Type I and Type III Collagen Metabolites as Predictors of Clinical Outcome in Epithelial Ovarian Cancer

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ABSTRACT

We evaluated the significance of biochemical tumor markers, i.e., aminoterminal propeptide of type III procollagen, trivalently cross-linked COOH-terminal telopeptide of type I collagen (ICTP), aminoterminal propeptide of type I procollagen, and CA 125 in the prediction of ovarian cancer outcome and compared them with several classical indicators of prognosis. The concentrations of biochemical markers were determined from the preoperative serum specimens of 55 patients with epithelial ovarian cancer. In the univariate analysis, all biochemical markers except PINP and all conventional prognostic indicators except histological subtype correlated significantly with survival. In the multivariate Cox analysis of biochemical markers, serum ICTP remained the only significant prognostic indicator of overall survival. Among all variables, clinical stage and ICTP were the only independent and significant determinants of prognosis. Because the content of trivalently cross-linked, mature type I collagen (the breakdown of which is detectable in the ICTP test) in malignant ovarian cancer tissue has been reported to be lower and that of bivalently cross-linked and non-cross-linked collagen has been reported to be higher than in benign tumors, the source of excess ICTP in the circulation of ovarian cancer patients is most likely the degradative damage of soft tissues surrounding the progressively growing malignant lesions. The serum ICTP concentration can thus be regarded as an indicator of the invasion of ovarian cancer. Such information is not available by conventional methods. Therefore, the ICTP test will improve the accuracy of predicting clinical outcome in this disease.

INTRODUCTION

About two thirds of the patients with epithelial ovarian cancer have a poor prognosis (1–2). A large number of clinico-pathological variables, such as clinical stage, histological subtype and grade, volume of residual tumor after surgical resec-

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2 The abbreviations used are: PIINP, aminoterminal propeptide of type III procollagen; ICTP, cross-linked carboxyterminal telopeptide of type I collagen; PINP, aminoterminal propeptide of type I procollagen.

There are also much data to suggest that the biological nature of the tumor is important for the aggressiveness, clinical behavior, and curability of ovarian carcinomas (4). The most important and widely used biochemical tumor marker among those identified thus far is CA 125. It reflects the activity of the epithelial cells of coelomic origin of the tumor and mesothelium (5, 6).

The fibrillar type I and type III collagens are essential for the integrity and consistency of soft tissues of all kinds, including soft tissue tumors. Our research group has investigated the metabolism (7–10) and clinical usefulness (7, 11, 12) of these collagens in ovarian cancer. PIINP2 (2), an indicator of type III collagen metabolism, has been a useful complement to CA 125 in monitoring the clinical behavior of ovarian cancer (13). ICTP (2), an indicator of the degradation of mature (trivalent cross-links) type I collagen, also reflects the clinical changes in this illness during cytotoxic chemotherapy (12).

In this study, we explored the clinical significance of the preoperative serum concentrations of PIINP and ICTP as predictors of the clinical outcome of ovarian cancer patients. Because the synthesis and breakdown of type I collagen are concomitantly enhanced in ovarian cancer (10), we also evaluated the clinical value of PINP (2), an indicator of type I collagen synthesis, in this function. We compared them first with the serum concentration of CA 125, a golden standard as a tumor marker in ovarian cancer, and then with the conventional clinical and histopathological indicators of prognosis.

MATERIALS AND METHODS

Fifty-five preoperative blood samples of ovarian cancer patients operated on in the Oulu University Hospital during 1989–1995 were available for PIINP, ICTP, PINP, and CA 125 determinations. The clinical records of the patients were reviewed for age, clinical stage, histological type and grade, presence of ascites, size of residual tumor after staging and debulking laparotomy, and therapy. The median age of the patients was 53 years (range, 24–73). The study was approved by the Ethics Committee of the University of Oulu.

All of the tumors were epithelial, the majority of them being serous (n = 32), whereas the rest were mucinous (n = 9), endometrioid (n = 6), clear-cell (n = 3), or undifferentiated (n = 5) tumors. According to the classification of the International Federation of Gynecology and Obstetrics, there were 21 stage I, 4 stage II, 24 stage III, and 6 stage IV tumors. Twelve
tumors were well-differentiated (grade 1), 18 were moderately (grade 2) differentiated, and 25 were poorly (grade 3) differentiated. The surgery was carried out using a vertical midline incision. Maximal possible cytoreduction was performed, and all palpable enlarged lymph nodes were removed. Extirpation of all visible or palpable tumor was possible in 28 patients. The cytoreduction was optimal (defined as the reduction of all tumor lesions to <2.0 cm in diameter) in 10 and suboptimal in 17 patients. Twenty-seven patients had ascitic fluid at primary laparotomy (defined as ≥100 ml fluid in the peritoneal cavity). At least four courses of platinum-based (cis-platinum 50 mg/m²) combination chemotherapy with cyclophosphamide (500 mg/m²) and epidoxorubicin (50 mg/m²) were given to 53 patients at 4-week intervals. Two patients died within 2 months after operation without any further therapy. None of the patients received radiotherapy. None of the patients had bone metastases at any phase of follow-up.

The median follow-up time was 35 (range, 1–107) months. At the end of the follow-up, 27 patients were alive (21 without any evidence of recurrent disease) with a median follow-up time of 50 (range, 29–107) months, 26 had died of ovarian cancer after a median follow-up time of 26 (range, 1–75) months, and 2 had died of concurrent diseases at follow-up times of 41 and 95 months. Follow-up data were available for all of the patients.

For statistical analyses, the patients with stage I and II as well as grade 1 and 2 tumors were combined and tested against those with stage III-IV and grade 3 carcinomas, respectively. For the same purpose, the patients were divided into two histopathological groups. For the biochemical indicators, PIIINP, ICTP, PINP, and CA 125, the most accurate cutoff values to discriminate between survivors and nonsurvivors, were calculated. These values were used in the univariate survival analysis.

The blood samples obtained before surgery were centrifuged, and the sera were immediately frozen and stored at −20°C until assayed. The PIIINP (14), ICTP (15), and PINP (16) concentrations were determined by equilibrium radioimmunoassays for the human antigens using kits from Orion Diagnostica (FIN-90460 Oulunsalo, Finland). The upper limits of the reference interval for serum PIIINP, ICTP, and PINP were 4.2 mg/l, 4.6 mg/l, and 79 mg/l, respectively. Serum CA 125 was assayed with kits from Centocor Europe (Tongeren, Belgium). Concentrations >35 units/ml were considered elevated. All assays were performed in duplicate.

Survival analyses were performed according to the Kaplan-Meier method (17). Survival was calculated as corrected survival from the date of laparotomy to the date of death or to the closing date of this study. Univariate analyses were carried out using the log-rank test, and multivariate analyses were carried out using the Cox model with backward variable elimination and the Wald statistic. In the multivariate analyses, the serum PIIINP, ICTP, and CA 125 concentrations and the patients’ ages were used as continuous variables, and the other prognostic indicators were used as categorical covariates.

RESULTS

The serum concentrations of PIIINP, ICTP, PINP, and CA 125 were higher than the upper reference limit in 40%, 49%,...
8%, and 82% of the cases, respectively. The serum concentrations of PIIINP, ICTP, and CA 125 were significantly higher in the patients with clinical stage III and IV disease, grade 3 carcinoma, and residual tumor after debulking laparotomy than in the respective reference groups (Table 1). The serum PINP concentrations were significantly higher in the patients with grade 3 carcinomas than in those with grade 1–2 carcinomas, whereas there was no substantial difference in the different clinical stage or residual disease categories.

The outcome of the patients was evaluated with respect to the serum PIIINP, ICTP, PINP, and CA 125 concentration and clinical and histopathological variables (Table 2). In the univariate analysis of the biochemical variables, PIIINP, ICTP (Fig. 1A), and CA 125 (Fig. 1B) were significant prognostic indicators of the patients’ clinical outcome (Table 2). For PINP, there was no cutoff concentration that would have significantly discriminated the low-risk patients from those with a poor prognosis. The conventional clinical and histopathological prognosticators, apart from histological subtype, also differentiated between the high-risk and low-risk patients (Table 2).

The median concentrations of serum PIIINP, ICTP, and CA 125 were 4.9 μg/l, 5.6 μg/l, and 250 units/ml, respectively. The median concentration of serum PINP was 47 μg/l. The cutoff value for ICTP (5.7 μg/liter) was slightly higher than that for PIIINP (3.9 μg/l), and the value for CA 125 (170 units/ml) was slightly lower than the median concentrations of the respective molecules.

The Cox multivariate stepwise method showed ICTP to be the only significant prognostic indicator of survival out of all of the biochemical markers evaluated here (Table 3A). In the analysis of only the patients with serous or anaplastic tumors ($n = 37$), again ICTP was the only significant indicator of prognosis [Exp(B) = 1.098 (95% confidence interval, 1.027–1.174)]. In each of the further analyses where biochemical markers were associated with one conventional indicator of prognosis (Table 3B), ICTP appeared to be a significant and independent prognosticator together with the conventional marker in question. In the analyses where two or more conventional prognosticators were associated with biological variables (Table 3C), the clinical stage and ICTP were significant indicators of prognosis, in this order. Among the conventional markers, clinical stage remained the only independent and significant determinant of prognosis (data not shown).

**DISCUSSION**

The most important finding in the present study was that an increased preoperative serum ICTP concentration predicts an

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**Table 2** The prognostic indicators$^a$ for the survival of ovarian cancer patients as evaluated by univariate analysis

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>No.</th>
<th>$P^b$</th>
</tr>
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<tbody>
<tr>
<td>Stage</td>
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<tr>
<td>I–II</td>
<td>25</td>
<td>0.0002</td>
</tr>
<tr>
<td>III–IV</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>30</td>
<td>0.0016</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Residual tumor</td>
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<td></td>
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<tr>
<td>no</td>
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</tr>
<tr>
<td>yes</td>
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</tr>
<tr>
<td>Ascites</td>
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<td></td>
</tr>
<tr>
<td>no</td>
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</tr>
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<tr>
<td>Age</td>
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<td></td>
</tr>
<tr>
<td>$\leq$50 yrs</td>
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</tr>
<tr>
<td>$&gt;$50 yrs</td>
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<tr>
<td>Histological type</td>
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<tr>
<td>serous</td>
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<td>0.3178</td>
</tr>
<tr>
<td>nonserous</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>PIIINP</td>
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<td></td>
</tr>
<tr>
<td>$\leq$3.9 μg/l</td>
<td>29</td>
<td>0.0001</td>
</tr>
<tr>
<td>$&gt;$3.9 μg/l</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>ICTP</td>
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</tr>
<tr>
<td>$\leq$5.7 μg/l</td>
<td>35</td>
<td>0.0005</td>
</tr>
<tr>
<td>$&gt;$5.7 μg/l</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>CA-125</td>
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<td>19</td>
<td>0.0041</td>
</tr>
<tr>
<td>$&gt;$170 units/ml</td>
<td>36</td>
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</table>

$^a$ For serum PINP, no cutoff concentration could be found.

$^b$ By using a log-rank test.
impaired prognosis of patients with ovarian cancer. This result was evident in the univariate analysis and in the Cox model, where serum ICTP was even more sensitive than CA 125. On the contrary, the PINP test failed in this function. Because the degradation and synthesis of type I collagen take place concomitantly in ovarian cancer (10), this difference warrants an explanation.

PINP molecules are cleaved off from type I procollagen molecules during the extracellular synthesis of type I collagen. PINP is thus an indicator of this synthesis. Type I collagen is abundant in both bone and soft tissues, and most of the PINP molecules in the blood are actually normally derived from bone (18). The possible malignancy-associated changes in type I collagen synthesis in soft tissue are thus very likely masked by the dominating bone metabolism of this collagen.

Recent studies have identified the detailed structure recognized by the human ICTP assay: it detects only such relatively large degradation products of type I collagen that contain two copies of a phenylalanine-rich region located between the triple-helical domain and the lysine-derived cross-link (19). Such a determinant is specific for the mature, trivalent cross-linked collagen and occurs neither in divalent cross-linked or non-cross-linked collagen molecules. The subtle differences in the maturation of type I collagen in bone and soft tissues are relevant in this respect; most of the molecules in the latter contain trivalent cross-links, whereas in bone, there are two to four times more divalent than trivalent cross-links (20).

In addition, type I collagen in bone is degraded by cathepsin K, which cleaves the COOH-terminal telopeptide of the α1-chain between the cross-link site and the phenylalanine-rich region. The products of this breakdown cannot be recognized by the ICTP assay. The major enzymes responsible for collagen turnover in soft tissues are the various matrix metalloproteinases. The degradation products formed in this process seem to contain the phenylalanine-rich regions of two COOH-terminal telopeptides and to be detectable in the ICTP assay. Thus, the ICTP test also measures the degradation of mature soft tissue type I collagen, although the antigen was originally isolated from bone (15), and its circulating concentration also increases in pathological bone degradation (18).

The cross-linking state of type I collagen has been recently studied in ovarian neoplasms (21). In malignant tissue, the content of trivalently cross-linked, mature type I collagen is low and that of insufficiently maturated fibers is higher than in benign lesions. For all these reasons, the excess ICTP in the serum of patients with progressive ovarian cancer cannot come from bone or tumor tissue but must originate from the enhanced breakdown of type I collagen in the soft tissue outside the tumor. This assumption is in line with our previous results showing that type I and type III collagen metabolites are present in higher concentrations in ascitic fluid than in the cyst fluid of malignant ovarian tumors (8) and that these collagens are insufficiently processed in the ascitic fluid (22). Several studies (23–28) have indicated that proteolytic processes promoting the invasive growth are common in ovarian cancer patients and have linked different matrix metalloproteases to these (29). The present
ICTP results suggest that such degradation is more prominent in women with poorly than with well-differentiated ovarian neoplasms. Enzymatic degradation of matrix proteins is the central factor that contributes to the spread and growth of malignant tumors (23) including ovarian cancer, and we conclude that the circulating ICTP concentration reflects this aspect of the invasive growth in ovarian cancer patients. ICTP therefore appears to be a more reliable variable than CA 125 in the prediction of prognosis in this disease. In diagnosing ovarian cancer serum, CA 125 is naturally superior to ICTP.

We have previously documented an inverse relation between the serum concentration of PIIINP and the survival of the patients with advanced ovarian cancer (13) as well as an accelerated synthesis of fibrillar collagen in these patients (10). Also in the present study, the preoperative PIIINP level could be identified as a prognostic indicator in the univariate analysis. In the multivariate Cox model, however, this indicator turned out not to be independent from the other biochemical or clinical variables.

The other determinants identified in the univariate analysis were in accordance with earlier findings (3, 4, 6, 30–33). The histopathological grade of differentiation correlated more closely with the serum concentrations of collagen metabolites than did the clinical stage of the disease (10), resulting in the loss of significance for the grade in the multivariate analysis. Residual tumor size and the presence of ascites correlated with the clinical stage and were thus also left out from the Cox analysis. However, the volume of residual tumor after debulking surgery was an important prognostic variable in the univariate analysis and one of the few prognostic factors over which the treating team had any control. In accordance with the present consensus (4), histological subtype (serous versus nonseroser or serous plus anaplasic versus others) had no prognostic significance. In several (34–36) but not all studies (37, 38), clinical stage has been the most accurate prognostic variable in ovarian cancer. The Federation of Gynecology and Obstetrics stage reflects the spread of the malignant neoplasm in general terms, whereas the preoperative circulating ICTP reflects an associated biological process. It is thus understandable that these parameters are complementary in the prediction of outcome in this malignancy.

REFERENCES
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