carcinomas (22 of 49; 45%; \( P < 0.01 \)). Similarly, LPB expression was found more frequently in ER-positive (43 of 53; 81.1%) than ER-negative samples (20 of 38; 52.6%; \( P = 0.007 \)). MGB, on the other hand, showed no relationship with ER status.

Of 24 nonmalignant nonbreast tissues, MGA was detected in only 1, i.e., in a sample of nonmalignant ovarian tissue. MGA was not found in any of eight nonbreast carcinomas (for details, see Tables 3 and 4 in the online data supplement). In contrast to MGA, MGB was detected in 4 of 15 nonmalignant breast tissues and in 1 of 7 nonbreast malignant samples, whereas LPB was present in 3 of 10 nonmalignant breast tissues and in 4 of 8 nonbreast malignant tissues.

In addition to sensitivity for early disease and specificity for cancer, organ specificity is a desirable property of a tumor marker. At present, prostate-specific antigen is a rare example of a marker with relative organ specificity. Our data presented here together with other reports \((4-6)\) suggest that expression of MGA is mostly, although not exclusively, confined to breast tissue. MGA may therefore be one of the first relatively mammary-specific markers. Its potential applications include \((a)\) the detection of breast cancer micrometastasis in lymph nodes, peripheral blood, and bone marrow; \((b)\) identification of metastasis of unknown origin, i.e., presence in such a metastasis would suggest a likely breast origin; and \((c)\) detection of early breast cancer. The last might be possible with the development of an ELISA for measuring MGA and its application to serum or urine.

References


Predicting Response of Ovarian Cancer to Paclitaxel Treatment Based on Trend Analysis of Serum CA125, Mirka Nekula, Ladislav Pecen, Renata Kalabova, Marta Simickova, Ondrej Topolcan, Richard Pikner, Vladimir Vondracek, and Dalibor Valik (Departments of 1 Laboratory Medicine and 3 Gynecology, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 65 Brno, The Czech Republic; 2 Institute of Computer Science, Pod Vodarenskou vezi 2, Prague, The Czech Republic; 4 Department of Nuclear Medicine, Charles University, E. Benes 13, 30599 Pilsen, The Czech Republic; * author for correspondence: fax 420-5-4321-1169, e-mail valik@mou.cz)

Recent randomized studies have reported significantly improved quality of life and survival in cancer patients who underwent tumor marker-directed treatment compared with patients treated according to Union Internationale Contre le Cancer (UIICC) criteria [see Ref. (1) and references therein]. According to these studies, systematic analysis of tumor markers may document whether therapy is effective and should be continued in spite of its ultimate adverse toxic effects or whether it should be terminated because of ineffectiveness. The advantages of biochemical assessment are objectivity and reproducibility as well as the fact that tumor marker assays are subjected to longitudinal external and internal quality control. We aimed to determine whether changes in serum concentrations of the tumor marker CA125 evaluated by a newly developed algorithm predict response to chemotherapy in paclitaxel-treated ovarian cancer patients.

Ovarian cancer patients with advanced disease (stage III at the time of diagnosis; \( n = 101 \); median age, 56 years; age range, 33–69 years) were enrolled in a prospective study monitoring changes in serum CA125 and the effect of therapy. Patients were treated with paclitaxel (Taxol; Bristol-Myers-Squibb) as part of a first-line combination chemotherapy. They received a median of six courses (range, three to nine) of chemotherapy. During a total of 689 chemotherapy cycles, serum CA125 concentrations were evaluated (AxSYM; Abbott Laboratories) and externally controlled through the CAPTMX tumor marker survey. Serum samples were obtained before starting therapy (specimen \( c_0 \)) and before each chemotherapy cycle (specimens \( c_1-c_n \)). The univariate Cox regression model and the stepwise Cox regression model were used for selecting the optimal variables that most accurately predicted disease-free interval. The statistical package SAS, release 8.22 (SAS, Inc.), was used. The time-to-progression (TTP) in different patient groups was compared using Wilcoxon and log-rank tests. CA125 concentrations between groups of responders and nonresponders to therapy were compared using the Wilcoxon test for unpaired data, the median test, and analysis of variance.

When we used 30 units/mL as a cutoff value for CA125, it indicated disease recurrence with the following parameters, based on our patient database \( n = 1378 \) ovarian cancers; clinical remission in 972 cases, progression in 406
PHREG procedure (SAS, release 8.22) for calculating optimal prediction of TTP. Cox model, variables stepwise Cox model (Table 1). In a multivariate stepwise regression analysis of CA125 concentrations in patients with ovarian cancer treated with paclitaxel. Significant health outcomes have been identified for use in making practice guidelines in clinical oncology based on overall survival, including disease-free survival; quality of life; reduced toxicity; and cost-effectiveness (2). The therapeutic efficacy evaluated in individual cancer cases according UICC criteria has several weaknesses: a lack of sensitivity in measuring the extent of metastatic disease and a lack of reproducibility between clinicians in interpreting changes seen from imaging methods (3). A recent American Society of Clinical Oncology statement (4) stipulated that tumor markers are the preferred method for monitoring disease progression in patients on systemic therapy when the disease is not easily assessable by imaging techniques (i.e., blastic bone metastases). The important advantage of marker measurement compared with imaging methods is that increases in tumor markers may draw attention to disease progression in distant organ(s) that may not have been studied by imaging methods. Some randomized studies have reported important improvements in quality of life and survival (1) for patients with marker-directed treatment compared with patients treated according to UICC criteria (5). Monitoring of therapy efficacy is emerging as a valuable clinical application of serum tumor markers, and positive correlations have been reported between changes in concentra-

Table 1. Comparison of univariate and multivariate models for TTP and variables \( c_0 - c_n / c_{n+1} \) (describing ratios of CA125) in patients with ovarian cancer treated with paclitaxel.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Cox model</th>
<th>Multivariate stepwise Cox model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( x^2 )</td>
<td>( P )</td>
</tr>
<tr>
<td>( c_0 )</td>
<td>19.0156</td>
<td>0.0001</td>
</tr>
<tr>
<td>( c_1 )</td>
<td>18.8399</td>
<td>0.0001</td>
</tr>
<tr>
<td>( c_2 )</td>
<td>19.0156</td>
<td>0.0001</td>
</tr>
<tr>
<td>( c_3 )</td>
<td>4.530</td>
<td>0.0329</td>
</tr>
<tr>
<td>( c_4 )</td>
<td>10.3017</td>
<td>0.0013</td>
</tr>
<tr>
<td>( c_5 )</td>
<td>3.4731</td>
<td>0.0624</td>
</tr>
<tr>
<td>( r_{c_1} )</td>
<td>21.6914</td>
<td>0.0001</td>
</tr>
<tr>
<td>( r_{c_2} )</td>
<td>20.1284</td>
<td>0.0001</td>
</tr>
<tr>
<td>( r_{c_3} )</td>
<td>4.0313</td>
<td>0.0447</td>
</tr>
<tr>
<td>( r_{c_4} )</td>
<td>2.0313</td>
<td>0.1543</td>
</tr>
<tr>
<td>( r_{c_5} )</td>
<td>0.6766</td>
<td>0.4108</td>
</tr>
<tr>
<td>( r_{c_1/r_{c_2}} )</td>
<td>10.6484</td>
<td>0.0011</td>
</tr>
<tr>
<td>( r_{c_2/r_{c_3}} )</td>
<td>0.3593</td>
<td>0.5489</td>
</tr>
<tr>
<td>( r_{c_3/r_{c_4}} )</td>
<td>0.2123</td>
<td>0.6450</td>
</tr>
<tr>
<td>( r_{c_4/r_{c_5}} )</td>
<td>2.0288</td>
<td>0.1543</td>
</tr>
</tbody>
</table>

Fig. 1. Kaplan–Meier analysis of TTP in a group of 76 patients with ovarian cancer who responded clinically by disease regression to administration of paclitaxel therapy. Patients were further segregated to subgroups of good responders (top line), who fulfilled the criteria \( r_{c_1/r_{c_2}} > 1.31 \) and \( r_{c_2} > 1.6 \) AND \( r_{c_3} > 1.26 \) (43 patients), and less favorable responders (bottom line) not fulfilling those criteria (33 patients). The true TTP is also shown in 17 censored patients (○), who are still undergoing chemotherapy.
tions of some markers and the response to systemic therapy in patients with cancer (6). A decrease in tumor marker concentrations to normal was reported to indicate remission of disease (7, 8) or to predict response to therapy (9).

Taxanes are, at present, established compounds considered effective in the treatment of ovarian cancer (10). Evaluation of CA125, but not carcinoembryonic antigen, was reported to be useful according to some studies (11, 12). Normalization of CA125 after the first course of treatment corresponded to a 53% survival rate, and the rate of decrease in CA125 concentrations was a sensitive predictor of the likelihood of achieving complete remission and prolonged survival (13). A decrease in CA125 was an independent prognostic factor for survival of women with advanced ovarian cancer and allowed identification of a high-risk population among patients with advanced ovarian cancer (providing the possibility of considering second-line chemotherapy earlier). The discrimination power of serial CA125 determinations for long-term survival seemed to be temporary, however, and prediction of the outcome for individual patients was much less precise (14). Several definitions for trends in serum CA 125 have been proposed (15–17), but the only definition that has been validated prospectively is that of Rustin et al. (18).

A recent analysis of 1396 patients involved in the testing of 14 investigational drugs for relapsed ovarian cancer in phase II clinical trials found that CA125 response rates were slightly higher than standard response rates, by a factor of 1.11, and could therefore provide a reliable, less expensive, and more available means of identifying active drugs worthy of further study (19). Some authors have raised concerns that specific drugs, such as paclitaxel, may render the CA125 concentration unreliable for indicating response (20). This question has been reexamined recently in 144 patients treated with paclitaxel in four different trials using the 50% and 75% response criteria defined above (10). Progression-free survival for responders compared with nonresponders was equivalent regardless of whether CA125-based criteria or standard response criteria were used. The false-positive rate for CA125 response was <3%, suggesting that if a decrease in the CA125 concentration indicates a response, then there is a response in 97% of cases and radiologic reassessment is probably not warranted. False-negative rates, however, were higher, reaching ~21%, and stopping treatment based on the absence of a response according to CA125 alone would therefore pose the risk of undertreating patients. Such undertreatment could be avoided if therapy was continued until there was evidence of progression by clinical, radiologic, and/or CA125 criteria.

In summary, we were interested whether sequential changes in tumor marker concentrations computed by a newly developed algorithm (21, 22) reflected a response to chemotherapy in paclitaxel-treated advanced ovarian cancer patients to predict further courses of disease. Our results support, in principle, those presented by Rustin and coworkers (18, 23) and Peters-Engl et al. (14).

In addition, we think that assessing therapy efficacy based on serum CA125 after the second cycle (and/or after the third cycle and radiologic confirmation of progressive disease) opens an option for clinicians to consider appropriate modification of chemotherapy. Such an approach may contribute to individualization of treatment of ovarian cancer patients.

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References

Allergy is estimated to be the sixth leading cause of chronic disease in the US, and the number of people exhibiting symptoms of an allergic response to various natural and synthetic compounds has increased dramatically in the last decade (1). The prevalence of food allergies is particularly evident in children; some reports estimate that the frequency of food allergies in infants is 2–5% (2). The identification of the specific IgE responsible for the clinical symptoms can be a costly and lengthy procedure. Various immunoassays, such as ELISA (3), the Radio Allergo Sorbent Test (4), and high-capacity solid-phase tests, e.g., the CAP system (Pharmacia) (5), are currently used in the diagnosis of allergies and have the inherent sensitivity and specificity to detect IgE in human serum. However, all of these assays are time-consuming, require large quantities of serum samples, and use poorly characterized antigen preparations. Moreover, none of the assays currently used in the diagnosis of allergies has the throughput to screen for the most common allergens, which are estimated to exceed 300.

High-density ordered arrays of molecules (microarrays) (6, 7) may circumvent most of the current limitations in the diagnosis of allergies by allowing simultaneous and multiparametric analysis of serum reactivity against a variety of antigens. Protein microarrays in combination with fluorochrome-labeled secondary antibodies have been used to reveal the presence in human serum of IgG and IgM directed against microbial antigens (8). However, this assay format is not suitable for the serodiagnosis of allergies. A signal enhancement procedure is required to reveal the presence of subnanomolar concentrations of analytes, such as serum-specific IgE. One such method has recently been described (9); here we report on an alternative methodology.

We developed a high-sensitivity assay to reveal the presence of specific IgE in human sera that combines allergen microarrays with tyramide signal amplification. Using contact printing with high-speed robotics (Total Array System; BioRobotics), we have arrayed on silanized glass microscope slides (CEL Associates) a variety of allergens together with human IgE at different concentrations. The allergens (Dermatophagoides pteronyssinus, D. farinae, Alternaria alternata, Olea europaea, Artemisia vulgaris, Dactylis glomerata, and house dust) were supplied by Radim S.p.A. Human IgE (purified from myeloma plasma) was purchased from Calbiochem Corporation. Allergens were extracted with 1× phosphate-buffered saline (PBS); 0.2 g/L KCl, 1.44 g/L Na2HPO4, 0.24 g/L KH2PO4, 8 g/L NaCl, pH 7.4) containing Tween 20 (0.1 mL/L) and printed using the same solution. Human IgE was printed using 1× PBS containing 0.1 mL/L Tween 20 and 1 g/L sodium dodecyl sulfate. Allergen preparations were spotted at 10 g/L, each spot being ~1 mL (10 ng). Arrays consisted of a 7×7 matrix that included the IgE internal calibration curves in duplicate and the allergens in quadruplicate (see Fig. 1B). Slides were handled and stored as described previously (8). Printed slides were incubated overnight at room temperature with a solution containing 20 g/L bovine albumin in PBS to block non-specific antibody binding. An adhesive tape (Abgene Limited) was used to contain samples/reagents within the array area. Sera (100 μL) were incubated at room temperature for 60 min. After washing (five times; each time with 1 mL of PBS containing 0.1 mL/L Tween 20), the following secondary antibodies were assessed for their ability to reveal serum IgE bound to the printed allergens:

1. Alexa 532-labeled anti-human IgE (OEM Concepts Inc.) at a final concentration of 27 mg/L in a solution of 2× PBS containing 10 g/L bovine serum albumin (BSA) and 0.1 mL/L Tween 20
2. A biotinylated anti-human IgE (KPL) at a final concentration of 10 mg/L in a solution of 2× PBS containing 10 g/L BSA and 0.1 mL/L Tween 20, followed by Alexa 546-streptavidin (Molecular Probes Inc.) at a dilution of 1:50 as detailed in the product data sheet
3. A horseradish peroxidase (HRP)-labeled anti-human IgE (KPL) at a final concentration of 10 mg/L in a solution of 2× PBS containing 10 g/L BSA and 0.1 mL/L Tween 20; bound secondary antibody was detected by incubating the slides with Alexa 546 tyramide conjugate (Molecular Probes) diluted 1:100 with Molecular Probes diluent, as detailed in the product information (10)
4. A biotinylated anti-human IgE at a final concentration of 1 mg/L, followed by incubation with HRP-streptavidin at a dilution of 1:100 as detailed in the product information (10); bound streptavidin-antibody complexes were detected by incubating the slides with Alexa 546 tyramide conjugate (Molecular Probes) diluted 1:100 with Molecular Probes diluent (10)

All incubations were conducted at room temperature: 60 min for the secondary antibodies and 15 min for the tyramide reagents. Before the fluorescence was read in the scanner (S5000; Packard Biosciences), the slides were washed and dried at 37 °C. Images were generated with the ScanArray™ software provided by Packard Bio-