High plasma YKL-40 level in patients with ovarian cancer stage III is related to shorter survival*

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Abstract. YKL-40 (human cartilage glycoprotein-39) is a member of family 18 glycosyl hydrolases. YKL-40 is a growth factor and is secreted by cancer cells. High serum levels of YKL-40 in patients with colorectal cancer and recurrent metastatic breast cancer have been associated with a poor prognosis. We evaluated the prognostic value of plasma YKL-40 in patients with primary ovarian cancer (OC). YKL-40 was determined by ELISA in plasma obtained preoperatively from 47 women with stage III OC and in plasma from 79 healthy females. The results showed that plasma YKL-40 was elevated compared to healthy females in 57% of the OC patients and was highest in the patients who died during the follow-up compared to the patients still alive (186 vs. 78 μg/l, p=0.002). Patients with high plasma YKL-40 (>130 μg/l) had significantly (p=0.0003) shorter survival than patients with normal plasma YKL-40. Multivariate Cox regression analysis showed that plasma YKL-40 (RH=3.95; 95% CI, 1.52-10.27; p=0.005) and radicality after primary surgery (RH=4.03; 95% CI, 1.81-8.97; p=0.001) were independent prognostic factors of survival, whereas age, histological type of tumour and serum CA125 had no independent prognostic value. In conclusion, plasma levels of YKL-40 proved of prognostic value in stage III OC patients.

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Abbreviations: OC, ovarian cancer; FIGO, The International Federation of Gynecology and Obstetrics; RH, relative hazard; CI, confidence interval; NOS, not otherwise specified

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Introduction

Ovarian cancer (OC) is the fifth most frequent female cancer type and the fourth most frequent cause of death from cancer among women in Denmark in spite of extensive therapy (1). At the time of diagnosis about 70% of patients have advanced cancer [The International Federation of Gynecology and Obstetrics (FIGO) stage III or IV]. Published five-year survival rates for OC patients range from 80% for stage I cancer to <20% for stage III and IV (2,3). Although many prognostic factors have been found in OC (4-7), no reliable method for identification of nonresponders to therapy has been described so far. Clearly, the need for useful prognostic factors in order to optimise treatment of the patients diagnosed with OC has to be emphasized.

YKL-40 is a mammalian member of family 18 glycosyl hydrolases (8-10). YKL-40 is a heparin and chitin binding protein (9,11) but has no chitinase activity (8,11). The exact function of YKL-40 is unknown, but it has recently been shown that YKL-40 is a growth factor for connective tissue cells (12) (Recklies AD, *et al*, Arthritis Rheum 43: abs. 1686, 2000) and is a potent migration factor for endothelial cells (13). Furthermore, the pattern of its expression in normal and disease state suggests a role in inflammation and remodeling of the extracellular matrix. The protein has been termed YKL-40 from its molecular weight (40 kDa) and the one-letter code for its three N-terminal amino acids (14). The gene has been sequenced, but promotor analysis and regulatory factors have not been described (15). YKL-40 is secreted in large amounts *in vitro* by the MG63 human osteosarcoma cell line (14) and is expressed selectively by murine mammary tumours initiated by *neu/ras* oncogenes (10). A search of the YKL-40 protein sequence against the dbest database at the NCBI using the BLAST program has shown that several types of cancer cells, including ovarian cancer, express the protein.

Furthermore, YKL-40 is synthesized by activated macrophages (11,15) and the protein is present in the specific granules of neutrophils and exocytosed by activation (16).

Increased serum levels of YKL-40 are found in patients with metastatic breast cancer (17) and preoperatively in patients with all stages of colorectal cancer (18) compared to the serum YKL-40 level in healthy controls. Interestingly, a significant association between increased serum YKL-40 level and short survival was found in both studies. Serum YKL-40 may therefore be used as a prognostic biochemical marker of survival (17,18). In the present study we have determined the level of plasma YKL-40 in healthy women and have assessed whether plasma YKL-40 can be used as a prognostic marker of survival in patients with stage III OC.

Materials and methods

Study design. According to the study design preoperative blood samples were left on the clot or on the blood cells at room temperature and then sent from all the participating hospitals to Statens Serum Institute in Copenhagen. The serum and plasma were separated by centrifugation at 2000 g for 10 min. Serum and plasma samples were stored at -80˚C in aliquots until analyses were performed. Serum and plasma YKL-40 levels are dependent on the method of sample collection (19). It was found that blood samples for YKL-40 analysis must be processed into plasma (EDTA) within 8 h at room temperature or into serum in less than 3 h at room temperature. If this is not possible, the blood samples must be stored at 4˚C until processed. In the MALOVA study (described below) a total of 383 patients with stage III OC were enrolled. However, blood samples of only 47 of the patients had been processed into plasma (EDTA) within 8 h from venipuncture.

Patients. The study comprise 47 women (median age 64 years, range 37-77) with a stage III epithelial OC included in the MALOVA study. The patients were included from December 1994 to May 1999 and at the end of follow-up November 2001, 34 patients had died of OC. Median follow-up time: 21 months, (range, 1-80) and 13 patients were still alive (median follow-up time, 55 months; range, 44-80). The MALOVA study ('MALignant OVArian cancer study') is a multidisciplinary Danish study on OC, covering epidemiology (lifestyle factors), biochemistry and molecular biology with the purpose to identify risk factors and prognostic factors for OC and the study is described in detail elsewhere (20). Preoperative blood samples as well as tumour tissue samples were obtained from the patients. Histopathological classifications of the ovarian tumours were based on the criteria of the WHO. Pathology reports and tissue specimens were collected from the different participating hospitals and reviewed blindly by one pathologist specialized in gynaecologic tumours. FIGO stages were obtained from clinical records and were reviewed by two gynaecologists, both specialized in OC. The study was approved by the Science Ethics committees in the study area, KF01-384/95.

Healthy volunteers. The normal range of plasma YKL-40 levels was determined in 79 apparently healthy females with a median age of 56 years (range 37-77 years). They were all healthy, were not taking any medicine, and had no clinical signs or symptoms of cancer, joint, liver, metabolic or hormonal disease (21).

Table I. Plasma YKL-40 levels in stage III OC patients according to histological type of tumour.

Histological type of tumour	No.	Plasma YKL-40 (μg/l) median (range)
Serous adenocarcinomas	33	117 (32-548)
Undifferentiated adenocarcinomas	4	576 (156-1808)
Papillary adenocarcinomas NOS	4	345 (102-672)
Mucinous adenocarcinomas	3	179 (102-856)
Endometroid adenocarcinomas	3	148 (73-186)

Biochemical analysis. Plasma YKL-40 concentrations were determined by ELISA (Quidel Corporation, Santa Clara, CA, USA) according to the manufacturer's instruction (22). The sensitivity of the assay was 10 μg/l, and the intra- and inter-assay coefficient of variations were 3.6% and 5.3%, respectively (n=67). Serum levels of the tumour-associated antigen CA125 were determined by immunoassay (EIA) (Abbott CA125-EIA, Abbott Laboratories, Chicago, IL) according to the manufacturer's instruction. The intra- and inter-assay coefficient of variations were 6.6% and 6.2%, respectively (n=60 and n=10).

Statistical analysis. Statistical comparisons between groups were carried out using the Mann-Whitney test. Survival differences were estimated by the method of Kaplan-Meier and tested by the log-rank test. Relative hazard (RH) as the exponential function of the respective regression coefficient and 95% confidence interval (95% CI) were derived from multivariate Cox analysis, used to test for independent prognostic variables (23). These analyses were performed using the SPSS 10.0 Statistical Software.

Results

The median preoperative level of plasma YKL-40 in the stage III OC patients was 168 μg/l (range 32-1808 μg/l) and significantly higher compared to the plasma level in age-matched healthy women (median 35 μg/l, range 20-130 μg/l) ($p<0.001$). In 57% (27/47) of the OC patients the plasma YKL-40 level was elevated above the upper normal range of age-matched healthy women (i.e., >130 μg/l).

Thirty-four of the patients (72%) died during the follow-up. The patients who died had significantly higher preoperative plasma YKL-40 levels compared to the patients still alive ($p=0.002$), whereas no significant difference in the serum CA125 levels was found between patients who died and the patients still alive. The patients who died of OC were also older than the patients still alive ($p=0.049$). Patients with serous adenocarcinomas (median 117 μg/l, range 32-548 μg/l,

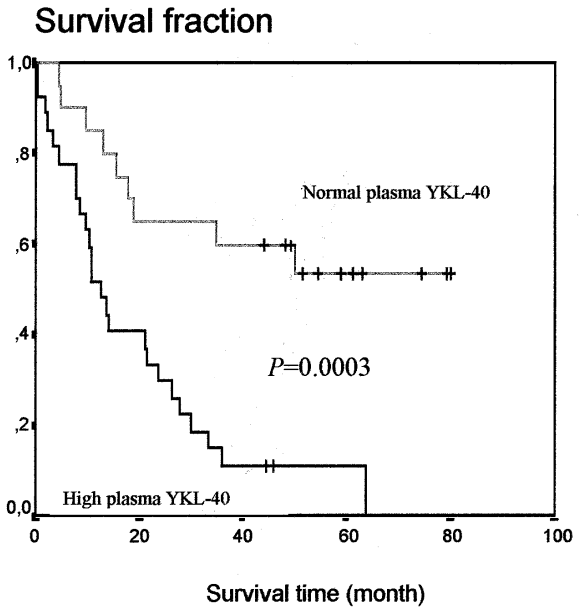

Figure 1. Kaplan-Meier survival curves. Stage III ovarian cancer patients with high plasma YKL-40 levels (>130 μg/l, n=27) and stage III ovarian cancer patients with normal plasma YKL-40 levels (≤130 μg/l, n=20).

n=33) had significantly (p=0.018) lower plasma YKL-40 levels compared to patients with undifferentiated adenocarcinomas, papillary adenocarcinomas NOS (not otherwise specified), mucinous and endometroid carcinomas (median 212 μg/l, range 73-1808 μg/l, n=14) (Table I).

Dividing the patients into two groups according to a preoperative plasma YKL-40 level at 130 μg/l (corresponding to the upper normal range of healthy age-matched females) a significant better survival was found for patients with normal plasma YKL-40 level (≤130 μg/l) compared to the survival for patients with elevated plasma YKL-40 level (>130 μg/l) (p=0.0003) (Fig. 1). The median survival time was 49 months for the patients with normal plasma YKL-40 level and only 13 months for the patients with elevated plasma YKL-40. If the cut-offs of plasma YKL-40 were set at 78 μg/l, 168 μg/l or 280 μg/l, corresponding to the lower quartile, the median and the upper quartile of the plasma YKL-40 levels in the OC patients, significant differences in survival could also be detected between patient groups (log-rank, p=0.014; p=0.0003 and p=0.026, respectively).

Multivariate Cox regression analysis including 41 of the stage III OC patients (in 6 patients there were not enough serum for CA125 analysis) showed, that the only independent prognostic factors were radicality after primary surgery (RH=4.03; 95% CI, 1.81-8.97; p=0.001) and plasma YKL-40 (cut-off value 130 μg/l, RH=3.95; 95% CI, 1.52-10.273; p=0.005), whereas age (p=0.58), histological type of the tumour (p=0.73) and serum CA125 (p=0.20) had no independent prognostic value of survival.

Discussion

The FIGO staging is a well-established strong prognostic marker of survival in patients suffering from OC. However, a considerable variation in prognosis has been demonstrated within each stage and therefore we selected only stage III OCs. This study was initiated as a consequence of two studies reporting serum YKL-40 levels as a prognostic marker in patients with metastatic breast cancer and colorectal cancer (17,18). No similar study has been performed on patients with primary OC. We found that 57% of the 47 patients with stage III OC had elevated plasma YKL-40 levels compared to healthy women. A strong association was found between short survival and high preoperative plasma YKL-40 levels in stage III OC patients. Furthermore, multivariate Cox regression analyses showed that plasma YKL-40 and radicality after primary operation of OC were independent prognostic variables of survival whereas serum CA125, histological type of tumour and age were not.

Patients with stage III OC have local metastases but no detectable metastases to the liver and lung. Other studies have reported high serum levels of YKL-40 in patients with metastases to liver and lung (17,18). Based on the review performed by two gynaecologists, both specialized in OC, our patients with stage III OC cancer had no detectable liver and lung metastases, but the high plasma YKL-40 level may indicate that these patients had undetectable distant metastases.

Patients with a serous tumour had lower plasma YKL-40 levels compared to patients with a tumour of another histology. The highest median plasma YKL-40 levels were found in patients with undifferentiated adenocarcinomas and papillary adenocarcinomas NOS, which are also known to be the histology group with the general worst prognosis (24). This finding could merely reflect that plasma YKL-40 is a prognostic marker elevated in the histological group of OC patients with the worst prognosis. However, multivariate Cox regression analysis showed, that the histological type of tumour had no independent prognostic value of survival.

Although plasma levels of YKL-40 may be prognostic of survival in patients with OC the mechanism by which plasma YKL-40 reflects disease status is unknown. YKL-40 is expressed and synthesized by various types of adenocarcinomas, including ovarian cancer (data not shown) but it will be important by immunohistochemical analysis to study if there is a relationship between positive staining of ovarian cancer cells and the plasma YKL-40 levels of the patients. We speculate that the YKL-40 positive cancer cells may have a different phenotype than the YKL-40 negative cancers, and thereby YKL-40 may reflect differences in the biology of various cancer cells.

The biological function of YKL-40 in cancer diseases is not known and YKL-40 production is not specific for cancer cells. *In vitro* studies have shown that YKL-40 is a growth factor for fibroblasts (Recklies AD, *et al*, Arthritis Rheum 43: abs. 1686, 2000) and chondrocytes (12). It has also been demonstrated that YKL-40 acts as a chemoattractant for human umbilical vein endothelial cells and stimulates migration of these cells at a level comparable to basic fibroblast growth factor (13). YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 may function in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells (13). High YKL-40 production in tissues and high circulating YKL-40 levels are associated with diseases characterized by high tissue remodeling or

inflammation such as rheumatoid arthritis (22,25,26), giant cell arthritis (27), bacterial infections (28), and liver fibrosis (29,30). It is unknown if YKL-40 has a function in cancer expansion and invasiveness and the elucidation of YKL-40 function in cancer diseases is an important objective of future studies.

In summary our study indicates that preoperative measurement of the YKL-40 level in plasma of stage III OC patients may be useful to identify a subgroup of patients with a poor prognosis. Future studies should clarify if plasma YKL-40 levels in the early stage of OC is useful as a prognostic marker of survival and recurrence.

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