ONCOLOGY

Circulating calprotectin in ovarian carcinomas and borderline tumors of the ovary

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OBJECTIVE: Recent studies indicate that circulating calprotectin may serve as a biomarker in some cancers. We investigated whether this is the case for ovarian neoplasms.

STUDY DESIGN: Calprotectin was analyzed with an enzyme-linked immunosorbent assay in EDTA-plasma collected prior to surgery from women with ovarian carcinomas (n = 89), borderline ovarian tumors (BOT, n = 39), and benign ovarian tumors (n = 71). Serum CA 125 was analyzed in the same study population.

RESULTS: Median plasma calprotectin concentration was elevated in ovarian carcinoma, compared with controls, as well as compared with

BOT (both $P < .001$). A positive correlation was found between CA 125 and calprotectin concentrations in ovarian carcinoma. Receiver operating characteristic curves demonstrated a larger area under the curve for CA 125 (0.85) as compared with calprotectin (0.70).

CONCLUSION: Plasma calprotectin is elevated in invasive ovarian cancer, but when used as a tumor marker, it is inferior to CA 125.

Key words: calprotectin, inflammation, ovarian cancer, ovarian neoplasm

Epithelial ovarian cancer is the most lethal cancer of the gynecological malignancies. Because of few early disease symptoms, most of the carcinomas are diagnosed when the disease is in the advanced stage (70%).[1] In contrast to the invasive carcinomas, borderline tumors of the ovaries (BOT), also named tumors of low malignant potential, have a low

recurrence rate (7%) and an excellent 10 year survival rate (95%).[2] The developmental tumor pathways are not definitively known, but a dualistic molecular model of epithelial ovarian cancer has been proposed, subdividing tumors into low-grade (type I tumor) and high-grade (type II tumor) types that have distinct pathways of tumorigenesis.[3] In this model, serous borderline tumors are putative precursors of invasive low-grade serous carcinoma (type I tumor) and are unrelated to high-grade serous carcinoma (type II tumor), for which morphologically recognizable precursor lesions have not been identified. There is a search for clinically useful biomarkers because the most commonly used serum biomarker, cancer antigen 125 (CA 125), is of limited value in diagnosing early clinical stages of ovarian cancer and is also hampered by being elevated in several noncancerous situations.

Calprotectin, also named human leukocyte protein L1,[4,5] MRP8/ MRP14,[6] S100A8/S100A9 complex,[7,8] and calgranulin,[9] is a member of the S-100 protein family. It is a calcium- and zinc-binding protein, mainly found in the cytosol of human neutro-

phil granulocytes and monocytes.[5] Calprotectin is released from activated neutrophils[4,5] and represents a marker of inflammation. Calprotectin has an antimicrobial action[10] and has proinflammatory cytokine functions[8] as well as chemotactic factor activity.[11] Augmented calprotectin concentration in feces is used in the diagnosis of inflammatory bowel disease.[12]

Calprotectin seems to be involved in tumor development, but the exact biological role remains to be defined.[8] Calprotectin inhibits intracellular enzymes that are important in cell proliferation[13] and also has broad apoptosis-inducing activity.[14] Calprotectin inhibits matrix metalloproteinases, which are involved in growth of primary and metastatic tumors.[15] A previous study has shown that calprotectin messenger ribonucleic acid was overexpressed in gastric cancer tissue.[16] In addition, calprotectin protein is overexpressed in hepatocellular carcinomas,[17] pulmonary adenocarcinomas, and invasive ductal carcinomas of the breast.[18] In these tumors, elevated protein expression of calprotectin was correlated with poor differentiation. Dale and Brandtzaeg[19] found overexpression

of calprotectin protein in squamous cell carcinomas of the lung. Calprotectin is elevated in serum and fecal tests from patients with colorectal carcinomas[20] and in serum as well as the primary tumor in prostate cancer.[7]

The only published calprotectin study, to our knowledge, including women with ovarian carcinomas, is a study by Ott et al,[9] demonstrating elevated calprotectin (named calgranulin A and calgranulin B in the actual study) protein concentration in ovarian cystic fluid as well as serum in women with ovarian neoplasia (n = 11), compared with women with benign ovarian cysts (n = 11).

There is a need for improved diagnostic circulating markers to differentiate between benign and malignant ovarian tumors. The objective of the current study was to investigate whether preoperative plasma concentration of calprotectin is altered in women with ovarian carcinomas as compared with women with BOT and benign ovarian tumors. We also wanted to explore any correlations between circulating calprotectin and CA 125 concentrations as well as between calprotectin and C-reactive protein (CRP), an established marker of inflammation. In addition, we wanted to assess whether calprotectin could represent an improved biomarker in diagnosing ovarian cancer as compared with CA 125.

MATERIALS AND METHODS
Patient selection and clinical samples
The study is based on a biobank with samples collected from January 2003 to December 2006 from women operated for suspected gynecological adnexal tumors at Ulleval University Hospital. The present study included 199 women with preoperatively suspected adnexal mass who were postoperatively diagnosed with benign ovarian cyst or tumor (also named controls, n = 71), BOT (n = 39), or ovarian carcinoma (n = 89). None of the included patients had a prior diagnosis of cancer or had received chemotherapy or surgery for the present disease. The study protocol was approved by the

Regional Committee of Medical Research Ethics in Eastern Norway, and informed written consent was obtained from each patient.

All tumors were histologically evaluated by 1 gynecological pathologist (V.E.) to confirm the diagnosis and tumor type. The tumors in the control group of 71 women with benign ovarian tumor included 22 serous cystadenomas, 17 mucinous cystadenomas, 3 mixed serous and mucinous cystadenomas, 5 dermoids, 5 endometriomas, 2 fibromas, 1 Brenner tumor, and 16 follicle cysts.

The tumors in the borderline group of women (n = 39) were diagnosed as 20 serous borderline tumors (17 International Federation of Gynecology and Obstetrics [FIGO] stage I, 2 stage II, and 1 stage III) and 18 mucinous borderline tumors (all FIGO stage I) and 1 mucinous cystic tumor with pseudomyxoma peritonei (FIGO stage III). The tumors in the carcinoma group (n = 89) included 61 serous adenocarcinomas (2 FIGO stage I, 4 stage II, 43 stage III, and 12 stage IV); 2 mucinous adenocarcinomas (1 FIGO stage I and 1 stage III); 12 endometrioid adenocarcinomas (6 FIGO stage I, 2 stage II, 3 stage III, and 1 stage IV); 9 clear cell tumors (5 FIGO stage I, 1 stage II, 2 stage III, and 1 stage IV); and 5 undifferentiated carcinomas (1 FIGO stage I, 2 stage III, and 2 stage IV). Clinical information was collected from hospital records and by additional patient interviews.

Fasting blood samples (serum and EDTA-plasma) were collected prior to operation. EDTA-blood vials for the calprotectin analyses were kept on ice for 30-120 minutes and centrifuged at 3400 revolutions per minute for 10 minutes at 4°C, and plasma was stored at −76°C until assay. Blood for serum was left in gel-coated vials for 30-60 minutes at room temperature, and serum was obtained by centrifugation at room temperature for 10 minutes at 3400 rpm and stored at −76°C until assay.

Laboratory analyses
Plasma calprotectin concentrations were analyzed in duplicates by enzyme-linked immunosorbent assay (ELISA) at the

Blood Bank and Department of Immunology and Transfusion Medicine, Ulleval University Hospital, where the assay was developed.[5] Briefly, the samples were diluted in assay buffer and added to microtiter plate wells previously coated with an IgG fraction of rabbit anticalprotectin. Standards and samples were incubated as previously described[20] and then washed 4 times with washing buffer. After adding alkaline phosphatase-conjugated affinity-purified rabbit anticalprotectin, the plates were incubated for further 45 minutes. After washing 4 times, the substrate (p-phenylphosphate) was added. Optical densities were recorded after 15-25 minutes. Interassay and intraassay coefficients of variation of the procedure are 5% and 13%, respectively.

High-sensitivity CRP was measured in serum with a Hitachi 917 instrument by particle-enhanced immunoturbidimetric assay (Tina-Quant CRP, Roche Diagnostics Corp, Indianapolis, IN), measuring the range of 0.1 to 300 mg/L. White cell count was assessed using a Sysmex SE 9500 (TOA Medical Electronics, Kobe, Japan). Serum CA 125 level was measured with an ARCHITECT system using a 2-step immunoassay technology (Abbot Laboratories, Abbott City, IL). High-sensitivity CRP, white cell count, and CA 125 were analyzed at the Department of Clinical Chemistry, Ulleval University Hospital.

Statistics
The clinical characteristics are presented as median values and range, or number of patients, and the laboratory results are presented as medians and 95% confidence interval of the medians. Because the variables were not normally distributed, a nonparametric Mann-Whitney U test was used when comparing continuous data. For the categorical data, a χ^2 test was used. Receiver operating characteristic (ROC) curves were constructed for calprotectin and CA 125. Sensitivity and specificity as well as a positive predictive value for calprotectin in identifying ovarian cancer vs benign tumors were calculated for a calprotectin threshold value of 900 μg/L as well as a CA 125