Chapter 17

BIOMARKERS IN DISEASE DIAGNOSIS AND TREATMENT

Integration of Biomarkers to Improve Patient Care

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Abstract: The concept of biomarkers is becoming more relevant in disease diagnosis and prognosis, as well as in pharmaceutical drug development. The particular role of these biomarkers is to improve the early diagnosis of human disorders, to give an individual prognosis of the stage and progression of a diagnosed disease, and to predict and monitor the effectiveness of an applied therapy. In medical compound development, their main impact will be on the prediction of adverse and toxic effects, and clinical efficacy of new chemical entities in man.

Keywords: Biomarkers, molecular markers, molecular diagnostics, molecular imaging, therapy, drug development, integration of biomarkers

1. RELEVANCE OF BIOMARKERS

In 2001, the Human Genome Consortium presented the first draft of the human genome sequence, and provided a final update of the draft sequence three years later[1,2]. Despite the enormous promise of its clinical relevance, the impact of the human genome sequence in terms of benefit in treating human disorders turned out to be limited, until we better understand how a genome translates into a phenotype and which changes on the molecular level give rise to a pathology on the macroscopic level.

Knowledge of the human genome sequence will accelerate the identification of genes, whose function is associated with a human disease. Several approaches can lead to the discovery of novel human disease genes, e.g., genetic linkage analysis[3], or expression profiling of transcripts or their corresponding protein products in the context of pathological conditions[4,5].

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Although we have seen a huge increase in knowledge of molecular biology of life, we still do not understand very well how to translate this plethora of information into relevant clinical applications. For instance, many putative biomarkers have been described for use in different medical applications and diseases, including different tumor disorders[6-14], neurological[15-17] and metabolic[18] as well as cardiovascular[19,20] conditions. However, most are not well accepted among clinicians, with the exception of a fairly low number of partly long-known molecules like PSA (Prostate Specific Antigen). This is consistent with the observation that the number of newly developed diagnostic assays based on new biomarkers seems to be declining over the last decade[21], despite the fact that an increasing number of entries in Medline in the context of the biomarkers and biomarker concepts can be found.

A recent investigation of Medline, while searching for publications including the term biomarker* (in titles and abstracts) between the period of 1995 - 2005, resulted in approximately 6000 records (only items with English abstract and work on humans have been selected).

Further investigation of these hits (Figure 17-1), showed that the terminology of biomarker(s) was used in 1995 in only <100 publications, whereas in 2004, we already counted around 1250 relevant entries, and it seems that this number is still increasing in 2005 (data not shown). When looking further into the context of the use of a biomarker concept, we identified a fairly significant number of records in the area of diagnostics (Dx) as well as therapeutics (Tx), whereas the number of citations in the context of imaging (Im) was only starting to increase in 2001/2002. This brief analysis at least indicates that the interest in the biomarker concept and terminology in the context of disease diagnosis and treatment has been strongly increasing during the last decade (although it should be noted that other terminology may have been used in similar research before 1995).

2. BIOMARKER CONCEPT: DEFINITIONS

As the interest in the concept of biomarkers for diagnosis and treatment of diseases is growing over the last years, a number of different definitions on the types and application of biomarkers have been used. Many terms with partly overlapping meaning like molecular markers, biological markers, biomarkers, diagnostic markers, surrogate markers, etc can be found in the relevant literature.

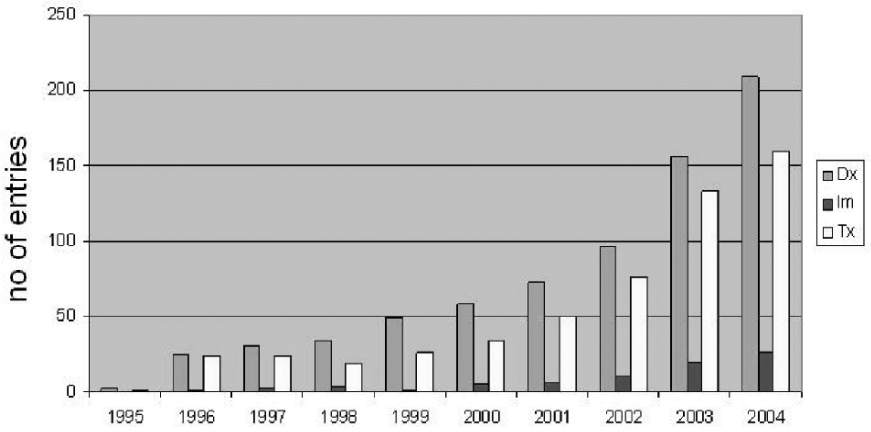

Figure 17-1. Medline entries 1995 - 2005 including the term ' biomarker*' in title or abstract (see text). Note that the presented analysis does obviously not cover all publications on biological markers - the intention was to get an idea on the introduction and use of the biomarker concept during the last decade. Dx - Diagnostics, Im - Imaging, Tx - Therapeutics.

In order to improve the discussion towards a consensus around types of biomarkers and use of the biomarker concepts in diagnosis or treatment of a disease, the NIH Biomarker Definitions Working Group suggest the use of the following definition[22]: *"Biological marker (biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention"*.

This broad definition of the term biomarker indicates that the nature of a biological marker can be rather diverse, ranging from a specific gene transcript, a peptide or a protein, which have been measured as being deregulated during the progression into a pathological status, but can also be an identified genetic mutation, or a physiological process by itself.

Typical examples of protein biomarkers include CA 125 (ovarian cancer), CA 15-3 and CA 27-29 (breast cancer), CEA (ovarian, lung, breast, pancreas, and gastrointestinal tract cancers), PSA (prostate cancer), or Aß42, (phospho)-tau (Alzheimer's Disease), CRP (Inflammation). Other examples would be blood pressure, LDL cholesterol, HIV load, FDG-PET imaging in Alzheimer's Disease, tumor shrinkage, bacterial/viral/fungal culture and sensitivity (infectious diseases), glucose, hemoglobin A1c (diabetes), or intra-ocular pressure (glaucoma).

Equally important in the context of biomarker concepts is the definition of the terms 'clinical endpoint' as well as 'surrogate endpoint'. According to

the 'NIH Biomarkers Definitions Working Group', the expressions clinical and surrogate endpoints are defined as[22]:

A **clinical endpoint** is a characteristic or variable that reflects how a patient feels, functions, or survives. Clinical endpoints are distinct measurements or analyses of disease characteristics observed in a study or a clinical trial that reflect the effect of a therapeutic intervention. Clinical endpoints are the most credible characteristics (e.g., survival, myocardial infarction, stroke, recurrence of cancer, etc) used in the assessment of the benefits and risks of a therapeutic intervention (e.g., application of a drug, surgery, device, etc) in randomized clinical trials.

A **surrogate endpoint** is a characteristic that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit) based on epidemiological, therapeutic, patho-physiologic, or other scientific evidence. The term *surrogate endpoint* applies primarily to endpoints in therapeutic intervention trials.

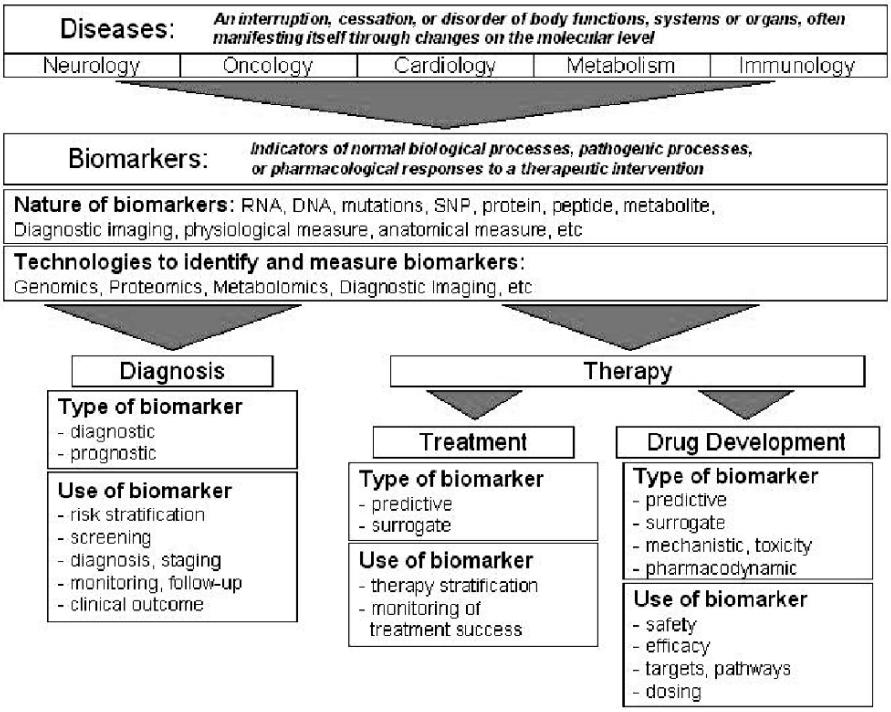

Figure 17-2: Overview of the types and use of biomarkers in diagnosis and treatment of disorders, as well as in drug development. The types of the biomarkers used for the detection of diseases are mostly diagnostic and prognostic, whereas in the area of treatment and compound development, the types of implemented biomarkers are predictive, surrogate, as well as mechanistic (including toxicity) and pharmacodynamic.

According to those definitions, several types of biomarkers can be distinguished related to their use in diagnostics of diseases, or therapeutic treatment, and pharmaceutical drug development (Figure 17-2)[23]:

Diagnostic biomarker:	Indicates the presence (and stage) of a disease
Prognostic biomarker:	Indicates the future behavior of a disease
Predictive biomarker:	Indicates the relative response to a treatment
Surrogate biomarker:	Substitutes for a clinical endpoint
Mechanistic biomarker:	Sndicates an effect on a desired target pathway
Toxicity biomarker:	Indicates a potentially toxic/adverse effect
Pharmacodynamic biomarker:	Indicates the highest effective dose of a drug

3. BIOMARKERS IN DIAGNOSTICS

Although the nature of a biomarker can be very diverse, ranging from an RNA or DNA sequence, a genetic mutation, a metabolite, an anatomical or physiological measurement, a diagnostic image, the protein biomarkers belong to the most relevant biochemical biomarkers in the clinical routine today.

Currently, there is enormous interest in the identification and validation of novel protein biomarkers for the early diagnosis of all kinds of tumor diseases, neurodegenerative disorders, cardiovascular conditions, and others. In particular, the introduction of what is usually referred to as 'Clinical Proteomics' has led to a dramatic increase in studies on clinical material like different types of body fluids (serum, plasma, urine, saliva, etc) for the presence of novel proteins or a multitude of proteins, which give a highly sensitive and specific indication if a pathological condition has developed in the past.

Until recently, a biomarker was a single event measurement, e.g. the putative presence of a disease was based on the detection and quantification a single protein like PSA in Prostate Cancer diagnosis.

Although the clinical introduction of PSA as a molecular marker has significantly impacted the diagnosis of Prostate Cancer[24], there are still major concerns in view of the specificity to discriminate between a benign and malignant prostate condition. It turned out in many clinical studies that the specificity (the probability that a diagnostic test result will be negative when the disease is not present) of PSA testing is relatively poor (in the order of 20-50%, depending on the details of the investigation)[25-27]. The expression of PSA is not a tumor-specific event, as its level can also be significantly increased in different benign conditions of the prostate, e.g.

hyperplasia, or inflammation. Obviously, the power of this biomarker molecule to discriminate between a malignant and a benign condition of the prostate organ is very low, and in consequence, a lot of false-positive results are generated based on solely PSA measurements in a serum sample of a patient.

According to a recent calculation[28], currently 8% of the male US population has increased PSA levels of >4 ng/ml, which is an indication to take a tissue biopsy from the prostate. In 2004, this would lead to the detection of around 760,000 malignancies compared to ca. 2,300,000 men (from a total of approx. 3 million men between the ages of 45 and 74) undergoing unnecessary biopsy due to low specificity of PSA testing.

Another serious problem with PSA has been reported to be in men with PSA levels <4 ng/ml, which so far has been considered as of low risk for Prostate Cancer[29]. Around 15% of men in this group were found to have malignancies in their prostate, from which another 15% showed advanced disease. There seems to be at least a significant risk of having a prostate tumor, even if the measured protein level in serum is below the usually used lower cut-off limit of 4 ng/ml PSA.

Therefore, there is a very strong interest for the search of proteins with an increased diagnostic power to discriminate between healthy/benign conditions versus malignancies of the male prostate. There is a whole range of proteins being suggested as potential biomarkers in prostate cancer care[9,30], but none of these has yet been successfully introduced into the clinical practice.

Only very recently, new technologies have been introduced into the biomarker discovery research, raising the possibility to not only identify single proteins as potential indicative event, but rather searching through the measured parameters for a combination of molecules, defining a diagnostic fingerprint or pattern[31]. The conceptual idea is that the individual proteins, coding for such a fingerprint, are not necessarily significant events to discriminate, e.g., a healthy from a diseased state, but the combination of these parameters will lead to a finally robust clinical assay. Using combinations of several biomarkers can help to make a diagnostic test more robust against the usual variability of diagnostic tests applied to a commonly quite heterogeneous patient population. Furthermore, single-events often suffer from technical errors, including sample variability in collection and preparation, measurement and instrumental errors, etc.

Several approaches suitable for the identification of novel biomarkers or biomarker fingerprints have been applied to patient body fluids (like urine, serum, plasma, prostatic fluid) to find improved answers for the diagnosis of prostate cancer (but also of many other diseases), in particular to improve the discrimination between benign and malignant lesions. The technologies

ranges from 2-DE (2-dimensional gel electrophoresis)[32-34], and SELDI (Surface Enhanced Laser Desorption Ionization)[35-37], to 2-D LC-MS (2-dimensional Liquid Chromatography coupled to Mass Spectrometry)[38].

Some first results obtained using the SELDI approach to identify biomarker profiles for diagnosis of Prostate Cancer indicate that it may be feasible to improve the diagnostic sensitivity and specificity by applying a multi-marker technology. Several studies reported sensitivities up to 95%, and specificities up to 97% [39-41].

It has to be mentioned however, that, according to a recent investigation[42], the individual results of different studies on Prostate Cancer profiling by SELDI, come to diverse models regarding the discrimination between the different types of samples analysed (e.g., normal vs. benign vs. malignant). The diagnostic value of each described fingerprint is therefore unclear, and has to wait for further validation on larger, multi-centered studies.

4. BIOMARKERS IN THERAPY

The pharmaceutical industry is currently facing a tremendous challenge, as the number of FDA approved new chemical entities (NCEs) has been considerably declining over the last 15 years[43]. The exact reason for this is not fully understood. Most common explanations are that companies have to deal with very complex disorders, with an increasing number of novel targets, or with currently not fully explored targets with an inherently bigger risk of failure, together with a limited range of predictive tools for decisions early in the development process, and finally, ever more demanding regulatory authorities.

Interestingly, the diagnostic industry seems to be in a quite analog situation as it has been reported that the number of newly developed and approved serum or plasma protein based diagnostic tests has been dropping in a similar way as the number of novel NCEs in the pharmaceutical industry over the last 15 years[21]. Also in the diagnostic area, it is speculated that the complexity of human physiology decreases the chances of success to accurately diagnose the status of a disease by using the body fluid level of a (single) protein.

Looking at the different reasons for attrition (or success) rates of a pharmaceutical development, three general trends are emerging[43]:

- Currently, the major reasons for compound failure are issues with clinical safety and/or toxicology (attrition rate ca. 10% and 15%, respectively), and lack of clinical efficacy (ca. 30% attrition rate).

- The attrition rate due to issue with PK (pharmacokinetics) and/or bioavailability has been dramatically decreased over the last 15 years from approx. 40% to now 10%.
- Whereas the average success rates from clinical phase 1 to achieve regulatory registration in different investigated indications is about 11%, there are significant differences between success rates of individual therapeutic areas like oncology and CNS (5-7%) versus, e.g., cardiovascular diseases or arthritis/pain (15-20%).

It seems that for diseases, where animal models are reasonably well able to represent the complex behavior of the human patho-physiology, the probability of a successful drug development is higher compared to those disorders, where valid and predictive animal models are lacking (e.g., CNS, oncology) - these areas are much more susceptible to attrition of a development compound.

Furthermore, whereas the prediction of *in vivo* characteristics of chemicals, like PK/bioavailability[44], which can be relatively well simulated by use of *in vitro* systems, has been clearly improved over the last decade this has not been achieved for the prediction of drug efficacy in man based on cell culture systems or animal models. A similar situation exists for the prediction of clinical safety/toxicology of compounds to be used in man.

This indicates, that the difficulty to predict the complex behavior of the human physiology, in particular with respect to expected negative or positive effects of a developmental compound, is currently the major hurdle for improvements in the drug development chain.

As the development of predictive biomarkers seems to be an attractive solution for these obstacles, it has to be mentioned that only relatively few biomarkers so far are accepted as being useful as surrogate endpoints in clinical trials. This is (again) mostly due to the fact that a single biomarker can hardly represent all possible effects of a therapeutic intervention in a very complex system like the human body.

Thus, it seems to be an obvious choice to go for multiple-biomarker strategies to predict clinical compound efficacy, as well as safety and toxicological aspects. Some first successful examples have emerged over the last few years already. For instance a recent study demonstrated that, based on a set of the expression values of a multitude of genes, it was possible to correctly classify >30 approved drugs used in three different therapeutic areas in CNS, namely antidepressants, antipsychotics, and opioid drugs. The analysis was performed on primary human neuronal cell cultures. It was possible to predict the correct drug class from those compounds, which has been excluded from the original training set with an accuracy in the higher 80% range[45]. It has to be noted that the used CNS compounds are often

highly related in terms of chemical structure as well as pharmacology, even for compounds used in different therapeutic areas (e.g., antidepressants and antipsychotics).

Also in the area of prediction of hepatotoxicity quite a number of recent reports were able to underscore the relevance of genomic and proteomic technologies to identify profiles or patterns of events, which are able to predict the toxicity of known hepatotoxicants in cultured primary human hepatocytes[46-50].

A very important aspect to be considered in this context is that the power of predicting desired or undesired effects of compounds by *in vitro* profiling technologies strongly depends on the breadth of the represented effects in the input training set: only those effects (desired or undesired), which are elicited by the chemicals in the group of drugs used for the selection of predictive biomarker patterns, can later be predicted for a test compound. This is in particular true for the prediction of therapeutic effects, as they are normally related to a very specific molecular mechanism. For instance, the numerous medications used today in the treatment of psychotic disorders, are mostly directed towards the dopamine D2 receptor. It has been shown in different reports that these compounds demonstrate a good correlation between their specific affinity to the dopamine D2 receptor and their antipsychotic potential[51,52]. In the case of antidepressive treatments, the molecular target of many developments in this area is focused around the serotonergic neurotransmitter system (e.g., the SSRI - Selective Serotonine Reuptake Inhibitors).

Furthermore, it has been shown that the activation of a specific molecular target leads to expression of characteristic down-stream effects. In consequence, the algorithms that are used to predict clinical efficacy for a certain drug can classify only those compounds, which induce similar up-stream and down-stream events compared to already known chemicals in the related therapeutic area. Drugs with a fully new mechanism of action cannot be recognized as belonging to a class of therapeutic compounds within the training set. This gives some clear limitations on the potential power of the biochemical marker profile approach to predict clinical efficacy in man.

In the case of biochemical markers, which are predictive for toxicologically adverse effects e.g. in the liver, this might be a slightly different situation. The toxic events of many pharmacologically active compounds have been characterized in the past. It is therefore expected that there is a relatively good chance that the induced toxicity of any new chemical entity tested is already (at least partially) represented within the chemical space of existing pharmaceutical drugs. In consequence, screening a large number of compounds with a known toxicity profile may suffice to prepare the learning tools of the used classification algorithms so that they

can pick up all relevant potential toxic side effects by the selection of an appropriate gene/protein profile.

5. INTEGRATED BIOMARKER APPROACH

As the underlying pathology of the development and progression of many human disorders is a very complex and dynamic process with many parameters changing temporally and spatially, depending on the stage of the disease, the concept of integrating several biomarkers of different types to more accurately diagnose and treat a human disorder will become more relevant in healthcare and disease management.

The main driving force for this development is that it cannot be expected that a single biomarker (or a biomarker pattern) will deliver an accurate diagnosis or prognosis of a disease and its outcome, and at the same time can predict the effectiveness of an applied therapeutic treatment.

Furthermore, the number of different possible treatment options for a certain disease makes it very unlikely that a single biomarker is able to represent all potential subsequent effects on the physiological or molecular level. For instance, in Alzheimer's Disease (AD), the major treatment concepts, which are currently under development in the pharmaceutical industry, are targeting the cholinergic neurotransmitter system on the one hand, and the amyloid plaque cascade on the other (Figure 17-3)[53].

Still, the most prominent target system is the CNS acetylcholine neurotransmitter pathway, not only in terms of numbers of development compounds, but in particular also concerning the development stage, which is far more advanced compared to other developments (not shown). Strongly increasing since a few years are the numbers of compounds to prevent the generation of beta-amyloid plaque deposition in some relevant brain regions. Although a few other targets are under therapeutic development for treatment of AD, the cholinergic and the amyloid pathways nearly contribute 2/3 of all compounds in clinical phases (Figure 17-3).

However, as the suggested cholinergic and amyloid cascades involve molecular targets from quite different neurological cells as well as different receptors or enzymes, it is easy to imagine that different biomarkers would be necessary in order to prove the effectiveness of a specific treatment in an individual patient. In the first case, the *in vivo* imaging of the forebrain cholinergic neurons by, e.g., targeting the nicotinic acetylcholine receptors, or the non-invasive monitoring of the activity of the acetylcholinesterase could be a way to follow treatment efficacy[54-58]. In the latter case, the direct imaging extra-cellular senile plaques by amyloid binding compounds may deliver a relevant biomarker for treatment monitoring[59-63].

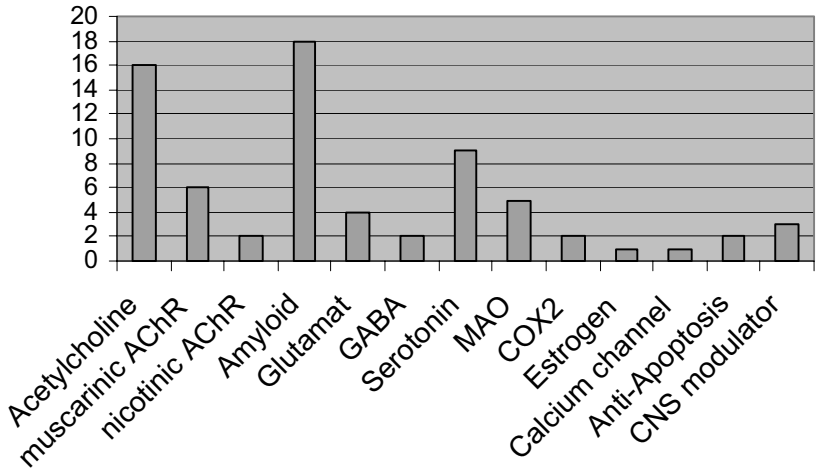

Figure 17-3. Lines of treatment options in AD, currently under development in the pharmaceutical industry (only compounds shown, which are at least in clinical phase 1).

In the case of early diagnosis of AD, the situation with respect to a useful biomarker is again different. The pathology of Alzheimer's Disease is characterized by the reduction of neurons in the temporal and frontal cortex, as well as the development of different extra- and intracellular depositions, in particular beta-amyloid (senile) plaques and neurofibrillary tangles[64]. However, the definitive diagnosis of AD is currently not possible in living subjects and is done by means of pathological examination of post-mortem biopsy material based on criteria established by the National Institute on Aging, and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Furthermore, neurodegenerative disorders are characterized by the fact, that patients remain asymptotic for many years after the pathological disease process has already started.

A variety of imaging-based as well as biochemical biomarkers have been studied over the last years in order to improve the positive predictive value for early diagnosis of AD and the differential diagnosis versus related dementia disorders. On the imaging side, this includes measurements like volumetric MRI of the complete brain and specific brain areas, like the hippocampus[65,66], MR spectroscopy[67-69], and PET imaging of amyloid plaques or inflammatory processes[70,71]. Only recently, the Centers for Medicare and Medicaid Services (CMS) approved FDG-PET for the differential diagnosis of AD for reimbursement in the USA[72].

On the biochemical path, a whole range of different putative biomarkers has been suggested in the past[15-17,73,74]. Although, some of these biomarkers

like beta-amyloid or the tau proteins seem to be involved in the pathological processes leading to AD, none of the currently discussed components are able neither to fully describe the different stages nor to predict the progression of the disease. Very recently, also a biomarker approach based on a fingerprint consisting of a multitude of serum proteins has been proposed to have diagnostic value in AD[75].

A very relevant clinical problem in the diagnosis of Alzheimer's Disease is only partially addressed by the currently available biomarkers - namely the differential diagnosis between AD and other forms of dementia. This is very relevant, as around one third of all dementia cases in elderly are accounted to conditions other than AD. Highly relevant among diseases to be discriminated from AD are for instance vascular dementia, frontotemporal dementia, Lewy body dementia, depression-related dementia, and some others. Although the clinical symptoms are similar, the molecular pathology of these disorders is quite different compared to AD, which has important implications for treatment decisions.

There is increasing evidence from recent studies that the latent (asymptomatic) stage of AD, which may develop very slowly and unrecognized over several decades, is progressing into MCI (Mild Cognitive Impairment) first, before moving into a more advanced stage of mild AD[76,77]. MCI compared to AD is characterized in that patients show less prominent cognitive deficits. There is still quite some debate on the exact definition of MCI, and in particular on the question of how many MCI patients will progress into AD, or if MCI can be a stable condition in some patients. There is some believe that virtually all patients with MCI may have pathological features of AD, whereas others do more believe that only a certain percentage of MCI patients will finally progress into Alzheimer's Disease pathology[78,79]. One recent study supporting the latter view has followed up a number of MCI patients and could show that around 10% of all patients yearly progressed into AD characteristics[80]. However, it is virtually impossible to predict, which patient is going to progress from MCI to AD and which MCI patients will be staying stable with their mild cognitive effects. This is obviously important information, as it is generally believed that an early treatment with available therapeutics today (i.e., mainly acetylcholinesterase inhibitors) may attenuate the progression of AD more effectively as compared to the usual (often too late) start of medication. This generates an urgent need to develop biomarkers, which are able to predict the progression into AD from early or pre-stages of this disease.

Figure 17-4 shows a schematic outline of the possible integration of different types of biomarkers in the clinical care of Alzheimer's Disease. During (late) asymptomatic phases of the disease or during phases of MCI, i.e., pathological changes have already become manifest but no or only mild

clinical symptoms can be diagnosed, it is very likely that only a combination of several biomarkers on the psychological, biochemical, as well as on the neuro-imaging level can deliver an accurate diagnosis of an existing neurodegenerative process, a differential diagnosis of AD vs. related disorders, as well as a predictive value for the progression of MCI into AD, or into other forms of dementia.

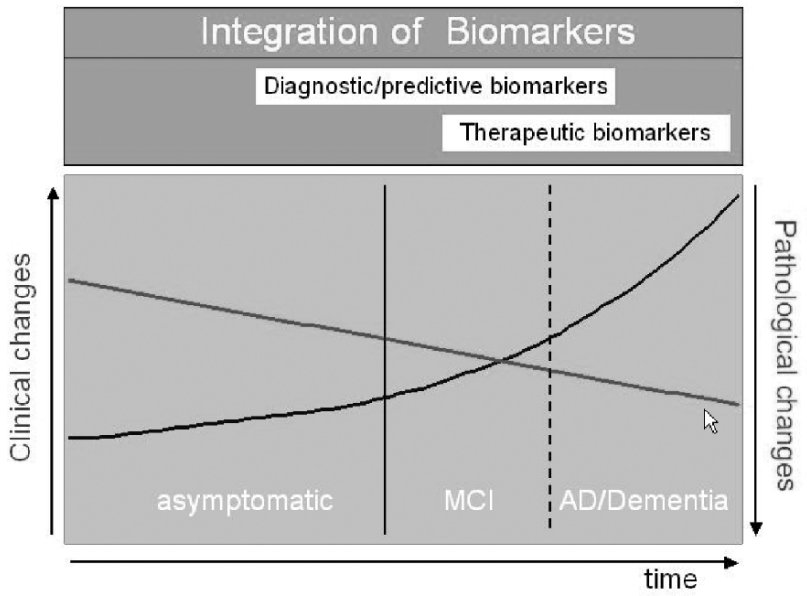

Figure 17-4. Integration of biomarkers in AD care (modified according to [76,77]). For details see text.

The usefulness of a biomarker for monitoring of therapeutic efficacy of an applied treatment clearly depends on the diagnosis and prognosis of the disease progression (e.g., AD vs. other forms of dementia), as well as the choice of a specific type of treatment (e.g., cholinergic vs. amyloid).

6. CONCLUSIONS

The concept of biomarkers to support the diagnosis of diseases, as well as the to support the development of therapeutic drugs is becoming more evident as our knowledge on the underlying molecular mechanisms of diseases is increasing.

The nature of biomarkers can be very diverse, ranging from a biochemical marker (e.g., DNA, RNA, protein, metabolite) to a physiological measurement like blood pressure. Non-invasive *in vivo* imaging is strongly emerging as one important nature of a relevant biomarker.

Different types of biomarkers exist, depending on their usefulness in diagnosis of diseases, or in pharmaceutical drug development (e.g., diagnostic, prognostic, surrogate, mechanistic, etc).

However, due to the complexity of many diseases, only the integration of different types of biomarkers will lead to an improved patient treatment on a personalized basis.

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