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Early Detection of Ovarian Cancer - An Enduring Challenge: Pro-Neurotensin and Procalcitonin as Promising Biomarkers

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Abstract

Background: Ovarian cancer is the leading cause of cancer death among female gynecologic malignancies, mainly because most of ovarian cancer diseases are diagnosed at an advanced stage. Hitherto, existing approaches for early detection were not able to reduce mortality. Therefore ongoing research has been focusing on the detection of new biomarkers, which have the potential of being used in screening programs, considering early detection as a premise to affect long-term survival.

Methods: 28 ovarian cancer patients and 68 non-cancer control subjects were enrolled into this cross-sectional study. A baseline survey was conducted and participants' fasting plasma was stored to measure the biomarkers Pro-Neurotensin (pro-NT), Procalcitonin (PCT) and CA125.

Logistic regression was used for uni- and multivariate analysis.

Results: Ovarian cancer patients showed significant lower concentrations of pro-NT and higher concentrations of PCT and CA 125 in fasting plasma (all p<0.0001). Within the ovarian cancer cases, pro-NT and CA125 respectively emerged to discriminate between early and advanced stage of ovarian cancer (pro-NT: p=0.0117; CA125: p=0.0361), and CA125 was associated with lymph node involvement (p=0.0461). Further analysis illustrated an increase of the predictive value of CA125 by combining pro-NT, PCT and CA125, resulting in a c index of 0.986 compared to the c index for CA125 of 0.950 (p=0.0004 for added value of proNT and PCT on top of CA125).

Conclusion: Not-withstanding the potential role as prognostic biomarkers, further studies with larger cohorts is necessary to confirm the findings.

Keywords: Biomarkers; Ovarian cancer; Pro-neurotensin; Procalcitonin; Early detection; Screening

Introduction

Ovarian cancer (OC) represents the second most common malignancy of the female genital organs and is the leading cause of death among female gynaecologic malignancies [1,2]. Despite the progress made in the understanding and treatment of ovarian cancer, its mortality rate has changed only marginally over the past 30 years. This is mainly due to the fact that nearly 67% of the patients diagnosed with high grade ovarian cancer are already at an advanced stage (Stage III and IV). However, the best basis for significant improvement in survival is an early detection of the disease [3].

In recent years there has been noticeable research in ovarian cancer screening, considering early detection as a premise to affect long-term survival.

In particular the assignments of new biomarkers, which have the potential of being used in screening programs, have gained increasing importance. Currently the cancer marker CA125 has been established in clinical routine. Yet its application is predominately considered as being useful in follow up rather than early detection as its specificity and sensitivity is still unsatisfactory [4-7]. Hence biological markers with high sensitivity and specificity are strongly needed for early detection of OC.

Promising in this regard is the tridecapeptide Neurotensin (NTS), which is a 13 amino-acid peptide that is produced from a single precursor, pre-pro-neurotensin [8]. It is well studied to act as neuromodulator in the central nervous system as well as a local hormone in the periphery [8,9]. By possessing numerous physiologic functions in the GI tract [9,10], it contributes to fat storage, obesity and metabolic disorders [8]. Overall existing data support broader biological effects assuming an involvement in growth stimulation of normal tissue such as bowel mucosa, pancreas, stomach, adrenal cortex as well as benign tumors like uterine leiomyomas [11,12].

Most importantly evidence also argues for a strategic role in carcinogenesis [8]. NTS is reported to have an effect at each step of tumor progression: cell proliferation, migration, invasion, and

neoangionesis [8,12,13]. All these cellular events are activated by the abnormal expression of NTS receptors at early stages of cell transformation [12,13]. NTS unfolds its effects by two different seven-transmembrane G-protein coupled receptors, NTSR1 as high affinity NTS receptor and NTSR2 as low affinity NTS receptor [8] and moreover, by a non-specific, single transmembrane sorting NTS receptor: NTSR3/sortilin [8].

Recent literature supports the use of NTS and NTSR1 as a potential biomarker for early detection and cancer progression [8]. Two Swedish population-based prospective cohort studies, the Malmö Diet and Cancer Study (MDC) and the Malmö Preventive Project (MPP), recently examined 3,498 women, whether NTS plasma concentration predicts the development of breast cancer during long-term follow-up [14,15]. By measuring fasting plasma concentration of pro-NT, a stable 117- amino acid fragment from the N-terminal part of the NTS precursor hormone and stoichiometrically equal to NTS, they were able to demonstrate a significantly positive correlation between fasting plasma level of pro-NT and later development of breast cancer [14,15]. To summarize, recent literature depicts the contribution of NTS and its receptors to tumor growth, migration and invasion in most solid tumors, e.g. breast cancer, prostate cancer, colorectal cancer, and pancreatic cancer [8]. So far, none is known about the validity for early detection and cancer progression in ovarian cancer.

Another potential biomarker described in literature is Procalcitonin (PCT), as prohormone of calcitonin. According to research, PCT has primarily emerged for predicting inflammatory conditions [16-19]. In a cross-sectional study, Matzaraki and colleagues reported increased serum PCT levels in cancer patients with metastatic disease [19]. Chaftari et al. confirmed these findings in their analysis depicting that PCT levels were significantly higher in cancer patients compared to non-cancer patients [16]. A recent prospective study suggested procalcitonin as a novel supplementary biomarker to increase the accuracy of prostate cancer screening. This is due to the fact that, significantly higher values of PCT were found in the prostate cancer group compared to the benign group [20]. Among cancer patients, advanced stage of disease was associated with significantly higher median PCT levels than early cancer disease [16], and thus attaches importance also to the prediction of disease progression.

This cross-sectional study aims to evaluate the potential role and the efficacy of plasma derived pro-NT and PCT as promising biomarkers in a cohort of ovarian cancer patients compared to non-affected subjects.

Materials and Methods

Assessment of clinical data

We conducted a cross-sectional clinical study that comprised 28 patients with ovarian cancer and 68 non-cancer control subjects who presented to the gynecological department of the Technical University in Munich and Rotkreuzklinikum in Munich respectively between August 2015 and January 2017. Included were cancer patients presenting with the primary diagnosis of ovarian cancer before any start of treatment. 21 patients presented with advanced stage of high grade serous ovarian cancer, which was defined as stage (FIGO) III-IV. Seven patients depicted early stage of disease comprising FIGO stage I-II (Table 1). 23 of 27 patients showed high grade serous tumor histology, three patients presented with endometrioid ovarian cancer and one patient respectively depicted low grade serous, clear cell and mucinous histology.

	N (%)
T-stage	
1a	3 (10.7)
1b	0
1c	3 (10.7)
2a	1 (3.6)
2b	1 (3.6)
3a	1 (3.6)
3b	7 (25)
3c	12 (42.8)
N-Status	
Positive	17 (60.7)
Negative	11 (39.3)
M-Status	
M1a	2 (7.1)
M1b	1 (3.6)
FIGO Stage (International Federation of Gynecology and Obstetrics system, 2016)	
IA	3 (10.7)
IB	0
IC	3 (10.7)
IIA	1(3.6)
IIB	0
IIIA	0
IIIB	8 (28.6)
IIIC	10 (35.7)
IVA	2 (7.1)
IVB	1(3.6)
Residual disease status	
R_0 (macroscopically no residual disease)	22 (78.6)
R_1 (residual disease <2 cm)	2 (7.1) (pt1/pt2: miliary dissemination along intestine)
R_2 (residual disease >2 cm)	4 (14.3) (pt1/pt2: with residual disease on mesenteric root; pt3: mediastinal lymph nodes; pt4: widespread residual disease along intestine)
Histology	
Serous high grade	22 (78.5)

Serous low grade	1(3.6)
Endometrioid	3 (10.7)
Clear cell	1(3.6)
Mucinous	1(3.6)

Table 1: Distribution of tumor stages.

To reduce the risk of recurrence, all patients received a guideline based treatment with a stage adapted surgery followed by chemotherapy with Carboplatin AUC5 and Paclitaxel 175 mg/m^2 q21. For advanced stage ovarian cancer cases (from FIGO IIIB) Bevacizumab 15 mg/kgKG q21 was additionally administered for 15 months. A patient with endometroid low grade carcinoma (pT1a G1) did not receive adjuvant chemotherapy. One patient presenting with mucinous ovarian carcinoma pT1a G2, a platin-based monotherapy was recommended.

Control subjects included healthy volunteers, who worked at the gynecological department of the Technical University in Munich during that period and patients who presented to both clinics without evidence of cancer. At study entry, vital parameters and temperature were recorded for all patients. There was no noticeable finding. All patients enrolled into this study underwent physical examination at study baseline. None of the patients presented with a sign of infection.

To avoid an adulteration of the biomarkers (especially pro-NT), subjects with severe kidney and cardiovascular disease, inflammatory disease as well as status after bowel resection, were excluded from the study. To exclude inflammation process we subsequently performed CRP analysis and evaluated leucocyte count which was administered within pre-surgical diagnostics. Further exclusion criteria were as follows: pregnancy, age < 18 years, participation in another clinical trial.

The local Ethical Committees of the Technical University of Munich approved the study protocol (reference number 146/14) and written informed consent was obtained from all participants before entry into the study.

At study enrollment a baseline survey was conducted. A questionnaire, developed for this study, was used to record the patients ´ age, BMI, medical history, disease status, and risk factors for carcinogenesis such as smoking, alcohol consumption, hormone replacement, reproduction, and behavioral factors of their lifestyle. Furthermore, the familiar and social background was encompassed.

In addition, medical records were reviewed to confirm admitting diagnosis as well as tumor characteristics. Tumor site was registered according to the International Classification of Diseases (ICD) version used at diagnosis. Moreover, baseline CT scan had to be allocated from all cancer patients within 45 days before first diagnosis. After registration to the study, participants were reexamined for blood collection after a 12-hour fasting period. Following, we immediately stored EDTA plasma aliquots at -80°C.

Biomarkers were measured in the stored fasting plasma. Pro-NT was quantified using a chemiluminometric sandwich immunoassay to detect a pro-Neurotensin precursor fragment (proNT 1–117) [14,15]. PCT levels were gauged using BRAHMS PCT sensitive KRYPTOR (BRAHMS GmbH, Hennigsdorf, Germany).

One year follow-up was conducted to obtain data on death and disease progression. Cancer progression had to be verified by imaging techniques; increase in peripheral tumor marker concentration alone was not considered as disease progression.

CT image analysis and body composition calculations

Hypothesizing that ovarian cancer patients suffer from cachexia, which could possibly influence the fasting plasma concentration of pro-NT, we additionally evaluated sarcopenia, as marker for paraneoplastic cancer cachexia [21].

For the assessment of sarcopenia as described by Bronger and Hederich et al., cross-sectional muscle area measurement in computed tomography slides of the L3 lumbar region was applied [21]. Precise defined muscle groups have been shown to be highly correlated with total body skeletal muscle [21].

For calculation muscle areas (cm^2) from 2 consecutive CT slides were averaged to give the muscle cross sectional area for the respective CT scan and was then related to the stature of the patients (muscle area/height^2) [21]. All measurements were performed by a trained person (P.H.) using the OsiriX software version 5.8.1 (Pixmeo, Geneva, Switzerland).

Statistical analysis

Values are expressed as means and standard deviations, medians and interquartile ranges (IQR), or counts and percentages, as appropriate. Group comparisons of continuous variables were performed using the Kruskal-Wallis test. Biomarker data were log-transformed. Logistic regression was used to evaluate proNT, PCT and CA125 for the diagnosis of ovarian cancer patients, both for uni- and multivariate analysis [22]. To demonstrate independence from the current state of the art, the added value of proNT and/or PCT on top of CA125 was evaluated based on the likelihood ratio chi-square test for nested models. The concordance index (C index or AUC) is given as an effect measure for uni- and multivariate models. For multivariate models, a bootstrap corrected version of the C index/AUC is given. 95% confidence intervals (CI) for risk factors and significance levels for chi-square are given. Receiver-operating-characteristic (ROC) curves were constructed for proNT, PCT and multivariate models to illustrate their ability to predict ovarian cancer.

All statistical tests were 2-tailed and a two-sided p-value of 0.05 was considered for significance. The statistical analyses were performed using R version 2.5.1 (http://www.r-project.org, library Design, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results

In our study we analyzed 28 patients with ovarian cancer and 68 non-cancer subjects. The mean age of the women in the study was 50 years (Table 2). With respect to possible confounders, cases were older than controls (66 yrs vs. 43 yrs). Further clinical characteristics of the study participants are shown in Table 2. In the scope of one year follow-up, 8 of 28 patients had developed recurrent disease including one patient that had died of her cancer disease.

Pro-neurotensin (pro-NT)

According to the analysis the median fasting plasma concentration in patients with ovarian cancer was 60.9 pmol/l (interquartile range (IQR) 41.9-71.9). In non-cancer subjects, median pro-NT was 94.0pmol/l (IQR 64.3-132.7, p<0.0001). In univariate logistic regression, fasting plasma concentration in ovarian cancer patients was found to be significantly lower as compared to non-cancer patients (p≤0.0001) (Table 3a). The c index (equivalent to the area under the ROC curve) was 0.794 with a 95% confidence interval of 0.708 to 0.882 (Table 3a). For illustration, an ROC plot differentiating ovarian-cancer patients vs. non-cancer individuals is plotted (Figure 1). To demonstrate the biomarker's ability to differentiate especially between early stage ovarian cancer patients and healthy controls, we performed post-hoc analysis. Assessment revealed a significant difference in concentrations comparing healthy controls and early stage ovarian cancer subjects (p<0.0001). The median pro-NT concentration in healthy controls was stated to be 94 pmol/l (IQR 64.3-132.7), whereas median concentration in early stage ovarian cancer was significantly lower, depicting a level of 42.2 pmol/l (IQR 32.3-52.5). Moreover analysis ensured a significant differentiation from advanced stage ovarian cancer patients respectively, constituting a median pro-NT concentration of 61.9pmol/l (IQR-49.7-73.6) (p<0.0001).

Demographics	All	Ovarian Cancer Pts (n=28)	Controls (n=68)
Age (years)	50 [37-64.3]	65.5 [58.5-72]	43 [32.8-53.3]
Height (cm)	166.5 [160-170.3]	165.5 [159.5-168]	168 [160-171.3]
Weight (kg)	63.5 [56-72.3]	66.5 [57.8-74]	62.5 [54.8-70]
BMI	23.1 [20.4-25.9]	24.2 [20.9-28.7]	22.5 [20.1-25.7]
Menopausal status			
Pre/perimenopausal		3	44
Postmenopausal		25	24

Table 2: Demographic and clinical characteristics.

Biomarkers	Model Chi^2	p-value	C index [95% CI]
PCT	16.5	<0.0001	0.721 [0.603-0.839]
pro-NT	24.3	<0.0001	0.794 [0.708-0.882]
CA125	79.8	<0.0001	0.950 [0.876-1.000]

Table 3a: Univariate logistic regression results.

Furthermore, correlation analysis confirmed that the novel biomarker pro- NT discriminates between early stage and late stage. To exclude age-dependency of the biomarkers we confirmed our results in a large control collective from the Malmö cancer and diet (MDC) [14] study with 1,929 (age 58 [IQR 53-63], Range 57-80) healthy subjects.

Pro-NT levels presented with a median of 107.7pmol/l [IQR 77.6-150.0]. Statistical analysis did not show a significant age-dependent correlation to pro-NT (r= 0.03, p=0.27, Spearman).

Procalcitonin (PCT)

The median PCT level in ovarian cancer patients was 0.053 ng/ml (IQR 0.045 ng/ml-0.071 ng/ml) versus 0.043 ng/ml (IQR 0.034 ng/ml-0.051 ng/ml) in the cohort of non-cancer subjects. According to logistic regression, PCT concentrations constitute a significant higher level in ovarian cancer patients as compared to non-cancer patients (p=0.0005), and a c index of 0.721 with a 95% confidence interval of 0.603 to 0.839.

Pursuing the same approach we engaged to illustrate its potential to differentiate between early stage ovarian cancer and healthy controls. Post-hoc analysis demonstrated a median PCT concentration of 0.04 ng/ml (IQR 0.03-0.05) in healthy controls, compared with this early stage ovarian cancers yielded a median concentration level of 0.06 ng/ml (IQR 0.05-0.07).

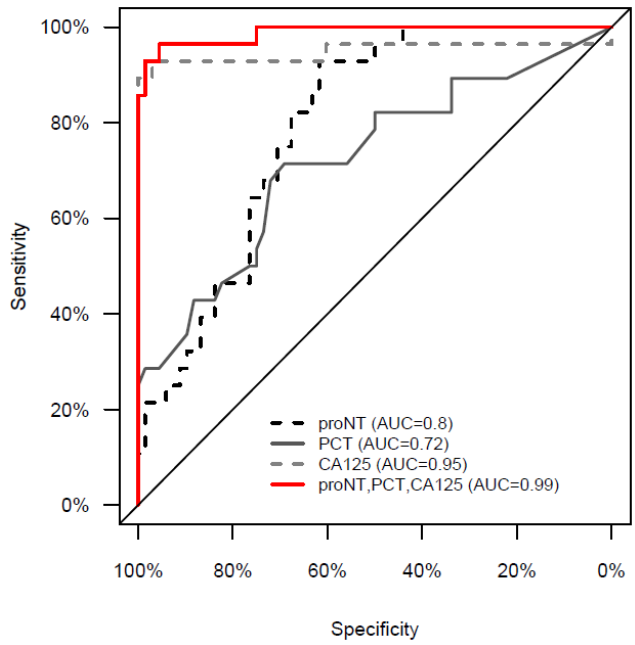

Figure 1: pro-NT, PCT, CA125 and combined analysis in ovarian cancer patients versus controls.

Also relative to PCT concentration in advanced stage ovarian cancer patients, stating a median level of of 0.05 ng/ml [IQR 0.04-0.07], PCT

significantly differentiates between healthy controls and early stage ovarian cancer patients (p=0.002).

With respect to age-dependency, PCT levels were additionally correlated in a larger cohort of healthy controls of the MDC-study. The median PCT level was 0.015 ng/ml [IQR 0.012-0.018]. There was no significant age-dependent correlation (r= 0.04, p=0.73).

To exclude an acute inflammatory process, considering PCT as inflammation related molecule, we examined the patients' CRP levels and peripheral leucocyte blood count as well as vital signs / physical status from study baseline and pre-surgical diagnostics. None of the patients presented with a sign of acute infection. Vital signs and temperature were recorded for all patients, there was no noticeable finding.

Leucocyte counts of each ovarian cancer patient represented the normal values. CRP analysis showed a median CRP of 0,93 mg/dl (IQR 0,93-2,67). Five cases that presented with extremely high tumor burden showed elevated CRP levels, thus these marker elevations are considered to be cancer related [23]. We did not find any correlations in vital signs and leucocyte counts, which remained within the normal range. Further analysis did not show any correlation between CRP and pro-NT levels (r=0.11). Yet, for CRP and PCT as inflammation related molecules, data assessment showed a moderate correlation (r=0.64).

CA125

For adjustment, the established biomarker CA 125 was additionally measured in our study collective consisting of cancer and non-cancer patients. The median CA125 level in ovarian cancer patients was 275.9 U/ml (IQR 104.7 U/ml - 512.2 U/ml), whereas in the control group the median was stated to be 11.4 U/ml (IQR 7.8 U/ml - 17.1 U/ml). The logistic regression analysis yielded a c index of 0.950 with a 95% confidence interval of 0.876 to 1, with significantly higher CA125 level in ovarian cancer patients ($p \leq 0.00001$) (Table 3a).

Combined analysis of pro-NT, PCT and CA125

The association of pro-NT and PCT with ovarian cancer was adjusted for CA125 levels using multivariate logistic regression. Due to further analysis pro-NT, PCT and CA125 presented statistically independent of each other regarding both PCT and pro-NT, individually added to CA125 (p=0.0040 and 0.0026, respectively), as did the combination of all three biomarkers (p= 0.0004 for added value on top of CA125) (Table 3b). The c index (bootstrap corrected for multivariable models) increased from 0.950 for CA125 to 0.973, 0.977 and 0.988 when adding PCT, pro-NT or both, respectively (Figure 1).

Model	Model Chi^2	Added Chi^2 (on top of CA125)	Added value p-value	Bootstrap corrected C index
PCT, CA125	88.1	8.3	0.004	0.973
pro-NT, CA125	88.9	9.1	0.0026	0.977
PCT, pro-NT, CA125	95.7	15.9	0.0004	0.986

Table 3b: Multivariable logistic regression results.

Among ovarian cancer patients, pro-NT and CA125 respectively emerged to discriminate between early and advanced stage of ovarian cancer (pro-NT: p=0.0117; CA125: p=0.0361) (Figures 2a and 2b). Yet all biomarkers failed to predict the risk of recurrent disease (n=8 (28.6%), data not shown), and only CA125 was associated with lymph node invasion by differencing between N0 and N1 situation (p=0.0416, Figures 3a-3c).

Scoping clinical characteristics, we performed an additional analysis to examine a possible association to pro-NT, CA125 and PCT. Overall data did not show any correlation between the biomarkers and patients' characteristics.

CT image analysis and body composition calculations

Baseline CT scans of all ovarian cancer patients were requested. 19 out of 28 baseline scans were available and appropriate for CT image analysis and cross-sectional muscle area measurement for the assessment of sarcopenia. All patients considered for analysis were tested negative for sarcopenia.

Discussion

PCT in a cohort of ovarian cancer patients. Both biomarkers were found to sufficiently discriminate between early stage ovarian cancer and healthy controls, confirmed by statistical significance. Particularly the combined assessment of pro-NT, PCT and CA125 revealed an increase of the predictive value of CA125 and thus might have a remarkable impact as indicators for ovarian cancer.

Ovarian cancer is the fifth most lethal cancer in women [24]. A primary cause for the high mortality rate is owned to the fact that the majority - more than 60% - of the patients are diagnosed with advanced stage disease (FIGO stage III/IV), since early ovarian cancer disease is mostly attendant by unspecific symptoms [24]. Additionally, early detections programs for ovarian cancer failed so far.

Currently the assessment of transvaginal ultrasound (TVS) and assignment of the biomarker CA125 is available in clinical routine. To examine the validity of these approaches as early detection methods, several studies have been initiated. To mention, the most recent and largest trials, the US PLCO Cancer Screening Trial as well as the UKCTOCS, were not able to show a mortality benefit by multimodal ovarian cancer screening [25,26], even though the UKCTOCS reported a significant stage shift to earlier diagnosis (stage I/II) in the multimodal (CA125 and TVS) arm [27].

Due to the missing data on mortality reduction, the German S3 Guidelines for malign ovarian tumors, the U.S. Preventative Task Force and the U.K National Screening Committee advance a consensus view that the screening for ovarian cancer with TVS and CA125 measurement in the general population as well as in women at high risk is not recommended. This recommendation was strengthened by

the corresponding increase of morbidity and mortality rates based on surgical intervention of false positive patients [28-30].

Several studies have been conducted on different additional ovarian cancer biomarkers. For example, aiming to enhance the distinction of malignant from benign pelvic masses, research has recently taken another approach by incorporating CA125 and HE4 into the "Risk of Ovarian Malignancy Algorithm" (ROMA) with menopausal status. However additional validation is required [31].

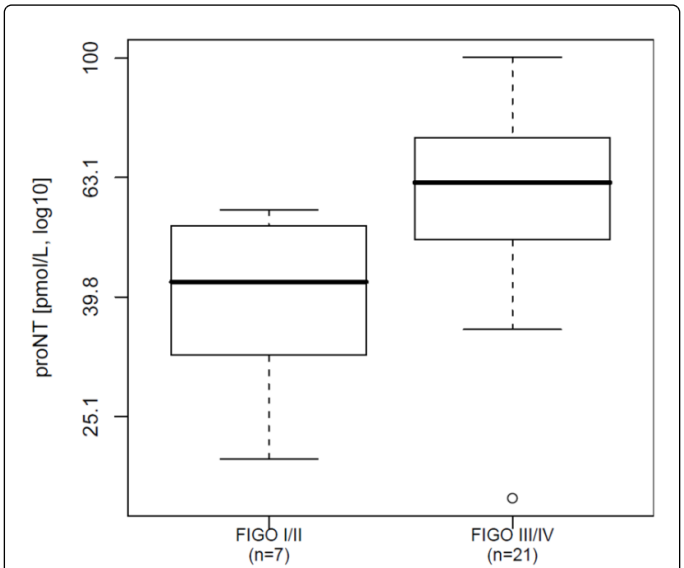

Figure 2a: Pro-NT in early (FIGO I, II) versus advanced stage (FIGO III, IV) of ovarian cancer.

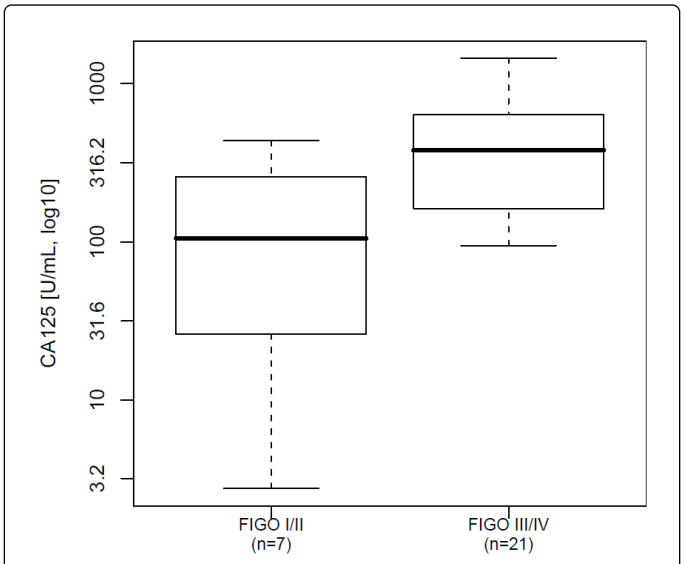

Figure 2b: CA125 in early (FIGO I, II) versus advanced stage (FIGO III, IV) ovarian cancer.

As mentioned above, evidence stresses the fundamental impact of the NTS/NTSR1 complex on carcinogenesis yielding that human cancer cell lines, e.g. ovarian cancer, express high levels of NTSR1 and exhibit growth responses to NTS [11]. NTS induces NTSR1 receptor synthesis and continuous receptor recycling, which results in permanent cell sensitivity to NTS and thus enhancement of tissue survival and proliferation [11].

Considering analysis Liu et al. identified a significantly higher NTS expression in tumor samples of high grade ovarian adenocarcinomas than in borderline tumors (low grade ovarian cancer) and normal ovaries [24].

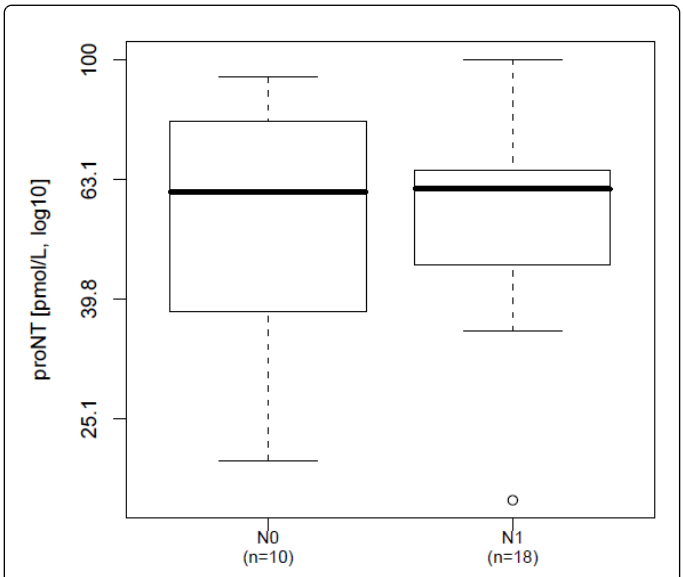

Figure 3a: Correlation of pro-NT and lymph node invasion (N0 versus N1).

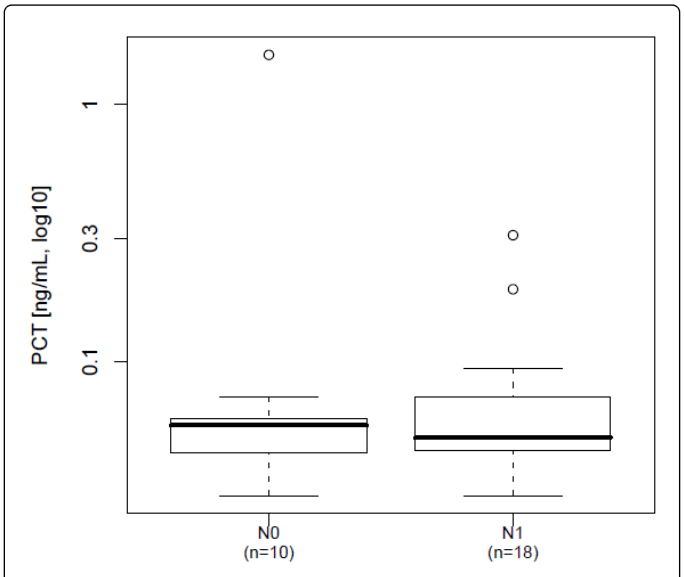

Figure 3b: Correlation of PCT and lymph node invasion (N0 versus N1).

In our analysis we investigated the fasting plasma concentration of pro-NT in ovarian cancer patients, which has not been examined elsewhere so far, according to the best of our knowledge. Opposing general findings on high NTS expression in tumor tissue, we observed

an inverse correlation concerning fasting plasma concentration of pro-NT in ovarian cancer patients. The pro-NT plasma concentration was significantly lower in ovarian cancer patients compared to non-cancer controls. The MDC/MPP study illustrated a significantly positive correlation between fasting plasma level of pro-NT and later development of breast cancer [14,15]. Melander et al. demonstrated a positive correlation between high levels of pro-NT in fasting plasma and increased risk of obesity-derived diseases as diabetes mellitus, cardiovascular disease and breast cancer [14]. Referring to the key metabolic actions of NST including digestion and metabolism of fat [14], we hypothesize that a possible explanation for the reduced pro-NT plasma level in ovarian cancer patients could be the catabolic turnover. For clarification we engaged in further analysis. Therefore we relied on a proven marker for the paraneoplastic cancer cachexia in ovarian cancer patients, namely sarcopenia. Contrary to our expectations, none of the 19 ovarian cancer patients considered for body composition analysis met the cutoff value, established for sarcopenia in ovarian cancer patients.

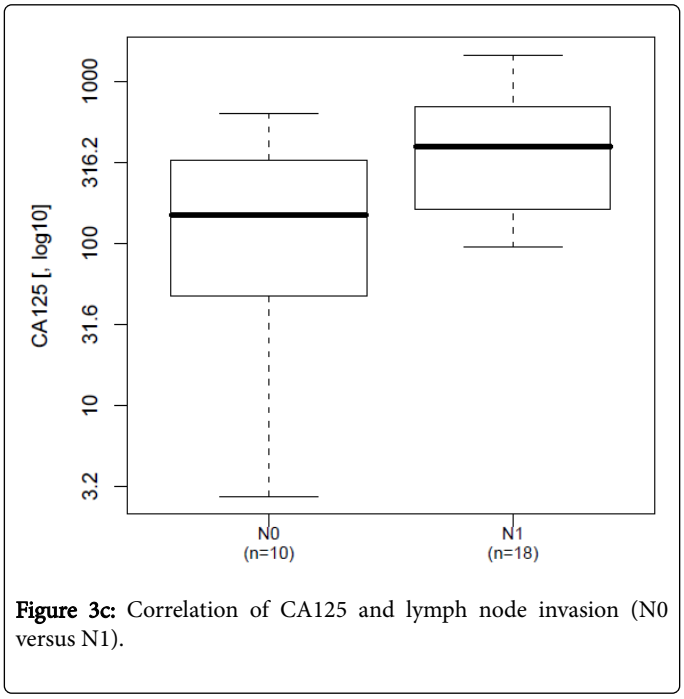

Figure 3c: Correlation of CA125 and lymph node invasion (N0 versus N1).

Accordingly the underlying relationship between pro-NT plasma concentration and ovarian cancer remains unclear so far. Nevertheless the importance of this finding remains unaffected. Moreover, analysis revealed significant higher PCT levels in ovarian cancer subjects compared to non-cancer subjects and thus confirmed previous research on this topic.

As mentioned before several studies reported increased serum PCT levels in cancer patients [30]. Among cancer patients, advanced stages of disease were associated with significantly higher median PCT levels than early cancer disease stages [16]. Thus it is important for prediction of disease progression as well as disease detection in lower tumor stages. In our cohort an acute inflammatory process, which could influence PCT levels as it is an inflammation related molecule, was excluded through clinical and laboratory diagnostics. However data has been indicating a role of chronic inflammation in ovarian carcinogenesis [30-33].

Conclusion

Concluding, PCT elevation might be limited by a reduced level of specifity, however the combination with inflammation-independent pro-NT strengthens its validity. Furthermore a very striking result to emerge is that pro-NT, besides CA125, significantly changed in concentration depending on tumor stage, early *vs.* advanced stage and may thus play a role in assessing progression of disease. Yet, in our study, only CA125 revealed to be correlated with lymph node invasion and none of the biomarkers showed evidence for predicting the risk of developing recurrent disease in the future.

However, our study needs to be interpreted in the light of its limitations. First it was a cross-sectional design with a small study population, resulting in a restricted ability to draw conclusions about the validity of pro-NT and PCT as biomarkers for early detection of ovarian cancer. Second, the medium average age differed significantly between cancer patients and non-cancer controls; yet we implemented further analysis within a large control collective of the MDC-study to confirm age-independency of the marker concentrations. Moreover patients' characteristics were gained from a retrospective chart review. Therefore, our current analysis must be considered as hypothesis generating study. Further data are necessary to confirm these findings in a larger cohort.

In summary, our study provides very striking findings about the relevance of pro-NT and PCT as biomarkers scoping early detection of ovarian cancer. Particularly the combined assessment of pro-NT, PCT and CA125 might have a remarkable impact as early detection indicators for ovarian cancer. Notwithstanding their role as predictive and prognostic biomarkers need to be further explored in larger cohorts. The datasets used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All procedures performed in the OMA study involving human participants were in accordance with the ethical standards of the ethics committee of the Technical University Munich and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards 146/14S, written informed consent was obtained from all individual participants included in the study.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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