Clinical Significance of Neopterin for Prognosis and Follow-up in Ovarian Cancer

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ABSTRACT

Neopterin, a pyrazinopyrimidine compound, is a marker of activation of cell-mediated immunity. The prognostic value of pretherapeutically and of serially measured urinary neopterin levels in patients with ovarian cancer was assessed, in a blinded manner, by analysis of 658 urine specimens from 74 women. The specimens were collected during a 5-year period (January 1981 to January 1986). Thirty-one deaths due to cancer were observed during the study period. By statistical analysis, a significant predictive value of pretherapeutic neopterin levels was found and compared with that of other possible prognostic clinical and laboratory findings. Multivariate analysis, using stratification by tumor stage, demonstrated that this predictive information was independent of other variables. A significant association was found between serial neopterin measurements and the current risk of death during follow-up (P < 0.0001). In addition, current death risk was correlated with neopterin levels measured 6 months previously (P = 0.026). The histological outcome at surgical reexamination was correlated with the current neopterin levels (P = 0.016). Further, normal neopterin levels in women with evidence of tumor at surgical reexamination were shown to be a sign of better prognosis than elevated levels. Measurement of urinary neopterin levels during follow-up of women with ovarian cancer appears to be a valuable adjunct to conventional techniques, particularly in patients refusing explorative surgery.

INTRODUCTION

Ovarian carcinoma is predominantly a disease of older white women of northern European extraction. A major problem in ovarian carcinoma is late detection of occult disease. Most patients present with tumor extension beyond the pelvis or positive retroperitoneal lymph nodes or with distant metastases. It is the only female genital malignancy that is surgically staged (1). Besides primary surgery at diagnosis, the role of surgical reexamination is established in the management of the disease to evaluate, after an adequate course of aggressive chemotherapy, the response to therapy in a patient thought to be clinically free of disease (2).

Diagnosis and monitoring of ovarian cancer present a difficult task, and there are few laboratory tests helpful for that purpose. A monoclonal antibody, OC 125, has been developed and has shown sensitivity for the management of this disease (3).

First results obtained for women with genital neoplasms have demonstrated that there is a close correlation between urinary levels of neopterin and course of disease (4). In carcinoma of the uterine cervix, preoperative levels of neopterin have been demonstrated to bear significant prognostic information (5). In vitro, neopterin, a pyrazinopyrimidine compound which is biosynthetically derived from GTP, is produced by human macrophages specifically upon induction by supernatants from activated T-lymphocytes, the active principle being γ-interferon (6, 7). In vivo, it contributes clinically significant information in diseases which regularly involve the activation of cellular immune mechanisms, e.g., infectious diseases (8, 9), allograft rejection episodes (10, 11), or autoimmune diseases (12, 13).

This study presents an analysis of the prognostic potential of pretherapeutic neopterin levels in urine of women with ovarian carcinoma. Further, an analysis is presented on the relationship between serial measurements of neopterin and the current risk of death during follow-up. Finally, in women having received surgical reexamination, the relationship between neopterin and the outcome of surgical reexamination as well as the prognostic significance of neopterin for subsequent survival after reexamination are studied.

PATIENTS AND METHODS

Patients. All women (n = 74) with histologically verified ovarian carcinoma who were diagnosed and under medical care in the University Hospital, Innsbruck, Austria, between January 1981 and January 1986 were included in the study. The closing date for follow-up was April 30, 1986.

Staging was performed according to the criteria of the International Federation of Gynecology and Obstetrics (1). The median follow-up time was 23.1 months. All of 11 patients with stage I carcinoma survived, the median survival time in 17 women with stage II carcinoma was 38.3 months (4 deaths), in 35 women with stage III carcinoma it was 21.0 months (18 deaths), and in 11 women with stage IV carcinoma survival was 13.3 months (9 deaths).

Histologically, 2 patients had germ cell tumor, 2 had granulosa cell carcinoma, and 70 were found to have epithelial ovarian carcinoma. For statistical analyses, all 3 histological types were combined.

The treatment schedule was essentially unchanged during the study period: Patients with stages Ia (n = 8) and Ib (n = 1) received hysterectomy and bilateral salpingooovarectomy and were irradiated 6 weeks later. All patients with higher stages were treated postoperatively with a polychemotherapy scheme (doxorubicin and cisplatin, each 50 mg/m² monthly. In January 1985, doxorubicin was replaced by epirubicin, a less cardiotoxic anthracycline analogue. In 17 patients, radical surgery was possible. In 36 women, the remaining tumor was smaller than 2 x 2 cm; these were classified as "subradically" operated. In 21 women, only palliative tumor reduction was possible. In these latter patients, early second look operation was performed following 5–6 courses of chemotherapy if they were responding well to the treatment. In general, after about 12 months of chemotherapy, responsive patients were surgically reexamined if the patients agreed. The second look operation was in strict adherence to the following schedule. In the absence of gross tumor, peritoneal washings were obtained for cytological examination. Multiple strips of peritoneum, biopsies of adhesions, and any other suspicious areas were removed; additionally the appendix was resected. If all specimens were negative, treatment was discontinued after 5–6 cycles of melphalan. Patients with evidence of disease received further treatment with cisplatin and doxorubicin (epirubicin). In no patient was cytostatic treatment delayed by severe side effects.

Laboratory Examinations. BC, thrombocyte number, hematocrit, hemoglobin level, and erythrocyte sedimentation rate were determined at diagnosis and during follow-up by commonly used procedures. Neopterin was determined by an optimized and fully automated high pressure liquid chromatography technique without previous oxidative treatment as described elsewhere (14); modifications are detailed in...
Ref. 12. Within-run precision of the method was 4.7% and day-to-day precision was 5.8%. Recovery experiments yielded a mean value of 99.3% (14). By this reverse-phase technique, urinary creatinine is simultaneously determined in the same urine specimen. To account for variations of urine concentrations, neopterin levels are related to these creatinine values and are expressed as μmol neopterin/mol creatinine. The native fluorescence of neopterin is used for detection (353 nm excitation, 438 nm emission wavelengths); creatinine is quantitated based on the UV absorption at 235 nm. The determinations were performed in first morning urine specimens. Either analyses were done immediately or specimens were stored at -20°C until analysis.

Pretherapeutic values of the laboratory variables were not always obtainable for all women. Therefore, the numbers of patients in different groups do not necessarily total 74.

Regarding neopterin, the study was performed in a blinded manner. All clinical decisions, diagnostic as well as therapeutic, were made without being aware of the current neopterin levels; the neopterin measurements were done without prior knowledge of the patient's clinical status. In general, determinations of neopterin were performed only at scheduled visits. In 7 of the patients that have died on ovarian carcinoma, however, 1 to at most 3 extra determinations were done during the 3 to 4 weeks preceding death. Laboratory normal values remained unchanged for all tests during the study period.

Statistical Procedures. The associations between pretherapeutic laboratory measurements and tumor stage were assessed by the Kruskal-Wallis rank sum test. Spearman’s rank correlation coefficients were computed for all possible pairs of pretherapeutic variables. The effect of individual pretherapeutic data on prognosis was examined by stratifying the patients on the basis of each variable in turn and computing the product-limit survival curves (15, 16) and the generalized Wilcoxon log rank statistic (Breslow test). Deaths from other causes were handled as censored observations throughout the study. For the continuously coded laboratory variables, the quartiles were used as strata limits. Thus, 4 strata were formed on the basis of each variable without prior knowledge of the number of deaths in each stratum. No age adjustments were used for the laboratory data. Stepwise multivariate regression analysis based on the proportional hazards model (16) was used to assess whether neopterin was an independent predictive factor.

To study the relationship between serial laboratory measurements and risk of death, a time-dependent version of the proportional hazards were undertaken. Tumor stage was used as a stratification variable. A time window for “valid” measurements was defined (17). The value at time t was taken to be the nearest prior measurement, provided that this value remained unchanged for all tests during the study period.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
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<tr>
<td>Neopterin (μmol/mol creatinine) (P = 0.0086)*</td>
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<td>299</td>
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<td>421</td>
<td>483</td>
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<td>Erythrocyte sedimentation rate (mm/h) (P = 0.22)</td>
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<td>63</td>
<td>35</td>
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<td>41</td>
<td>52</td>
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<td>8</td>
<td>57</td>
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<td>253</td>
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<td>30</td>
<td>10</td>
<td>63</td>
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<td>10</td>
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<tr>
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<td>10</td>
<td>63</td>
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<td>14</td>
<td>30</td>
<td>10</td>
<td>63</td>
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</tbody>
</table>

* Level of significance of difference among stage groups; Kruskal-Wallis test.

RESULTS

Pretherapeutic laboratory measurements are shown in Table 1, dependent on tumor stage. Of these 6 variables, only neopterin levels were significantly associated with tumor stage. Of the clinical characteristics, age at diagnosis and the body weight per length ratio were not correlated with stage (not shown). Pretherapeutic neopterin levels were weakly correlated with erythrocyte sedimentation rates (Spearman’s rank correlation coefficient, 0.34; P < 0.05); no significant correlations were observed with the remaining laboratory tests as well as with age and the body weight per length ratio (P > 0.05).

Analysis of Pretherapeutic Clinical and Laboratory Measurements. When the patients were stratified by the quartiles of each pretherapeutic laboratory or clinical variable in turn or by stage (4 levels) or surgery (3 levels), a significant prognostic effect was found only for neopterin (P = 0.015, Breslow test), and age was of borderline significance (P = 0.060). Stage (P > 0.13) as well as surgery (P = 0.23) showed a weak but not significant effect, whereas the other variables were not associated with survival probability (P = 0.30). Fig. 1 shows the survival curves for patients grouped by neopterin, age, stage, or surgery. For clarity, only two subgroups were formed for each variable by median values.

Multiple stepwise regression analyses using the assumption of proportional hazards were undertaken. Tumor stage was used for stratification. Age and laboratory data were coded according to their quartiles. As in univariate analysis, only neopterin was found as a significant predictor of survival, independent of stage (regression coefficient, 0.487 ± 0.227 (SE); P = 0.025, test of the “improvement x²”). Fig. 2 shows
Patients were stratified by either tumor stage, primary surgery, pre-therapeutic neopterin levels, or age at diagnosis. The medians of observed distributions were used for classification.

Patients were divided into subgroups according to tumor stage or primary surgery and within these subgroups stratified according to pretherapeutic neopterin levels. Carcinoma were not eligible for these analyses since no pretherapeutic data were recorded. The remaining 2 women with nonepithelial tumors were alive at the end of the study period and were thus treated as censored observations.

Analysis of Serial Laboratory Measurements. The data base of serial levels of laboratory tests was composed of 400 hematocrit values (median, 36%; 10th percentile, 30%; 25th percentile, 33%), 452 erythrocyte sedimentation rates (median, 30 mm/h; 75th percentile, 50 mm/h; 90th percentile, 80 mm/h), 658 neopterin values (median, 235 μmol/mol creatinine; 75th percentile, 375 μmol/mol creatinine; 90th percentile, 640 μmol/mol creatinine), 1009 platelet counts (median, 190/ni; 75th percentile, 235/ni; 90th percentile, 290/ni), 1024 WBC counts (median, 5000/μl; 75th percentile, 6500/μl; 90th percentile, 8000/μl), and 1025 hemoglobins (median, 117 g/liter; 10th percentile, 100 g/liter; 25th percentile, 107 g/liter), determined at 1051 patient examinations.

Assessing the association between current risk of death and each variable separately, significance was found for neopterin (regression coefficient b, 1.127 ± 0.232; P < 0.0001, two-sided test of the ratio regression coefficient to its standard error), leukocyte count (b, 0.978 ± 0.221; P < 0.0001), and thrombocyte number (b = 0.528 ± 0.213; P = 0.013). Stepwise multivariate extension of this analysis showed that only neopterin levels and leukocyte numbers were simultaneously significant indicators of increased current risk of death. For neopterin, b = 1.0075 ± 0.2637 and P = 0.0002; for leukocytes, b = 0.6391 ± 0.2341 and P = 0.0064.

When the levels of the laboratory variables taken were those measured 6 months before, a significant association with current death risk was found only for neopterin (b = 0.5168 ± 0.2335; P = 0.026). No other variable was significant, either analyzed separately or in combination, at a lag time of 6 months.

Serial Laboratory Measurements and Second Look Laparotomy. Of the 30 women with surgical reexamination, 28 had a neopterin measurement immediately (i.e., less than 30 days) before surgery. In 14 of these no evidence of tumor was found histologically, and in 13 of the latter neopterin levels were below the median value of serial neopterin data, at 235 μmol/mol creatinine. Of the 14 women with histological evidence of tumor, 7 had a neopterin level above this value. Thus, neopterin levels were significantly associated with absence or presence of tumor (P = 0.016, Fisher's exact test of the corresponding two-way contingency table). Of the remaining 5 laboratory variables, however, none was significantly correlated with the histological evaluation at second look (P > 0.1).

A product limit analysis of survival subsequent to reexamination was possible for 25 women (3 patients were followed just until reexamination). Fig. 3 shows the survival functions observed for these women divided into four categories (no evidence of disease or evidence of disease and normal neopterin or elevated neopterin). A significant difference of the survival curves was found. Considering only women with evidence of disease at reexamination (Fig. 3, Curves C and D) a significant difference was also found for women with normal (Curve C)

Fig. 1. Cumulative survival functions observed in 52 women with ovarian carcinoma. Patients were divided into subgroups according to tumor stage or primary surgery and within these subgroups stratified according to pretherapeutic neopterin levels below (—) versus above (---) the median value of 275 μmol/mol creatinine.

Fig. 2. Cumulative survival functions observed in 52 women with ovarian carcinoma. Patients were divided into subgroups according to tumor stage or primary surgery and within these subgroups stratified according to pretherapeutic neopterin levels below (—) versus above (---) the median value of 275 μmol/mol creatinine.

Fig. 3. Cumulative survival functions observed in 25 women subsequent to second look laparotomy. Patients were divided into subgroups according to result of second look surgery (evidence or no evidence of tumor) and neopterin level at second look (below or above the median of serial neopterin measurements, 275 μmol/mol creatinine). Curve A, no evidence of tumor, elevated neopterin at second look (7 patients, 6 deaths); Curve B, no evidence of tumor, normal neopterin at second look (10 patients, 1 death); Curve C, evidence of tumor, normal neopterin at second look (7 patients, 2 deaths); Curve D, evidence of tumor, elevated neopterin at second look (7 patients, 6 deaths).
versus elevated neopterin levels (Curve D) at reexamination (\( P = 0.016 \), Breslow test).

The neopterin patterns observed in 7 women that had refused second look laparotomy are shown in Fig. 4, a and b. Three women were alive at the closing date of the study (Fig. 4a); they exhibited normal (2 cases) or slightly elevated neopterin (1 case) values throughout. In the remaining 4 women who had died during the observation period, elevated neopterin levels indicated progression of disease at least 5 months preceding death (Fig. 4b). Fig. 4, c–e details neopterin levels seen in the 3 women that correspond to the deaths in Fig. 3, Curve B (both normal neopterin and no evidence of tumor at reexamination, and Fig. 3, Curve C (evidence of tumor, but normal neopterin at reexamination). In these women, neopterin levels were elevated during at least 6 months preceding death.

The neopterin levels immediately before death among the 31 women that had died during the study period were all elevated. Dependent on the interval between last neopterin measurement and death, the values showed a marked increase immediately before death (not shown).

DISCUSSION

Neopterin is not a tumor-specific marker. Rather, it was shown in in vitro experiments that neopterin is produced in large amounts by human monocyte/macrophages (7). At present, no other cell type has been identified which is capable of neopterin production, even by a large variety of stimuli. Recombinant human \( \gamma \)-interferon is as potent in inducing the phenomenon as are supernatants from allogeneically activated T-cells. Addition of monoclonal antibodies directed against human \( \gamma \)-interferon to supernatants from stimulated T-cells completely suppressed neopterin release from monocyte/macrophages (7). In vivo, conditions known to be associated with activation of cell-mediated immunity lead to enhanced neopterin excretion in urine (8–13). Simultaneous determination of \( \gamma \)-interferon and neopterin levels in recipients of renal allografts during cellular rejection episodes demonstrated a good correlation between the 2 parameters (18). According to the present knowledge, neopterin can be regarded as a sensitive tool for monitoring the activation of cellular immune reactions. It is not, however, simply a marker identifying very sick patients. For example, clinically healthy infants after receipt of live measles-mumps vaccines showed very sharply increasing neopterin levels that normalized rapidly when antibodies were formed (19). None of the investigated children presented with clinical symptoms. In Tanzanians who had low grade malarial parasitemia, highly elevated neopterin levels were found, again in the absence of clinical disease (9).

In the present study which was stimulated by the finding of a prognostic value of neopterin in cervical carcinoma (5), it was found that, after correction for stage or therapeutic modalities, pretherapeutic neopterin levels provide a significant information on prognosis whereas 5 comparable laboratory tests that were selected because they are easily accessible and not tumor specific failed to yield predictive information. The immunological marker CA 125, as defined by the monoclonal antibody OC 125 (3), was not investigated since during the first years of this study the test was not available to us.

An indicator of a malignant process should not only give predictive information at time of diagnosis but should enable continuous monitoring of the patient’s state. Thus, serial levels of neopterin and the 5 other laboratory variables were also analyzed. Neopterin levels were found to provide the most significant information concerning risk of death due to the carcinoma. Of the other tests, WBC number was found to be significant in combination with neopterin as an indicator of current risk of death. Since there was neither a relationship between pretherapeutic WBC and tumor stage or prognosis nor a significant association with the result of surgical reexamination, it seems likely that the observed elevation of WBC numbers in patients shortly before death is due to secondary endstage complications causing leukocytosis as infectious or inflammatory phenomena.

The analysis of neopterin patterns in women who refused surgical reexamination and in those who died in spite of normal neopterin levels at surgical reexamination revealed two arguments in favor of neopterin measurements for monitoring ovarian cancer: (a) a normal neopterin level combined with evidence of tumor at second look indicates a better prognosis whereas a high neopterin value and evidence of tumor seem to predict a rapidly progressive process; (b) probably even more important, neopterin measurement appears to provide a noninvasive diagnostic alternative particularly in those patients who, for several reasons, refuse explorative surgery.

Several limitations and possible weaknesses of the statistical analyses should be noted. In the analysis of pretherapeutic neopterin levels, the crossing hazards (Figs. 1 and 2) might invalidate the assumption of proportional hazards. On the other hand, the good agreement of the results obtained with this
model and the results of the log rank tests in the subgroup analysis (Fig. 2) argues that the prognostic effect of pretherapeutic neopterin levels should be real. A further source of possible bias is the inclusion of data from (few) unscheduled examinations of patients during the 3 to 4 weeks preceding death. Since there is a significant association of elevated neopterin levels measured 6 months before time t with an elevated death risk at time t, however, this problem does not appear to invalidate the conclusions. Prior studies in patients with gynecological tumors have failed to detect a significant influence of histological type on neopterin levels (4); therefore a bias due to the inclusion of 4 women with nonepithelial tumors seems unlikely. No attempts have been made to compute correlation coefficients between serial levels of neopterin and serial determinations of clinical variables as weight loss since the intervals during examinations of the patients were not strictly regular nor was there a constant frequency of determinations per patient.

At first glance, the poor prognosis associated with high levels of an indicator of cellular immunity might seem surprising. For example, cancer patients often present with reduced levels of most in vitro immune functional tests. A possible explanation for this apparently paradoxical situation might be that the permanently high neopterin levels in cancer patients indicate permanent presence and, hence, continued recognition of antigenic structures by the immune system. This permanently activated immune state, associated obviously with the inability of the immune system to efficiently clear the host organism from tumor might be coupled with depressed in vitro tests. Thus, antigen recognition appears to be intact in cancer but effector functions appear to be at least ineffective. Interestingly, recent research has demonstrated the in vivo presence of preactivated T-cells in conditions known to be characterized by reduced in vitro functional tests (20). Additionally, activated macrophages might even promote tumor cell growth by secreting growth factors or helping in angiogenesis (21). Other evidence that activation of immune processes need not necessarily be of benefit for the host of a tumor stems from similarities between malignant and autoimmune disorders (22).

Summarizing the results, the study shows that the determination of urinary neopterin levels may be a valuable additional help for management of women with ovarian cancer. Particularly in patients refusing exploratory surgery, this test can be regarded as a sensitive and noninvasive alternative to conventional methods.

REFERENCES
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