Biomarkers in the treatment of cancer: opportunities and pitfalls

There can be no doubt that laboratory medicine currently plays an essential role in the management of cancer patients [1]. The determination of circulating biomarkers has been used in clinical oncology for decades as an integral part of patient management. Circulating biomarkers are used in screening, contribute to the diagnosis, predict the prognosis, may help with the selection of therapy, can be used to assess the response to therapy or to detect disease recurrence. Nine papers in the current issue of *Clinical Chemistry and Laboratory Medicine* describe different aspects of the utilization of circulating tumor biomarkers in the management of cancer patients across a spectrum of tumors [2-10].

The opportunities and pitfalls in the use of circulating biomarkers can be well demonstrated with the example of carbohydrate antigen (CA) 19-9. As suggested by the title of the review by Galli et al. [9] in the current issue of *Clinical Chemistry and Laboratory Medicine*, CA19-9 certainly represents a biomarker to be handled with great care. It has been known for decades that malignant transformation is accompanied by disturbances of glycosylation [11]. In particular, abnormal glycosylation products are synthesized by the tumor cells, e.g., sialyl-Lewis *a* antigen (CA19-9). However, the synthesis and metabolism of sialyl residues are disturbed not only in tumor cells, but also in a number of inflammatory conditions, liver or pulmonary disorders. For example, because CA19-9 is catabolized in the liver, cholestasis or liver dysfunction may be associated with increased concentrations of this glycoprotein. As a result CA19-9 concentrations are elevated above the threshold in a substantial proportion of patients without malignancy. Moreover, the presence of rheumatoid factor or other antibodies may result in spurious elevation of CA19-9 levels [9]. Thus, even an apparent increase of CA19-9 levels several orders of magnitude above the normal range does not necessarily signify the presence of malignant disease.

Increased concentrations of CA19-9, whether caused by benign conditions or spurious elevations, are obviously cause of anguish for the patient and his family, lead to more investigations or even invasive interventional procedures, accompanied by complications with resulting morbidity and other sequels. The utilization of CA19-9 in clinical practice is further complicated by methodological issues like lack of correlation and interchangeability of the results obtained using different assays.

Highest concentrations of CA19-9 are encountered in patients with pancreatic cancer (pancreatic ductal adenocarcinoma), a tumor with an extremely poor prognosis. As reviewed by Galli et al. [9] serum CA19-9 concentrations are an independent prognostic parameter in patients with pancreatic cancer. However, only a small proportion of patients with pancreatic cancer present with tumors amenable to surgical resection, and only a small proportion of patients undergoing surgery are actually cured. As a result, the incidence of pancreatic cancer essentially equals mortality. The lack of really effective therapeutic options for the majority of patients with pancreatic cancer puts into question the utility of the determination of circulating biomarkers, including CA19-9, to detect recurrence or progression as the therapeutic options in these patients are limited. The utilization of CA19-9 as a tumor biomarker in other gastrointestinal tumors is complicated by the lack of specificity outlined above. Mildly increased CA19-9 concentrations are observed in a substantial proportion of patients without any malignancy, and, similarly, mild elevation of this biomarker is encountered in many patients with history of cancer and no other signs of disease activity. This ambiguity puts into question the very rationale to use serial determination of CA19-9 in tumors other than pancreatic cancer. Moreover, with the exception of isolated liver or lung metastases in patients with colorectal carcinoma, recurrences of gastrointestinal cancers, similar to pancreatic cancer, are mostly incurable, and the advantage of early diagnosis of recurrent disease is questionable in these cases.

The pitfalls and dangers of using circulating biomarkers in general, and CA19-9 in particular, for cancer screening are illustrated in the paper by Tong et al. [10]. The authors retrospectively reviewed CA19-9 concentrations in a large population coming for health check-ups. Among more than 33,000 individuals <2% had an increased CA19-9 concentration. However, only <2% of
these individuals with increased CA19-9 levels were subsequently diagnosed with cancer, and high concentrations of this biomarker were caused in the overwhelming majority of the subjects by non-malignant conditions, including fatty liver, cholecystolithiasis or chronic hepatitis B. In other words, among more than 33,000 individuals, only nine cases of cancer were discovered, and only five patients could be treated with a curative intent, meaning that for each curatively treated case of cancer, CA19-9 has to be measured in more than 6000 individuals. It would have been interesting to see how many tumors were diagnosed, e.g., during the next 6 months in patients with normal CA19-9 concentrations, but this information was apparently not collected. The authors split the patients with increased CA19-9 and no evidence of malignant disease according to the kinetics of CA19-9. During the follow-up, CA19-9 concentrations normalized in two thirds of the subjects while persistent elevation was evident in the remaining third. No significant difference was observed in serum CA19-9 concentrations between subjects with a benign condition and persistently elevated CA19-9 concentrations and the nine patients with cancer. The results of this paper based on the data from a real-world practice emphasize the problems and sometimes even dangers of using circulating tumor biomarkers for cancer screening. Only a small proportion of patients who have increased CA19-9 concentrations harbor an occult malignancy, and many of these tumors may be poorly treatable. However, the prevalence of increased CA19-9 concentrations in the general population is relatively high and these patients and their families may spend weeks in anxiety waiting for the results of further examinations. The utilization of CA19-9 for screening purposes is certainly not justified by the available data. Some clinicians may even feel that the topic of tumor biomarkers has been hijacked for commercial activities that are not supported by medical science. The fears that many people have about being affected with an incurable disease are understandable. While screening (using radiology methods) is effective in lowering mortality in some tumors, notably breast cancer, we have to find the courage to say honestly to the potential patients that currently we do not have any reliable method for screening or even early diagnosis of pancreatic cancer. Moreover, the current success rate in the treatment of patients diagnosed with pancreatic cancer even in operable cases is rather low.

An early stage of development biomarkers associated with protein glycosylation is described in the paper by Kuzmanov et al. [2]. As outlined above, aberrant protein glycosylation is a feature common to almost all cancers. The authors report a pilot study of the sialome, sialic acid containing glycoproteins, in supernatants of epithelial ovarian carcinoma (EOC) cell lines as well as biological fluid of EOC patients using a tandem mass spectrometry-based approach. From a total of 333 proteins and 579 sialylated glycosylation sites that were identified 21 candidate biomarkers were found that should be examined in further studies. EOC is an example of a tumor that is managed by a multimodality approach that heavily relies on the determination of tumor biomarkers both in the diagnostic setting and in the patient follow-up. Circulating biomarkers are essential in helping to preoperatively establish the diagnosis as well as in the assessment of the disease course and the response to therapy by the serial measurement of serum biomarker concentrations, including CA125 and human epididymis protein 4 (HE4) [12–16]. In clinical practice, the concentrations of circulating biomarkers are often measured serially to follow the course of the disease. Although the clinical significance of serial determination of CA125 is controversial [13], and a prospective trial has been interpreted as demonstrating the absence of benefit of serial CA125 measurement to detect early recurrence [17], in another study the detection of CA125 rise increased the chance of optimal secondary surgical cytoreduction that was associated with an improvement of survival [18].

While determination of circulating biomarkers represents an integral part of the management of patients with EOC the role of circulating biomarkers in the most common malignancy in women, breast cancer, is less well defined. Pedersen et al. describe a retrospective experience with three circulating tumor biomarkers, the glycoprotein CA15-3, carcinoembryonic antigen (CEA) and human epidermal growth factor receptor (HER)-2 in the detection of recurrent disease in patients with breast cancer [7]. This study clearly demonstrates the limitations of tumor biomarkers to detect recurrence in this tumor. Less than half of the patients with recurrence had increased concentrations of one of these biomarkers, and even when the three biomarkers were combined less than two thirds of the patients had elevated levels of any of these molecules. Many clinicians would probably disagree with the conclusions of the paper that recommend the utilization of this panel of biomarkers for the diagnosis of recurrence. In fact, the recommendations from laboratory professionals and the clinicians are conflicting with respect to the use of biomarkers to diagnose recurrence. While the European Group on Tumor Markers recommends serial determinations of CEA and CA15-3, the American Society of Clinical Oncology guidelines state the contrary [19]. To understand the clinical point of view one has to realize that there are currently no data proving that early diagnosis of distant
metastases improves survival in patients with breast cancer. Distant metastases are in most cases incurable, and early diagnosis of metastatic disease may numerically improve the prognosis only as a result of the lead-time bias. From a practical point of view the only curable recurrence is the local recurrence, but as also shown in the report by Pedersen et al. [7] increased concentrations of tumor biomarkers are rare in patients with isolated local recurrence. Thus, many clinicians see serial determination of tumor biomarkers as being of little help in the practical management of the patient with breast cancer. The proactive approach with serial determination of circulating tumor biomarkers may have a role in the management of patients when the metastases detected would be amenable to a curative therapy like EOC of liver metastases in colorectal cancer [20].

The advent of targeted therapy had a profound effect on the disease course in patients with HER-2-positive breast cancer [21]. HER-2 is not only an important therapeutic target in breast cancer, but as reported by Pedersen et al. [7] high circulating concentrations of this protein may indicate the presence of distant metastases. Moreover, circulating HER-2 may also be used to assess the response to treatment. The predictive significance of the kinetics of serum HER-2 concentrations is the focus of the report by Petersen et al. [6]. The authors demonstrate that the decrease of HER-2 concentrations was correlated with disease control, while an increase predicted disease recurrence. An interesting observation that may have relevance to the therapeutic scenario was made with regard to the patients with very high circulating HER-2 concentrations. As Petersen et al. [6] indicate, standard doses of trastuzumab may not be sufficient in these patients. Obviously, this needs to be prospectively tested in a clinical trial, especially in view of the cardiotoxicity of trastuzumab [22], but the paper by Petersen et al. demonstrates yet another potential mechanism of trastuzumab resistance. New anti-HER-2 drugs, pertuzumab [23] and trastuzumab emtansine (T-DM1) [24] are currently entering clinical practice and the prediction of response or early detection of the lack of response to anti-HER-2 therapy are of considerable significance.

Most circulating biomarkers currently used are proteins or glycoproteins. Only relatively recently biomarkers that are measured using methods based on determination of circulating nucleic acids have been introduced into the clinic. These biomarkers include cell-free DNA, microRNAs or epigenetic modifications of DNA [25–30]. The significance of epigenetic modifications in carcinogenesis, tumor progression and metastasis is being increasingly recognized [31]. Among different epigenetic modifications, most studies so far have focused on DNA methylation [28–30]. In another paper in the present issue of Clinical Chemistry and Laboratory Medicine Balgkouranidou et al. report the results of the investigation of SOX17 promoter methylation status in 73 patients with gastric carcinoma [8]. SOX17 promoter methylation was detected in 59% of gastric cancer patients, but in none of the control subjects. Moreover, SOX17 promoter methylation was associated with poor prognosis. These results not only offer insight into the molecular pathogenesis of gastric cancer, but also identify a potential diagnostic and prognostic biomarker, or even a potential therapeutic target in this common malignancy that ranks among the tumors considered most difficult to treat.

While, for obvious reasons, the brunt of the research efforts is directed at the commonly encountered tumors like breast cancer, pancreatic cancer or EOC, it is equally important to have biomarkers for rare neoplastic disorders. There are literally hundreds of rare tumors, and the patients with these disorders add up to form a significant proportion of cancer patients. Medullary carcinoma of the thyroid is a rare tumor associated with distinct biomarkers. The measurement of serum calcitonin is used for the diagnosis and follow-up in patients with this uncommon malignancy. However, increased serum calcitonin concentrations are observed in tumors other than the medullary carcinoma of the thyroid as well as in patients with some benign conditions. Giovanella et al. [3] present an interesting experience indicating that simultaneous determination of procalcitonin may help to differentiate among the patients with increased serum calcitonin concentrations those who harbor medullary carcinoma of the thyroid.

In addition to the paper by Tong et al. [10], two other papers in current issue of Clinical Chemistry and Laboratory Medicine are dealing with the issue of false-positive elevation of circulating tumor biomarkers and add to the growing list of non-neoplastic disorders characterized by increased concentration of circulating tumor biomarkers [32]. Lee et al. [4] report on the association of CEA concentrations with non-alcoholic fatty liver disease in healthy Korean non-smokers. Higher CEA was more common in subjects with non-alcoholic fatty liver disease and concentrations increased with severity of steatosis. However, the CEA concentrations were mostly within the normal range in subjects with steatosis. Liver toxicity that may manifest as steatohepatitis is a common side effect of systemic or regional chemotherapy in patients with metastatic colorectal carcinoma, mostly liver metastases [20]. Although it remains to be confirmed whether the steatohepatitis associated with chemotherapy is also accompanied by increased CEA
concentrations, the CEA concentrations observed in steatosis are usually not in a range that would confound the diagnosis of recurrence.

Pulmonary alveolar proteinosis is a rare disorder caused by abnormal clearance of the surfactant in the lung alveoli. In a retrospective analysis of 38 patients with this rare disease Fang et al. report increased concentrations of CEA and cytokeratin 19 fragment (CYFRA21-1) that were above the cut-off established for healthy populations [5]. As mentioned above, serum concentrations of other circulating tumor biomarkers are also increased in non-neoplastic lung disorders, including CA19-9. The interpretation of circulating biomarkers in patients with lung disorders therefore requires great caution.

The papers in the current issue of Clinical Chemistry and Laboratory Medicine have concentrated only on one aspect of cancer biomarkers. This reflects certain imbalance in the research priorities that focus on biomarkers produced by or associated with tumor cells and to some degree neglect other potential areas of biomarker use, e.g., the measurement of host response to tumors [33]. This lack of interest and resulting lack of predictive biomarkers may be one of the key factors responsible for insufficient progress in the use of immune system manipulations in the treatment of cancer. Similarly, while some side effects of anticancer drugs like skin or eye toxicity may be easily monitored in the use of immune system manipulations, determination of biomarker measurements should be tailored for each primary tumor based not only on the sensitivity or specificity of a given biomarker in detecting the recurrence, but also on the availability of therapeutic options in the case of the detection of recurrence.

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