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**Title:** Risk factors and predictors for recurrent venous thrombosis: building blocks for a prognostic model
**Issue Date:** 2016-05-12
Chapter 5

Coagulation factor levels in relation to venous thrombosis and cancer: Results from the MEGA study


Submitted for publication
Abstract

The pathophysiology underlying the association between cancer and subsequent venous thrombosis (VT) is not well known. Furthermore, it is not known in what way patients with cancer who develop VT are different from those who do not.

We aimed to study plasma coagulation factor levels (procoagulant, anticoagulant, fibrinolytic) in four groups of individuals with and without cancer and with and without VT.

From the MEGA case-control study (n=11253) four groups of participants with or without cancer (CA+ or CA-) and with or without VT (VT+ or VT-) were included, and plasma coagulation factors measured after the initial anticoagulant treatment for the thrombotic event. Cancer diagnoses were objectively verified. Median levels of coagulation factors, with 95% confidence intervals, were estimated, as well as geometric mean differences in factor levels over the groups of participants with the VT-CA- group as the reference.

Median levels of coagulation factors were generally lowest in the VT-CA- group (n=2825). Compared with this group, levels of fibrinogen, factor VIII, von Willebrand factor and factor XI were increased in the VT+CA- participants (n=2166) and highest in the VT+CA+ participants (n=147). Results were most pronounced for factor VIII and von Willebrand factor. Levels of factor V, IX, total and free protein S and TFPI were increased only in the VT+CA+ participants.

To conclude, increased levels of procoagulant coagulation factors in participants with both VT and cancer suggest a generalized role of procoagulant pathways in patients with cancer and suggest the importance of a procoagulant state in cancer-associated VT.

Introduction

An association between cancer and venous thrombosis was first described by Bouillaud and Trousseau in the 19th century already.[1,2] Since then, the strong relation between cancer and venous thrombosis has been confirmed in various studies. It is estimated that a fifth of all venous thrombotic events are cancer associated.[3-5] Cancer is reported to increase the risk of venous thrombosis about four- to seven-fold[3,6], and venous thrombotic events are a major cause of morbidity and mortality in patients with cancer.[7]

The pathophysiology underlying the association between cancer and venous thrombosis is largely unknown. It is likely to be multifaceted and to involve interactions of tumor cells, the hemostatic system, cancer treatment measures and characteristics of the patient. General procoagulant effects are exerted by the host response to cancer (acute-phase reaction, paraprotein production, inflammation, necrosis and hemodynamic disorders) and by anticancer therapies.[8] In addition, several substances released by and activities directly associated with tumor cells (including tissue factor, tumor derived cytokines, inhibitors of fibrinolysis and cell adhesion molecules) play a prominent role.[8-10]

Progression of cancer is accompanied by the development of a hypercoagulable state. It is cited in literature that about 50% of all patients with cancer and up to 90% of patients with metastasised cancer exhibit abnormalities in one or more routine coagulation parameters.[11-16] The most commonly described hemostatic changes in patients with cancer are an increase in plasma levels of clotting factors I (fibrinogen), V, VIII, IX and XI as well as in fibrinogen degradation products and platelet count.[17] Most of these studies were, however, conducted a long time ago.

Cancer treatment strategies, and therefore the prognosis of patients, have changed considerably. Furthermore, it is not well known how patients with cancer who develop venous thrombotic events differ from those patients with cancer without thrombosis. Few studies have linked the coagulation profile in patients with cancer with the clinical occurrence of venous thrombosis.[18-20] Neither has this, as far as we know, been done for a wide range of procoagulant and anticoagulant factors.

We aimed to study several plasma coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) in four groups of individuals with and without cancer and with and without venous thrombosis to determine to what extent the coagulation profile differs between these groups. For this purpose, we used data from the MEGA case-control study (n>10 000) in which for more than half of the participants blood was sampled and factors of the hemostatic system were measured.
Methods

Participants
This study was performed within the MEGA- (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study, which is a large case-control study aimed at identifying risk factors for venous thrombosis. Details of this study have been described previously.[6] In brief, from March 1999 until September 2004, 4956 consecutive patients with a first objectively diagnosed venous thrombotic event were included from six anticoagulation clinics in the Netherlands. Anticoagulation clinics monitor all patients taking vitamin K antagonists in a well-defined geographical area. Detailed diagnostic information was obtained from hospital discharge reports and general practitioners. 3297 Partner controls of the patients, who had no history of venous thrombosis, were included in the study. Additionally from January 2002 to September 2004, 3000 random-digit-dialing controls (RDD), with no history of venous thrombosis, were included. All participants were 18 to 70 years of age. All participants completed the informed consent process prior to enrolment, and the study was approved by the ethics committee of the Leiden University Medical Center.

Between 2007 and 2009 the vital status of all MEGA participants was acquired from the Dutch population register, as has been described previously.[21] For the participants who died, a cause of death (encoded according to International Classification of Diseases ICD-10-CM) was obtained from the national register of death certificates.

Cancer diagnosis
All participants were asked to complete a detailed questionnaire on acquired risk factors for venous thrombosis. All items in the questionnaire referred to the period before the index date. We used the date of diagnosis of venous thrombosis as the index date for patients as well as their partner controls. For the RDD controls the index date was the date of completing the questionnaire. Participants were asked to report on the presence of acquired risk factors, amongst others any type of diagnosed malignancy, date of diagnosis and type of malignancy diagnosed. Approximately three months after discontinuation of anticoagulant treatment, or one year after the event in case anticoagulant treatment was continued for more than a year, cases and their partner controls were interviewed. The RDD group was invited for the interview at time of returning the questionnaire. In the interview, participants were again asked to report on any malignancies diagnosed after inclusion in the study, date of diagnosis and type of cancer diagnosed. Self-reported cancer diagnoses were verified by means of discharge letters from the primary physician or hospital where patients were treated. Details of this verification process were described previously.[6] For the current study, participants with a cancer diagnosis within five years before the index date, or a cancer diagnosis within six months after the index date, were included. Participants with a cancer diagnosis outside this period were excluded from analyses.

Blood collection and laboratory analyses
At the time of the interview blood was sampled from both cases and controls. Details of collection and processing of blood samples have been described previously.[22,23] For logistic reasons, blood sampling for measurement of coagulation proteins was done in patients diagnosed with venous thrombosis before June 1, 2002. Blood samples were drawn at least three months after discontinuation of oral anticoagulant therapy, or during anticoagulant therapy in patients who continued this therapy for more than one year. Partner controls visited the anticoagulation clinic for blood sampling at the same time as their partner and therefore blood samples were available only for partner controls recruited before June 2002. The additional group of controls recruited via RDD were invited for a blood sample irrespective of their time of enrolment. Participants who were unable or unwilling to provide blood samples or were patients and partner controls recruited after June 1, 2002, were sent buccal swabs to collect DNA for genetic profiling.

The levels of natural anticoagulants (antithrombin, protein S, protein C levels and TFPI), procoagulant factors (fibrinogen, factor II, factor V, factor VII, factor VIII, von Willebrand factor, factor IX, factor X, and factor XI), and the fibrinolytic marker D-dimer were assessed in the blood samples. All assays were performed in automated machines by laboratory technicians who were unaware of the case–control status of the samples. For details on the measurements of coagulation factors, see the Supplement.

Statistical analyses
Blood samples were available for 2377 participants with venous thrombosis (48% (2377/4956)) and 2939 controls (47% (2939/6297)). 123 Participants (64 cases, 59 controls) were excluded from analyses because of a cancer diagnosis more than five years before inclusion in the study, because of a cancer diagnosis more than six months after the index date or because information regarding a possible cancer diagnosis or cancer diagnosis date was missing. In total, 2313 participants with venous thrombosis and 2880 controls without venous thrombosis were included for the current analyses.

Median coagulation factor levels with corresponding 95% confidence intervals were estimated for participants with neither venous thrombosis nor cancer (VT-CA-; n=2825), participants with cancer but without venous thrombosis (VT-CA+; n=55), participants with venous thrombosis but no cancer (VT+CA-; n=2166) and for participants with both cancer and venous thrombosis (VT+CA+ group; n=147). Boxplots with medians and corresponding 95% confidence intervals were constructed for every coagulation factor separately to visually show the spread of coagulation factor levels over the groups of participants (Supplementary Figure 1). Levels of D-dimer were plotted on the 10log scale, because of the wide range of D-dimer measurements.

Mean differences in coagulation factor levels (and corresponding 95% confidence intervals) were estimated between the groups of participants, with the VT-CA- group as the reference category. Mean differences were adjusted for age and sex by means of multivariate linear regression analysis. Mean differences between the groups
of participants were estimated with a natural logarithmic transformation, thus
providing geometric mean differences, since mean differences without a logarithmic
transformation are substantially influenced by extremely high or low values of
coagulation factors for some of the participants. The interpretation of a geometric
mean difference is different from the interpretation of a mean difference. Instead of an
absolute difference in factor levels it represents a relative difference with in our case
the VT-CA- group as the reference. For example, a geometric mean difference for factor
VIII of 1.10 in the VT+CA+ group means that on average factor VIII levels are 1.10 times
higher (10% higher) in the VT+CA+ group than in the VT-CA- group. We chose a cut-off
of five percent to define levels of coagulation factors as increased.

Participants with a cancer diagnosis within five years before their thrombotic event
could have gone into remission in the meantime and might have a different coagulation
profile than participants whose malignancy was still active at time of blood sampling.
For this reason, we estimated median coagulation factor levels in the VT+CA+ group
separately for participants who died of cancer in the years after the index date (as
registered by the national register of death certificates) and for participants who
survived the years following the index event. The first group of participants was
classified as ‘active cancer’ while the second group of participants was classified as
‘unknown activity’. Geometric mean differences, adjusted for age and sex, in
coagulation factor levels between the two groups of participants with cancer (active
cancer vs activity unknown) were estimated.

At the time of blood collection 304 participants (275 individuals with venous
thrombosis; 29 controls without venous thrombosis) were on anticoagulant treatment.
These participants were excluded from all analyses concerning vitamin K dependent
coaulation factors (factor II, VII, IX and X, protein C activity, total protein S antigen, free
protein S antigen) and factors that are otherwise affected by anticoagulant treatment
(D-dimer).

Results

The clinical characteristics of the participants are shown in Table 1, for all participants
included and for the four groups of participants separately. Mean age of all participants
was 48 years. Mean age was higher in participants with cancer, i.e. 55 years in the
VT+CA+ group and 58 years in the VT-CA+ group. The most common types of cancer
were breast, prostate, colorectal and a hematological type of cancer.

Median levels of coagulation factors differed considerably across the four groups
of participants and were generally lowest in the group of participants without venous
thrombosis and without cancer (VT-CA-) (Table 2). Differences in median levels over
the groups of participants were most pronounced for levels of factor VIII (activity and
antigen), von Willebrand factor and D-dimer. Levels were lowest in the VT-CA-
group, higher in the other groups of participants and highest in the VT+CA+ group.
Levels of factor V, factor VII, protein C activity and total protein S antigen were increased
in both cancer groups (VT-CA+ and VT+CA+) as compared with the groups of participants
without cancer (VT-CA- and VT+CA-).

Geometric mean differences in coagulation factor levels, adjusted for age and sex,
between the VT-CA- group and the VT-CA+, VT+CA- and VT+CA+ groups are presented in
Table 3. The VT-CA- group was used as the reference category and the geometric mean
differences for the other groups of participants represent the relative increase in levels
of the coagulation factors. Following a cut-off of five percent to define an increase in
coagulation factor level, we identified four patterns for the coagulation factors over the
four groups. For the first pattern, levels were increased by at least 5% for the VT+CA+
participants only, and not for the other groups. We identified pattern 1 for factor V,
factor IX, total and free protein S and TFPI. Fibrinogen, factor VIII activity and antigen,
von Willebrand factor, factor XI and D-dimer levels were increased by at least 5% in the
VT+CA- group and highest in the VT+CA+ group (pattern 2). Only D-dimer levels were
increased to the same extent both in the VT+CA- and VT+CA+ groups (by approximately
40%). Levels of factor VII were increased to about the same extent in both groups of
participants with cancer (VT-CA+ and VT+CA+) (pattern 3). The fourth pattern showed
no clear difference in factor levels over the groups of participants, which was the case
for factor II, factor X, antithrombin and protein C. In all of abovementioned analyses
adjustment for age had a larger effect on mean differences in coagulation factor levels
than adjustment for sex (results not shown).

In Table 4, the median coagulation factor levels are shown for participants who
died of cancer in the years following their thrombotic event (n=39) and for participants
who survived in the years following thrombosis (n=97) (‘active cancer’ vs ‘unknown
activity’). The geometric mean differences in coagulation factor levels, adjusted for age
and sex, between the two groups are additionally shown. After adjustments, levels of
factor VIII activity, factor VIII antigen and von Willebrand factor were increased by at
least 20% in the active cancer group as compared with the group of participants
with cancer with unknown activity. Levels of both TFPI and D-dimer were increased in
the active cancer patients as compared with the cancer patients with unknown activity by
approximately 15%.
Table 1. Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>VT* - Cancer -</th>
<th>VT - Cancer +</th>
<th>VT + Cancer -</th>
<th>VT + Cancer +</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>2825</td>
<td>55</td>
<td>2166</td>
<td>147</td>
<td>5193</td>
</tr>
<tr>
<td>Male sex n, (%)</td>
<td>1351 (48%)</td>
<td>26 (47%)</td>
<td>986 (46%)</td>
<td>72 (49%)</td>
<td>2435 (47%)</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>48 (18-70)</td>
<td>58 (31-70)</td>
<td>47 (18-70)</td>
<td>55 (18-70)</td>
<td>48 (18-70)</td>
</tr>
</tbody>
</table>

*VT denotes: venous thrombosis

Table 2. Median coagulation factor levels for participants with or without cancer and with or without venous thrombosis

<table>
<thead>
<tr>
<th>Coagulation factor</th>
<th>VT* - Cancer - median (95%CI)</th>
<th>VT - Cancer + median (95%CI)</th>
<th>VT + Cancer - median (95%CI)</th>
<th>VT + Cancer + median (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procoagulant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen activity (g/L)</td>
<td>3.2 (3.2-3.3)</td>
<td>3.5 (3.2-3.7)</td>
<td>3.4 (3.4-3.4)</td>
<td>3.7 (3.5-3.8)</td>
</tr>
<tr>
<td>Factor II activity (IU/dL)†</td>
<td>109 (109-110)</td>
<td>113 (107-119)</td>
<td>112 (111-112)</td>
<td>111 (109-115)</td>
</tr>
<tr>
<td>Factor V (IU/dL)</td>
<td>92 (91-93)</td>
<td>97 (91-103)</td>
<td>93 (92-94)</td>
<td>101 (96-104)</td>
</tr>
<tr>
<td>Factor VII activity (IU/dL)†</td>
<td>109 (108-110)</td>
<td>124 (109-130)</td>
<td>112 (110-113)</td>
<td>119 (116-128)</td>
</tr>
<tr>
<td>Factor VIII activity (IU/dL)</td>
<td>106 (104-107)</td>
<td>110 (103-132)</td>
<td>134 (131-136)</td>
<td>148 (139-152)</td>
</tr>
<tr>
<td>Factor VIII antigen (IU/dL)</td>
<td>108 (107-110)</td>
<td>123 (115-132)</td>
<td>146 (143-148)</td>
<td>162 (152-175)</td>
</tr>
<tr>
<td>Von Willebrand factor antigen (IU/dL)</td>
<td>105 (103-105)</td>
<td>110 (102-123)</td>
<td>138 (136-140)</td>
<td>158 (148-164)</td>
</tr>
<tr>
<td>Factor IX antigen (IU/dL)†</td>
<td>103 (102-104)</td>
<td>109 (101-117)</td>
<td>107 (106-109)</td>
<td>113 (109-117)</td>
</tr>
<tr>
<td>Factor X activity (IU/dL)†</td>
<td>116 (115-117)</td>
<td>126 (115-129)</td>
<td>118 (117-119)</td>
<td>116 (111-120)</td>
</tr>
<tr>
<td>Factor XI activity (IU/dL)</td>
<td>98 (97-99)</td>
<td>106 (101-112)</td>
<td>104 (102-105)</td>
<td>105 (103-111)</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (IU/dL)</td>
<td>105 (105-106)</td>
<td>105 (101-109)</td>
<td>105 (105-106)</td>
<td>107 (105-109)</td>
</tr>
<tr>
<td>Protein C activity (IU/dL)†</td>
<td>116 (115-117)</td>
<td>124 (115-131)</td>
<td>115 (113-116)</td>
<td>122 (117-127)</td>
</tr>
<tr>
<td>Total protein S antigen (IU/dL)†</td>
<td>101 (100-102)</td>
<td>113 (100-117)</td>
<td>102 (101-103)</td>
<td>109 (105-112)</td>
</tr>
<tr>
<td>Free protein S antigen (IU/dL)†</td>
<td>90 (89-91)</td>
<td>88 (82-105)</td>
<td>92 (91-94)</td>
<td>97 (92-102)</td>
</tr>
<tr>
<td>TFPI (U/mL)</td>
<td>1.7 (1.7-1.7)</td>
<td>1.9 (1.7-2.0)</td>
<td>1.7 (1.7-1.7)</td>
<td>1.9 (1.9-2.0)</td>
</tr>
<tr>
<td>Fibrinolytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-dimer (ng/mL)†</td>
<td>236 (230-240)</td>
<td>263 (230-312)</td>
<td>327 (315-339)</td>
<td>347 (303-400)</td>
</tr>
</tbody>
</table>

*VT denotes: venous thrombosis, CI: confidence interval
†Participants on anticoagulant treatment during blood sampling were excluded

Table 3. Geometric mean differences in coagulation factor levels between groups of participants with and without cancer and with and without venous thrombosis

<table>
<thead>
<tr>
<th>Coagulation factor</th>
<th>VT - Cancer - GMD (95%CI)†</th>
<th>VT - Cancer + GMD (95%CI)†</th>
<th>VT + Cancer - GMD (95%CI)†</th>
<th>VT + Cancer + GMD (95%CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procoagulant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen activity (g/L)</td>
<td>reference</td>
<td>1.00 (0.95-1.06)</td>
<td>1.05 (1.04-1.06)</td>
<td>1.11 (1.07-1.14)</td>
</tr>
<tr>
<td>Factor II activity (IU/dL)†</td>
<td>reference</td>
<td>1.01 (0.97-1.05)</td>
<td>1.01 (1.00-1.02)</td>
<td>1.03 (1.00-1.05)</td>
</tr>
<tr>
<td>Factor V (IU/dL)</td>
<td>reference</td>
<td>1.02 (0.97-1.07)</td>
<td>1.02 (1.01-1.03)</td>
<td>1.06 (1.03-1.09)</td>
</tr>
<tr>
<td>Factor VII activity (IU/dL)†</td>
<td>reference</td>
<td>1.05 (0.99-1.12)</td>
<td>1.02 (1.01-1.04)</td>
<td>1.08 (1.04-1.13)</td>
</tr>
<tr>
<td>Factor VIII activity (IU/dL)</td>
<td>reference</td>
<td>1.04 (0.95-1.13)</td>
<td>1.25 (1.23-1.27)</td>
<td>1.30 (1.23-1.37)</td>
</tr>
<tr>
<td>Factor VIII antigen (IU/dL)</td>
<td>reference</td>
<td>1.04 (0.95-1.14)</td>
<td>1.34 (1.32-1.37)</td>
<td>1.42 (1.34-1.50)</td>
</tr>
<tr>
<td>Von Willebrand Factor antigen (IU/dL)</td>
<td>reference</td>
<td>1.02 (0.93-1.12)</td>
<td>1.33 (1.30-1.36)</td>
<td>1.43 (1.35-1.52)</td>
</tr>
<tr>
<td>Factor IX antigen (IU/dL)†</td>
<td>reference</td>
<td>1.03 (0.98-1.08)</td>
<td>1.04 (1.03-1.05)</td>
<td>1.09 (1.06-1.12)</td>
</tr>
<tr>
<td>Factor X activity (IU/dL)†</td>
<td>reference</td>
<td>1.04 (0.99-1.09)</td>
<td>1.02 (1.01-1.03)</td>
<td>1.01 (0.98-1.04)</td>
</tr>
<tr>
<td>Factor XI activity (IU/dL)</td>
<td>reference</td>
<td>1.00 (0.95-1.06)</td>
<td>1.05 (1.04-1.06)</td>
<td>1.09 (1.05-1.12)</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin (IU/dL)</td>
<td>reference</td>
<td>1.00 (0.97-1.03)</td>
<td>1.00 (0.99-1.00)</td>
<td>1.03 (1.01-1.05)</td>
</tr>
<tr>
<td>Protein C activity (IU/dL)†</td>
<td>reference</td>
<td>1.02 (0.97-1.07)</td>
<td>0.99 (0.98-1.01)</td>
<td>1.03 (1.00-1.07)</td>
</tr>
<tr>
<td>Total protein S antigen (IU/dL)†</td>
<td>reference</td>
<td>1.04 (0.99-1.09)</td>
<td>1.01 (1.00-1.02)</td>
<td>1.05 (1.01-1.08)</td>
</tr>
<tr>
<td>Free protein S antigen (IU/dL)†</td>
<td>reference</td>
<td>1.01 (0.93-1.10)</td>
<td>1.04 (1.03-1.06)</td>
<td>1.05 (1.01-1.10)</td>
</tr>
<tr>
<td>TFPI (U/mL)</td>
<td>reference</td>
<td>1.03 (0.95-1.10)</td>
<td>1.00 (0.99-1.02)</td>
<td>1.08 (1.03-1.13)</td>
</tr>
<tr>
<td>Fibrinolytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-dimer (ng/mL)†</td>
<td>reference</td>
<td>0.97 (0.81-1.15)</td>
<td>1.42 (1.37-1.47)</td>
<td>1.41 (1.25-1.58)</td>
</tr>
</tbody>
</table>

*VT denotes: venous thrombosis, GMD: geometric mean difference, CI: confidence interval
†Adjusted for age and sex
‡Participants on anticoagulant treatment during blood sampling were excluded

Discussion

In this study, in which we studied plasma coagulation factor levels in participants with and without cancer and with and without venous thrombosis, we found that all coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) were lowest in individuals without venous thrombosis and without cancer. Compared with this group of participants, levels of fibrinogen, factor VIII activity and antigen, von Willebrand factor and factor XI were increased in participants with venous thrombosis without cancer and were highest in participants with both venous thrombosis and cancer. These findings were most pronounced for factor VIII and von Willebrand factor (30-40% increase). Levels of factor V, factor IX, total and free protein S and TFPI were increased only in the group of participants with both venous thrombosis and cancer. Levels of factor VII were increased in participants with cancer and were unaffected by the presence or absence of venous thrombosis.

Our findings of increased levels of procoagulant coagulation factors in participants with venous thrombosis without cancer and even higher levels of these factors in participants with both venous thrombosis and cancer support prior observations of a generalized role of procoagulant pathways in patients with cancer and thrombosis and emphasize the importance of the coagulation system in cancer-associated venous thrombosis. These findings were most pronounced for levels of factor VIII and von Willebrand factor. Our finding of slightly increased levels of anticoagulant proteins, free protein S and TFPI, in participants with cancer and venous thrombosis is suggestive of an additional effect of cancer on anticoagulant pathways.

Although previous studies have compared coagulation profiles for individuals with and without cancer,[11,16,19,24], few studies have linked these profiles with venous thrombotic events in patients with cancer.[18,19] Johnson et al compared coagulation profiles between 98 (hospice) patients with advanced cancer either with or without deep vein thrombosis (identified on screening) with a group of control participants without cancer.[19] Goldenberg et al studied coagulation factor levels in 36 patients with cancer-only, 58 patients with venous thrombosis-only and 32 patients with both cancer and venous thrombosis.[18] Some of our findings are in accordance with these studies, while others are not. Similar to our observations, Johnson et al found increased levels of fibrinogen, factor VIII and D-dimer in cancer patients as compared with healthy controls. However, cancer patients with DVT had somewhat lower levels of fibrinogen and factor VIII than cancer patients without DVT, which is contrary to our findings. Goldenberg et al reported an increased level of von Willebrand factor in the group of cancer patients with DVT as compared with the group of patients with cancer or DVT alone. This is in line with our findings. These studies were, however, not comparable with ours with respect to patient selection and study design. For example, Johnson et al only included hospice-in patients with advanced cancer. Furthermore, none of the results were adjusted for age and sex.

We found somewhat increased levels of the anticoagulant proteins total and free protein S and TFPI in the group of participants with cancer and venous thrombosis. In

Table 4. Median coagulation factor levels and geometric mean differences according to activity of cancer

<table>
<thead>
<tr>
<th>Coagulation factor</th>
<th>Median (95%CI)</th>
<th>GMD (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VT patients with cancer with unknown activity (n=97)</td>
<td>VT patients with active cancer (n=39)</td>
</tr>
<tr>
<td>Fibrinogen activity (g/L)</td>
<td>3.7 (3.5-3.8)</td>
<td>3.7 (3.4-3.9)</td>
</tr>
<tr>
<td>Factor II activity (IU/dL)†</td>
<td>111 (107-116)</td>
<td>112 (109-116)</td>
</tr>
<tr>
<td>factor V (IU/dL)</td>
<td>97 (93-102)</td>
<td>106 (97-111)</td>
</tr>
<tr>
<td>Factor VII activity (IU/dL)†</td>
<td>120 (116-128)</td>
<td>119 (107-138)</td>
</tr>
<tr>
<td>Factor VIII activity (IU/dL)</td>
<td>141 (132-152)</td>
<td>151 (147-195)</td>
</tr>
<tr>
<td>Factor VIII antigen (IU/dL)</td>
<td>155 (143-166)</td>
<td>198 (157-222)</td>
</tr>
<tr>
<td>Von Willebrand factor antigen (IU/dL)</td>
<td>152 (136-162)</td>
<td>192 (154-226)</td>
</tr>
<tr>
<td>Factor IX antigen (IU/dL)†</td>
<td>113 (108-120)</td>
<td>112 (102-123)</td>
</tr>
<tr>
<td>Factor X activity (IU/dL)†</td>
<td>115 (110-121)</td>
<td>120 (110-128)</td>
</tr>
<tr>
<td>Factor XI activity (IU/dL)</td>
<td>107 (103-112)</td>
<td>105 (94-115)</td>
</tr>
<tr>
<td>Antithrombin (IU/dL)</td>
<td>106 (105-110)</td>
<td>108 (103-110)</td>
</tr>
<tr>
<td>Protein C activity (IU/dL)†</td>
<td>123 (117-127)</td>
<td>116 (111-137)</td>
</tr>
<tr>
<td>Total protein S antigen (IU/dL)†</td>
<td>108 (103-113)</td>
<td>110 (105-120)</td>
</tr>
<tr>
<td>Free protein S antigen (IU/dL)†</td>
<td>97 (92-103)</td>
<td>103 (92-107)</td>
</tr>
<tr>
<td>TFPI (U/mL)</td>
<td>1.9 (1.7-2.0)</td>
<td>2.2 (1.9-2.3)</td>
</tr>
</tbody>
</table>

* CI denotes: confidence interval, VT: venous thrombosis, GMD: geometric mean difference, for which the patients with cancer with unknown activity were set as reference.
† Adjusted for age and sex
‡ Participants on anticoagulant treatment during blood sampling excluded
general, levels of anticoagulant proteins, such as antithrombin, protein C and protein S are assumed to be lower in patients with cancer than in non-cancer individuals due to a decreased hepatic synthesis of such anticoagulant proteins.[17] Decreased levels of both protein C and protein S in patients with cancer as compared with healthy controls have indeed been shown in several studies.[25-27] A possible explanation for these conflicting results is that previous studies included patients with more advanced disease. In patients with advanced disease, consumption of these proteins (as seen in sepsis)[28] or active liver disease may have led to decreased levels of these proteins.

We found increased levels of factor VII in patients with cancer, independent of the presence of VT, which is in line with a study by Kakkar et al.[29] In this study in over 100 patients with solid tumors and a comparison group of healthy volunteers, plasma levels of factor VII were found to be 46% higher in patients with cancer. These results were, however, not corrected for age and sex.

In our study, factor VIII, von Willebrand factor and D-dimer showed the highest rise in levels in participants with cancer. High factor VIII and von Willebrand factor levels have been described before in different types of cancer patients.[30-32] Levels of these factors were largely determined by age in our study, which is in accordance with studies showing progressive increase in plasma coagulation factors with age.[33] Levels of factor VIII (activity and antigen) and von Willebrand factor were substantially increased in participants with both cancer and venous thrombosis but were not increased in VT-CA+ participants after adjustment for age and sex. An explanation for these findings could be that the more aggressive types of cancer and advanced stages of cancer are associated with venous thrombosis.[6,34-37] Perhaps these types and advanced stages of cancer induce higher levels of coagulation factors, which subsequently induce a higher risk of venous thrombosis than other types of cancer. Indeed, Vormittag and colleagues observed a significant difference in factor VIII levels according to tumor site,[38] which were highest in patients with tumor sites associated with a high risk of venous thrombosis. Auwerda et al. reported an association between factor VIII and von Willebrand factor levels and disease stage, with highest levels in patients with stage III disease (vs stage I or stage II disease).[39] The same can be concluded from our analysis in which we found that venous thrombosis patients who died from cancer had much higher factor VIII and von Willebrand factor levels than VT patients with cancer who survived.

Strengths of our study are that we studied coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) in four groups of participants: participants without venous thrombosis and without a cancer diagnosis, a cancer-only group, a venous thrombosis-only group and a group with both venous thrombosis and cancer. Furthermore, the plasma levels of a wide range of procoagulant and anticoagulant factors that are essential to the coagulation system were measured at the same time and with the same standardized assays for each factor. In addition, the levels were adjusted for age and sex.

Some limitations of this study have to be mentioned as well. First of all, blood was sampled at least three months after inclusion in the study. For this reason some of the MEGA-study participants who died after inclusion into the study but before the moment of blood collection are missing in our analyses. Also, participants with an advanced stage of disease and who were therefore unable to visit the hospital for blood sampling or participants who were not willing to visit the hospital for other reasons are not included in our analyses. Of 3227 cases in the MEGA study eligible for blood sampling, 851 (26%) did not provide a blood sample. For the partner controls this was 32% and for the RDD controls this was 51%. For abovementioned reasons, participants with cancer included in our analyses may have been less ill than those who did not participate, which can have diluted our results. Secondly, a drawback of this study is the relatively small sample size for some groups of participants, which did not allow us to study coagulation factor levels for different types of cancer. Furthermore, we missed some clinical details on the cancers diagnosed, such as stage of cancer and information on cancer treatment. However, a recent longitudinal study from Austria (n=112) in which hemostatic factors were measured in patients with various types of cancer at multiple time points showed that several coagulant factors were increased in patients with malignancy, at diagnosis, but also during the course of antineoplastic treatment with little difference in coagulation factor concentrations before and during antineoplastic treatment.[20]

Overall, we found increased levels of procoagulant coagulation factors in individuals with venous thrombosis without cancer and even higher levels of these factors in individuals with both venous thrombosis and cancer, suggesting a generalized role of procoagulant pathways in patients with cancer. These findings were most pronounced for levels of factor VIII and von Willebrand factor. Our finding of slightly increased levels of anticoagulant proteins, free protein S and TFPI in participants with cancer and venous thrombosis is suggestive of an additional role of anticoagulant pathways in cancer. For further studies it would be useful to study coagulation factor levels in relation to cancer and venous thrombosis for different types and stages of cancer and in patients with different cancer treatments in large enough numbers and sufficient follow-up.
Supplemental Data

Supplemental Methods

Prothrombin (factor II) activity, factor VII activity and factor VIII activity were measured with a mechanical clot detection method on a STA-R coagulation analyser following the instructions of the manufacturer (Diagnostica Stago, Asnieres, France). Levels of factor IX antigen, factor X antigen, factor VIII antigen, factor V antigen, factor XI antigen and total protein S levels were determined by enzyme-linked immunosorbent assay (ELISA). Fibrinogen activity was measured on the STA-R analyzer according to methods of Clauss. Von Willebrand factor (VWF) antigen was measured with the immunoturbidimetric method, using the STA Liatest kit (rabbit anti-hum VWF antibodies), following the instructions of the manufacturer. Measurement of antithrombin and protein C levels was performed with a chromogenic assay on the STA-R analyser. Free protein S was measured by an immune-turbidimetric method (Diagnostica Stago) accordingly to the manufacturer instructions. TFPI activity in plasma was measured by a chromogenic assay using the ACTICHROME TFPI activity assay (Sekisui Diagnostics, Stamford, Connecticut, USA) following the instructions of the manufacturer. TFPI activity was measured by inhibition of cleavage of a chromogenic substrate (spectrozyme Xa, Sekisui diagnostics) by factor Xa, after initiation of coagulation with an excess of factor X and Tissue Factor-Factor VIIa complex. D-dimer was assayed using the D-dimer HemosIL assay (Instrumentation Laboratory). The HemosIL D-Dimer HS is an automated latex enhanced immunoassay performed on the ACL TOP 700CTS (Instrumentation Laboratory, Warrington, UK).
Predictive value of factor VIII levels for recurrent venous thrombosis: Results from the MEGA follow-up study


*J Thromb Haemost 2015; 13(10):1823-1832*