Neutrophil extracellular traps (NETs) as marker for thrombus formation and early detection of cancer

Background:

Ovarian cancer (ovarian-/tubal-/peritoneal) is the sixth most frequent type of cancer and the fourth leading cause of cancer related death among Swedish women. Due to mild symptoms, two thirds of patients with ovarian cancer are diagnosed with advanced stage of the disease, and this is reflected in poor outcome. Since thrombus formation in ovarian cancer is very common studies on ovarian cancer patients is suitable when looking at the relation between thrombosis and cancer.

In ovarian cancer a variety of tumor markers in peripheral blood, most notably CA–125, have been found inadequate as screening test for ovarian cancer mainly because of poor sensitivity in early stage ovarian cancer. RMI (risk of malignancy index) is an algorithm incorporating CA-125, ultrasound findings and menopause status for the preoperative assessment of adnexal tumors (Jacobs et al 1990). It has been demonstrated to distinguish ovarian cancer from benign ovarian masses with a sensitivity of 92% and a specificity of 82% (cut-off = 200) when used in a tertiary center (Hakaansson et al 2012). Human epididymis protein 4 (HE4) is a novel tumor marker approved by the FDA for monitoring recurrence or progressive disease in patients with epithelial ovarian cancer (Moore et al 2009). Over expression of HE4 has been found in serous and endometrioid ovarian carcinomas. HE4 is included together with CA-125 in the ROMA algorithm (risk of ovarian malignancy algorithm) (Moore et al 2010) for preoperative assesment of suspected ovarian cancer. The biomarker panel suPAR(II-III), HE4, CA125 and age in premenopausal women improved discrimination of malignant and benign ovarian tumors (Leandersson et al 2016) and high preoperative blood levels of HE4 has shown to predict poor prognosis in patients with ovarian cancer (Kalapotharakos et al 2012).

Inflammation is considered one of the hallmarks of cancer, and emerging light is now being shed on the neutrophil release of nuclear chromatin, referred to as *neutrophil extracellular traps* (NETs) in cancer and cancer-associated thrombosis. NETs were first discovered by Brinkman et al in 2004 (Brinkman et al 2004) as a mechanism for trapping and killing bacteria by the innate immune system, but has recently been shown to play a central role in non-infectious conditions such as arterial (Qi H 2017) and venous (Kimball 2016) thrombosis and cancer. Driven by the tumor environment, NETs have been implicated in tumor progression (Demers Oncoimmunology 2016, Guglietta S Nat Coomun 2016) by interacting with cancer cells and enhancing immune escape and angiogenesis, metastatic spread (Cools-Lartigue 2013, Park 2016) by establishing a seeding bed for metastatic cells and promoting tumor cell migration, and in cancer-associated thrombosis (Demers, 2012, Thalin, 2016) by activation of platelets and coagulation factors as well as by providing a scaffold for platelets and red blood cells. In line with this, we have now found elevated levels of the NET-specific marker H3Cit in plasma from patients with a variety of malignancies (Thalin 2018) as well as from patients with cancer-associated thrombosis (Thalin 2016), suggesting the potential of NET markers, such as H3Cit, as novel cancer biomarkers. Notably, NETs have also been shown to accumulate in peripheral blood vessels, compromising organ function in tumor-bearing mice (Cedervall 2015), and markers of NETs have been detected in widespread microthrombi in cancer patients (Thalin 2016). We furthermore found associations between high levels of circulating H3Cit and mortality in patients with advanced cancer. These data not only suggest that markers of NETs may be useful in cancer diagnostics and prognostics, but also that a NET-induced microthrombotic state may contribute to multi-organ failure and death in patients with advanced cancer, providing possible therapeutic targets.

AIMS and planned studies:
1: Analysis of NET markers (primary marker H3Cit, secondary markers NE, cfDNA, IL-8) in plasma from 200 patients with benign, borderline and malignant ovarian tumors. Our objective is to analyze if H3Cit discriminate malignant from benign ovarian tumors.

2: Analysis of NET markers (H3Cit, NE, histone H3, histone 2B, DNA) in tumor tissue and (if present) micro-thrombi via histopathological evaluation from above 200 patients. The objective is to analyze correlations between NET-positive tumor tissue/thrombi and NET markers in plasma.

3. Analysis of markers of coagulation (D-dimer, TAT, sP-Selectin, PAI-1, TF) in plasma from above 200 patients. The objective is to analyze correlations between markers of NETs and markers of coagulation in plasma.

4. Analysis of markers reflecting multi organ failure (troponin, eGFR) in plasma from above 200 patients. The objective is to analyze correlations between markers of NETs and markers of organ failure in plasma.

5: Analysis if H3Cit levels in relation to overall survival may predict prognosis in ovarian cancer patients.

Ethical approval is granted by the Ethical Committee of Lund University. Dnr 558 2004, Dnr 94 2006, Dnr 495 2016.

Expected result / benefit for the society
In a broader perspective, markers of a pro-thrombotic state, such as markers of NETosis, such as H3Cit, could aid in revealing cancer in patients with thrombosis. These markers could also be useful in screening for susceptibility to thrombosis in cancer patients.If it appears that levels of NET biomarkers especially H3cit correlate to the risk of thrombosis in ovarian tumors and especially cancer, H3cit measurement may be used as a plasma biomarker for thrombosis prevention.
If H3cit appears to be a general biomarker for micro-thrombosis development in all types of inflammation, H3cit may also be used as a general plasma biomarker for thrombosis prophylaxis. Repeated measurements of H3cit may indicate if the risk of thrombosis persists and predict how long the thrombosis prophylaxis should be prolonged. Prolonged unnecessary usage of thrombus prevention put the patient at avoidable risk for bleeding complications, which is a large problem for the individual patient and in the health care system.

If plasma NET biomarkers especially H3cit correlate to the stage of cancer, these markers may be used in different biomarker panels to indicate early diagnosis of different types of cancer.

Material, methods and time schedule:
Frozen plasma and tumor tissue from 200 patients from the biobank GUNNEL at Lund University, Medicon Village will be selected by Arturas Dobilas (AD) and Christer Borgfeldt (CB). Patient records will be extracted to the study data base (AD). Preliminary plasma from benign n=25, borderline n =25 and malignant ovarian tumor n=150 patients will be analyzed by AD and Charlotte Thålin (CH) at the clinical laboratory at Danderyd hospital with ELISA (Thalin et al 2017). Tumor tissue will be analyzed by immunohistochemistry (Brinkman et al 2016) by AD and CT. The supervisors CB and Håkan Wallén (HW) will support project and the statistical analyses as well as the manuscript writing will be performed by all of the involved researchers in the group.

The time plan is to start autumn 2019 to extract patient material from the biobank and get the patients records. In 2020, the laboratory work will be performed at the laboratory in Danderyd hospital and late 2020 and in 2021; the statistical analyses and manuscript writing will be performed.

Participants and collaboration:
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Originality of the project and news value

Emerging light has recently been shed on the neutrophil release of nuclear chromatin, referred to as *neutrophil extracellular traps* (NETs) in cancer and cancer-associated thrombosis. Only few clinical studies have so far been made in this research field. The research group lead by Professor Håkan Wallén has developed new in house ELISAs and been able to study NET in cardiovascular patients. The collaboration with the team in Lund will allow studies of NET in ovarian cancer patients in different stages. Furthermore, NET and coagulation measurements will explore the risk for thrombosis and the association to early cancer.

Publication of results

The results will be published in a scientific peer reviewed English spoken journal, as well as in Swedish journal relating to medical science and popular science journal. If the results keep what the studies published so far indicate, contact will also be made with radio and television channels.

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