Editorial

**Human epididymis protein 4: the start of a post-ROMAn era?**

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As the result of advances in the understanding of molecular bases of cancer and introduction of targeted therapy, laboratory medicine currently plays an indispensable role in the management of cancer patients (1). Traditionally, the measurement of tumor markers has been a topic of major interest for both medical oncology and clinical chemistry/laboratory medicine. With time the well-established paradigm of utilizing tumor marker determination for the diagnosis of cancer or tumor recurrence has evolved into a more diversified concept that also encompasses the prediction of patient prognosis or response to therapy (2). Moreover, peripheral blood is no longer the exclusive sample matrix used for assessment of tumor markers, and other sample sources including urine, breath, malignant effusions, secretions or tumor tissue are being used with increasing frequency. The term biomarker has been coined to reflect the increasing diversity of this concept.

Epithelial ovarian carcinoma (EOC) is the leading cause of death for gynecological cancer. The high mortality rate of EOC is due to the fact that the tumor is usually diagnosed at a late stage, when the chance of a definitive cure is quite low. However, the survival of patients affected with EOC has improved substantially over the recent decades with the introduction of multimodality treatment approach that includes radical surgery and systemic or regional (intraperitoneal) chemotherapy. Consequently, rather than a rapidly fatal malignant disorder, EOC now often takes a chronic course characterized by episodes of recurrent disease that may be repeatedly controlled by systemic chemotherapy (3).

For the past several decades, the determination of tumor markers has played an important role in the management of patients affected with EOC. The utilization of tumor markers is best documented in establishing the diagnosis of EOC. As a general rule histological confirmation is required for diagnosis of cancer across the spectrum of different primary tumors. Unlike other tumors, e.g., breast cancer, endometrial cancer or most gastrointestinal tumors, EOC is not easily accessible to endoscopic or percutaneous biopsy. The biopsy in patients presenting with suspected EOC can usually be performed only during surgical procedure using laparoscopic approach or even laparotomy. However, the quality of the primary surgery represents the principal factor determining the prognosis in EOC patients, and poorly planned procedure with unexpected or even surprising diagnosis of EOC that ends up only as diagnostic operation will jeopardize the patient prognosis. It has been demonstrated that the patient outcome is substantially improved if the patient is operated in a high-volume center by an experienced surgeon (4). Thus, it is critically important that the diagnosis of EOC be established before the surgery and the patient could be referred for the procedure to a center that can deliver multidisciplinary care (5).

Different algorithms have been proposed over time for non-invasive preoperative diagnosis of EOC. The introduction of human epididymis protein 4 (HE4) has provided another biomarker that could improve the preoperative diagnosis of epithelial ovarian carcinoma (6–9). The simultaneous determination of carbohydrate antigen (CA) 125, imaging findings or HE4 represents a relatively reliable estimate indicating the probability of the presence of epithelial ovarian carcinoma (7, 8, 10, 11). The Risk of Malignancy Index (RMI) is an algorithm combining ultrasound findings of the pelvic mass, CA125 concentrations and menopausal status, while the Risk of Ovarian Malignancy Algorithm (ROMA) combines menopausal status with the serum concentrations of CA125 and HE4 (10, 11). It has been demonstrated that the sensitivity of ROMA is better than the sensitivity of RMI is detection of EOC (11). However, it has been also demonstrated that in premenopausal women the diagnostic performance of ROMA was not better than HE4 alone (7). Although most studies examining the role of HE4 in diagnosis EOC were performed in the Western world, the utility of the determination of serum HE4 in the diagnosis of EOC is not limited to Western countries as indicated by a recent study demonstrating increased HE4 concentration in Korean patients affected with EOC (6).

The utilization of biomarkers in the management of patients with EOC is not limited to establishing the diagnosis. As outlined above, the course of EOC now resembles more or less a chronic disorder. This course is often characterized by repeated recurrences of the disease that are controlled by chemotherapy before recurring again (3). The problems associated with the diagnosis of recurrence, estimation of prognosis and prediction of response are of obvious importance. In patients with EOC, the peritoneal cavity represents the most common site of tumor spread, and the disease is frequently limited to the peritoneum. However, the marked prolongation of survival resulting from the multimodality treatment appears to be changing (or rather revealing) the natural history of EOC, and more patients live long enough to develop distant metastases. Among sites of distant relapse, central nervous system metastases, once considered extremely rare, are now being diagnosed with an increasing frequency (12). As with other chronic disorders, chronic complications are becoming manifest in cancer patients that result from the...
long-term toxicity of therapy, including accelerated atherosclerosis (13, 14), second primary tumors (15) or the natural evolution of the disease, e.g., metastases in hitherto unusual sites such as the central nervous system (12).

The utilization of tumor markers in monitoring the course of disease, including the assessment of response to treatment or the detection of recurrence is more controversial. Although serial determinations of serum CA125 concentrations have been routinely used in the surveillance of EOC patients, in a clinical trial that examined the timing of institution of second-line chemotherapy and randomized between initiation of therapy at the detection of CA125 relapse versus delaying the initiation of therapy until symptomatic or clinical relapse in patients with EOC in complete remission after first-line chemotherapy, no survival difference was found between patients treated immediately after detection of CA125 rise and patients treated at the time of symptomatic or clinical relapse (16). This result has been interpreted as meaning that using CA125 measurements in surveillance has no practical significance. Consequently, the practice of serial determination of CA125 in patients previously diagnosed with EOC has been abandoned by many centers. However, the randomized trial reported by Rustin et al. (16) has been subjected to wide criticism. One of the points raised was that this trial used as the treatment of recurrence only chemotherapy and not secondary cytoreductive surgery. Moreover, there was considerable heterogeneity in the chemotherapy regimens used, and the choice of drugs and/or schedules may be regarded as suboptimal in a large proportion of patients in that trial. In a retrospective study analyzing patients undergoing secondary cytoreductive surgery at a single institution, early surgery after a CA125 rise was associated with increased chance of optimal secondary surgical cytoreduction (17). Optimal secondary surgical cytoreduction was associated with significantly improved survival (median 47 months vs. 23 months in patients with suboptimal resection), and the chance of optimal cytoreduction decreased by 3% with each week of delay after the detection of increased CA125 concentrations (17).

The predominant pattern of spread of EOC in the peritoneal cavity may result in difficulties of response assessment using imaging techniques, e.g., computed tomography or magnetic resonance imaging, that are commonly used to define the response to therapy in patients affected with other solid tumors. The response to chemotherapy is associated with a rapid decrease of CA125 concentrations. It has been demonstrated that the results assessed using the response defined by standard radiological criteria or by CA125 decrease correlate (18). Thus, similarly to prostate specific antigen in patients with prostate cancer, CA125 may be used to define the response to therapy in a portion of patients with EOC. The utility of HE4 in the assessment of therapeutic response remains to be defined.

The utilization of HE4 as a tumor biomarker is not limited to EOC. Increased concentrations of HE4 have also been reported in other tumors, notably endometrial cancer. In the spectrum of gynecologic malignancies, endometrial cancer is in many respects an antipode of EOC. Unlike EOC, endometrial cancer manifests early by vaginal bleeding, resulting in timely diagnosis and surgical therapy that is curative in most cases. Therefore, in contrast to EOC, most patients with endometrial cancer are cured by surgery alone or surgery combined with external beam radiation and/or brachytherapy. Therefore, although in developed countries endometrial cancer is the most common gynecological malignancy, patients with advanced/metastatic disease are rare to the extent that metastatic endometrial cancer may be regarded almost as an “orphan” disease and prospective trials in metastatic endometrial cancer are difficult to perform. The prognosis of patients with advanced/metastatic endometrial cancer is poor, and the need for new treatments and biomarkers that would allow the prediction of prognosis and response to therapy is being urgently felt. However, in contrast to EOC the data on the systemic treatments and utilization of biomarkers are much more limited in endometrial cancer. Both hormonal therapy and cytotoxic chemotherapy have shown efficacy in metastatic endometrial cancer. Hormonal therapy with gestagens such as megestrol acetate can lead to long-term disease control in individual patients, but the algorithm for how to select therapy between hormonal and cytotoxic treatments has not been completely defined. Disease control with cytotoxic chemotherapy is low and the duration of response usually short, and more prospective clinical trials are needed that would also include new targeted agents.

The opinion paper by Plebani (9) in the current issue of Clinical Chemistry and Laboratory Medicine (CCLM) covering wide range of research topics also provides a comprehensive state-of-the-art summary of current utilization as well as front-line research investigations on the topics related to use of HE4 in cancer diagnosis and management. The data indicate that the concentrations of HE4 are age-dependent and affected by smoking or renal function. In many studies, HE4 was determined simultaneously with CA125. Interestingly, HE4, unlike CA125 is not increased in patients with endometriosis, offering a potential in differential diagnosis between this benign disorder and EOC. In EOC patients increased HE4 is associated with poor prognosis. Some data indicate that serial determination of serum HE4 concentration may reflect the response to therapy better than the concentrations of CA125 (9). The potential role of changes of HE4 concentrations in the assessment of response to chemotherapy is one of the aspects that certainly deserve further study. Importantly, although a large part of investigations reviewed in the opinion paper by Plebani (9) focused on ROMA, the paper also shows that the utilization of HE4 goes beyond ROMA, both in finding use in patients with EOC for other purposes like prediction of prognosis or monitoring after primary therapy, or being used as a biomarker in tumors other than EOC. Only time and further studies will tell whether these investigations really herald the advent of a “post-ROMAn” era in HE4 research.

Practically all tumor markers currently used in clinical practice, including CA125 and HE4, are known to be increased in patients with benign disorders and no evidence of malignancy (19). Elevation of concentrations of tumor markers in patients with benign conditions represents an important limitation when using tumor marker measurement in the diagnostic work-up. This topic is also addressed by the paper
by Hertlein et al. (20) in the current issue of this journal. The authors examined serum HE4 concentrations in a large cohort of patients with cancer and patients suffering from non-cancerous conditions (the use of the term “benign” may not be appropriate when speaking about potentially fatal disorders such as liver cirrhosis or chronic obstructive lung disease). As expected, increased HE4 concentrations were observed in patients with EOC, with differences being observed between EOC cases of serous and mucinous histology. High HE4 concentrations were also observed in patients with lung cancer (20). Among patients with non-cancerous disorders, very high concentrations were observed in patients with renal failure and liver cirrhosis (20).

The utilization of HE4 as a diagnostic and prognostic biomarker in patients with endometrial cancer is reported by Zanotti et al. on a large cohort of patients (21). Serum HE4 concentrations were significantly increased in patients with endometrial cancer compared to normal controls. Serum CA125 was also increased in endometrial cancer patients, but when specificity was set at 95%, sensitivity in detecting endometrial cancer was 66% for HE4, 33% for CA125 and 64% for the combination of both biomarkers. HE4 concentrations were associated with menopausal status, age, stage, depth of tumor invasion, presence of cervical invasion, lymph node involvement, adnexal involvement, positive peritoneal cytology, lymphovascular invasion and grade. Importantly, increased serum HE4 concentrations were predictive of disease-free survival and overall survival in univariate as well as multivariate analyses.

Along with “classical” tumor markers, other biomarkers play a role in the management of EOC, including BRCA mutations or parameters associated with the immune response against the tumor. Mutations of BRCA-1 gene are associated with very high risk of breast cancer and EOC (22). The presence of BRCA-1 mutations is associated with increased sensitivity to some agents, e.g., platinum compounds, and targeted therapy with poly-(ADP-ribose)-polymerase 1 (PARP1) inhibitors is currently under investigation for the treatment of BRCA-1-associated tumors. In the present issue of CCLM Vietri et al. (23) describe the experience with analysis of mutations of BRCA-1 and BRCA-2 genes in the Italian population, including the description of a new mutation. The detection of mutations in the BRCA-1 and BRCA-2 genes is important not only in identifying the persons at high risk of cancer, e.g., EOC, that may be prevented by bilateral oophorectomy, but there is cumulative evidence that EOC associated with BRCA-1 germ-line mutations is more responsive to platinum compounds, the backbone of chemotherapy regimens in this tumor, and EOC patients harboring BRCA-1 mutations have better prognosis (3, 24–27). Thus, determination of BRCA mutation status is an indispensable part of the diagnostic work-up in EOC patients.

There are data suggesting that in cancer patients biomarkers associated with the immune and inflammatory response may be of comparable significance to the circulating tumor markers, e.g., HE4 or CA125, or the presence of BRCA mutations, but for various reasons this topic has for long been relatively neglected. The most convincing proof of existence of an immune response against the tumor is the detection of cells mediating the immune system in the tumor tissue or tumor microenvironment. The presence of tumor-infiltrating lymphocytes (TIL) may be determined immunohistochemically and represents an independent prognostic factor in EOC (28). The phenotype and function of leukocyte populations, including lymphocytes and monocytes/macrophages in the tumor microenvironment may be studied even more easily in malignant ascites that is commonly encountered in patients with advanced EOC (29, 30). Serial measurements of biomarkers of the host response against the tumor may be used for the assessment of response to therapy in the tumor microenvironment (31). Among molecules associated with systemic response of the host to neoplasia, neopterin, a pteridine compound produced from guanosine triphosphate by macrophages activated with interferon-gamma, has been extensively studied in EOC and endometrial cancer (32). Neopterin can be measured in serum or urine using immunoassay or high-performance liquid chromatography (33, 34). Although increased neopterin concentrations are encountered in different disorders ranging from acute myocardial infarction (35, 36), infections or autoimmune disorders (37) to malignant tumors (32, 38), and, therefore, increased concentrations of this marker are non-specific, neopterin represents a powerful prognostic indicator. High neopterin concentrations in cancer patients are predictive of poor prognosis across the spectrum of different primary tumors, including EOC or endometrial cancer (32, 38). In patients with advanced cancer, increased neopterin concentrations are correlated with decreased circulating CD4+ T-lymphocyte counts (39) and decreased lymphocyte proliferation (40). Interestingly, neopterin concentrations may also increase after administration of systemic chemotherapy (41). The possibility to measure neopterin in urine offers a potential of repeated sequential measurements of this biomarker. It has been demonstrated in patients with EOC that changes of urinary neopterin concentrations reflect the disease course (42). It may be hypothesized that, similarly to the combined indices such as RMI or ROMA, that are used in the diagnostic setting, the combination of measurements of serum biomarkers CA125 and HE4 with the parameters of immune response like TIL counts and/or serum or urinary neopterin could lead to improved prognostication in patients with EOC. This approach certainly deserved further investigation in prospective studies.

The utilization of cancer biomarkers has increased markedly with the advent of targeted therapy. Targeted agents are now entering into the therapeutic armamentarium for gynecological malignancies. Bevacizumab, the monoclonal antibody against vascular endothelial growth factor, is currently being introduced into the treatment of advanced EOC. Other targeted agents have shown promising results in EOC or endometrial cancer. High circulating concentrations of VEGF are associated with the response to bevacizumab (43), but the measurement of VEGF concentrations is fraught with some methodological problems (44) and is still not used in routine practice. VEGF is a molecule with powerful immunosuppressive activity (45), and one of the mechanisms of action of anti-VEGF therapy may be the restoration of the host immune function.
response to the tumor. However, the data on biomarkers of the host response, e.g., neopterin, during bevacizumab therapy are very limited (46).

In conclusion, HE4 now represents an established biomarker used in the management of EOC. The utility of serum HE4 measurements is by no means limited to establishing the diagnosis of EOC, as a component of complex algorithms such as ROMA, but may also include the assessment of response to therapy. The potential of this biomarker in the management of not only EOC, but also other malignant tumors, e.g., endometrial cancer, has not yet been fully realized. Only future prospective studies will determine whether the promise of HE4 will be maintained in the “post-ROMAn” era.

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