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Full Length Article

Procoagulant activity in gynaecological cancer patients; the effect of surgery and chemotherapy

F. Abu Saadeh [a,*], R. Langhe [c], D.M. Galvin [a], S.A. O Toole [c], D.M. O'Donnell [b], N. Gleeson [a,c], L.A. Norris [c]

[a] Department of Gynaecology Oncology, St James's Hospital, Dublin 8, Ireland
[b] Department of Medical Oncology, St James's Hospital, Dublin 8, Ireland
[c] Trinity College Department of Obstetrics and Gynaecology, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland.

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ABSTRACT

Background: Gynaecological cancers are associated with high rates of venous thromboembolism (VTE). Studies on ambulatory cancer patients do not support thromboprophylaxis during chemotherapy. Approximately 6–7% of gynaecological cancer patients suffer a postoperative VTE despite Low Molecular Weight Heparin prophylaxis (LMWH). Large cancer studies have shown that Calibrated Automated Thrombogram (CAT) and Microparticles (MP) assays may be useful in predicting VTE but data on gynaecological cancer patients is scarce.
Objective: Our objective was to identify whether the CAT assay and MP functional assays have potential as biomarkers predictive of VTE in gynaecological cancer patients.
Patients and methods: Gynaecological cancer patients were investigated before surgery (n = 146) and at 5, 14 and 42 days post-surgery (n = 78). Fourteen additional patients were investigated before chemotherapy and after 3 and 6 cycles of therapy. Thrombin generation was measured before and after addition of thrombomodulin.
Results: Patients with clear cell cancer (CCC) of the ovary and patients with endometrial cancer had higher ETP and peak thrombin compared with patients with benign disease. Patients who developed VTE (n = 8) following surgery had enhanced thrombin generation prior to surgery which persisted during the post-operative period despite LMWH prophylaxis. Both neoadjuvant and adjuvant chemotherapy showed increased thrombin generation following addition of thrombomodulin. There were no differences in MP levels during the study.
Conclusions: CAT assay shows potential as a promising biomarker for the prediction of VTE in gynaecological cancer patients. The identification of high risk patients combined with individualised LMWH prophylaxis might reduce VTE in this high risk group.

1. Introduction

Gynaecological cancers, and in particular adenocarcinomas are associated with a very high risk of VTE; with rates as high as 42% reported for clear cell cancer of the ovary [1]. Patients are particularly at risk during the postoperative period where VTE occurs in 6–7% of patients despite LMWH prophylaxis, [2]. Recent international guidelines recommend extended thromboprophylaxis beyond hospital stay for cancer patients having major pelvic and/or abdominal surgery [3]. This strategy has decreased postoperative VTE events, but confers a risk of substantial bleeding [4]. An increasing number of ovarian cancer patients are treated with neoadjuvant chemotherapy. The cumulative effects of chemotherapy and surgery may further increase the risk of VTE. Chemotherapy releases procoagulant material from the tumour and is also associated with endothelial damage, which may result in disruption of key regulatory pathways in haemostasis including the activated protein C (APC) pathway [5, 6]. Predictive biomarkers for VTE in ambulatory cancer patients have been proposed but, the utility of these biomarkers for VTE in gynaecological patients has not been investigated [7]. Previous studies from our group have shown that ovarian tumours from patients who developed cancer related VTE express increased amounts of Tissue Factor (TF), the primary initiator of blood coagulation, compared to those from matched patients with gynaecological cancer who remained thrombosis free [8]. This suggests that procoagulant material emanating from the tumour could contribute to a hypercoagulable state in these patients. Plasma assays to detect such hypercoagulability may therefore be useful in identifying patients at high risk of VTE and facilitate individualised LMWH prophylaxis.

Tumour derived Microparticles (MPs) are negatively charged small membrane vesicles which are highly procoagulant and are associated with TF activity. A recent study showed that tumour derived TF-MPs can trigger VTE in an animal model [9]. This suggests that tumour derived MPs could be a useful biomarker for cancer associated VTE [10]. Results of studies on MP in cancer are inconclusive, largely due to the variety of methods used for detection. The Vienna Cancer and Thrombosis Study (CATS) has shown that although elevated MP-TF levels are found in patients with un-resectable, poorly differentiated

* Corresponding author.
E-mail address: ferasabusaadeh@dubgyn.org (F. Abu Saadeh).

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adenocarcinomas, MP-TF activity was not predictive of VTE [11]. A randomised trial of LMWH prophylaxis based on levels of MP-TF showed that MP-TF might have potential as a screening tool in pancreatic cancer, however the low rate of VTE events in the study precluded definitive conclusions [12].

Calibrated automated thrombography (CAT) is a global coagulation assay that measures the potential thrombin production following stimulation with TF. The CAT assay has been shown to be predictive of increased risk of VTE in ambulatory cancer patients and also predict recurrent thrombosis [13]. The CAT test can also be adapted to increase its sensitivity to APC [14]. Recent studies have shown that it may be superior to anti-Xa as a surrogate marker of LMWH activity [15].

We used the CAT assay and a functional MP assay to measure the procoagulant activity of patients with gynaecological cancer compared with those with benign disease. We also examined procoagulant activity in a group of ovarian cancer patients undergoing neoadjuvant (preoperative) chemotherapy, surgery and adjuvant (postoperative) chemotherapy. Our aim was to identify whether the CAT assay and the MP assay have potential as biomarkers for VTE in gynaecological cancer patients.

2. Methods

2.1. Study design and setting

All patients with adenocarcinoma of ovary or endometrium diagnosed and treated in St James's Hospital, Dublin, Ireland (a tertiary cancer centre) over an 18-months period were invited to participate in this study. Patients undergoing surgery for benign gynaecological conditions were recruited as controls. Patients with known thrombophilia (defined as Protein C, S or antithrombin deficiency, Factor V Leiden, Prothombin 20210 mutation or Lupus anticoagulant positive) or a personal history of VTE prior to the diagnosis of cancer were excluded. Ethical approval was obtained from the local ethics committee.

2.2. Patients

Following full and informed written consent, venous blood samples were taken from patients with suspected gynaecological malignancy 1–2 days prior to surgery for removal of their tumour and from patients undergoing surgery for benign tumours. Patients were classified according to the histological subtype of tumour. Clinical information was obtained from a review of hospital chart including; age, surgery, debulking status, tumour histology, stage and grade of the cancer. Patients were followed up for 1 year after surgery. All patients had LMWH (Tinzaparin 75 IU/kg daily) for the duration of their hospital stay. Symptomatic VTE following surgery was objectively confirmed by CT pulmonary angiogram or venous Doppler. A subgroup of patients also had blood samples taken at day 5 (4 h post-prophylactic LMWH), 14 days and 42 days post-surgery. Patients undergoing chemotherapy were treated with carboplatin/paclitaxel. Patients undergoing adjuvant chemotherapy had blood samples taken at the start of therapy, and following cycle 3 and cycle 6 of treatment.

2.2.1. Blood sampling

At each time point, venous blood (4.5 ml) was taken from the antecubital fossa with minimum venous stasis using 3.13% sodium citrate as anticoagulant. For CAT analysis, samples were centrifuged at 4 °C for 20 min at 2000 *g*. The resulting platelet poor plasma was carefully removed, aliquoted into cryotubes and snap frozen at −80 °C until assay. For MPs analysis, blood was centrifuged at 1500 g at room temperature for 15 min; the resulting supernatant was recentrifuged at 13,000 *g* for 2 min. The final supernatant was carefully removed and stored at −80 °C until assay. All samples were processed and stored within 1 h of phlebotomy.

2.3. CAT assay

Endogenous thrombin potential (Thrombinoscope™, Synapse BV, Maastricht, Netherlands), was measured as previously described [16]. Briefly, 80 μl of plasma was incubated with 20 μl of platelet poor plasma reagent containing 5 pM of Tissue Factor. Thrombin generation was initiated by addition of fluorogenic thrombin substrate (Fluca® Thrombinoscope™, Maastricht, Netherlands) and quantified by thrombin calibration standard. Fluorescence was measured at 20 s intervals for 60 min or until thrombin generation was completed. Peak thrombin production and area under the thrombin generation curve (ETP), lag time and time to peak were determined and reported for each sample. In some experiments, thrombomodulin (10 nM) (Haematologic Technologies Inc., Vermont, USA) was added to the plasma prior to assay. The interassay coefficient of variation (based on 10 normal control samples) for peak thrombin production was 4.85% and for thrombomodulin treated plasma, 10.74%.

2.4. MPs assay

MPs were detected using a commercially available functional assay (Zymutest MP-activity, Hyphen Biomed, Neuville sur Oise, France) according to the manufacturer's instructions. Results are expressed in nM phosphatidyl serine (PS). The limit of detection for the assay was 0.05 nM PS.

2.5. Statistical analysis

Distribution of each parameter was checked for normality using Normal Probability Plot or formal tests such as the Kolmogorov-Smirnov test and the Shapiro-Wilk test and log transformed where criteria for normal distribution were not met. GLM-ANOVA and repeated measures ANOVA were used to measure the effects within and between groups as appropriate. Post hoc tests compare differences between groups and time points. In all cases $P < 0.05$ was considered significant. Data is presented as box and whisker plots (figures) and median and interquartile range (tables).

3. Results

3.1. Patients

One hundred and forty six (n = 146) patients were recruited for this study between Jan 2011 and June 2012. Table 1 shows the demographic details for all patients. These patients were divided into three groups; patients with benign histology (n = 50), patients with malignant disease (ovarian and endometrial cancer) who did not experience post-operative VTE (n = 88) and patients with malignant histology (ovarian and endometrial cancer) who developed symptomatic VTE post-operatively (n = 8). Tumours from patients who did not develop VTE (malignant (VTE −), were classified histologically as high grade serous (HGS) (n = 17), endometrioid (n = 7), clear cell cancer (n = 9), and endometrial cancer (n = 18). In the group who developed VTE, tumours were classified as clear cell cancer (n = 2), high grade serous (n = 3), high grade serous treated with neoadjuvant chemotherapy (n = 1), and endometrial cancer (n = 2). Thirty ovarian cancer patients undergoing chemotherapy were investigated, 16 patients receiving neoadjuvant chemotherapy and 14 patients receiving adjuvant chemotherapy.

All patients undergoing surgery were invited to donate additional samples for post-operative study. Patients on therapeutic doses of LMWH were excluded. A subgroup of 78 patients agreed to participate and was followed post-operatively (30 patients with benign disease, 43 patients with malignant disease (thrombosis free) and 5 patients with a malignancy who suffered post-operative VTE. Blood was taken serially at fixed time points (day 1 (pre-operative), day 5(4 h post-