with consequent osteoporosis and osteopenia, respectively, as well as decreased flexibility. These changes can result in fractures due to falls and sternal and rib fractures during cardiopulmonary resuscitation. Fractures seen in accidental trauma occur most often in the femoral neck, vertebrae, proximal humerus, and ribs. Muscle mass is decreased with age but can also be due to wasting secondary to immobility, chronic disease, malabsorption, dysphagia, and lack of proper caloric intake and nutrition [36, 37, 41].

Cardiovascular System With age comes increased incidence of or risk for atherosclerosis, valve stenosis, and cardiovascular effects of long-term systemic hypertension. The cardiac output is also decreased. Long-term systemic hypertension can lead to myocyte hypertrophy and replacement fibrosis. The elder may develop acquired thrombotic tendencies. Decreased peripheral vasoconstriction and vasodilation, often coupled with decreased sweat response in the skin, can lead to hypothermia and hyperthermia, as well.

Respiratory System The lungs have decreased elastic recoil, decreased pulmonary reserve, and overall decreased lung mechanics with decreased chest compliance and strength of respiratory muscles. Therefore, elders are prone to pneumonia. Underlying pulmonary emphysema is not uncommon in this population, as well.

Gastrointestinal System Elders often have dry mouth, or xerostomia, secondary to aging or to certain medications. Xerostomia can result in periodontal disease, cavi-

ties, and difficulty swallowing. Decreased gag reflex can also cause dysphagia and lead to aspiration. The senses of taste and smell are decreased. Medications can also alter the sense of taste. Appetite decreases with aging as does the sense of thirst. Elders have slower gastrointestinal peristalsis which can lead to fecal impaction. Decreased gastric secretions and intestinal enzymes can result in malabsorption. Colonic diverticular disease and diverticulitis are also prevalent in this population.

Genitourinary System The decreased cardiac output and atherosclerosis result in decreased blood flow to the kidneys. The kidneys have a decreased number of nephrons, decreased glomerular filtration rate, impaired water absorption, decreased urine concentration ability, and decreased creatinine clearance [36, 37, 41]. In women, decreased estrogens lead to vaginal atrophy. Increased vaginal pH can promote infections. The pelvic wall structures weaken and can result in a cystocele or uterine prolapse.

Hepatobiliary System The liver volume is decreased, at least partly due to decreased blood flow to the liver. Microscopically, hepatocytes often have glycogenated nuclei, not only in those individuals with diabetes mellitus but also as a marker of hepatocyte senescence in most aging individuals. Hepatocytes also have lipofuscin accumulation which can interfere with cellular pathways. Decreased hepatic metabolism, especially decrease in the P450 enzyme activity, can interfere with drug metabolism. Liver regeneration capacity is also decreased [43].

Central Nervous System The elder brain is atrophied with decreased weight. The cerebral ventricles can be enlarged due to the atrophy of the surrounding parenchyma. An enlarged subdural space puts the elder at risk for subdural hemorrhage secondary to a fall. The brain has decreased blood flow and decreased neurotransmitters. It is not uncommon to see atherosclerosis of the basilar artery and circle of Willis, meningeal edema, remote infarctions, and lacunar infarctions in the basal ganglia. Age-related increase in neurofibrillary tangles can be seen, including in the parahippocampal gyrus and amygdala. Elders can have impaired gait, decreased proprioception, and slower reaction times, putting them at risk for falls and resultant intracranial injury [36].

Performing the Elder Autopsy

Body Measurements

Weigh the body unclothed and measure body length [36–39, 41]. Find out if the weight has changed dramatically over the past 12 months or if there has been a noticeable trend (especially weight loss) over the past few years. It is important to

calculate a body mass index (BMI), but pathologists must also understand that a loss of weight can also accompany other pathologies such as dementia.

Radiographs

Full-body radiographs can be considered if there is any history of or findings suspicious for trauma or neglect. Radiographs will detect fractures (acute, healing, and possibly remote), reveal abnormal mineralization, and suggest osteomyelitis (especially beneath pressure ulcers).

Toxicology

The vitreous should be analyzed for electrolytes, glucose, and ketones [2, 4, 14]. Dehydration, renal failure, diabetes mellitus, and diabetic ketoacidosis are some entities that may be discovered. An elder's medication level may be elevated due to decreased metabolism and clearance. The level may be low if the medications have not been taken or administered.

Autopsy Procedures

Below are a few remarks or "tips" about autopsy procedures in the elderly. After a thorough external and internal examination possibly including radiographs, the pathologist may want to alter what would ordinarily be the "routine" autopsy procedure.

- Measure the thickness of the abdominal panniculus to correlate with weight in cases of cachexia, malnutrition, or an otherwise underweight elder.
- Perform and document an oral examination.
- If trauma is identified grossly or radiographically, it is worthwhile to do a posterior Y incision exposing the back and buttocks to identify any occult subcutaneous or muscular injury.
- Native coronary arteries and any bypass grafts should be dissected, examined, and submitted for histology.
- If the small and large bowels are not routinely opened, they should be in elder cases. Document and appropriately evaluate the consistency of fecal material, volume of bowel contents including feces in cases of suspected neglect, and any pathology such as tumors or diverticuli.
- If the decedent has pressure sores, the ulcer can be cultured and sectioned for histology, especially the bone to evaluate for underlying osteomyelitis.
- Full neuropathologic examination with relevant sections should be performed in cases of suspected or known dementia.

The elder autopsy is often complicated by diseases in multiple organ systems which can confuse the diagnostic picture. Remember that many elders will die with their diseases and not necessarily of their diseases. Take time to differentiate disease that is relevant from disease that is irrelevant to the cause of death [36, 40].

Autopsy of Individuals with Eating Disorders

Deaths can result from eating disorders including obesity, anorexia nervosa, and bulimia nervosa with or without anorexia nervosa. Death due to the use of supplements and steroids can also fall into this category.

Obesity

Worldwide obesity has nearly tripled since 1975 [44–62]. In fact, in 2016 almost two billion adults worldwide age 18 years and older were overweight, and over 650 million were diagnosed as obese. Also, in 2016, 41 million children under the age of 5 years fell into the categories of overweight or obese. From 5 to 9 years of age, over 340 million children were overweight or obese [49–52]. In the United States in 2016, almost 40% of adults were considered obese [45–47]. Between the ages of 2 and 19 years, 18.5% of children were obese [45–47, 50–55].

Overweight and obese conditions are defined as an abnormal or excessive fat accumulation, respectively, that may impair health [48, 49]. Since these definitions are subjective, methods have been devised to allow physicians and the public to better categorize disorders of fat accumulation. BMI, body mass index, is an anthropometric index of weight and height that is defined as body mass in kilograms divided by the height in meters squared [54]. BMI is a screening tool to classify adiposity. In other words, BMI assists the clinician and pathologist in categorizing a patient as being of normal weight, underweight, overweight, or obese. The BMI does have its limitations as it uses height and weight and does not directly measure body adiposity. More definitive measures of body fat are skinfold thickness, bioelectrical impedance analysis, and dual energy X-ray absorptiometry (considered the gold standard).

Because adiposity varies with age and gender, the BMI changes substantially between the age of 2 years up to 20 years [50–56]. Therefore, BMI-for-age charting based on gender should be used. BMI-for-age is better than the standard pediatric weight-height charts in assessing underweight and overweight children as it takes into account age-related changes that occur in early childhood and in puberty. BMI in children correlates with cardiovascular disease, systemic hypertension, hyperlipidemia, and hyperinsulinemia later in life. BMI under the age of 2 years has a weak association with adiposity and future obesity and, therefore, is not used in this age group.

Obesity is associated with comorbidities and a decreased life expectancy [57–62]. The life expectancy is decreased by 6 years in obese men and by 7 years in obese females. Obese individuals are more likely to die before the age of 70 years.

The top four causes of death in obese individuals are malignancy, infection, heart disease, and pulmonary thromboembolus.

Obese individuals should be weighed and measured (unclothed). The BMI can then be calculated. Make sure that when the body length is measured, that the tape measure is flat on the table and not over the decedent's protuberant abdomen, creating a false increase in length. Radiographs can be helpful to assess degenerative changes to the bones and joints, even in obese children. The CDC BMI-for-age growth charts show BMI as a percentile ranking for children 2–19 years of age. BMI can be calculated, but the distribution of fat is also of concern since intra-abdominal (visceral) fat is associated with cardiovascular disease. BMI is a screening evaluation, and, in some circumstances such as users of anabolic androgenic steroids (discussed later in this chapter), a high BMI does not correlate with adiposity.

Effects of Obesity

Organs most often adversely affected by obesity are the heart, liver, and kidneys [58]. The heart is usually increased in weight secondary to increased blood volume and systemic hypertension, as well as fat deposition externally and within the myocytes. In men, the enlarged heart often shows an increase in the left ventricular thickness, whereas in women the heart weight is increased with an increase in both the left and right ventricular thicknesses [58]. Cardiac uptake and oxidation of fats are not balanced, and the heart accumulates lipid leading to lipotoxicity. The fat deposits can directly alter cellular structures, are toxic to myocytes, and alter systolic and diastolic function. Obesity can also be associated with accelerated atherosclerosis. The liver is often enlarged with steatosis accompanied by ballooning and degenerating hepatocytes and acute inflammation. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis can occur secondary to obesity. Obese individuals and those with type II diabetes mellitus (DM) commonly have glycogen accumulation in the hepatic nuclei. Kidneys can be scarred secondary to glomerulosclerosis, arteriolosclerosis, and arteriosclerosis resulting in chronic renal failure. For some reason, in men with an elevated BMI, the thyroid gland is often increased in weight [58].

Obese individuals can develop type II diabetes mellitus (DM) due to a progressive defect in insulin secretion coupled with a progressive rise in insulin resistance [62]. Obese individuals with type II DM are at risk for hyperglycemic hyperosmolar nonketotic sudden death, evidenced by elevated glucose but absence of ketones or ketone bodies in the vitreous.

Anorexia Nervosa

Anorexia nervosa (AN) is a psychological eating disorder characterized by a distorted body image and intentional weight loss [63–70]. Individuals with AN have an obsessive fear of weight gain, a distorted body image, refuse to eat to maintain a healthy body weight, and deny the serious consequences of such behavior. Most

patients are women, and the average age of onset is 17 years. In the United States, 1–3% of women and 0.5% of men will experience AN during their lifetime [63–70]. Left untreated, 20–50% with AN will die. If treated, 15% will still likely die from complications of the disorder. One in five AN deaths is due to suicide. The anorexic decedent is markedly underweight with a BMI usually under 17.5. The decrease in body adipose tissue and muscle atrophy are apparent at autopsy. Vitreous chemistry may be positive for ketones as the body switches from carbohydrate to lipid energy sources [4]. If vomiting is induced, vitreous chloride will be decreased, as well. Some anorexic individuals abuse laxatives resulting in a low potassium level.

These individuals often have gastrointestinal disturbances including delayed motility and gastric dilatation as well as gastric smooth muscle atrophy. An acute gastric dilatation can result in gastric necrosis and perforation. Delayed gastric emptying followed by dilatation has also been reported to compress the inferior vena cava and superior mesenteric vein resulting in circulatory collapse.

One third of anorexic deaths are due to cardiac causes [65, 68]. Hypotension, bradycardia, repolarization abnormalities, arrhythmias, and prolonged QT interval are very common. Electrolyte imbalances, including low phosphorous and magnesium levels, can cause arrhythmias. Within the heart, one can see muscular atrophy including decreased left ventricular wall thickness resulting in mitral valve prolapse. Microscopically, myocytes are small, fragmented, display contraction bands, and have cytoplasmic accumulations of lipofuscin pigment.

Besides electrolyte abnormalities, severe muscle weakness, bulbar muscle dysfunction, and depressed diaphragmatic contractility can result in acute respiratory distress, aspiration, and sudden death. The immune system is markedly depressed and infections, sometimes fatal, are not uncommon. Death is usually caused by electrolyte imbalances and/or cardiac failure. Common findings at autopsy include low weight, lanugo hair, dry skin, acrocyanosis, and sometimes, hypercarotenemia (yellow skin). There may be osteoporosis, pressure ulcers, or malnutrition-induced hepatitis.

Bulimia Nervosa

Bulimia nervosa (BN) is an eating disorder characterized by periods of binging followed by purging [69, 71–73]. Clinically, it is defined as at least two binge eating episodes a week for a period of 3–6 months. BN is more common than AN but has a lower mortality of approximately 4%. Ten to fifteen percent of bulimics are males, and 85–95% are females [69, 71–73]. The age of onset is in the late teens. In the United States, 1.5% of people will suffer from BN. BN usually does not result in weight loss and can even cause weight gain. These individuals binge on an excessive amount of food, usually within a short time period, and, with a fear of gaining weight, then purge the consumed food. Purging can be accomplished by induced vomiting as well as the use of ipecac, laxatives, enemas, and diuretics.

Induced vomiting using one's hand can result in cuts, scars, and calluses on the dorsal middle phalanges (knuckles) from the teeth. The retching during vomiting

can cause facial and ocular petechiae as small venules and capillaries rupture. Repeated vomiting erodes the enamel of the teeth leading to loss of enamel and tooth decay. Chronic gastric reflux with esophageal inflammation can also be seen. Repeated binging stretches the stomach and can actually damage the stomach lining resulting in tears and chemical peritonitis, or conversely, gastroparesis can result. The kidneys of individuals with longstanding bulimia nervosa can show chronic interstitial nephritis, proximal tubular swelling, and diffuse glomerulosclerosis. Death is usually the result of electrolyte imbalance.

Supplements and Anabolic Steroids

Many individuals take supplements in an attempt to change their body habitus, avoid medical procedures, substitute for traditional chemotherapy, treat medical conditions, and improve overall health. Unfortunately, too many of these supplements are not regulated, and the consumer may be unaware of the exact contents and/or side effects [74–79]. Besides often being ineffective, some contain harmful chemicals that can damage the body and even result in death [74, 76, 79]. For instance, supplements for weight loss may contain ephedrine, an arrhythmogenic agent. If a decedent is known to have taken supplements, the pathologist should obtain the exact supplement so that it can be used to interpret postmortem toxicology results.

Appearance and performance enhancing drugs (APEDs) are most often used by males to improve appearance by building muscle mass or to enhance athletic performance; however, they are also being used by females. Anabolic androgenic steroids are the best studied of the APEDs. These steroids are synthetic variations of the male sex hormone testosterone. People, men and women, take the steroid orally, inject them into veins or into the musculature, or apply them to the skin as creams, gels, or patches. Injection puts the user at risk for contracting and transmitting infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

Steroids act on the brain and are addictive. They can produce mood swings including anger ("roid rage"), paranoia, and delusions. They do not trigger a rapid increase in the brain's dopamine, but, over time, they can affect the dopamine-serotonin-opiate sites and receptors [75, 77, 78]. At autopsy, the increase in muscle mass in both men and women is usually very obvious. The BMI of these individuals can be in the "obese" range because of the increased muscle mass. Below are other findings which may be seen at autopsy.

Integumentary System Men may show oily skin and hair and alopecia, as well as increased length and thickness of non-scalp hair, while women may show male pattern baldness or hirsutism and growth of facial hair.

Musculoskeletal System There is increased muscle mass and elevated BMI. Only in teens, stunted overall growth or height may be seen. There is often swelling of the

hands and feet. Evidence of rhabdomyolysis, such as pigmented casts in the renal tubules, may be noted.

Cardiovascular System Cardiomegaly and left ventricular hypertrophy can occur. Accelerated atherosclerosis, coronary thrombosis, and patchy or interstitial myocardial fibrosis are often present.

Hepatobiliary System Cholestatic hepatitis may be found, as well as hepatic cysts and hepatocellular adenomas and carcinomas.

Genitourinary System Incidence of renal cell carcinoma may be increased, as well as prostatic hypertrophy and cancer in men. Spermatogenesis may be decreased. The clitoris may be enlarged in women, and there may be uterine and breast atrophy. Other microscopic findings in the kidney may include glomerulosclerosis, tubular atrophy or acute necrosis, and interstitial fibrosis.

The Present and the Future

Challenging postmortem cases are often those of sudden or unexpected deaths in which the routine autopsy is no longer the "routine." Cases with an uncommon cause of death, those which have nonspecific gross and microscopic findings, and cases involving unusual case histories can leave the pathologist perplexed and frustrated. Fortunately, advances have been made in several areas of laboratory medicine as well as non-pathology medicine, and many of these are applicable to the autopsy [80–82]. Also, a better understanding of complex pathophysiology such as infectious diseases and SIRS can help unravel what at first seems to be a web of unrelated signs, symptoms, and autopsy findings. Pathology and its many subspecialty branches are continuously evolving and advancing; autopsy pathology should change, as well.

In 1971, the Joint Commission on Accreditation of Hospitals dropped the hospital accreditation standard requiring a 20–25% autopsy rate for deaths occurring in the hospital [83–86]. In 1964, the US hospital autopsy rate was approximately 41%. By 2003, this rate had dropped to 11%, and by 2007 it was at 8.5% [85–95]. The effects of this drop on resident education, in particular pathology residents, are detailed in Chap. 6 of this book. Exposure to pathology for medical students is also decreasing as it is no longer taught as a stand-alone basic science course during the first 2 years of medical school. Unfortunately, while breaking down barriers between the basic and clinical sciences, this approach has helped to make pathology a poorly understood specialty. Very few medical students have ever seen an autopsy before graduating from medical school [85]. It is no wonder that they are not well equipped to inform and counsel their patients on requesting an autopsy. The declining autopsy

rate also creates a negative cycle with unfamiliar clinicians seeking consent in fewer cases. To confound matters, the autopsy, a medical procedure, is costly and not an economic priority in pathology departments.

So where do we see the future of autopsy pathology? The advances in imaging modalities have led to the false perception that the autopsy will be unable to add any additional information regarding a deceased patient's clinical condition [80]. Radiography complements the autopsy but is certainly no replacement for all the autopsy can accomplish. The autopsy remains the gold standard of quality assurance and public health [81]. Though human anatomy remains unchanged over the millennia, insults to the human body do change with time. These insults include toxicities, harmful social trends, changes in cultures with negative body image and expectations, novel instruments of violence, social changes, environmental conditions, and microbiological mutations, and the list goes on. Such changes/insults and their effects on the human body can only thoroughly be appreciated, followed, studied, and eventually prevented through the autopsy. Only by correlating the "world" as we currently understand it with its effects on the human body can we learn and move forward. The autopsy will always be part of the ultimate answer to health-related questions. If conducted thoroughly using appropriate techniques and incorporating knowledge of those often-challenging populations, the autopsy will continue to contribute to science, medicine, and public health.

References

1. Sanchez H. Historical perspective on the autopsy. In: Collins KA, editor. Chapter 2 in autopsy performance and reporting. 3rd ed. Northfield: College of American Pathologists Press; 2017. p. 17–22.
2. Collins KA, Cina SJ, Hooper JE. Getting the most out of the autopsy: ancillary studies. In: Collins KA, editor. Chapter 28 in autopsy performance and reporting. 3rd ed. Northfield/Illinois: College of American Pathologists; 2017.
3. Belsey SL, Flanagan RJ. Postmortem biochemistry update: current applications. J Forensic Legal Med. 2016;40:49–57.
4. Collins KA. Postmortem vitreous analyses. NewsPath. updated 2016. https://emedicine.medscape.com/article/1966150-overview.
5. Palmiere C. Postmortem diagnosis of diabetes mellitus and its complications. Croat Med J. 2015;56:181–93.
6. Elisaf M, Milionis H, Siamopoulos KC. Hypomagnesemic hypokalemia and hypocalcemia: clinical and laboratory characteristics. Miner Electrolyte Metab. 1997;23(2):105–12.
7. Elisaf M, Liberopoulos E, Bairaktari E, Siamopoulos K. Hypokalemia in alcoholic patients. Drug Alcohol Rev. 2002;21(1):73–6.
8. Elisaf M, Merkouropoulos M, Tsianos EV, Siamopoulos KC. Pathogenetic mechanisms of hypomagnesemia in alcoholic patients. J Trace Elem Med Biol. 1995;9(4):210–4.
9. Girard C, Scarpelli MP, Tettamanti C, Palmiere C. Postmortem evaluation of cholesterol, triglyceride, and apolipoprotein levels. Int J Legal Med. 2017;131(6):1777–82.
10. Schirripa ML, Scarpelli MP, Palmiere C. Mediators of inflammation of asthma: a forensic perspective. Am J Forensic Med Pathol. 2017;38(2):153–8.
11. Palmiere C, Bardy D, Mangin P, Werner D. Postmortem diagnosis of unsuspected diabetes mellitus. Forensic Sci Int. 2013;226(1–3):160–7.

12. Khuu HM, Robinson CA, Brissie RM, Konrad RJ. Postmortem diagnosis of unsuspected diabetes mellitus established by determination of decedent's hemoglobin A1c level. J Forensic Sci. 1999;44(3):643–6.
13. Palmiere C, Augsburger M. The postmortem diagnosis of alcoholic ketoacidosis. Alcohol Alcohol. 2014;49(3):271–81.
14. Collins KA. In: Collins KA, editor. Special procedures appendix in special autopsy dissections step-by-step diagrams. Northfield: College of American Pathologists Press; 2010. p. 46–8.
15. Low Blood Pressure (Hypotension). Mayo clinic. www.mayoclinic.org/diseases-conditions. Mayo foundation for medical education and research, 2018. Accessed 21 Mar 2018.
16. Maggio PM. Sepsis and septic shock. Merck manual. www.merckmanuals.com/professional/critical-care-medicine/sepsis-and-septic-shock. Accessed 21 Mar 2018.
17. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA. 2016;315:801–10.
18. Marik PE, Abdalsamih MT. SIRS, qSOFA, and new sepsis definitions. J Thorac Dis. 2017;9(4):943–5.
19. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. N Engl J Med. 2015;372:1629–38.
20. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Cooper DJ, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012. JAMA. 2014;311(3):1308–16.
21. Peach BC. Implications of the new sepsis definition on research and practice. J Crit Care. 2017;38:259–62.
22. Balk RA. Systemic inflammatory response syndrome (SIRS): where did it come from and is it still relent today? Virulence. 2014;5(1):20–6.
23. Simpson SQ. New sepsis criteria: a change we should not make. Chest. 2016;149(5):1117–8.
24. Bell M, Collins KA. Postmortem microbiologic testing. In: Collins KA, editor. Chapter 29 in autopsy performance and reporting. 3rd ed. Northfield: College of American Pathologists Press; 2017.
25. Riedel S. The value of the postmortem microbiology cultures. J Clin Microbiol. 2014;52(4):1028–32.
26. Tsokos M, Puschel K. Postmortem bacteriology in forensic pathology: diagnostic value and interpretation. Legal Med. 2001;3:15–22.
27. Carpenter HM, Wilkins RM. Autopsy bacteriology: review of 2,033 cases. Arch Pathol. 1964;77:73–81.
28. Morris JA, Harrison LM, Partridge SM. Practical and theoretical aspect of postmortem bacteriology. Curr Diagn Pathol. 2007;13:65–74.
29. Kavita I, Michael G, Jie W, Jaime G, Jerome L, Joseph L, Tao H. Identification of Bacteria in formalin-fixed, paraffin-embedded heart valve tissue via 16S rRNA gene nucleotide sequencing. J Clin Microbiol. 2006;44(7):2609–11.
30. Martinez RM. Genes in your tissue: probe identification and sequencing microbial targets from formalin-fixed, paraffin-embedded tissue. Clin Microbiol Newslett. 2014;36(18):139–47.
31. Rapid Influenza Diagnostic Tests. Centers for disease control and prevention, national center for immunization and respiratory diseases (NCIRD), Center for Disease Control and Prevention (CDC) Atlanta. https://www.cdc.gov/flu/professionals/diagnosis/clinician_guidance_ridt.htm. Last accessed 2018.
32. DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. Clin Microbiol Rev. 2004;17(4):903–25.
33. Hans R, Marwaha N. Nucleic acid testing-benefits and constraints. Asian J Transfus Sci. 2014;8(1):2–3.
34. Lucia HL, Kangwanpong D. Identification of dengue virus-infected cells in paraffin-embedded tissue using in situ polymerase chain reaction and DNA hybridization. J Virol Methods. 1994;48(1):1–8.

35. Health, United States 2016. Table 20. US department of health and human services, center for disease control and prevention. National Center for Health Statistics. May 2017. DHHS Publication No. 2017–1232.
36. Collins KA, Presnell SE. Elder neglect and the pathophysiology of aging. Am J Forensic Med Pathol. 2007;28(2):157–62.
37. Collins KA. Elder Abuse. In: Seigel JA, Saukko PJ, editors. Chapter 171 in encyclopedia of forensic sciences. 2nd ed. Waltham: Elsevier Publishing; 2013.
38. Collins KA. The underweight elder: maltreatment, self-neglect, or normal aging? Acad Forensic Pathol. 2013;3(1):40–5.
39. Howe JA, Collins KA, King PS, Yeoman C. Investigation of elder deaths. Acad Forensic Pathol. 2014;4(3):290–304.
40. Collins KA. The negative older adult autopsy: why do they die? Acad Forensic Pathol. 2015;5(1):83–90.
41. Collins KA. Elder maltreatment. In: Payne-James J, Byard RW, editors. Chapter 161 in encyclopedia of forensic and legal medicine. 2nd ed. Waltham: Elsevier; 2014.
42. National Pressure Ulcer Advisory Panel (NPUAP) announces a change in terminology from pressure ulcer to pressure injury and updates the stages of pressure injury. NPUAP, 13 Apr 2016.
43. Tajiri K, Shimizu Y. Liver physiology and liver diseases in the elderly. World J Gastroenterol. 2013;19(46):8459–67.
44. Hoyert DL, Xu J. Deaths: preliminary data for 2011. Natl Vital Stat Rep. 2012;61:1–52.
45. The health effects of overweight and obesity. Center for disease control and prevention. www.cdc.gov/healthyweight/effects/index.html. Accessed 21 Mar 2018.
46. NHLBI. 2013. Managing overweight and obesity in adults: systematic evidence review from the obesity expert panel. www.nhlbi.nih.gov/sites/www.nhlbi.nih.gov/files/obesity-evidence-review.pdf.
47. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. www.nhlbi.nih.gov/files/docs/guildelines/ob_gdlns.pdf. Accessed 21 Mar 2018.
48. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon Dam Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet. 2014;384(9945):755–65.
49. Overweight and obesity fact sheet. World Health Organization. February 2018. www.who.int/mediacentre/factsheets/fs311en.
50. Dietz WH, Bellizzi MC. Introduction: the use of BMI to assess obesity in children. Am J Clin Nutr. 1999;70(suppl):123s–5s.
51. Guo SS, Roche AF, Chumlea WC, Gardner JD, Siervogel RM. The predictive value of childhood body mass index values for overweight at age 35 years. Am J Clin Nutr. 1994;59:810–9.
52. Hammer LD, Kraemer HC, Wilson DM, Ritter PL, Dornbusch SM. Standardized percentile curves of body mass index for children and adolescents. Am J Dis Child. 1991;145:259–63.
53. Himes JH, Dietz WH. Guidelines for overweight in adolescent preventive services: recommendations from an expert committee. Am J Clin Nutr. 1994;59:307–16.
54. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. J Chronic Dis. 1972;25:329–43.
55. Petrobelli A, Faith MS, Allison DB, Gallagher D, Chiumello G, Heymsfield SB. Body mass index as a measure of adiposity among children and adolescents: a validation study. J Pediatr. 1998;132:204–10.
56. CDC table for calculated body mass index values for selected heights and weights for ages 2 to 20. http://www.cdc.gov/nccdphp/dnpa/bmi/00binaries/bmi-tables.pdf.
57. Khaodhiar L, McCowen KC, Blackburn GL. Obesity and its comorbid conditions. Clin Cornerstone. 1999;2(3):17–31.
58. Mandel R, Loeffler AG, Salamat S, Fritsch MK. Organ weight changes associated with body mass index determined from a medical autopsy population. Am J Forensic Med Pathol. 2012;33(4):382–9.
59. National health and nutrition examination survey. NCHS fact sheet. December 2017. National Center for Health Statistics 2017.

60. Peeters A, Barendregt JJ, Willekens F, Mackkenbach JP, Mamun AA, Bonneux L. Obesity in adulthood and it consequences for life expectancy: a life-table analysis. Ann Intern Med. 2003;138:24–32.
61. Strawbridge WJ, Wallagen MI, Sherma SJ. New NHLBI clinical guidelines for obesity and overweight: will they promote health? Am J Pub Health. 2000;90:340–3.
62. Golay A, Ybarra J. Link between obesity and type 2 diabetes. Best Pract Res Clin Endocrinol Metab. 2005;19(4):649–63.
63. Jauregui-Garrido B, Jauregui-Lobera I. Sudden death in eating disorders. Vasc Health Risk Manag. 2012;8:91–8.
64. Hewitt PL, Coren S, Steel GD. Death from anorexia nervosa: age span and sex differences. Aging Ment Health. 2001;5(1):41–6.
65. Ratnasuriya RH, Eisler I, Szmuhter GL, Russell GF. Anorexia nervosa: outcome and prognostic factors after 20 years. Br J Psychiatry. 1991;158:495–502.
66. Hudson JI, Hiripi E, Pope HG, Kessler RC. The prevalence and correlates of eating disorders in the national comorbidity survey replications. Biol Psychiatry. 2007;61(3):348–58.
67. Le Grange D, Swanson SA, Crow SJ, Merikangas KR. Eating disorder not otherwise specified presentation in the US population. Int J Eat Disord. 2012;45(5):711–8.
68. Arcelus J, Mitchell AJ, Wales J, Nielsen S. Mortality rates in patients with anorexia nervosa and other eating disorders: a meta-analysis of 36 studies. Arch Gen Psychiatry. 2011;68(7):724–31.
69. Citrome L. Binge eating disorder and comorbid conditions: differential diagnosis and implications for treatment. Clin Psychiatry. 2017;78(suppl 1):9–13.
70. Marques L, Alegria M, Becker AE, Chen C, Fang A, Chosak A, Diniz JB. Comparative prevalence, correlates of impairment, and service utilization for eating disorders across US ethnic groups: implications for reducing ethnic disparities in health care access for eating disorders. Int J Eat Disord. 2011;44(5):412–20.
71. Rushing JM, Jones LE, Carney C. Bulimia nervosa: a primary care review. Prim Care Companion J Clin Psychiatry. 2003;5(5):217–24.
72. Smink FRE, van Hoeken D, Hoek HW. Epidemiology for eating disorders: incidence, prevalence and mortality rates. Curr Psychiatry Rep. 2012;14(4):406–14.
73. Ouellette JD. Statistic on Bulimia. http://www.mirror-mirror.org/bulimia/statistics-on-bulimia.htm. Accessed 21 Mar 2018.
74. Frati P, Busardo FP, Cipolloni L, De Dominicis E, Fineschi V. Anabolic androgenic steroid (AAS) related deaths: autoptic, histopathological and toxicological findings. Curr Neuropharmacol. 2015;13:146–59.
75. Turillazzi E, Perilli G, Di Paolo M, Neri M, Riezzo I, Fineschi V. Side effects of AAS abuse: an overview. Mini Rev Med Chem. 2011;11(5):374–89.
76. Montisci M, El Mazloum R, Cecchetto G, Terranova C, Ferrara SD, Thiene G, Basso C. Anabolic steroids abuse and cardiac death in athletes: morphological and toxicological findings in four fatal cases. Forensic Sci Int. 2012;217(1–3):e13–8.
77. Kutscher EC, Lund BC, Perry PJ. Anabolic steroids: a review for the clinician. Sports Med. 2002;32(5):285–96.
78. Anabolic steroids. National Institute on drug abuse. National Institutes of Health. US Department of Health and Human Services. 2016. www.drugabuse.gov.
79. Solimini R, Rotolo MC, Mastrobattista L, Mortali C, Minutillo A, Pichini S, PAcifici R, Palmi L. Hepatotoxicity associated with illicit use of anabolic androgenic steroids in doping. Eur Rev Med Pharmocol Sci. 2017;21:7–16.
80. Shojania KG, Burton EC, McDonald KM, Goldman L. Changes in rates of autopsy-detected errors over time. JAMA. 2003;289:2849–56.
81. Burton EC. The autopsy: a professional responsibility in assuring quality of care. Am J Med Qual. 2002;17:56–60.
82. Collins KA. Beyond the autopsy: special procedures performed postmortem. Acad Forensic Pathol. 2015;5(2):280–90.
83. Bean SM, Nagler A, Buckley PJ. What are the pathology education requirements for all nonpathology ACGME-accredited programs in an academic center? Am J Clin Pathol. 2012;138:327–31.

84. Oluwasola OA, Fawole OI, Otegbayo AJ, Ogun GO, Adebamowo CA, Bamigboye AE. The autopsy: knowledge, attitude, and perceptions of doctors and relatives of the deceased. Arch Pathol Lab Med. 2009;133:78–82.
85. O'Grady G. Death of the teaching autopsy. BMJ. 2003;327(7418):802–3.
86. Hull MJ, Nazarian RM, Wheeler AE, et al. Resident physician opinions on autopsy importance and procurement. Hum Pathol. 2007;38:342–50.
87. Harrington DE, Sayre EA. Managed care and measuring medical outcomes: did the rise of HOMs contribute to the fall in the autopsy rate? Soc Sci Med. 2010;70:191–8.
88. Lowe JA, Davis CJ. Need we revive falling autopsy rates? PAS Rep. 1977;15:1–7.
89. McPhee SJ, Bottles K. Autopsy moribund art of vital science? Am J Med. 1985;78:107–13.
90. Heller J, Friedlander D, Celler SA. Autopsy findings as a modifier of diagnosis related group classification and hospital reimbursement. Mt Sinai J Med. 1985;52(5):311–4.
91. Turnbull A, Osborn M, Nicholas N. Hospital autopsy: endangered or extinct? J Clin Pathol. 2015;0:104. http://jcp.bmj.com/. Accessed 18 Mar 2018.
92. Bamber AR, Quince TA. The value of postmortem experience in undergraduate medical education: current perspectives. Adv Med Educ Pract. 2015;6:159–70.
93. Hoyert DL. The changing profile of autopsied deaths in the United States, 1972–2007. NCHC data brief no. 67. Hyattsville: National Center for Health Statistics; 2011.
94. Hoyert DL, Kung HC, Xu J. Autopsy patterns in 2003. National center for health statistics. Vital Health Stat. 2007;20(32):1–27.
95. Xu J, Kockanek KD, Murphy SL, Tejada-Vera B. Deaths: final data for 2007, National vital statistics reports;58(19). Hyattsville: national Center for Health Statistics; 2010.