Venous thrombosis in cancer patients: Prediction, diagnosis and management
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Prediction of venous thromboembolism in cancer patients by tissue factor dependent microparticle coagulant activity, biomarkers and a clinical score

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In preparation
Chapter 7

| ABSTRACT |

**Introduction**
Cancer patients receiving chemotherapy in an ambulatory setting have a risk for venous thromboembolism of approximately 3 to 4% per year, which is, however, not high enough to warrant thromboprophylaxis. Previous studies have focused on tools for selection of cancer patients with a high risk for VTE. In the present study, we aimed to study tissue factor (TF)-dependent coagulant activity as an alternative predictor for VTE, and to compare it to previously studied biomarkers and the Khorana score.

**Methods**
In six hospitals blood was obtained from ambulatory cancer patients receiving chemotherapy and patients were followed for 6 months for the development of VTE. TF-dependent coagulant activity was measured in a fibrin generation test (FGT) on fresh plasma in the local laboratories. Plasma was frozen for central measurement of the other biomarkers. Clinical data were gathered for calculation of the Khorana score. Test characteristics including sensitivity and positive predictive value (PPV) were calculated taking into account death as a competing risk.

**Results**
This prospective cohort consists of 443 patients, with a mean age of 61 years, of which 49% women. In total, 23 patients developed VTE after a mean time of 2.1 months (5.2%). The FGT had a sensitivity of 61% (95% CI 31-84%) and a PPV 4.7% (1.9-9.3%); D-dimer a sensitivity of 84% (61-97) and a PPV of 6.9% (3.5-12); P-selectin a sensitivity of 85% (56-96) and PPV of 14% (4.8-27). For factor VIII the sensitivity was 44% (61-97) and the PPV 11% (4.5-21) and for prothrombin fragment 1+2 sensitivity was 70% (36-89) and PPV 5.9% (2.4-12). Lastly, a high Khorana score had a sensitivity of 63% (28-83) and PPV of 5.1% (1.6-12).

**Discussion**
None of the studied biomarkers or the Khorana score was superior in prediction for VTE. In future, combinations of biomarkers or scores should be studied.
INTRODUCTION

Venous thromboembolism (VTE) complicates the clinical course of 10-20% of all cancer patients and represents a major cause of morbidity and mortality in this patient group (1-4). Moreover, the management of VTE in cancer patients is particularly challenging, because these patients not only have an increased risk for a recurrent event but also a higher bleeding frequency during anticoagulant treatment compared to patients without cancer (5). Prevention of VTE in cancer patients using thromboprophylaxis may therefore increase survival and quality of life. Thromboprophylaxis has been shown to be effective and safe for other indications, including hospitalized cancer patients (6;7).

Two large randomized controlled clinical trials and a systematic review of the literature have shown that thromboprophylaxis with low-molecular-weight-heparin (LMWH) is able to reduce the occurrence of VTE in cancer patients, but the overall incidence is around 4%, and therefore too low to justify thromboprophylaxis in all cancer patients (8-10). Additional concerns are the need for daily injections and the associated risk of bleeding. The next step therefore is to identify cancer patients with a high risk for VTE, where thromboprophylaxis would have a more favourable benefit-risk ratio. Thus far, two main approaches have been used for this purpose. First, clinical prediction scores - combining clinical and laboratory parameters - have been or are being developed (11-13). Of these scores, the one developed by Khorana and colleagues is the best validated (11). This score consists of 5 items, including site of cancer, pre-chemotherapy platelet and leukocyte count, body mass index, and hemoglobin value or the use of erythropoiesis stimulating factors. Although easy to use, extrapolation to other patient populations is difficult as cancer cohorts can vary considerably in clinical characteristics, including differences in cancer types and treatments. A second approach has been the use of several biomarkers, including D-dimer, P-selectin, prothrombin fragment 1+2 and factor VIII (14-16). Most of these biomarkers seem to predict the development of VTE in cancer patients and, at least some of them, may have the potential to increase the predictive value of the Khorana score (17).

Another promising candidate biomarker is tissue factor (TF) bearing microparticles (18;19). Microparticles are procoagulant cell-derived vesicles, which circulate in the peripheral blood (20;21). Compared to healthy controls, cancer patients have increased levels of microparticles, which may expose TF on their surface, the protein that initiates coagulation (22;23). Therefore, we hypothesize that the coagulant activity of TF-exposing microparticles can predict the development of VTE in cancer. To determine the coagulant activity of tissue factor bearing microparticles, an easy and robust coagulation test called the fibrin generation test (FGT) was developed, which specifically measures the coagulant activity of microparticles in autologous plasma (24).

The aim of this study was to compare the predictive power of the FGT, biomarkers (D-dimer, P-selectin, F1+2 and factor VIII), and the Khorana score for the development of VTE in a large prospective cohort of cancer patients.
Chapter 7

| METHODS |

This international multicenter prospective study was conducted in six hospitals, namely the Academic Medical Center in Amsterdam, the VU Medical Center in Amsterdam, the Slotervaart hospital in Amsterdam (the Netherlands), Hospital d’Annunziata in Chieti (Italy), Hôpital Lariboisière in Paris (France) and the National Cancer Institute in Mexico City (Mexico). In all participating hospitals, approval of the institutional review boards was obtained. Consecutive patients with gastro-intestinal, breast, pancreatic, bladder, ovarian, lung or hormone refractory prostate cancers, all stage III or IV, were included, after written informed consent. These patients received chemotherapy or were scheduled to start chemotherapy within 7 days from inclusion. An exclusion criterium was ongoing anticoagulant treatment in therapeutic doses.

Clinical data were retrieved from the patient files or by an interview with the patient, and were collected in standardized case report forms (CRF). All patients were followed-up for six months. The primary outcome was objectively verified symptomatic or asymptomatic VTE, i.e. deep venous thrombosis, pulmonary embolism (PE) or abdominal vein thrombosis. Asymptomatic venous thrombosis was included in the main outcome, taking into account the recent clinical evidence pointing to the clinical significance of these unsuspected cases of venous thrombosis (25). No routine screening was performed to search for venous thrombosis in the included patients. In case of venous thrombosis, patients were treated with anticoagulant treatment according to local practice. When patients died during the 6 months of follow-up, the treating physicians were requested to check the autopsy results, where available, and to evaluate if PE was a (possible) cause of death.

At inclusion, blood was collected via a single blood withdrawal either directly out of a venous catheter (within 5 minutes after placement) or by vena puncture, using standardized procedures. Measurement of the TF-microparticle dependent coagulant activity was performed on fresh plasma, whereas the remaining plasma was stored for later measurement of other biomarkers. Platelet poor plasma (PPP) was prepared by centrifugation at 1560 x g for 20 minutes within 1 hour after blood withdrawal. PPP was stored on melting ice to stabilize coagulation factors, and was used within 2 hours for the FGT, as described earlier (24).

Briefly, PPP was recalcified, and the time until fibrin formation, i.e. clotting of the plasma, was monitored in a spectrophotometer in the absence and presence of an inhibitory antibody to activated factor VII (anti-VIIa). The prolongation of the clotting time in the presence of anti-VIIa, which is expressed as percentage of the clotting time in the absence of anti-VIIa, reflects the TF coagulant activity that is associated with MP. Based on receiver operating curves analysis from the first 50 consecutive cancer patients included at the Academic Medical Center, Amsterdam, a cut-off set at 13% showed the best discriminating power. Therefore, prolongation of the clotting time in the presence of anti-VIIa ≥13% was considered abnormal, and the FGT was positive when ≥13% (19).
For all other biomarkers and the Khorana score, cut-offs from the original publications were used. D-dimer (Innovance), factor VIII activity, and pro-thrombin fragment 1+2 (F1+2; Enzygnost monoclonal, ELISA) were obtained from Siemens Healthcare Diagnostics (Deerfield, IL), and P-selectin (ELISA) from R&D Systems Inc. (Minneapolis, MN). These cut-off values are: D-dimer (> 1.44 μg/mL), factor VIII (> 232%), F1+2 (> 358 pmol/L), soluble P-selectin (> 53.1 ng/mL) (14;16;26). According to the Khorana score patients were assigned to the three risk categories low risk (0 points), intermediate risk (1 or 2 points) and high risk (3 points or more) (11).

The samples from one center showed a very distinctive pattern, with very high levels of prothrombin fragment 1+2, very low factor VIII and P-selectin levels. All biomarkers were measured centrally and we measured a subset of these samples a second time to see whether the transport of samples might have caused these changes, which was not the case. The protocol for measurement and blood withdrawal was followed and the researchers were experienced in participation in thrombosis studies, therefore, it remains unclear what might have caused the defect in these samples. For us, this was reason not to include these biomarker measurements in the analyses.

Statistical analyses
For the present analysis, the sensitivity and positive predictive value (PPV) for VTE were determined for the FGT, D-dimer, P-selectin, F1+2, and the Khorana score. In patients with advanced cancer, a high rate of death is anticipated to limit the validity of the traditional Kaplan Meier analyses which use non-informative censoring (27;28). Patients who die have had no chance to develop VTE during follow-up, and above that, the predicting factors might be linked to VTE and survival, which would introduce bias. As a consequence Kaplan Meier analyses commonly overestimate predictive values. For these reasons, a more conservative approach was chosen using competing risk analyses with informative censoring (27;29).

We used the method of Saha and Heagerty to calculate the prognostic accuracy of the various markers in predicting VTE, with mortality considered as competing risk (29). Estimations of sensitivity, specificity, and the AUC (area under the ROC curve) are based on the nearest neighbor estimation of the bi-variate distribution function of the marker and the event time. We determined the AUC as a global summary of the discriminatory capacities of markers. To accompany the point estimates, we estimated 95% confidence intervals based on bootstrap re-sampling using 1000 samples. To express predictive values, we calculated the 6-month cumulative incidence, once again considering mortality as a competing risk (30). Analyses were performed with the packages cuminc and CompRisksROC in R version 2.15.1 (31). As a comparison, Kaplan Meier analyses using log rank testing, and binary logistic regression for calculation of the unadjusted hazard ratio were performed.

We used multiple imputation as a technique to deal with missing data for the calculation of the Khorana score (<10% of data were imputed). Ultimately, per individual biomarker or clinical score, two by two tables were reconstructed and test characteristics were
calculated. All statistical analyses were performed in PASW statistics version 19 (Inc., 2009, Chicago, Ill), with the exception of the competing risk analyses which were performed in R.

| RESULTS |

Patient characteristics

In total, 443 patients were included with a mean age of 61 years, of which 49% women (Table 1). Twenty-three patients developed VTE (5.2%; 95% CI 3.3-7.8) after a mean time of 2.1 months (SD 1.2). A total of 77 patients (18%) died after a mean follow-up of 3.3 months (SD 1.5). In none of these patients, VTE was considered the most probable cause of death. All other patients were followed up for 6 months, except one patient who moved abroad after 1 month and was subsequently lost to follow-up.

Table 1. Patient’s characteristics (n=443)

<table>
<thead>
<tr>
<th>N (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
</tr>
<tr>
<td>Sex (% women)</td>
</tr>
<tr>
<td>Adjuvant treatment for cancer</td>
</tr>
<tr>
<td>Type of cancer</td>
</tr>
<tr>
<td>Esophageal / stomach cancer</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td>Breast</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Intestinal cancer</td>
</tr>
<tr>
<td>Genito-urinary tract cancer</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>Locally limited disease</td>
</tr>
<tr>
<td>Locally unresectable disease</td>
</tr>
<tr>
<td>Metastasized disease</td>
</tr>
<tr>
<td>Other staging system</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>First cycle of chemotherapy</td>
</tr>
<tr>
<td>Second</td>
</tr>
<tr>
<td>Third or fourth cycle</td>
</tr>
<tr>
<td>Survival</td>
</tr>
<tr>
<td>Died within 6 months</td>
</tr>
<tr>
<td>Time between inclusion and death, months (SD)</td>
</tr>
<tr>
<td>Venous thrombosis</td>
</tr>
<tr>
<td>DVT/PE/both</td>
</tr>
<tr>
<td>Abdominal</td>
</tr>
<tr>
<td>UEDVT</td>
</tr>
<tr>
<td>Symptomatic</td>
</tr>
</tbody>
</table>
**TF-dependent coagulant activity (FGT)**

The FGT was performed in 428 patients, while in the remaining 15 patients (3.4%) the measurement failed for technical reasons. A positive test, i.e. equal to or more than 13% prolongation of the clotting time in the presence of anti-VIIa, was found in 130 patients (30%). Of the 23 patients with VTE, 13 patients had a positive test, the remaining 10 patients tested negative. Unadjusted for death, the sensitivity was 57% (95%CI 35-78) and the PPV was 10% (95%CI 4.9-15). In Kaplan Meier analysis, the probability of VTE was higher in the patients with a positive test (p=0.004, log rank testing), corresponding with a hazard ratio for VTE of 3.2 (95%CI 1.4-7.6).

Taking into account death as a competing risk, the sensitivity was 61% (95%CI 31-84%), the PPV 4.7% (1.9-9.3%) and the AUC was 0.54 (0.37-0.69; Table 2).

**Biomarkers**

A D-dimer test was performed in 437 patients (99%) and 144 (30%) of them had a high D-dimer. Of the patients with VTE, the measurement failed in 1 patient, 15 patients had a high, and 7 patients a low D-dimer. Unadjusted for death, the sensitivity was 65% (43-84) and the PPV 10% (5.2-15). In Kaplan Meier analyses, the probability for VTE was higher among the patients with a high D-dimer (p<0.001), corresponding with a hazard ratio of 4.7 (1.9-12). When taking into account death as a competing risk, the sensitivity was 84% (61-97) and the PPV 6.9% (3.5-12; Table 2).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity</th>
<th>95%CI</th>
<th>Specificity</th>
<th>95%CI</th>
<th>PPV</th>
<th>95%CI</th>
<th>NPV</th>
<th>95%CI</th>
<th>AUC</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII</td>
<td>87</td>
<td>61-97</td>
<td>61</td>
<td>55-67</td>
<td>11</td>
<td>4.5-21</td>
<td>1.7</td>
<td>0.6-4.0</td>
<td>0.81</td>
<td>0.47-0.97</td>
</tr>
<tr>
<td>D-dimer</td>
<td>84</td>
<td>58-95</td>
<td>55</td>
<td>52-58</td>
<td>6.9</td>
<td>3.5-12</td>
<td>1.4</td>
<td>0.5-3.3</td>
<td>0.66</td>
<td>0.49-0.78</td>
</tr>
<tr>
<td>F1+2</td>
<td>70</td>
<td>56-89</td>
<td>54</td>
<td>51-58</td>
<td>5.9</td>
<td>2.4-12</td>
<td>2.5</td>
<td>1.1-5.2</td>
<td>0.58</td>
<td>0.38-0.65</td>
</tr>
<tr>
<td>P-selectin</td>
<td>85</td>
<td>56-96</td>
<td>62</td>
<td>54-70</td>
<td>14</td>
<td>4.8-27</td>
<td>2.3</td>
<td>1.0-4.5</td>
<td>0.59</td>
<td>0.39-0.70</td>
</tr>
<tr>
<td>FGT</td>
<td>61</td>
<td>31-84</td>
<td>54</td>
<td>51-57</td>
<td>4.7</td>
<td>1.9-9.3</td>
<td>3.0</td>
<td>1.5-5.4</td>
<td>0.54</td>
<td>0.37-0.69</td>
</tr>
<tr>
<td>Khorana</td>
<td>63</td>
<td>28-83</td>
<td>58</td>
<td>23-81</td>
<td>5.1</td>
<td>1.6-12</td>
<td>3.0</td>
<td>1.6-5.2</td>
<td>0.58</td>
<td>0.37-0.74</td>
</tr>
</tbody>
</table>

P-selectin was measured in 338 patients (76%). In 105 patients included in one center, a technical withdrawal problem led to invalid samples and therefore P-selectin, prothrombin fragment 1+2 and factor VIII measurements were considered unreliable and excluded. Of the remaining 338 patients, 37 (11%) had a positive test. Of the patients with VTE, in 3 patients P-selectin was not measured, 6 patients scored positive and 14 scored normal, an unadjusted sensitivity of 30% (12-54) and a PPV of 16% (6.2-32). In Kaplan Meier analyses, patients with a positive P-selectin test had a higher probability for VTE (p=0.002), and a hazard ratio of 3.9 (1.4-11). After adjusting for death, sensitivity was 85% (56-96) and the PPV 14% (4.8-27).
Pro-thrombin fragment 1+2 (F1+2) was measured in 338 patients (76%), of which 101 were elevated (30%). Of the 23 patients with VTE, no F1+2 was available in 3 patients, 12 patients had increased levels of F1+2 and 8 had normal F1+2; an unadjusted sensitivity of 60% and a PPV of 12%. In Kaplan Meier analyses, patients with an elevated F1+2 had a higher probability for VTE (p=0.002), with a hazard ratio of 3.8 (95%CI 1.5-9.7). After adjusting for death, sensitivity was 70% (36-89) and the PPV 5.9% (2.4-12).

Factor VIII was measured in 294 patients (66%), of which 54 patients (18%) had an abnormal test result. Of the 23 patients with VTE, in 7 patients had no factor VIII measurement was available, 7 patients had an abnormal, and 9 patients had a normal factor VIII, hence, a sensitivity of 44% (20-70) and a PPV of 13% (5.4-25). Unadjusted survival analyses revealed a p-value of 0.002 and logistic regression revealed a hazard ratio of 3.8 (95%CI 1.4-11). Adjusted for death as competing risk, the sensitivity was 44% (61-97) and the PPV 11% (4.5-21).

Khorana score
The Khorana score was available for all patients, of whom 114 had a low score (26%), 250 an intermediate (56%) and 79 a high score (18%). Of the 23 patients with VTE, 4 had a low score, 14 patients an intermediate and 5 patients had a high score. When dichotomising the score into high versus low/intermediate, the Khorana score had an unadjusted sensitivity of 22% (7.5-44) and a PPV of 6.3% (2.1-14). The probability for VTE was not higher among patients with a higher Khorana score (p=0.34). Taking into account death as competing risk, the sensitivity was 63% (28-83) and the PPV 5.1% (1.6-12).

| DISCUSSION |

The present study evaluated the predictive value of an in-house developed TF-microparticle dependent clotting test (FGT), previously established biomarkers and the Khorana score for the occurrence of VTE in patients with cancer. None of the studied predictors seemed to be significantly better than the other with large overlaps of the confidence intervals.

The biomarkers were measured with the same methods as in the original publications and we used the same cut-off values, which enables a direct comparison between the historical data and the present results. Hazard ratio’s in patients with high biomarker levels are somewhat higher in the present study, e.g. a HR of 4.7 (1.9-12) for D-dimer compared to 2.8 (1.7-4.6) in the original paper (15). Positive predictive values are not commonly reported in the other studies, which makes a comparison difficult. In the present study, the sensitivity and PPV of the Khorana score are lower than in the initial derivation and validation cohort of Khorana as well as in the cohort of the CATS study (11;17). The latter study reported a PPV of 22%, whereas we found a much lower PPV of 6.3% (unadjusted) or 5.1% (adjusted).

Several aspects of the design and findings of the present study require comment. First, the FGT as a measure for TF-dependent microparticle coagulant activity did not perform better or worse than the other individual biomarkers. The question is whether the FGT
is suitable for use in a large clinical trial, or in clinical practice. When compared to other microparticle clotting tests, the FGT is a straightforward test, easy to learn and relatively inexpensive. The test has extensively been validated, and no problems were encountered while implementing the test in other (also routine) laboratories.

Second, the 95% confidence intervals around the estimates were relatively large which reduces the confidence in the estimates and in their possible clinical relevance. In the present study, previously established cut-off values were used, whereas an alternative approach could have been to derive new cut-off values of these biomarkers and the Khorana score. Lastly, combinations of tests have the potential to be more sensitive, which is worthwhile to investigate further.

Third, we analyzed the predictive values, taking into account death as a competing risk in this group of advanced cancer patients. Standard Kaplan Meier analysis with log rank testing censors patients when they die, which implies that they – in theory – could still develop the event of interest. This is obviously not a correct assumption as death ‘competes’ with the event of interest. Recently, Campigotto and colleagues stressed the importance of accounting for death as a competing risk in cancer-associated thrombosis studies (28). In the present investigation, death occurred more frequently than VTE and the biomarkers were indeed able to predict death (AUC up to 0.67; data not shown), which confirms the importance of this modified type of analysis. In the present study, the point estimates of the PPV were considerably lower after correction for death as a competing risk, whereas sensitivities generally were somewhat higher. To our knowledge, none of the previous studies have taken death into account as a competing risk, which may have overestimated the predictive value of the tests.

Obviously, prediction only will not save patient’s lives, but is merely a step towards targeted thromboprophylaxis. Ongoing and new studies will need to sort out whether selection of high risk cancer patients for thromboprophylaxis is effective and safe. Furthermore these studies will need to establish the most optimal duration, the preferred type of anticoagulant and the burden thromboprophylaxis has on the quality of life.

**Conclusion**

In the present study, a ‘head to head’ comparison of different established and new predictors for VTE in cancer, none of the studied markers are superior to other markers or scores. The positive predictive values of the studied markers and the Khorana score do not exceed 15%; therefore, a combination of different tests may be preferable. Future prospective cohort studies in this field should account for death as a competing risk in order to avoid bias.
**REFERENCE LIST**


