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Risk factors of thrombosis in cancer : the role of microparticles
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Summary and general discussion

The incidence of cancer is growing with increased ageing of people and cancer is now the leading cause of death in the West European countries and the US. As described in the introduction of this thesis, hypercoagulability seems to contribute to the two most frequent causes of death in cancer patients, metastasis and venous thrombosis. The poor prognosis of cancer patients who develop thrombosis forms a challenge to clinicians to select cancer patients at highest risk for development of thrombosis and to develop effective prophylactic strategies to prevent thrombosis and hopefully also improve survival.

Although the relationship between cancer and thrombosis has been known for more than a century, the mechanism by which tumour predispose to thrombosis has not been elucidated. The activation of blood coagulation in patients with cancer may well have several causes. Prothrombotic mechanisms may be related to the host's response to cancer and other factors to procoagulant properties of the cancer cells themselves.

The aim of this thesis is to investigate risk factors for cancer-related thrombosis (part I) and the role for microparticles in the pathogenesis of cancer-related thrombosis (part II).

Part I

Chapter 2. We investigated the risk factors for venous thrombosis in cancer patients with implantable ports (arm or chest ports) who underwent chemotherapy in our hospital between 1994 and 2003. In this 10-year period, clinically overt thrombotic events, which were all confirmed by ultrasound or angiography, were counted. Catheter-related thrombosis cumulative incidence in patients who did not receive anticoagulants was 28% in patients with arm ports and 33% in patients with chest ports. In contrast, in patients who did receive anticoagulants the incidence was 32% in patients with arm ports and only 1% in patients with chest ports (odds ratio (OR) 34.8 95% confidence interval (CI) 7.3-165). Left-sided placement as compared to right-sided and catheter tip position in the superior *vena cava* as compared to placement in the right atrium were associated with a 3.5- respectively 2.6-fold increased risk of thrombosis. Blood samples were obtained from 101 patients for analysis of plasma concentrations of factor VIII, IX, XI, and for gene mutation analysis, namely FV G1691A and FII G20210A mutation. Thrombosis was associated with elevated homocysteine levels (OR = 3.8, 95% CI 1.3-11.3), but not

with factor V Leiden or prothrombin 20210A gene mutations, or high concentration of factor VIII, IX or XI. Based on our data, we concluded that prophylaxis with anticoagulants should be recommended when using chest ports, and that the use of arm ports for the administration of chemotherapy in cancer patients should be avoided. Determination of plasma homocysteine levels may identify patients at an increased risk for thrombosis but further research is needed to further substantiate these data and its clinical relevance.

Chapter 3. This chapter concerns a review of the incidence and risk factors of catheter-related thrombosis. Cancer as underlying disease, but also type and material of catheters are risk factors in the development of catheter-related thrombosis. Since the introduction of other materials for the production of central venous catheters, which seem to be less thrombogenic, and the increased use of oral anti-cancer agents precluding the need for permanent venous catheters, the incidence of catheter-related thrombosis is likely to decline. The issue of prophylactic anticoagulants therefore becomes less important in cancer patients.

Chapter 4. Risk factors of venous thrombosis in lung cancer patients are reviewed in this chapter. Lung cancer is the second most common cancer in Western countries and the leading cause of cancer death in men, but strikingly few papers on the phenomenon of venous thrombosis in lung cancer patients are found and data on mortality due to thrombosis are limited. Risk factors of venous thrombosis which were identified in lung cancer patients are histology, namely adenocarcinoma, presence of metastatic disease, pneumonectomy and anti-cancer therapy including chemotherapy and the use of anti-VEGF targeted drugs. Other risk factors identified include platelet counts (prior to systemic treatment) and in one case report active TF expressing circulating microparticles.

Chapter 5. Although adenocarcinoma was identified by us as a risk factor for venous thrombosis in lung cancer patients, no other studies were ever performed to estimate the risk of venous thrombosis in patients with either adenocarcinoma or squamous carcinoma originating in the same organ site. We therefore performed a follow-up study in patients with upper gastro-intestinal cancer, i.e. gastric and oesophageal carcinoma, to study the incidence of venous thrombosis in adenocarcinomas and squamous carcinomas arising in this organ site as described in this chapter. We performed a study in 1000 consecutive patients diagnosed with upper-gastrointestinal cancer in our hospital in the

period between 1980 and 2000. In these 535 oesophageal carcinoma patients, of whom 216 (40%) had an adenocarcinomas and 319 (60%) a squamous cell carcinoma, and 465 patients with adenocarcinomas originating in the stomach, the incidence of thrombosis was 10 respectively 15 times higher than in the Dutch population. The risk of thrombosis in patients with adenocarcinoma was 2.6-fold increased (HR 2.63, 95% CI: 1.38-5.00) compared to patients with squamous cell carcinoma. Survival was markedly decreased in patients who developed thrombosis (HR 2.13, 95 % CI: 1.64-2.88), irrespective of whether the tumour originated in the stomach or the oesophagus as compared to that in patients who did not develop thrombosis.

Part II describes several studies on microparticles, isolated from blood of cancer patients, and their role in the pathogenesis of cancer-related thrombosis. Microparticles or apoptotic bodies vary in size between 100-1000 nm, and their numbers, cellular origin and chemico-physical properties seem to be dependent on the type of disease and in cancer patients on type of cancer, stage of the as well as factors contributing to the thrombotic event. For a long time, microparticles were considered to be cellular debris reflecting cellular activation or damage, but these microparticles are now known to interact with other cells and acquire a pathophysiologic potential. There are several lines of evidence supporting the procoagulant activity of microparticles and several small studies have demonstrated that microparticles levels are elevated in individuals suffering from thrombotic events. So far little is known about the role of microparticles in cancer-related thrombosis. Most of the data on numbers and cellular origin of microparticles have been obtained using flow cytometry. This technique offers the possibility to label microparticles with several fluorochrome-labelled antibodies and annexin-V and thus determine the cellular origin of the microparticles. However, different laboratories use different methods for isolation and preservation of microparticles, precluding direct comparison of data obtained, which may in part explain the sometimes seemingly inconsistent or conflicting data. More recently, various groups are exploring the use of other techniques to detect MP isolated from blood of different individuals in order to quantify MP number and determine their characteristics.

Chapter 6. We investigated procoagulant properties of circulating microparticles isolated from blood of unselected pancreatic and breast adenocarcinoma patients, from consecutive subjects without cancer who presented with ultrasound or CT-scan confirmed

venous thrombosis, and from healthy subjects. Microparticles were examined by FACS analysis and microparticle-associated tissuefactor (TF) activity measured by determination of factor VIIa-dependent factor generation of Xa. Patients with disseminated breast and pancreatic cancer had increased levels of microparticles-associated TF activity as compared to healthy controls, subjects with idiopathic acute venous thrombosis and cancer patients without metastatic disease. Patients with elevated microparticles-associated TF-activity and microparticles-expressing the epithelial mucin antigen MUC1 had a lower survival at 3-9 months follow-up than those with low MP-TF activity and no expression of mucin antigen on their MP: the likelihood of survival was 0.42 (95% CI: 0.19-0.94) for an individual with these two predictor variables present, after adjustment for other factors (age, type of cancer, venous thrombosis) in a Cox proportional hazards model. Our results suggest an important role for microparticles-associated TF and mucin in the pathogenesis of thrombosis in disseminated mucinous adenocarcinoma patients.

Chapter 7. In a case-control study, Microparticle-associated TF activity in blood isolated from 100 consecutive cancer patients was investigated. Cases were 51 unselected cancer patients who presented with radiologically confirmed venous thrombosis and controls were 49 cancer patients without thrombosis, who were matched for age, type and stage of cancer and type of cancer-specific treatment, including the precise chemotherapy regimens as well as previous cancer-specific treatments. An additional risk factor for development of venous thrombosis was defined as concurrent chemotherapy or compression of veins by tumour. Of the 51 cases, i.e. the cancer patients with venous thrombosis, 24 patients did not have an additional thrombosis risk factor, whereas 27 patients did have an additional risk factor. The median microparticle-associated TF activity in patients with additional risk factors was low and did not differ from that in the 49 control cancer patients without venous thrombosis. In contrast, microparticle-associated TF activity was elevated in all 24 cancer patients without additional risk factors and differed significantly from that found in all other cancer patients. Median survival in patients with thrombosis but without additional risk factor was significantly shorter than in patients with additional risk factors and control cancer patients, namely 1.9, respectively 13.1 and 12.2 months.

Chapter 8. The role of tissue factor was further investigated in 62 patients with pancreatic cancer in whom we assessed circulating MP-TF activity. We also assessed expression of TF by immunohistochemistry in the original tumours, of 27 patients of whom

tumour biopsies were available to us. Both factors were correlated with development of venous thrombosis and survival. Of the 62 patients, all with ductal adenocarcinoma of the pancreas, 22 patients had locoregional disease and 40 distant metastases. Thrombotic events developed in 12 (19%) of the 62 pancreatic carcinoma patients and was confirmed in all cases by ultrasound or CT-scan. Circulating microparticle-associated TF activity were analysed to assess predictors of survival following diagnosis. There was no difference between the total number of microparticles, platelet-derived microparticles and platelets in pancreatic cancer patients with or without thrombosis. All patients with venous thrombosis had elevated MP-TF activity, whereas only 6 (13.6%) patients without VTE had elevated microparticles, and MP-TF activity was significantly higher in patients who presented with VTE than in the other patients. In the 27 tumour specimens available for immunohistochemistry, only in a small percentage of tumours TF expression was observed and the extent of TF expression was not clearly associated with the grade of malignancy. Furthermore, no clear association was found between TF expression in the tumour and MP-TF activity. Mortality was associated with elevated microparticle-associated-TF activity and both MP-TF activity and occurrence of venous thrombosis significantly predicted poor survival.

In summary, in this thesis risk factors of thrombosis such as central venous catheters, the use of chemotherapy, FV Leiden and prothrombin gene mutation and abnormal levels of proteins involved in the clotting cascade were investigated in various groups of cancer patients. Although none of the studies performed in **part I** of this thesis explored the use of thrombosis prophylaxis, this should be considered in patients identified to be at highest risk for development of thrombosis, namely patients with an adenocarcinoma of the lung or gastro-intestinal tract, patients with a central venous catheters undergoing anti-cancer therapy, and the advantage of such a strategy should be weighed against the risk for bleeding episodes in this group of patients. In part II of this thesis, the presence of epithelial cell-derived, mucin-bearing microparticles in the circulation of cancer patients has been demonstrated and microparticles bearing active tissue factor were linked to cancer-related thrombosis and poor prognosis in patients with various types of carcinoma. In the coming years, additional studies in larger cohorts of cancer patients may help to define the role of circulating microparticle-associated TF activity as a potential biomarker for cancer-related thrombosis risk and poor prognosis in cancer patients. Such research is also needed to investigate whether active TF-bearing microparticles in cancer

patients are indeed the cause of thrombosis or a marker of a prothrombotic state and to unequivocally demonstrate whether the source of TF-bearing microparticles in cancer patients is the tumour cells themselves or other cells, including platelets, monocytes, activated endothelial cells or even fibroblasts.

