Editorial

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Biomarkers, inflammation and cancer: where to go?

DOI 10.1515/cclm-2016-0769

Laboratory medicine plays an important role in the management of cancer patients [1]. The success in the treatment of cancer is critically dependent on the collaboration of the multidisciplinary team that obviously includes also specialists in clinical chemistry and laboratory medicine. Information from the laboratory has far-reaching consequences that help in guiding the management of the patient from the moments preceding the diagnosis throughout the multidisciplinary therapy to subsequent follow-up or palliative care, and is crucial for the diagnosis, prediction of response and prognosis, monitoring of treatment effect, or detection of toxicity. The introduction of new agents, specifically the drugs targeting defined molecular pathways responsible for malignant transformation, is becoming increasingly dependent on simultaneous development of companion diagnostics to guide the patient selection. Several papers in the current issue of *Clinical Chemistry and Laboratory Medicine* document how laboratory medicine (possibly defined more broadly than we are used) shapes (or may shape) the practice of clinical oncology.

The determination of circulating biomarkers has been an integral part of the management of cancer patients for several decades, and tumor biomarkers such as α-fetoprotein (AFP), carcinoembryonic antigen (CEA), or prostate specific antigen (PSA) are currently widely used in the diagnosis as well as monitoring of cancer patients during the course of disease or treatment. However, the availability and widespread use of tumor biomarkers has also resulted in some misconceptions and misuses. Many of the tumor biomarkers are associated not only with malignant disease, but increased concentrations of these molecules also accompany many other benign disorders, and high concentrations are frequently encountered in patients without a malignant tumor. Therefore, inappropriate testing can result in unnecessary investigations, including invasive procedures, with associated cost, risk of complications and patient anxiety. These issues have been, for some time, widely discussed in the medical community [2–4].

Moreno-Campoy et al. [5] present a retrospective analysis of patterns of requests for tumor markers in a setting of a large center. These descriptive data are of paramount interest with regard to the characterization of clinical practice. As the authors point out, the majority of tumor markers currently used are characterized by variable sensitivity and specificity that is usually suboptimal for a reliable diagnosis. This paper documents widespread use of tumor markers other than PSA or AFP for diagnostic purposes. Using very liberal criteria, only about 40% of requests could be classified as appropriate. As expected, most investigations were negative, but the high percentage of positive results in an absence of tumor pathology is certainly troubling. Confused ordering of tumor marker does not resolve, but rather increases diagnostic uncertainty, leading to further unnecessary investigations, including invasive procedures. Physicians are usually very diligent in selecting medication, and prescribing a drug without clear indication and justification would be viewed as negligence, if not worse. It is therefore paradoxical that the practice of indiscriminate ordering of laboratory test without any justification that may be in its consequences equally harmful to the patient as the prescription of wrong or unnecessary medication remains a widely overlooked issue.

The paper by Moreno-Campoy et al. may serve as a warning against this widespread and dangerous trend. Specifically, some tumor biomarkers like carbohydrate antigen 19-9 (CA19-9) should be ordered with great caution. CA19-9 is increased in many different non-neoplastic disorders. Meanwhile the chance to cure cancers associated with high CA19-9 levels like pancreatic carcinoma is still relatively low. As recently demonstrated in a study that summarized the results of using CA19-9 for screening of a large cohort of apparently healthy subjects, the overwhelming majority of increased CA19-9 concentrations were observed in patients in whom no malignancy could be subsequently identified [6]. Cancer was diagnosed only in anecdotal cases in this large cohort, and only in some of these cases could the tumor be cured. Thus, from a cohort of more than 33,000 subjects who had CA19-9 level determined, only nine patients were diagnosed with cancer, of whom only five could be treated with curative intent [6].

As mentioned above, circulating (mostly protein) tumor biomarkers have been for a long time an indispensable part of the diagnostic armamentarium in cancer patients. Because of relatively low sensitivity and specificity of these protein biomarkers, the search for new biomarkers is ongoing. Yang et al. [7] present a review on exosomal RNAs as a biomarker in cancer. As exosomes play an important role in the pathogenesis of cancer, it is not surprising that the concentrations of exosomal non-coding RNAs such as microRNAs, long non-coding RNAs, and circular RNAs are increased in patients across of spectrum of malignancies. Thus, similarly to tissue or serum concentrations of non-coding RNAs [8–10], exosomal non-coding RNAs are emerging as potential biomarkers across a range of neoplastic disorders, including glioblastoma, non-small cell lung cancer, gastric cancer, colorectal cancer, pancreatic cancer, esophageal cancer, bladder cancer, prostate cancer, breast cancer, melanoma and hepatocellular carcinoma. Importantly, exosomal non-coding RNAs have been analyzed not only in the circulation (serum or plasma), but also in urine of patients with bladder or prostate cancer. Urine (and, possibly, other body fluids) may represent an important sample matrix in this approach [11]. In a sense, the analysis of exosomes could constitute an important step in the direction toward a liquid biopsy.

Hepatocellular carcinoma is a tumor that is particularly difficult to treat. Surgery, the only curative therapy, is possible only in a minority of patients. A major limitation for the surgical treatment of hepatocellular carcinoma is the frequent presence of liver cirrhosis. This limitation can be circumvented by liver transplantation [12], but this approach is for medical or logistical reasons not feasible in the majority of patients. Other liver-directed therapeutic approaches like chemoembolization [13] or hepatic arterial infusion [14] and targeted therapies like sorafenib [15] may prolong life, but are not curative. Therefore, we are still searching for optimal therapy for this tumor that in the world-wide perspective counts among the most common malignant disorders. The presence of cirrhosis not only limits therapeutic options, but also frequently modifies the strategy of performing diagnostic procedures. In fact, because of associated risk, in contrast to cancers of other primary location, tumor biopsy is not considered mandatory in hepatocellular carcinoma if certain conditions, including radiological findings and pre-specified high circulating concentrations of AFP are met. The utilization of AFP measurement to establish the diagnosis of hepatocellular carcinoma in the absence of histological confirmation therefore preceded and to some extent heralded the concept of liquid biopsy. Obviously, the utilization of liquid biopsy as an approach to obtain diagnostic, predictive, and prognostic information is still at a very primordial stage of development in solid tumors, including hepatocellular carcinoma, and we are only starting to estimate the potential implications of this approach.

Searching for new biomarkers, Mžik et al. [16] compared DNA methylation patterns in normal tissue and in hepatocellular carcinoma and found differences in promoter methylation of several genes, some of which were associated with patient prognosis. These differences could serve as basis of the development of biomarkers for the diagnosis and monitoring of therapy in hepatocellular carcinoma. The advancements of liquid biopsy in hepatocellular carcinoma that could follow would be useful not only in the diagnosis, but also for the monitoring of disease course during the treatment, possibly helping to introduce new therapies, including targeted agents.

Gastrointestinal medical oncology remains an arena of unmet medical need. Simultaneous with seeking therapies for mostly incurable malignancies such as advanced hepatocellular carcinoma, gastric cancer, or pancreatic cancer, we should also develop companion diagnostics that would allow us to select the right agent to the right patient, specifically for targeted drugs. In fact, looking back at many of negative trials of new agents published recently, the lack of reliable predictive biomarker could represent one of factors responsible for the failure of a new drug [17–20]. Replicating stories of successful biomarkers like HER-2 expression for anti-HER-2 therapy in breast cancer proved to be difficult [21], and new innovative approaches aiming at biomarker research are needed.

So far, the research on biomarkers has been primarily focused on the biomarkers associated with the properties of the tumor cells. It has, however, been realized that the host response to neoplasia may have similar significance for the outcome as the properties of the tumor cell [22]. Consequently, biomarkers of the host response also need to be investigated and followed.

Over the last decades, a great number of molecules that are associated with the host immune and inflammatory responses have been identified, but only few of these molecules, such as C-reactive protein, are currently being routinely used in the clinical practice. The associated costs, the need for expertise and lack of standardization have been among the principal factors barring the introduction of other biomarkers, most of which, in fact, correlate in some extent with C-reactive protein and, consequently, add limited information. More complex biomarkers have also been investigated. Peripheral blood cell count-derived ratios like neutrophil-to-lymphocyte ratio,

lymphocyte-to-monocyte ratio, platelet-to-lymphocyte ratio, or systemic inflammatory index have been studied extensively in the last few years, mainly because these indices can be calculated retrospectively from the laboratory data available for virtually all patients. Despite obvious advantages associated with the use of peripheral blood cell count-derived ratios, many issues should be kept in mind while using these biomarkers which are nonspecific, and abnormal values are encountered not only in cancer patients, but also in patients with benign disorders. Moreover, significant differences in peripheral blood cell count-derived ratios obtained from automated or manual differential counts have been observed [23]. Interestingly, the peripheral blood cell count-derived ratios do not correlate with some other biomarkers of inflammatory response, e.g. neopterin [23], despite the fact that the circulating biomarkers of inflammatory response like neopterin and peripheral blood cell count-derived ratios are increased in a similar spectrum of disorders ranging from cancer to atherosclerosis and its complications, infections, or autoimmune disorders [24–27]. Most importantly, peripheral blood cell count-derived ratios like other circulating biomarkers may not necessarily reflect the situation in the tumor microenvironment. In fact, the data on biomarkers of inflammatory and immune response in the tumor microenvironment are limited, partly because it is difficult to obtain a sample from the microenvironment [28–30]. Among the biomarkers in the tumor microenvironment, the presence of lymphocytes, the so-called tumor-infiltrating lymphocytes, has been proven as an independent prognostic and predictive biomarker across a spectrum of solid tumors [31, 32]. However, a wider utilization of tumor-infiltrating lymphocytes is still hampered by issues including lack of standardization, potential differences in lymphocyte infiltration among different regions of the tumor or between primary and metastatic lesions and, obviously, the need for repeated biopsy. Peripheral blood cell count-derived ratios are, in essence, relative lymphocyte counts that may represent a form of liquid biopsy assessing the host immune response to the tumor although, as mentioned above, the difference between the local response in the tumor microenvironment and systemic response represents an obvious limitation here. However, lymphocyte counts expressed as ratios to neutrophil, platelet or monocyte counts have been shown, similarly to tumor-infiltrating lymphocytes, to predict prognosis across a wide range of neoplastic disorders [23, 33–36].

The utilization of this approach is demonstrated, again on an example of hepatocellular carcinoma, by Wang et al. [37]. The authors bring the concept of using peripheral blood cell count-derived ratios, in this case systemic inflammatory index, as prognostic biomarkers one step further and examine the prognostic significance of dynamic change of this index before and after resection with curative intent. In the present study, stable low or decreasing value of the systemic inflammatory index was associated with better prognosis after resection. The dynamic change of systemic inflammatory index was an independent predictor of recurrence in the multivariate analysis.

More investigations should be devoted to the biomarkers of inflammatory and immune response elicited by tumor growth. These biomarkers may guide therapy not only by serving as prognostic biomarkers or predictors of therapeutic response, but may also be used to monitor or predict the toxicity of treatment. For example, it has been recently observed that increased concentrations of inflammatory biomarkers like neopterin are associated with gastrointestinal toxicity of anticancer therapy [38]. Gastrointestinal toxicity that is, along with hematologic toxicity, the most common side effect of anticancer therapy is still assessed mostly by patient-reported symptoms. The utilization of laboratory biomarkers in the monitoring of the gastrointestinal side effects is still limited [39, 40]. More attention should be focused on inflammatory biomarkers as predictors of toxicity of anticancer treatment or even on the investigation of direct toxicity induced by these molecules [41].

As outlined above, the tumor elicits a host response that can be detected and has important implications for the management of patients. The host immune response may result in the elimination of malignant cells. In some cases, the tumor cells are not totally eliminated and an equilibrium state between tumor growth and immune response will ensue. Subsequently, malignant cells may escape from the control by the immune system. Inflammatory reaction not only accompanies tumor cell elimination, but also plays an indispensable part in supporting the growth of malignant cells. It may be, however, more difficult to examine the question whether the inflammatory response can primarily contribute to the development of cancer.

Oikonomopoulou et al. [42] present an interesting study reporting a positive association between *Echinococcus granulosus* infection and long-term risk of cancer. Using a Cyprus national registry, the authors report that the incidence of cancer is increased in patients with history of *Echinococcus granulosus* infection. These findings are not surprising as other infectious diseases have been linked to increased cancer risk. However, increased cancer risk has mostly been associated with infections

localized at the primary tumor site, e.g. *Helicobacter pylori* infection in the case of gastric cancer, putatively in association with oxidative stress in the organ microenvironment [43, 44]. In contrast, the increased cancer risk associated with *Echinococcus granulosus* infection seems not to be organ-restricted. The mechanism(s) behind this association remain currently speculative.

Still as a retrospective study, the report by Oikonomopoulou et al. has several potential limitations that have to be acknowledged here. Only a minority of subjects in the registry were finally investigated. Among the cancer types, prostate cancer is prominent. The authors report an increase in cancer incidence in late age. It is well known that the prevalence of prostate cancer in elderly men is high, these tumors tend to be indolent, and over-diagnosis is a real issue here. It may be possible that prostate cancer was diagnosed more frequently in patients with the history of infection because these individuals were followed more diligently. Moreover, the statistical significance is relatively weak. Meanwhile, although this may seem to be only an epidemiological study, it has implications for how we view inflammatory response in patients with cancer. There could be several implications for the pathogenesis of cancer as well as the interpretation of inflammatory phenomena in cancer patients. It is now realized that immune response is crucial in the pathogenesis of cancer that may result in both tumor elimination and enhanced tumor growth mediated by tumor-promoting inflammation [22]. Moreover, the advent of drugs that manipulate the immune response is rapidly changing the landscape of medical oncology. We need to know more about the relation between immune response and cancer, and the study of immune phenomena in cancer patients should not be limited only to identifying prognostic and predictive biomarkers, like peripheral blood cell count-derived ratios, but it might also help us to gain important insights about potential new therapies for this still deadly disease. While deciphering the role of immune system in cancer pathogenesis we should not rely only on increasingly sophisticated laboratory methods, but epidemiological studies could bring new information essential for understanding the role of immunity in cancer.

In conclusion, the papers in the present issue of *Clinical Chemistry and Laboratory Medicine* illustrate how the advances in the broadly defined field of laboratory medicine continue to shape the practice of clinical oncology, including medical, radiation, and surgical oncology. This progress should not only result in better diagnosis, but might also increase our understanding of the pathogenesis of cancer, leading to new therapeutic approaches.

Author contributions: The author has accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

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