Venous thrombosis in cancer patients: Prediction, diagnosis and management
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14.

General discussion

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This thesis focuses on venous thromboembolism (VTE) in patients with cancer. In the first part, we studied the roles of microparticles in cancer progression and venous thrombosis, and potential applications of microparticles as a predictive biomarker. Part two addressed the diagnosis of upper extremity deep vein thrombosis, whereas the last part concerned the management of venous thromboembolism in cancer patients.

**PART I: PREDICTION**

*Chapter 2* reviewed the different roles of microvesicles (microparticles and exosomes) in cancer, with a focus on cancer progression and the development of venous thrombosis. Intricately, microvesicles can contain active molecules including receptors or genetic material, or carry these molecules on their surface. Hereby, these small vesicles are able to convey messages or molecules to other cells, or can be used by a cell as a way to get eliminate toxic substances (e.g. chemotherapeutics). In the (near) future, the list with various functions and roles of microvesicles in cancer will probably become much longer. Interesting, not only from a scientific point of view, but even more so if we can find ways to intervene with the production of (certain type of) microvesicles and thereby would be able to inhibit tumour growth, to prevent the development of venous thrombosis, or to increase the vulnerability of cancer cells to chemotherapeutics. Closer to implementation is the use of subtypes of microvesicles as a prognostic biomarker, e.g. the use of tissue factor bearing microparticles to predict the occurrence of VTE.

In *chapter 3*, we focussed on a specific subtype of microparticles, namely CD24-bearing microparticles. Cancer patients have increased levels of CD24-bearing microparticles, when compared to healthy subjects (median 2.9 versus 1.2 x 10^5; p<0.001). Patients with high and low levels of CD24-bearing microparticles did not show differences in coagulation markers, nor in VTE, but the prognosis of patients with high CD24-bearing microparticles was poor (odds ratio to die within 15 months: 4.6; p=0.016), when compared to patients with low CD24-bearing microparticles. Therefore, these microparticles might prove a useful prognostic biomarker, if these findings can be confirmed in a larger population of cancer patients.

*Chapter 4* was an exploratory study in which patients with cancer were prospectively followed, to see whether markers of coagulation activation and microparticle dependent coagulant activity would be associated with the development of venous thrombosis. At baseline, markers of *in vivo* coagulation and the total number of microparticles were significantly elevated in cancer patients, when compared to healthy subjects. In those patients who developed VTE within 6 months, the baseline levels of coagulation activation markers and phospholipid dependent coagulant activity were comparable, however, the tissue factor and activated factor VII dependent microparticle coagulant activity were higher in the patients who developed VTE, when compared to those who did not. This suggests that the TF/factor VII dependent coagulant activity can be used to predict VTE.
Chapter 5 described the characteristics and validation of the fibrin generation test (FGT), a plasma recalcification test. The test measured the presence of procoagulant phospholipids as well as procoagulant tissue factor. The FGT formed the result of an equilibrium reaction; therefore the reproducibility of the FGT was dependent on the type of test plasma employed. Using test plasma with high levels of endogenous TF, the intra-assay variability was 276 (SD ± 13.5 seconds) with a variation coefficient of 4.9%. In the presence of antibody to activated factor VII, the variation coefficient increased to 9.9%. The inter-assay variation coefficients were 11.7% and 17.6% in the absence and presence of the antibody to factor VIIa, respectively. In normal pool plasma, high clotting times were measured with large variation coefficients. As this plasma recalcification test is easy to perform, cheap and the reproducibility is fair, it might become useful in clinical practise for measuring the inherent ability of the plasma to clot and the contribution of TF-bearing microparticles to haemostasis and thrombosis.

Chapter 6 aimed to elucidate the relationship between number and coagulant activity of tissue factor (TF) bearing microparticles, and the cellular origin of these specific microparticles. Overall, we found no correlation between the number of TF-bearing microparticles and the TF-dependent coagulant activity (r=0.029; p=0.69). Even more so, in patients, a high number of TF-bearing microparticles and a high coagulant activity seemed almost mutually exclusive. Therefore, we postulated that there are two types of circulating TF-bearing microparticles, a coagulant and a non-coagulant form. More research is needed to clarify our findings on the cellular origin of TF-bearing microparticles, as there was a marked variation between patients. We could identify tumour derived TF-bearing microparticles in 5 patients, however, these microparticles also stained double positive for blood cell markers. In 8 other patients, the cellular origin of only a minority of the TF-bearing microparticles could be determined; these microparticles originated from platelets. Multiple research groups are currently evaluating the use of the number and coagulant activity of TF-bearing microparticles as predictive biomarkers for VTE in cancer. The future will learn us whether one of these measurements proves useful.

In chapter 7 we aimed to study the value of TF-dependent coagulant activity (via the FGT) in the prediction of VTE in cancer, and to compare it to previously studied biomarkers. The prospective cohort consisted of 443 patients, of whom in total 23 patients developed VTE within 6 months (incidence 5.2%). The FGT had a sensitivity of 61% (95%CI 31-84%) and a positive predictive value (PPV) of 4.7% (1.9-9.3%). None of the studied biomarkers or the Khorana clinical score was superior in the prediction for VTE, with sensitivities ranging from 44 (factor VIII; 61-97%) to 85% (P-selectin; 56-96%) and PPV’s ranging from 4.7% (high Khorana score; 1.6-12) to 14% (P-selectin; 4.8-27). This chapter concerned an interim analysis of an ongoing study. Hopefully, the increase in sample size will provide more power to see differences among the studied biomarkers. Also, with the larger sample size, combinations of biomarkers, the Khorana score and the FGT can be analysed. As the various biomarkers and the score have a different pathophysiological background, a
combination of tests might prove a fruitful way to lift the PPV to a value above e.g. 20%, which would enable giving thromboprophylaxis in a randomized controlled trial to those patients with a high risk for VTE, the ultimate goal.

Finally, chapter 8 explored the effect of chemotherapy and anti-angiogenesis agents on the coagulant activity and composition of circulating microparticles. Treatment did not affect the overall procoagulant activity of the microparticles. The composition of microparticles did change after therapy: levels of microparticles bearing the receptor for vascular endothelial growth factor (VEGFR-1) decreased after therapy in glioma patients (p=0.021), whereas levels of endothelial microparticles tended to increase in glioma patients (p=0.041). These observations are merely hypothesis generating, as they suggest an association with prognosis or a response to treatment, however, this needs to be established by future studies.

**PART II: DIAGNOSIS**

In chapter 9 we systematically reviewed the current literature for studies reporting on the diagnostic accuracy of ultrasonography for clinically suspected upper extremity deep vein thrombosis (UEDVT). In the analysis, 9 studies with 687 patients were included. Overall, the methodological quality was considered low, sample sizes were small and large between-study differences were observed. In most hospitals worldwide, ultrasonography has substituted venography as the first choice imaging technique. However, this review also revealed that the evidence concerning the diagnostic accuracy and therefore also the safety of ultrasonography is rather scarce, with wide confidence intervals around the summary estimates. Data on the diagnostic accuracy of ultrasonography for the detection of lower extremity DVT cannot be extrapolated to suspected upper extremity DVT without further studies, as the anatomy of the upper extremity might pose additional problems for ultrasonography and the patient population with suspected UEDVT differs from that with suspected leg DVT. Therefore, the use of ultrasonography for the diagnosis of UEDVT warrants more research.

Chapter 10 described the Armour study, a multicenter diagnostic management study which aimed to evaluate the safety and efficacy of a new diagnostic algorithm for suspected UEDVT. This algorithm combined a clinical score (the Constans’ score), D-dimer and ultrasonography. The study population comprised of 406 patients with suspected UEDVT. The algorithm was feasible and completed in 390 of the 406 patients, i.e. 96%. In 87 patients (21%) an unlikely score combined with a normal D-dimer test result excluded UEDVT. In 103 (25%) and 54 (13%) patients a diagnosis of UEDVT or superficial vein thrombosis was made, respectively. Of the 249 patients with a normal diagnostic work-up, one developed UEDVT during follow-up for an overall failure rate of 0.40% (95%CI: 0.0-2.2%), which is below the previously defined maximally allowed upper confidence interval of 3.0%. Therefore, this algorithm can safely and effectively exclude venous thrombosis of the upper extremity. Provided the safety can be confirmed by future studies, this approach would be very attractive for use in clinical practise, as it is relatively simple and resembles the algorithm for suspected leg DVT.
Chapter 14

We concluded this part of the thesis with a planned sub-study of the Trousseau study. The main study demonstrated that extensive screening for cancer in patients with idiopathic venous thrombosis (using CT-scans of the chest and abdomen, plus mammography in women) did not increase overall survival, when compared to limited screening. Chapter 11 revealed that in as much as 30% of all extensively screened patients, abnormalities found by imaging necessitated further diagnostic work-up. This yielded 6 malignancies and resulted in a positive predictive value of 6.6%, a sensitivity of 33% and a specificity of 70%. The mean costs for the total screening were €165 for the routine and €331 for the extensive screening. These findings underscore the problems associated with screening using CT-scans, namely the high proportion of abnormalities found which advocate further imaging and of which only a minority are relevant. In our present society, the costs which arise from such a strategy form an increasingly important topic.

PART III: MANAGEMENT

In chapter 12, we aimed to assess strategies used for the treatment of patients with cancer and venous thrombosis among physicians worldwide, experts on thrombosis as well as non-experts. A total of 229 invitations were sent, and 141 physicians completed the survey (60%). LMWH was indicated as the first choice for the long-term treatment by 82% of the respondents, of whom 60% used full therapeutic doses and 40% chose a dose reduction. When continuing anticoagulants after the long-term treatment period, 44% of respondents preferred LMWH, 10% VKA, while the remaining 45% chose per individual patient for either LMWH or VKA.

Finally, 600 cancer patients with pulmonary embolism (PE) and 1200 matched patients with PE without cancer were identified within a large pharmacy database in chapter 13. From 1998 to 2008, we observed a clear increase in the use of long-term LMWH in cancer patients, but not in controls (p<0.001 and p=0.76, respectively). In 2007/2008, LMWH was prescribed to 32% of the patients with cancer and PE, compared to 1.7% of the patients in 1998/1999.

Taken together, these two chapters indicate that the intended use of LMWH for the long-term treatment of cancer patients is relatively high, especially among experts on thrombosis. In contrast, at least in 2007/2008, the actual use of LMWH is still far from optimal. The question rises what can explain this underuse. Therefore, future studies should focus on the impact of long-term use of LMWH on the quality of life, and reasons for physicians and patients to choose for vitamin K antagonists (VKA) rather than LMWH. Also, the optimal dose of LMWH and the type of anticoagulant chosen varied substantially, probably reflecting the limited available evidence. In the future, the ongoing Longheva study might provide an answer to the question whether or not LMWH is also superior to VKA after completion of the first 6 months of anticoagulant treatment. Concerning the optimal dose of LMWH, probably, this will remain to be a decision made by individual physicians, in view of the unlikely possibility of a trial on this topic. The various new anticoagulants...
are emerging factors in the treatment of VTE in the non-cancer population. However, the percentage of cancer patients enrolled in the trials with new anticoagulants is modest, with the last trials even excluding cancer patients. Therefore, studies specifically aimed at cancer patients with VTE need to provide an answer to the question whether the new anticoagulants are superior in efficacy and safety, when compared to long-term LMWH. Until then, long-term LMWH remains the drug of choice for the treatment of VTE in cancer patients, with all the associated advantages and disadvantages.