Review

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Laboratory medicine and medical oncology: the tale of two Cinderellas

Abstract

Cancer represents a leading cause of death in the developed countries. The past 50 years have witnessed major progress in both laboratory medicine and clinical oncology that has translated into improved prognosis of cancer patients. From the humble beginnings as unrelated specialties, major advances in the understanding of molecular bases of cancer progression led to increased interactions between laboratory medicine and clinical (mostly medical) oncology. Laboratory medicine is now an integral part of the management of cancer patients. The many aspects of the role of laboratory medicine in clinical oncology include the determination of biomarkers that are used in establishing the diagnosis, predicting response to therapy or prognosis, study of the host response to tumor growth, detection of treatment toxicity and determining the concentrations of anticancer drugs.

Keywords: biomarkers; cancer; laboratory medicine; medical oncology.

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Introduction

In the developed countries, cancer is currently the second leading cause of death. It is estimated that every third person will be affected with cancer during his or her lifetime, and every fourth person will die of cancer. Historically, cancer as a leading cause of death and a major public health issue is a relatively recent phenomenon. Despite the fact that malignant tumors have been known since antiquity, only a few generations back, cancer was relatively rare. The incidence of most malignant tumors has increased sharply during the 20th century as a result of lifestyle changes and the prolongation of life expectancy.

The history of laboratory medicine also dates back more than thousand years [1, 2]. Fifty years ago the standing of what was then medical oncology and clinical chemistry/laboratory medicine in the medical community was quite similar. Both specialties were at that time mostly outside the perimeter of interest of society, as well as the medical community. Since then, medical oncology and laboratory medicine have undergone a remarkable metamorphosis not unlike that from Cinderella to a princess. Despite the fact that 50 years ago in most institutions departments of oncology or laboratory medicine were hidden somewhere in the basements, today these departments represent the leading edge of the medical profession and occupy extensive space on separate floors or even in separate buildings. Thus, the following review may be more a tale of two Cinderellas and a journal that sprouted in the shadow of the Berlin Wall.

The essential feature of tumor growth is its systemic nature. While the assessment of local tumor extent using imaging or even histological examination may reveal only one aspect of neoplastic growth and progression, the assessment of systemic behavior of cancer is predominantly in the realm of laboratory medicine. The role of laboratory medicine in oncology also increased because of the need to define the changes associated with the tumor growth and progression at the molecular level. The rise of laboratory medicine as an independent specialty is also a relatively recent development. As mentioned above, the developments of medical oncology and laboratory medicine in the past 50 years have much in common. Both specialties rose from humble beginnings to being currently the fastest growing areas of medicine. There were times when the paths of medical oncology and laboratory medicine might have seemed to wind in parallel, but now, with these paths more resembling two busy speedways, there appear to be multiple crossings.

Our understanding of cancer pathogenesis has evolved enormously during the past 50 years. In a seminal paper published in 2000, Hanahan and Weinberg defined...
the six hallmarks of cancer, defined as functional properties responsible for malignant phenotype that include sustained proliferative signals, evasion of growth suppression, resistance to apoptosis, replicative immortality, induction of angiogenesis or invasion and formation of metastases [3]. The acquisition of these properties is made possible by two characteristics of malignant tissues, i.e., genomic instability of cancer cells and elicitation of the inflammatory response. Both genomic instability and inflammation then promote tumor progression. In a review revisiting the same topic published in 2011, two more hallmarks of cancer were added, including alteration of energy metabolism of cancer cells and evasion of immune response [4]. The importance of the concept of tumor microenvironment encompassing tumor cells, stromal cells and multiple interactions between the numerous cellular populations in the tumor is also being increasingly recognized [4].

For obvious reasons, the introduction of new therapies into the clinical practice has lagged significantly behind the discoveries in the basic research. In fact, in many cancers considerable progress of research of the molecular basis of tumor growth and progression has still failed to translate into the clinic. Laboratory medicine as a specialty has been instrumental in translating the progress in basic science into clinical medicine and has been bridging the gap between basic and clinical research. The role of laboratory medicine in medical, surgical or radiation oncology is by no means limited to the assessment of the tumor burden or dynamics. An at least equally important, although still underestimated aspect of laboratory medicine in oncology, is the detection of toxicity. Today, clinical oncology and laboratory medicine may be regarded as indispensable partners in the care of cancer patients [5].

During the past decades, with the expanding role of laboratory medicine in oncology and the introduction of new medical treatment options, the number of papers dealing with these topics has increased sharply. Oncology has also been increasingly covered in Clinical Chemistry and Laboratory Medicine. The present review aims at highlighting both the currently established and potential contribution of laboratory medicine in oncology, mainly medical oncology. As outlined above, both medical oncology and laboratory medicine are relatively new specialties, and the interaction between the two disciplines is even more recent, therefore the focus in the present review is on more recent papers defining a field that was almost non-existent only 50 years ago.

50 Years of Clinical Chemistry and Laboratory Medicine in the changing world of laboratory medicine and oncology

Numerous parallels may come to the mind when looking back at the past 50 years since the first issue of Clinical Chemistry and Laboratory Medicine. The journal has changed as the world was changing. What began as a German language journal (with some papers in English, and, exceptionally, in French) has been transformed into an international journal publishing exclusively English language papers. The name of the journal changed four times from the original Zeitschrift für Klinische Chemie to Zeitschrift für Klinische Chemie und Klinische Biochemie in 1967, putting the English translation Journal of Clinical Chemistry and Clinical Biochemistry ahead of the German title in 1976, in 1991 changing the title to European Journal of Clinical Chemistry and Clinical Biochemistry and, finally, in 1998 to Clinical Chemistry and Laboratory Medicine. The outside world was changing, too. The journal is still being published in Berlin as it was 50 years ago. The first issue appeared less than 18 months after the construction of the Berlin Wall, just few hundred meters east from the building that serves as the editorial office for the journal. Similarly to this infamous structure that divided the city, Germany, Europe and, finally, the world into two parts that had limited communication, the topics we encountered in the first issues of the Journal were similarly separated from the practice of then nascent specialty of medical oncology. In fact, only a few papers in the first years were dedicated to topics with any relation to cancer or oncology (one paper each in 1963 and 1964, three papers in 1965 and three papers in 1966). Thus, in the first 3 years of the journal (under the title Zeitschrift für Klinische Chemie) only five papers related to cancer were published, much less than in many single monthly issues in recent years. This can be contrasted, e.g., with nine papers accompanied by an editorial covering oncology topics in the October 2011 issue of Clinical Chemistry and Laboratory Medicine [5–14]. Moreover, the predominant topic of communications in the early issues of the journal, the experimental studies on the Ehrlich mouse ascites tumor had little to say to the physicians practicing clinical oncology at that time [15–17].

The science and practice of medicine changed even more rapidly than society. Like the Berlin Wall, the separation between the worlds of clinical chemistry and
laboratory medicine on one side and clinical oncology in the broad sense (encompassing medical, surgical and radiation oncology) has also disappeared, probably less conspicuously, but by no means less permanently. Fortunately, the advances of clinical oncology also resulted in improved prognosis of cancer patients. In one of the papers of relevance to clinical practice, a brief report describing elevations of lactate dehydrogenase published in 1967, the final sentence of the paragraph describing a case of a patient with leukemia is “Tod 14 Tage später” (Death 14 days later) [18]. In contrast, in Clinical Chemistry and Laboratory Medicine, volume 50, we can read a case report of a patient with breast cancer presenting with liver metastases and hyperbilirubinemia who is alive and well on targeted therapy with trastuzumab 7 years after the diagnosis [19].

With time, the number of papers on topics related to oncology in Zeitschrift für Klinische Chemie und Klinische Biochemie, Journal of Clinical Chemistry and Clinical Biochemistry, European Journal of Clinical Chemistry and Clinical Biochemistry and, finally, Clinical Chemistry and Laboratory Medicine increased sharply. The number of papers on topics related to oncology almost tripled in Clinical Chemistry and Laboratory Medicine during the 15 years of the journal bearing this name (Figure 1). Starting from 2009 many issues of the journal include a section “Cancer diagnostics” featuring papers focusing on oncology.

The current role of laboratory medicine in the management of cancer patient

From its humble beginnings, evidenced by scarce papers in the first years of the journal, the role of laboratory medicine in the management of cancer patients has expanded enormously. Molecular changes associated with the tumor arise in the complex interactions of tumor microenvironment, and subsequently spread systemically. Laboratory methods may be used both to study the tumor microenvironment as well as to study tumor effects on the systemic level. Four major areas of clinical chemistry/laboratory medicine in clinical (mostly medical) oncology may be defined, including tumor biomarkers, detection of the host response to tumor, biomarkers of toxicity and measurement/prediction of anticancer drug concentrations.

Tumor markers – the old and the new

The measurement of tumor markers is a traditional topic that has been providing a link between oncology and laboratory medicine for decades. During that time, the well-established paradigm of using tumor marker detection for

Figure 1  Number of papers on oncological topics in Clinical Chemistry and Laboratory Medicine. Shown is the number of papers dealing with oncological topics from 1998 (volume 36, first volume under the title of Clinical Chemistry and Laboratory Medicine) to 2012 (volume 50).
early diagnosis of cancer or cancer recurrence has evolved into a more diversified utilization. The traditional concept of tumor marker, a protein or glycoprotein, has been expanded to cover a wide range of molecules [20], and the term biomarker is now preferred to reflect this molecular diversity. Also peripheral blood is no longer the exclusive sample matrix used for assessment of these biomarkers, and other sample matrices including urine, breath, malignant effusions, secretions or tumor tissue are now being increasingly used.

Increased concentrations of circulating protein or carbohydrate biomarkers ("tumor markers") have been detected across the range of tumors, from common tumors, mainly of epithelial origin (carcinomas) [11, 21–28] to more rare tumors, e.g., meningioma [29]. Despite the many controversies, the advent of tumor markers has virtually transformed the management of some malignant disorders, including prostate cancer, epithelial ovarian carcinoma and hepatocellular carcinoma. The determination of prostate specific antigen (PSA) plays an indispensable role in the early diagnosis as well as the management of prostate cancer [30]. In fact, the introduction of PSA has transformed the way this tumor is treated and, consequently, may have changed the course of this disease. The evaluation of disease course in patients with prostate cancer is largely based on serial PSA measurements. The detection of a sustained rise of PSA is often the basis for the diagnosis of relapse, termed biochemical relapse, and leads to institution of therapy.

Determination of tumor markers also plays an important role in the management of epithelial ovarian carcinoma. Since surgery is the principal prognostic factor in patients affected with this tumor it is important that the diagnosis is established before the procedure and the patient may be referred to an experienced surgeon [31]. The introduction of human epididymis protein 4 (HE4) has provided another biomarker to improve the preoperative diagnosis of epithelial ovarian carcinoma [21–24]. The simultaneous determination of carbohydrate antigen (CA) 125 and HE4 with imaging represents a reliable estimate of the probability of the presence of epithelial ovarian carcinoma [22, 23]. Determination of α-fetoprotein plays an essential role in the diagnosis of hepatocellular carcinoma. In fact, hepatocellular carcinoma represents an exception in the rule that histological or cytological verification is always required for cancer diagnosis. In patients with hepatocellular carcinoma, the detection of high serum concentrations of α-fetoprotein in association with specific imaging findings can be a substitute for histological diagnosis because the biopsy could be associated with considerable risk in some patients. Measurement of α-fetoprotein is used for early diagnosis of hepatocellular carcinoma in patients at risk, to detect recurrence and to follow the patients during or after the therapy [27]. Des-γ-carboxyprothrombin and fucosylated α-fetoprotein are sometimes used along with α-fetoprotein [27].

The role of tumor markers in most other tumors is far less established compared to prostate cancer, hepatocellular carcinoma or epithelial ovarian carcinoma. Much has been said about misuses of tumor marker determination in clinical practice [32], sometimes culminating in what has been termed as "tumor marker terrorism". In fact, virtually all tumor markers currently used in routine practice may be increased in a range of benign disorders as recently comprehensively reviewed by Trape et al. [8]. Increased tumor marker concentrations in subjects with benign disorders represent an important limitation that has to be considered when using tumor marker determination as a diagnostic aid.

While the utility of the determination of "classical" tumor markers may still be, in many instances, controversial, the potential of non-protein molecules as cancer biomarkers is being increasingly recognized. In fact, there has been a constant movement from assessments based on biomarkers only indirectly linked with the pathogenesis of cancer to the detection of biomarkers associated with causal events in the tumor growth and progression. In this respect, the analysis of nucleic acids has been of major importance.

Pathogenesis of cancer involves a sequence of events caused by the alterations in the genome of the cancer cells. Typically, mutations of multiple genes are found that are responsible for the aberrant behavior of the cell. Gene mutations may be associated with a specific tumor phenotype, and, most importantly, the response to therapy. The determination of specific gene mutations has entered routine practice. Some of these mutations are germ-line mutations and indicate high risk for developing a specific tumor, e.g., BRCA-1 mutations associated with breast and ovarian carcinoma [33]. Interestingly, the presence of BRCA1 mutations is associated with specific tumor phenotype and sensitivity to some agents, e.g., platinum compound, and targeted therapy with poly-(ADP-ribose)-polymerase 1 (PARP1) is currently under investigation for the treatment of these tumors. Other mutations are acquired mutations present only in tumor cells. Among these mutations, Kirsten RAS (KRAS) mutations are used to predict the efficacy of monoclonal antibodies targeting epidermal growth factor receptor (EGFR), cetuximab and panitumumab, in metastatic colorectal carcinoma, BRAF mutations are associated with the response to
vemurafenib, a BRAF inhibitor, in malignant melanoma, and EGFR mutations predict the efficacy of low-molecular-weight EGFR inhibitors erlotinib and gefitinib in non-small cell lung cancer [34, 35].

In addition to the detection of DNA mutations, the role of epigenetic aberrations [36], mainly DNA methylation [37–39], but also histone acetylation [40] and microRNAs [6, 41], in the tumor growth and progression has been increasingly recognized in recent years. Although current methods of investigation of epigenetic processes in daily practice have been limited almost entirely to the study of DNA methylation, with the advent of drugs targeting epigenetic pathways, mainly histone deacetylase inhibitors, the applications may spread to the study of histone modifications to follow the efficacy of therapy [42]. Among other classes of nucleic acids, considerable attention has been devoted in the past few years to microRNAs. MicroRNAs are small non-coding RNA molecules that post-transcriptionally modulate gene expression, and are involved in the epigenetic regulation of different biological processes relevant to tumor growth and metastasis, including cell proliferation, apoptosis, differentiation and epithelial-mesenchymal transition [6]. Specifically cell-free circulating microRNAs represent promising new biomarkers, both in aiding the diagnosis and determining the prognosis, in a range of malignant tumors, including lung cancer or epithelial ovarian carcinoma [6, 41, 43].

The role of molecular biomarkers is well-established in predictive diagnostics in relation with targeted therapies. In fact, the utilization of many targeted agents that are active in 5%–15% of patients affected with a particular tumor would not be possible without the use of predictive diagnostics that not only identifies patients likely to benefit from the targeted agents, but also excludes patients in whom there would be no benefit, incurring only cost and toxicity. This concept can be illustrated on the example of human epidermal growth factor receptor (HER)-2-positive breast carcinoma. Historically, in patients with breast carcinoma the over-expression of HER-2 has been associated with poor outcome. The introduction of drugs targeting HER-2 has not only transformed the management of HER-2-positive breast carcinoma, but also served as a paradigm for the introduction of targeted therapy in medical oncology [19]. HER-2 is a 185-kDa transmembrane tyrosine kinase receptor, member of the HER family of receptors that also includes HER-1, HER-3 and HER-4. No ligand has been described for the HER-2 receptor. HER-2 is constitutively active and may undergo ligand-independent dimerization with other HER receptors, leading to tyrosine phosphorylation and activation of downstream signaling [44]. As the over-expression of HER-2 is encountered only in about 15% of patients with breast carcinoma, the determination of tumor HER-2 status is of critical importance. From the methods used to examine HER-2 status of the tumor, immunohistochemistry and fluorescence in situ hybridization (FISH) are used in clinical practice [44]. HER-2-targeted agents are effective only in breast cancer patients with tumors over-expressing HER-2. Given the relatively low prevalence of HER-2 over-expressing tumors, only the identification of a reliable predictive biomarker, in this case the determination of HER-2 over-expression, means that the activity of HER-2 targeted agents could be established. Currently, a number of anti-HER-2 therapies are available or are about to be registered, including humanized monoclonal antibody trastuzumab, low-molecular-weight inhibitor lapatinib, another antibody pertuzumab and an antibody-toxin conjugate trastuzumab emtansine (T-DM1). Recently, trastuzumab has been introduced into the therapy of HER-2 over-expressing metastatic gastric carcinoma. HER-2 protein may also be measured in serum, but in contrast to the tumor HER-2 status, serum HER-2 determination has so far found little use in clinical practice [45, 46]. Other important predictive biomarkers used in medical oncology recently identified, including the presence KRAS mutations in gastrointestinal carcinomas [47], BRAF mutations [35, 48], and EGFR mutations in non-small cell lung cancer [34], have been mentioned above. Complex assays utilizing multigene expression profiles, e.g., Oncotype Dx, that determine the expression of a panel of genes associated with tumor progression and metastases have been introduced into the clinical practice and are used in therapeutic decisions [9].

The technological advances have also allowed the identification and measurement of circulating cancer cells in patients with solid tumors [7]. While the presence of circulating neoplastic cells has been used for decades in the diagnosis and monitoring of the effect of therapy in patients with leukemia, and it has been known for more than a century that circulating tumor cells are present in patients with solid tumors, the detection of these cells has been hampered by an extremely low frequency with approximately one circulating tumor cells in 10^4–10^7 leukocytes [7]. Moreover, the prognostic significance of detection of circulating tumor cells is limited by the phenomenon of metastatic inefficiency, with only one circulating tumor cell in many thousands being able to initiate metastasis [49]. Despite these problems, circulating tumor cells represent an emerging class of cancer biomarkers that allow the most direct assessment of the tumor, close to a “liquid biopsy”. However, a number of studies illustrate the difficulties associated with the detection of circulating tumor cells [50, 51].
Another aspect of laboratory medicine of importance in clinical practice is the detection of infection by oncogenic viruses, e.g., human papilloma viruses that cause carcinoma of the uterine cervix as well as other tumors, including anal cancer and some cases of head and neck carcinomas [52, 53]. Among other biomarkers, the detection of parameters associated with angiogenesis, one of the hallmarks of cancer, is being slowly introduced into the laboratory and the clinic [54]. Serum concentrations of vascular endothelial growth factor (VEGF) and its receptors are increased in patients with advanced cancer [55]. Some data indicate that high circulating VEGF concentrations may predict the response to anti-VEGF therapy, e.g., bevacizumab [56]. Similarly, the determination of matrix metalloproteinases, enzymes that play an important role in tumor invasion and formation of metastases has been introduced [57].

There are many issues in the field of cancer biomarkers that urgently need to be addressed. For example, apart from circulating VEGF there are still no predictive biomarkers for drugs targeting VEGF or VEGF receptors that currently represent the backbone of therapy in some tumors, e.g., metastatic renal cell carcinoma [58], and these therapies are currently prescribed more or less indiscriminately. While most assays used either tumor tissue or peripheral blood (including plasma or serum) as the sample matrix for biomarker determination, methods have been introduced to determine tumor biomarkers in cerebrospinal fluid [59–61], urine [62–64], stool [65], secretions [66] or breath [67–69]. Breath analysis has been in the forefront of research in recent years and volatile organic compounds have been identified as potential tumor biomarkers [67–69]. The presence of volatile compounds associated with tumor growth may also represent an explanation for canine olfactory detection of cancer, a phenomenon that has been well documented [70, 71].

Detection of the host response against tumor

Tumor growth is not only the result of proliferation of populations of malignant cells, but an equally important role may be played by the host response. Until recently, this aspect has been mostly neglected or underestimated. The changing view of the role of the host response can be illustrated by the two reviews by Hanahan and Weinberg that have been already discussed above [3, 4]. It is symptomatic that the earlier review enumerated six hallmarks mostly associated with the properties of tumor cells, while revisiting the topic one decade later the authors included evasion of immune response as an important hallmark and elicitation of inflammatory response as one of the enabling characteristics [4]. It is now evident that tumor growth elicits host response mediated both by specific (immune) and non-specific (inflammatory) signals that can be detected using different laboratory methods. It is being increasingly recognized that immune response in relation to tumor growth is a double-edge sword, and immune and inflammatory phenomena may result in both tumor elimination and progression. Unlike in infectious disorders, the detection of specific immune response (formation of antibodies) is mostly not being utilized diagnostically, but to assess host-tumor interactions. Although the practical significance of the measurement of host response to tumor may be presently viewed as limited, the study of these phenomena is important for the understanding of the pathogenesis of tumor progression and metastatic spread. Moreover, there is cumulative evidence indicating that the host response to neoplasia is an important prognostic factor. With the expanding role of targeted therapies, including agents that modulate immune response, e.g., ipilimumab, in the management of cancer patients, the role of laboratory assessment of immune or inflammatory response is bound to increase.

The specific (immune) and non-specific (inflammatory) phenomena associated with the host response against the tumor may be difficult to separate and many parameters currently used reflect both aspects of the host response. Parameters used to assess the host response include the phenotypic and functional characterization of leukocyte populations, determination of antibodies, cytokines that regulate the host response or small molecules produced or metabolized as the result of host response, and the host response may be studied both at the local and systemic levels. Tumors may, e.g., induce the formation of specific antibodies, such as antibodies against HER-2 in patients with breast cancer [72]. There are reports indicating that the detection of autoantibodies in patients with cancer could be useful diagnostically as demonstrated for anti-survivin antibodies in patients with colorectal cancer [73]. Interestingly, the presence of autoimmune response may be a positive prognostic factor, e.g., the presence of anti-thyroid peroxidase antibodies in patients with breast cancer [74].

The systemic immune and inflammatory phenomena associated with the host response to neoplasia result in marked changes of molecules that may be measured in the laboratory and used to assess the host response. It has been known for many years that cancer progression...
is associated with acute phase response that results in elevations of some circulating proteins, e.g., C-reactive protein, or the decrease of other proteins, e.g., albumin [75, 76]. Among other laboratory parameters, malignant tumors have also been associated with an increase of concentrations of neopterin or changes of tryptophan metabolism.

Neopterin is a pteridine produced by macrophages activated with interferon-γ from guanosine triphosphate (GTP) in a reaction catalyzed by GTP cyclohydrolase I as a first step in a pathway leading to the production of 5,6,7,8-tetrahydrobiopterin. For a reason that is still not fully clarified activated human macrophages possess only the activity of GTP cyclohydrolase I while lacking the activity of enzymes distal to this pathway leading to the accumulation of the large amounts of neopterin. Neopterin may be determined in the serum or in the urine by immunoassay or by high-performance liquid chromatography [77, 78]. Increased neopterin concentrations are observed across a spectrum of disorders ranging from acute myocardial infarction [79, 80], infectious or autoimmune disorders [81] to malignant tumors [82]. Increased urinary or serum neopterin concentrations in patients are also associated with poor prognosis across the range of primary tumors [82, 83]. Moreover, increased neopterin concentrations are associated with the decrease of circulating CD4+ T-lymphocytes [84] and a decline of lymphocyte function [85]. Neopterin may also serve as a biomarker of surgical stress, e.g., in patients undergoing liver resection for cancer or benign disorders [86]. Neopterin concentrations may also increase after administration of systemic chemotherapy [87].

Another enzyme induced by interferon-γ is indoleamine 2,3-dioxygenase that catalyzes the production of kynurenine from tryptophan [88]. Indoleamine 2,3-dioxygenase may be expressed by both tumor cells and monocytes infiltrating the tumor. Tryptophan depletion results in cell growth suppression, affecting both the tumor cell proliferation [89] as well as the proliferation of lymphocytes [90]. Experimental data indicate that the induction of indoleamine 2,3-dioxygenase may result in the suppression of immune response in the tumor microenvironment. Moreover, kynurenine and its metabolite hydroxykynurenine have antiproliferative activity [91].

Activation of the immune response may also be associated with increased production of nitric oxide, although to lesser extent in humans compared to rodents. The production of nitric oxide may be assessed by measuring serum or urinary nitrate concentrations [92]. Increased nitrate concentrations have been described in patients with disorders associated with the activation of the immune system, e.g., inflammatory bowel disease [93], but, in contrast to neopterin or kynurenine, data on nitrate in cancer patients are scanty.

The utility of the concept of tumor microenvironment is evident in relation to the study of the host response against the tumor [94]. At the local level, the presence of leukocytes may be detected histologically in tumors. It has been demonstrated that of particular interest is the presence of lymphocytes that are termed tumor-infiltrating lymphocytes. Across a spectrum of tumors of different primary locations, the presence of tumor-infiltrating lymphocytes has been associated with prognosis [95], or response to chemotherapy [96]. Populations of leukocytes, including lymphocytes and monocytes/macrophages in the tumor microenvironment may be studied even more easily in malignant effusions, e.g., ascites [97, 98], and these measurements may be used for the determination of response to therapy in the tumor microenvironment [99].

The concentrations of molecules associated with immune and inflammatory response, e.g., neopterin or tryptophan, have also been studied in malignant ascites.

### Biomarkers of treatment toxicity

Unless adequately treated cancer is a deadly disease. Therefore, aggressive therapies need to be prescribed for cancer patients. Local treatments, including surgery or radiation therapy, may be very effective in the control of localized tumors, but what kills the patient is usually distant spread resulting in systemic metastases. Systemic disease can usually be treated only by a systemic approach, i.e., drug therapy. Given the catastrophic consequences when systemic treatment fails, anti-cancer drugs are usually administered at maximum tolerated doses. Only in selected cases the problem of systemic toxicity may be circumvented by regional administration of the drug, e.g., hepatic arterial infusion [100]. Due to individual differences in treatment tolerance, the administration of maximum tolerated dose results in serious toxicity in a significant proportion of patients. Therefore, the prediction and early detection of treatment toxicity is of major importance in clinical practice.

The problem of detection of treatment toxicity has been studied for all agents, but is of particular importance for cytotoxic drugs, a class of drugs characterized by low therapeutic index. These drugs damage not only the neoplastic cells, but also normal tissues. The toxicity of cytotoxic drugs may be, in general, divided into toxic effects that are common to most cytotoxic drugs and result from
toxicity on rapidly dividing tissues, and to toxic effects that are specific to a given drug. The toxic effects common to most cytotoxic drugs include toxic effects resulting from the suppression of bone marrow and gastrointestinal mucosa.

The myelotoxicity may easily be detected with simple peripheral blood cell count that can be performed in almost any laboratory. The detection of the second most common side effect of chemotherapy, gastrointestinal toxicity, still relies heavily on complaints reported by the patient that may be unreliable. For some agents, e.g., paclitaxel, gastrointestinal toxicity is the principal adverse event determining treatment tolerance [101]. Currently, there are no laboratory methods used in daily practice that would detect or could be used to follow gastrointestinal disturbances associated with administration of anticancer agents. Promising results have been reported with the measurement of gastrointestinal permeability or determination of serum citrulline concentrations. Testing of intestinal permeability using differential sugar absorption measured as urinary recovery has been established as a useful test in the assessment of disorders of the small intestine, with the results being expressed as a ratio [102]. Changes of intestinal permeability in cancer patients may be detected by determining the disaccharide/monosaccharide (e.g., lactulose/mannitol) ratio [103]. Parameters of intestinal permeability are increased in cancer patients even before the therapy, and it has been demonstrated that the lactulose/mannitol ratio is markedly increased in cancer patients experiencing gastrointestinal toxicity [103, 104]. Gastrointestinal mucosal damage may also be detected by measuring serum citrulline. Low citrulline concentrations have been reported in patients treated with intensive chemotherapy [105]. Gastrointestinal toxicity is also one of the principal manifestations of the graft vs. host disease in patients after allogeneic hematopoietic stem cell transplantation. The results of a pilot study indicate that serum cytokeratin 18 concentrations may represent an early biomarker of gastrointestinal graft vs. host disease [10].

The toxic effects on other organs are less common, but the consequences may still be catastrophic. Prominent among these side effects is cardiotoxicity, a toxicity that typically manifests late after the therapy. Concerns about the devastating consequences of cardiotoxicity of anticancer agents and efforts to identify patients at risk and detect early signs of cardiac dysfunction have led to the constitution of an interdisciplinary field of cardioncology. Early biomarkers of cardiotoxicity have been identified, including troponins, natriuretic peptides and markers of endothelial dysfunction or myocardial ischemia [106]. Cardiotoxicity as a result of anthracycline exposure is relatively rare. Emerging data indicate that a more common problem is the acceleration of atherosclerosis in young patients treated with curative chemotherapy. This phenomenon was first documented in survivors of germ cell tumors. Germ cell tumors typically affect young adults and can be cured even at the metastatic stage. Several studies have noted an increased prevalence of cardiovascular risk factors and a higher incidence of cardiovascular events in survivors of germ cell tumors [107–109]. Laboratory risk factors of atherosclerosis in patients with cancer are, similar to the general population, associated with alterations of parameters of immune and inflammatory response [110]. A negative impact of chemotherapy on the risk factors of atherosclerosis has also been described in patients with breast cancer [111]. Another example of late toxicity with devastating consequences is represented by second primary tumors, including myeloid neoplasms, that may be associated with genetic predisposition [112], e.g., BRCA-1 mutations, that are being increasingly used in testing persons at risk [33, 113].

New therapies are associated with hitherto unknown toxicities. This can be demonstrated in the case of hypomagnesemia induced by the antibodies against EGFR [114]. Hypomagnesemia is, in general, an underestimated problem in clinical medicine [115]. Severe hypomagnesemia that may be observed after anticancer agents, including anti-EGFR antibodies or platinum compounds could be manifested by serious symptoms. Therefore, serum magnesium determination is essential in the management of cancer patients treated with these drugs.

### Monitoring or predicting the concentrations of anticancer drugs

For the drugs with a narrow therapeutic index, e.g., cytotoxic agents, the optimal approach for dosing involves measuring of systemic drug concentrations. With the advent of new technological approaches, the possibilities of monitoring concentrations of anticancer drugs have expanded significantly in recent years. While in clinical practice, dosing based on body surface area is usually used, the actual doses obtained vary significantly in individual patients. The technological advances in liquid chromatography, gas chromatography and mass spectrometry made possible the development of therapeutic drug monitoring in patients treated with cytotoxic chemotherapy [116]. Monitoring of serum concentrations may also be useful in cancer patients treated with targeted
agents. Highly sensitive methods have been developed for a number of agents, e.g., mammalian targeted of rapamycin inhibitors [117]. However, monitoring of therapeutic drug concentrations has still not entered routine use in medical oncology. A number of factors limit the use of therapeutic drug monitoring, including the complexity of drug-tumor interactions, cumulation of side effects or late toxicity, instability of anticancer drugs, heterogeneity and instability of tumor cells, local bioactivation that is not reflected in systemic drug concentrations or variable tumor blood supply [116]. One of the principal reasons for limited utilization of therapeutic drug monitoring is also that in the daily practice the information on drug concentrations may arrive at a moment when it is too late to adjust the medication dose. From this perspective, the prediction of drug concentrations based on pharmacogenetic information seems to be a better strategy compared to serial drug monitoring.

The marked inter-individual differences in the metabolism of anticancer agents have sparked the fast advancement of pharmacogenomic approaches in medical oncology [9]. Broadly speaking, pharmacogenetic analyses in medical oncology may be distinguished into the determination of the presence of appropriate target in the tumor, or to the study of genetic polymorphisms associated with drug metabolism. Genetic analyses of the tumor were introduced to predict response to targeted agents as outlined above. There is some evidence, that tumors associated with germ-line mutations of some genes, e.g., BRCA-1, may be more responsive to certain agents, such as platinum compounds [118]. Of equal importance is the pharmacogenetic analysis of genes involved in the drug metabolism to predict both treatment efficacy and toxicity [9]. The polymorphisms of genes coding for the cytochrome P450 enzymes have been studied for more than a decade [119]. For example, the significance of polymorphisms of the cytochrome P450 (CYP) 2D6 the enzyme responsible for biotransformation of tamoxifen to its active metabolite, endoxifen, is well characterized [9]. Prediction of treatment toxicity may be even more important. 5-Fluorouracil represents a drug constituting the backbone of chemotherapy regimens in gastrointestinal cancer. 5-Fluorouracil is well-tolerated in the majority of patients, but may rarely induce serious, sometimes even fatal toxicity that is associated with polymorphisms of the gene coding for dihydropyrimidine dehydrogenase, the enzyme responsible for 5-fluorouracil degradation. Genotyping may identify patients who are at risk of serious toxicity [120].

Measurement of drug concentrations may be useful not only in anticancer therapy, but also in symptomatic treatment of patients with advanced cancer. A method allowing for simultaneous measurement of urinary concentrations of 19 drugs or metabolites using liquid chromatography-tandem mass spectrometry has recently been introduced [121].

Conclusions

The role of laboratory medicine in the management of cancer patients is rapidly expanding. In the spectrum of different presentations of malignant disorders we can hardly find a situation in which determination of a diagnostic, predictive or prognostic biomarker, a parameter reflecting the host response and/or treatment toxicity, or a study of pharmacokinetic or pharmacodynamic parameters would not play an indispensable role in patient management. The emancipation of clinical chemistry/laboratory medicine as well as medical oncology has been accomplished simultaneously in mutual symbiosis. Instead of the two Cinderellas of 50 years ago we have now two princesses ready to work together to take the leading role in the most prestigious endeavor, saving human lives. As Brugsch and Schütte, the first publishers of this journal, wrote in an article introducing its first issue “...die Aufgabe des Wissenschaftlers ist, zum Wohle der Mitmenschen Wissen und Werte zu schaffen, denn nur dann wird die Wissenschaft bestehen” (“the role of the scientist is to create knowledge and values for the benefit of the fellow humans because only then it is science”) [122]. This sentence is as valid today as it was 50 years ago. The cooperation of physicians and other scientists of different specialties is the only way forward in the global effort to combat cancer.

Conflict of interest statement

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Professor Bohuslav Melichar, MD, PhD was born on March 6, 1965 in Hradec Králové (then Czechoslovakia). After attending, between 1983 and 1989, the Charles University Medical School in Hradec Králové, Czechoslovakia, he graduated in 1989. Between 1989 and 1992 he was a resident in internal medicine at 2nd Department of Internal Medicine, Charles University Medical School and Teaching Hospital in Hradec Králové, Czechoslovakia. In 1992 he entered a Ph.D. program in medical immunology and worked simultaneously as a physician at the 2nd Department of Internal Medicine, Charles University Medical School and Teaching Hospital in Hradec Králové. In 1992 he was a visiting scholar in the University of Innsbruck, Austria, working with Professor Wachter and Professor Fuchs on the pteridines as indicators of immune response. In 1993–1994 he was a visiting scholar in the University of Bordeaux, France, working in the laboratory of Professor Mégraud, focusing on *Helicobacter pylori* infection. Between 1994 and 1996 he was a Fulbright scholar at the M.D. Anderson Cancer Center, Houston, Texas, United States, working first on antitumor activity of macrophages in experimental colorectal cancer, and subsequently in the laboratory of Professor Ralph S. Freedman on immunology of peritoneal carcinomatosis and intraperitoneal immunotherapy. After coming back from the United States he continued working as a physician in the 2nd Department of Internal Medicine and, later, in the Section of Hematology/Oncology of the Charles University Medical School and Teaching Hospital in Hradec Králové, Czech Republic. Simultaneously, he served as an instructor in internal medicine at the Charles University Medical School. In October 1998 he defended Ph.D. thesis “Increased production of neopterin and other indicators of immune activation in patients with malignant tumors and its biologic significance.”

In March 1999 he passed a specialization examination in medical oncology. Since January 2000 he was working as medical oncologist in the Department of Oncology and Radiotherapy of the Charles University Medical School and Teaching Hospital in Hradec Králové. Between March 2001 and August 2001 he was (on a NATO scholarship) visiting scientist in the Department of Gynecologic Oncology of the University of Texas, M.D. Anderson Cancer Center, Houston (working again with Professor Ralph S. Freedman). In 2001 he was appointed associate professor of medicine and in November 2006 full professor. In February 2008 Professor Melichar was appointed the Head of the Department of Oncology, Palacký University Medical School and Teaching Hospital, Olomouc, Czech Republic.

In January 2009 Professor Melichar was appointed associate editor for oncology of *Clinical Chemistry and Laboratory Medicine*. Professor Melichar is currently the president-elect of the International Society of Pteridinology and scientific secretary of the Czech Oncology Society. He is a member of Czech Society of Clinical Biochemistry, American Society of Clinical Oncology and European Society of Medical Oncology. The main areas of interest of Professor Melichar are the study of biomarkers of immune response in cancer patients and the study of new medical therapies for solid tumors, including breast cancer, renal cell carcinoma, ovarian cancer and gastrointestinal malignancies. Professor Melichar is the author of 147 papers in journals with the impact factor, including 93 papers as the first author.