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Early diagnosis of epithelial ovarian cancer - Analysis of novel biomarkers

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Fakultetsopponent: **Associate Professor Christer Borgfeldt**
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Avhandlingen baseras på följande arbeten:

I. **Ovarian cyst fluid is a rich proteome resource for detection of new tumor biomarkers**
Kristjansdottir B, Partheen K, Fung ET, Marcickiewicz J, Yip C, Brännström M, Sundfeldt K
Clinical Proteomics, 2012 Dec 27; 9(1):1, doi:10.1186/1559-0275-9-14

II. **Early inflammatory response in epithelial ovarian tumor cyst fluid**
Kristjansdottir B, Partheen K, Fung ET, Yip C, Levan K, Sundfeldt K
Manuscript

III. **Potential tumor biomarkers identified in ovarian cyst fluid by quantitative proteomic analysis, iTRAQ**
Kristjansdottir B, Levan K, Partheen K, Carlsohn E, Sundfeldt K
Clinical Proteomics, 2013 Apr 4; 10(1):4, doi: 10.1186/1559-0275-10-4

IV. **Evaluation of ovarian cancer biomarkers HE4 and CA125 in women presenting with a suspicious cystic ovarian mass**
Partheen K, Kristjansdottir B, Sundfeldt K
Journal of Gynecologic Oncology, 2011 Dec 22; 4:244-252
doi: 10.3802/ jgo.2011.22.4.244

V. **Diagnostic performance of the biomarkers HE4 and CA125 in type I and type II epithelial ovarian cancer**
Kristjansdottir B, Partheen K, Levan K, Sundfeldt
Gynecologic Oncology 2013 Jul 25; 131:52-58
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Early Diagnosis of Epithelial Ovarian Cancer - Analysis of Novel Biomarkers

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ABSTRACT

Background: Majority of epithelial ovarian cancer (EOC) is detected in advanced stage with bad prognosis and high mortality. Reliable diagnostic markers are lacking, pre-cancerous lesions in the more aggressive tumors are not clearly defined, vague or unspecific early symptoms, and the localization of the ovaries, deep in the pelvis contributes to late diagnose. Heterogeneity, not only different type of histology, but also different intrinsic biology and behavior characterizes ovarian cancer. Invasive surgery with histological examination is needed to confirm the diagnosis. Less than 25% EOC are diagnosed early, when there is great possibility to cure and 5-year survival >90%, in contrast to 20-30% 5-year survival in late stage EOC. Thus, early detection is of utmost importance. Proximal fluids, like ovarian cyst fluid, are promising in the search for early markers. Cancer antigen 125 (CA125), the most used biomarker since 30 years, and a promising marker human epididymis 4 (HE4) have recently been approved by FDA to be used in the prediction of malignancy in women with a pelvic mass.

Aims: To explore ovarian cyst fluid as a source mining for new diagnostic biomarkers for EOC, and to validate the markers found together with CA125 *(Paper I-III);* and to evaluate the diagnostic performance of HE4 and CA125, to distinguish between benign cysts and EOC, and EOC divided into slow growing type I and the aggressive type II EOC *(Paper IV-V)*.

Method: Cross sectional, observational, explorative, and diagnostic clinical studies, with prospective and consecutive collection of cystic fluid, blood and tumor tissue at the time of operation and retrospective analysis. Women with suspicious malignant pelvic cysts, already scheduled for operation at our clinic for tumor surgery were included. High throughput proteomic analyses were used for searching for novel markers, and selected proteins were validated with ELISA or immunoblot. *Paper I:* The cyst fluid proteome was mined with surface-enhanced laser desorption/ionization time of flight (SELDI-TOF) mass spectrometry (MS) (n=192). *Paper II:* Enrichment of a selection of known cancer antigens to overcome high abundant proteins, and with focus on inflammation, was followed by Immunoprecipitation MS (n=38). Significantly differently expressed chemokines were validated (n=256). *Paper III:* Serous cystadenoma (n=5) and serous adenocarcinoma (n=10) of different stages were analyzed with isobaric tag for relative and absolute quantification (iTRAQ), followed by immunoblot validation (n=68). *Paper IV-V*: HE4 and CA125 levels in plasma were analyzed with ELISA and Risk of Ovarian Malignancy Algorithm (ROMA) was calculated (n=393). Significant differences, receiver operator characteristics (ROC) area under the curve (AUC), cut-off levels, sensitivity and specificity were estimated with regard to malignancy, grade, stage histologic subtype and type I and type II.

Results: *Paper I:* Combination of Apolipoprotein CIII and Protein C inhibitor had the best AUC (0.91) in cyst fluid, and improved by CA125 (0.94). Abundant proteins were a problem in the cyst fluid analyses. *Paper II:* Interleukin-8 and Chemoattractant Protein-I were highly significantly increased expressed in cyst fluid. Increased inflammatory response was present in early tumor development and earlier than in blood. *Paper III:* Two of 87 differentially expressed proteins in cyst fluid, with high significance and fold change, Serum Amyloid A-4 (SAA4) and astacin-like metalloendopeptidase (ASTL) were validated, and SAA4 was significantly increased in cyst fluid, but not in blood. *Paper IV***:** HE4 complemented CA125 in the diagnosis of ovarian cysts, especially in the premenopausal women. Sensitivity for ROMA at set specificity of 75% was highest in the postmenopausal cohort (87%). *Paper V:* HE4 and CA125 diagnosed the aggressive type II EOC most correctly (AUC 0.93), but the results were not acceptable in early stage type II (AUC 0.85) or in type I EOC (AUC 0.79) respective early type I AUC 0.73).

Conclusion: Ovarian cyst fluid is an excellent source for the search of novel biomarkers for early diagnosis of EOC. Early events are found near the tumor in the early phase, like the inflammatory response and later on in the peripheral circulation. HE4 complements CA125 in predicting malignancy in cystic ovarian tumors. The result from this thesis support, that EOC should be looked upon as several different diseases. Finding early markers that are specific for each histology subgroup will be the future challenge. Combination of such markers in a panel could improve the early diagnosis of EOC.

Keywords: EOC; ovarian adenocarcinoma; ovarian cyst fluid; pelvic mass; tumor biomarker; mass spectrometry; SELDI-TOF MS; iTRAQ;

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