Evaluation of soluble tumour necrosis factor alpha receptors p55 and p75 in ovarian cancer patients

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Summary

Purpose: Evaluation of serum TNFα receptor 1 (p55) and 2 (p75) concentrations preoperatively in patients with ovarian masses.
Methods: Estimation by ELISA assay in 51 women with ovarian cancer and 16 healthy controls. Mean values and correlations with CA-125, tumour volume index, morphological score, pathological finding and cytoreduction were estimated.
Results: Mean concentrations of p55 and p75 in cancer patients were 2006 ± 1030 pg/ml and 2849 ± 1092 pg/ml, respectively, whereby for controls 1323 ± 291 pg/ml and 2386 ± 475 pg/ml, respectively. The area under the ROC curve for CA-125, p55 and p75 for cancer (FIGO Stages I-IV) were: 0.85 (95% CI 0.75-0.92), 0.73 (95% CI 0.60-0.83) and 0.65 (95% CI 0.50-0.77), respectively. Serum p55 correlated with morphological ultrasound score and CA-125 but not with FIGO stage, tumour grade or tumour volume index. No correlations of p75 with these parameters were observed.
Conclusion: Estimation of p55 and p75 provide little information in ovarian cancer patients and have poor detecting power.

Key words: TNFα; Receptor 1; Receptor 2; Ovarian; Cancer; Tumour marker.

Introduction

In spite of diagnostic development, 75% of all detected ovarian cancers are in more advanced stages (III and IV according to the Federation of Gynecology and Obstetrics International (FIGO) with obvious impact on therapy and poor prognosis. The early stages of this neoplasm are typically asymptomatic and the research of new potential markers is needed worldwide.

Tumor necrosis factor alpha (TNFα) is a common mediator of apoptosis, inflammation and immune response [1]. Its role in ovarian biology remains unclear. The effect of TNFα is regulated by its two receptors; receptor 1 (p55, CD120a) and receptor 2 (p75, CD120b). The signal is transferred into the cell via a complicated protein system to target transcription proteins: nuclear factor κB (NF-κB) and c-Jun. This pathway results in regulations of cell growth, death, carcinogenesis and stress response [1, 2]. Many studies support the dominating role of receptor I (p55) in signal transduction, whereby receptor II (p75) plays a modulatory role, being incapable of transducing signals alone [1-5]. However it was hypothesised that both receptors could be agonists and antagonists depending on their concentrations. [6] Thus more important to tumour biology seem to be the expressions and concentrations of TNFα receptors p55 and p75 in determining the final effects [7].

Expression of both receptors differs depending on the kind of cell and is not regulated by their ligand [2, 7, 8]. Higher concentrations of p75 is typical for monocytes and lymphocytes, whereas receptor p55 is quite typical for epithelial cells [8].

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Receptors dissociate from cell surfaces becoming free molecules in the blood. Serum p55 and p75 interact with TNFα in the same manner as if bound to the cell surface. The significance of serum complex formation is the blocking of the appropriate biological effect of TNFα [2, 7, 9, 10]. The significance of these interactions is still unclear and the subject of few studies. Some qualitative data are not clearly followed by quantitative studies. In a few studies it has been suggested that there are changes in p55 and p75 concentrations on cell surfaces as well as serum levels in patients with benign and malignant ovarian tumors [11-13].

The aim of our study was to evaluate the serum concentrations of p55 and p75 receptors in patients with ovarian cancer and healthy controls. Special attention was paid to the possible detecting potential and the comparison with a well known marker for serous ovarian cancer – CA-125.

Materials and Methods

We performed a prospective study of 51 patients with ovarian cancer treated surgically in the Department of Mother's and Children's Health and Department of Gynecologic Surgery, Karol Marcinkowski University of Medical Sciences in Poznan, Poland between June 2000 and November 2002. The protocol of the study included preoperative gynaecologic examination with transvaginal ultrasound (7,5 MHz, Aloka SSD5000, Japan). The morphological score according to Ferrazzi *et al.* [14] and tumour volume index (TVI = 0.523xAxBxC) were estimated at ultrasound examination. Preoperatively blood was collected and centrifuged for five minutes at 3,000 rpm, the serum frozen and kept at -70°C until the whole material was completed. The concentrations of p55 and p75 were assayed with commercially available kits (Quantakine, R&D Systems, USA) in duplicate. The extinction was read on Dynex MRX

Endpoint 1.33 (UK). The sensitivity of p55 and p75 tests were 3.0 pg/ml and 1.0 pg/ml, respectively, according to the manufacturer. The intraassay precision was maintained within 8.2%. The cut-off values for receptor 1 and receptor 2 were estimated on the receiver operating characteristic curve (ROC) at the point of maximum sum of sensitivity and specificity. We also estimated CA-125 concentrations on a Axsym2000 (Abbot Lab, USA), with the reference cut-off level of 35 U/ml. Material was verified after operation by pathological examination, with estimation of histological type, staging according to FIGO and grading of malignant tumours. Among cancer patients 14 were FIGO Stage I, one patient Stage II, 29 patients Stage III and seven Stage IV. Histologically 25 cancers were serous, eight mucous, eight solid, five endometrioid, three clear cell and two indifferentiated.

The control group consisted of 16 patients, examined according to the same protocol and additionally by an internist. They did not report any chronic disease in anamnesis. They also had multiple laboratory tests to exclude current disease (blood morphology, CRP, AlAT, AspAT). Gynaecological examination with ultrasound yielded no abnormalities.

Statistical analysis was performed using SigmaStat version 2.0 (Jandel Corp, USA). Differences in mean values were analysed with the Mann-Whitney test. We also estimated the area under the ROC curve (AROC) with methodology and software according to Metz *et al.* (ROCkit version 0.9) [15]. The correlations were estimated by Spearman's test (Rs) and Pearson's correlation coefficient. The results are presented as means ± standard deviation (SD). We considered $p < 0.05$ as statistically significant.

Our study received approval from the local bioethics committee and all patients gave their written consent for participation in the study.

Results

Mean ages in ovarian cancer patients and controls were: 51.5 ± 11.5 (range 23-80) and 27.8 ± 12.6 (range 20-63), respectively. To delineate discrimination for statistical analysis we calculated the cut-off level for p55 and p75 by the ROC curve including healthy women and those with malignant ovarian tumours. The cut-off value for TNFα receptor 1 was 1663 pg/ml, corresponding to 54.9% sensitivity and 93.8% specificity. The same calculated parameter for TNFα receptor 2 was 2837 pg/ml, corresponding to 43.1% sensitivity and 81.3% specificity. The sensitivity and specificity of CA-125 estimation in detecting ovarian cancer was 74.5% and 93.8%, respectively. Mean concentrations of CA-125, receptors p55 and p75 are presented in Table 1 with only statistically significant differences pointed out. Correlations of p55 and p75 concentrations with FIGO stage, grading, tumour volume index, morphological index and CA-125 are presented in Table 2. Even when serous and non-serous tumours were analysed separately no above-mentioned correlation was estimated.

Table 1. — *Mean concentrations of CA-125, p55 and p75.*

	Ovarian cancer		Controls	
	mean	range	mean	range
CA-125 (U/ml)	713.0 ± 1159.9[a]	6.2 - 6001.0	14.8 ± 8.5[a]	3.2 - 38.0
p55 (pg/ml)	2006 ± 1030[b]	369 - 4920	1323 ± 291[b]	983 - 2070
p75 (pg/ml)	2849 ± 1092	814 - 7919	2386 ± 475	1711 - 3430

[a]$p < 0.001$; [b]$p = 0.007$.

Table 2. — *Correlations of p55 and p75 with disease stage, tumour grade, volume index and morphological score.*

Spearman's test	p55	p75
FIGO stage	NS	NS
Grading (G)	NS	NS
TVI	NS	NS
Morphological score	Rs = 0.34, p = 0.003[a]	NS
CA-125	Rs = 0.36, p = 0.009[b]	NS

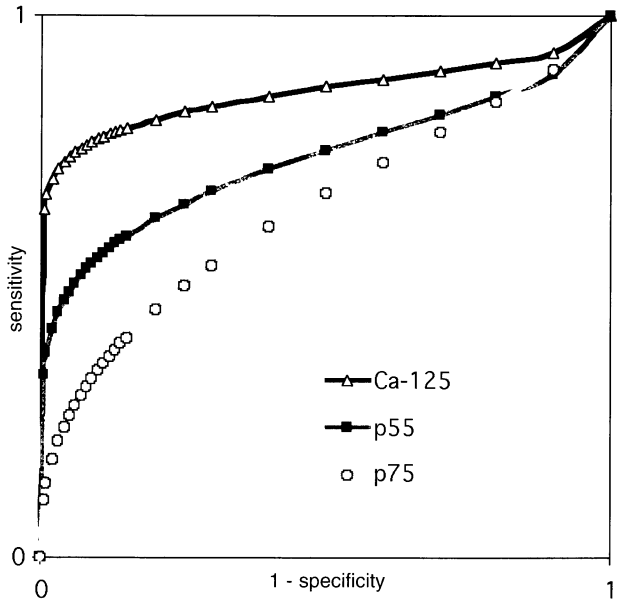
NS - not significant.
[a]Pearson's correlation coefficient r = 0.33, p = 0.004.
[b]Pearson's correlation coefficient r = 0.18, p = 0.20 - not significant.

The discrimination power in detecting malignancy independently from FIGO stage is presented as a ROC curve in Figure 1. The AROC (area under curve) for CA-125, p55 and p75 is: 0.85 (95% CI 0.75-0.92), 0.73 (95% CI 0.60-0.83) and 0.65 (95% CI 0.50-0.77), respectively. In the same manner the ROC curve for discrimination between FIGO Stage I of disease and controls was drawn and is presented in Figure 2. Area under the ROC curve for CA-125, p55 and p75 is 0.66 (95% CI 0.43-0.84), 0.52 (95% CI 0.29-0.76) and 0.60 (95% CI 0.37-0.80), respectively.

The concentrations of p55 differed in patients with ovarian cancer depending on optimal cytoreduction. Mean concentrations of p55 in patients who underwent hysterectomy with omentectomy (n = 29) and patients without optimal cytoredution (n = 22) were 1777.9 ± 1108.8 pg/ml vs 2308.8 ± 847.8 pg/ml ($p = 0.034$). The same values calculated for p75 were 2693.6 ± 765.6 pg/ml vs 3054.6 ± 1408.5 pg/ml and the difference was not statistically significant. The values for CA-125 were 645.1 ± 1331.2 U/ml vs 803.3 ± 908.7 U/ml ($p = 0.015$).

Discussion

The prognosis in patients with ovarian cancer is a great cause of concern worldwide. Unfortunately the typical patient with detected ovarian cancer has advanced disease, approximately 75% are in Stage III and IV due to unsuccessful early detection methods [5, 16]. In our investigation the percentage of advanced stages (70.6%) was similar to that found in the literature. The difficulties in early stage diagnosis are typical for this cancer and alternative methods must be developed. Some suggested alternative biochemical methods are based on estimation of CA-125 [17-19]. We found CA-125 to be elevated in patients with ovarian cancer, although the mean value is strongly influenced by a few high values in women with malignancy of advanced stage. Similar results were found in literature [16, 20] The satisfactory value of CA-125 estimation in patients with FIGO Stages I to IV (AROC = 0.85) was not so impressive in patients with early stage of the disease. In FIGO Stage I the area under ROC curve classifies this test as a poor detecting method (AROC = 0.66). The low value of CA-125 in early diagnosis is one of the reasons preventing it from becoming a widespread screening tool. In our study low sensitivity with good specificity of this test, confirmed by ROC analysis in FIGO Stage I, also supports the opinion of its limited application in early diagnosis. Early stage results similar to our own are reported in the literature, but higher values of sensitiv-



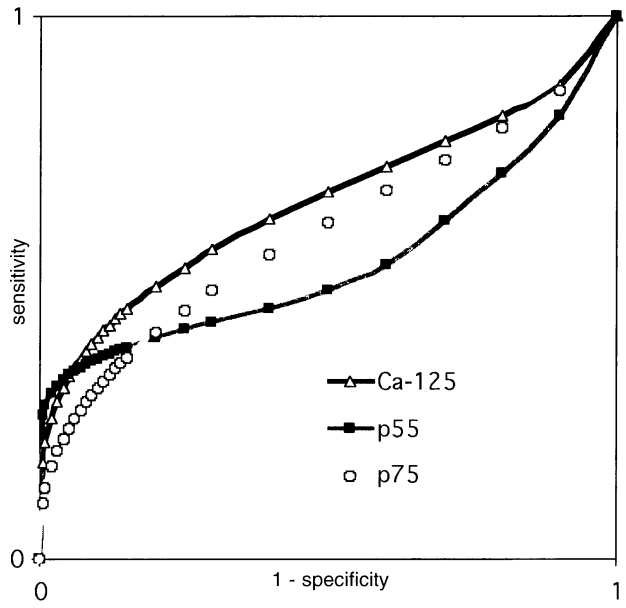


Figure 1. — ROC curves for CA-125, p55 and p75 in detecting ovarian cancer in all FIGO stages.
Figure 2. — ROC curves for CA-125, p55 and p75 in detecting ovarian cancer in FIGO Stage I.

ity and/or specificity were reported only in studies with a higher percentage of advanced stages [16-18, 20, 21].

The concentrations of soluble p55 and p75 have been reported to be higher in many malignant diseases. Mean serum concentrations of p55 in melanoma patients and in the control group were 1451 ± 384 pg/ml and 887 ± 219 pg/ml, respectively. Also concentrations of p75 receptor were higher in cases of malignancy (5109±2759 pg/ml vs 1926 ± 481 pg/ml) [22]. Higher concentrations of both receptors are more typical of metastases. In our study higher concentrations of p55 and p75 were observed among patients with ovarian cancer, but only in case of receptor 1 did it reach statistical significance. Gadducci *et al.* [11] reported similar results to our study and additionally p55 and p75 also correlated with FIGO stage, but not with histologic type, grade, CA-125 levels and possible operative cytoreduction. In our study not many correlations of p55 and p75 concentrations were observed except a weak one between p55 and morphologic score as well as CA-125. Interestingly mean p55 concentrations preoperatively as well as CA-125 were lower in patients with possible optimal cytoreduction compared to the group where only partial debulking or explorative laparotomy was possible. In our study no correlation with histologic type of cancer was found, but in some studies such dependence has been reported [13].

Serum TNFα receptor 1 concentrations in our study showed a stronger relationship to clinical status (morphologic score, CA-125, sensitivity, specificity, AROC, optimal cytoreduction) than receptor 2. It supports the theory that the serum source of p55 is more likely to originate from ovarian cancer cells. It has been reported that practically all ovarian cancer types produce p55 and its distribution in tumours is almost constant. Receptor 2 is typically produced by stromal cells [12].

Conclusion

1. Serum concentrations of TNFα receptor 1 (p55) correlate moderately with morphologic ultrasound score and CA-125 concentrations, but not with cancer stage, grade and histology.

2. Estimation of serum TNFα receptor 2 (p75) concentrations does not provide important information in ovarian cancer patients.

3. Receptors p55 and p75 failed to detect ovarian cancer independently from FIGO stage, but p55 has a moderate and comparable to CA-125 power in detecting early stages of the disease.

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