The handle http://hdl.handle.net/1887/20565 holds various files of this Leiden University dissertation.

**Author:** Hengel, Lisa Gery van den  
**Title:** Tissue factor isoforms and signaling receptors in (non-)hemostatic processes  
**Issue Date:** 2013-02-26
MICROPARTICLE-ASSOCIATED TISSUE FACTOR ACTIVITY IN PLASMA IS UNAFFECTED BY CYTOTOXIC CHEMOTHERAPY TREATMENT IN METASTATIC TESTICULAR CANCER PATIENTS

Lisa G. van den Hengel, A.Q.M. Jeanne van Steijn-van Tol, Rogier M. Bertina, Henri H. Versteeg, Susanne Osanto

Submitted for publication.
Cancer is associated with an increased risk of thromboembolic events. Incidence and risk depend on tumor type with mucinous adenocarcinomas giving the highest risk. Chemotherapy is an independent risk factor for vascular complications, both venous thromboembolism (VTE) and arterial thrombosis\textsuperscript{1,2}. Testicular cancer is a malignancy that often occurs in young and mostly healthy men with a low risk for systemic hypercoagulability. However, these patients are at high risk for vascular complications during or immediately after the three-months of curative chemotherapy\textsuperscript{3}.

It has been hypothesized that cytotoxic chemotherapy induces thrombosis by triggering the release of blood microparticles (MPs) carrying procoagulant tissue factor (TF) from apoptotic tumor and vascular endothelial cells\textsuperscript{4}. However, little evidence is available regarding this hypothesis. Khorana \textit{et al.}\textsuperscript{5} showed that 2 of the 11 pancreatic cancer patients who developed VTE during chemotherapy have progressively increasing plasma levels of MP-associated TF (MP-TF) antigen and activity. Tesselaar\textsuperscript{6} reported normal MP-TF activity in 20 of the 24 cancer patients with chemotherapy-induced VTE. Further studies are required to provide more information on the effect of chemotherapy on plasma levels of MPs expressing procoagulant TF.

Cisplatin-based chemotherapy rapidly induces cancer cell death as well as vascular injury in testicular cancer patients. This might result in the release of procoagulant TF-bearing MPs from the malignant tumor cells and (sub)endothelial tissues\textsuperscript{7}. Immunohistochemical staining showed that TF was expressed in non-malignant testicular ducts in the orchidectomy specimen of testis carcinoma patients (Fig. 1A). Strikingly, we observed moderate to high TF expression in the testicular tumors of these patients (Fig. 1A). Together, these data suggest that during effective chemotherapy, MPs released from apoptotic testicular cancer cells into the circulation might carry tumor cell-derived TF.

We performed an exploratory, prospective study to investigate whether standard chemotherapy in metastatic testicular cancer patients indeed induces the release of MPs carrying procoagulant TF. Thirteen metastatic testicular cancer patients (mean age 40±11 years) were treated with 3 to 4 courses of standard (B)EP chemotherapy consisting of three-weekly intravenously (i.v.) administered etoposide (100 mg/m\textsuperscript{2}, at days 1-5), cisplatin (20 mg/m\textsuperscript{2}, at days 1-5) with (n=11) or without (n=2) bleomycin (30 IUSP, at day 2) (Fig. 1B). To assess a direct effect of chemotherapy-induced tumor cell death on plasma MP-TF activity, we collected blood from these patients via a peripheral intravenous catheter on day 1, 2 and 4 during the first 2 chemotherapy courses (Fig. 1B). EDTA was used as anticoagulant, because it prevents both coagulation and platelet activation. Previously, we have shown that similar MP-TF activities were found in EDTA and citrate anticoagulated plasma of healthy subjects. For practical reasons, we collected blood not during but after completion of each infusion of a single chemotherapy agent, the earliest blood sample being 1.5 hours
after the start of chemotherapy (Fig. 1B). The study was approved by the Medical Ethical Committee at the LUMC and all patients provided written consent. Platelet poor plasma was immediately centrifuged at 2700g for 10 min and subsequently stored at -20°C until used. MPs were isolated from stored plasma by centrifugation at 18,890g for 30 min. MP-TF activity was subsequently determined by measuring the generation of active factor X (FXa) in a TF- and factor VII (FVII)-dependent fashion, as reported previously 6].

Before the first course of chemotherapy, the mean MP-TF activity level was 16±8 fM Xa/min (range 10-30 fM Xa/min), which lies within the range of MP-TF activity levels measured in EDTA-anticoagulated plasma of 20 healthy controls (8-230 fM Xa/min) (F.J.S.H. Woei-A-jin, et al., unpublished observations). The MP-TF activity levels did not significantly increase after subsequent etoposide and cisplatin administration on day 1 (Fig. 1C). Similarly, no changes were detected in MP-TF activity in the blood samples obtained during the following days of the first course (Fig. 1C). However, one patient showed elevated MP-TF activity (305 fM Xa/min) before etoposide infusion on day 2. Because we might have missed a peak in MP-TF activity prior to the second day of the first course, we collected additional blood samples from two patients overnight at four-hour intervals, but no changes in MP-TF activity were observed (data not shown).

Three weeks later, prior to the start of the second chemotherapy cycle, the average MP-TF activity level (26±15 fM Xa/min, range 10-44 fM Xa/min) was not significantly different from the average MP-TF activity level measured before the start of the first chemotherapy cycle (p=0.093). The MP-TF activity levels did not significantly change over the course of the second cycle when compared to the corresponding baseline level (Fig. 1C). The individual measurements of MP-TF activity of both courses are all lower than the maximal MP-TF activity level in healthy controls (<230 fM Xa/min), except for one. The reproducible measurement of an increased MP-TF activity (>230 fM Xa/min) in one patient on day 2 of the first course (Fig. 1C) appears to be a spurious finding.

Next, we determined whether the vascular endothelium in these testicular cancer patients was activated in response to standard chemotherapy infusion, using Von Willebrand Factor (VWF) as a plasma marker of endothelial activation. VWF antigen levels were measured prior and during chemotherapy administration by ELISA using rabbit polyclonal anti-human VWF and horseradish peroxidase-conjugated rabbit anti-human VWF antibodies (Fig. 1B)8. A gradual increase in the mean VWF levels during chemotherapy was observed (Fig. 1D), suggesting that chemotherapy induced endothelial activation.

Chemotherapy induced a complete regression of metastases in all 13 testicular cancer patients included in this study, indicating that chemotherapy caused destruction and apoptosis of the tumor cells. None of these cancer patients developed VTE during or immediately after chemotherapy treatment. However, 2 of the 13 patients (15%)
developed severe arterial complications. One patient was diagnosed with angina pectoris immediately after the 1st week of the second course of chemotherapy and developed a myocardial infarction within 4 months after chemotherapy. The other patient was also diagnosed with angina pectoris 2 weeks after the start of the second course and developed a myocardial infarction on day 6 of the third chemotherapy course.

In conclusion, testicular cancer cells are a potential source of TF, but this study provides evidence that chemotherapy-induced tumor cell destruction is not associated with a rise in blood MP-TF activity. However, we cannot exclude a possible peak in MP-TF activity within 1.5 hours after chemotherapy reflecting an immediate transient response to treatment.

**Acknowledgements**

This work was supported in part by the Dutch Cancer Society (KWF UL 2006-3618). HHV is the recipient of a NWO-VIDI award (#17.106.329).

**References**

Figure 1. (A) Expression of TF in healthy and tumorigenic human testicular tissues. Immunohistochemical staining of paraffin sections of tumorigenic (I, III, IV) testicular tissues with isotype control (I) and mouse anti-human TF antibody (III, IV) and TF staining of healthy (II) testicular tissue. Representative images are shown with 20x magnification. (B) Chemotherapy infusion time table representing the first and second course. Arrows indicate the time-points for collecting blood for MP-TF activity measurements prior to chemotherapy infusion (control, C), and after etoposide (E), bleomycin (B) and cisplatin (P) administration at day 1, 2 and 4. Asterisks indicate time-points for VWF analysis. (C) Levels of MP-TF activity in plasma of testicular cancer patients during chemotherapy. Mean MP-TF activity levels (fM Xa/min) are shown prior to chemotherapy. The Wilcoxon Signed Rank test was used to compare the MP-TF activity levels prior to the start of each chemotherapy course with those during chemotherapy. (D) Levels of VWF antigen in plasma of testicular cancer patients during chemotherapy. Mean VWF antigen levels (% of normal pool plasma (npp)) are shown prior to chemotherapy infusion at day 1, 2 and 4 (C) and after cisplatin infusion at day 1 (P) during the first 2 chemotherapy courses. The Wilcoxon Signed Rank test was used to compare the VWF antigen levels prior to the start of each chemotherapy course with those during chemotherapy. * p<0.05.