Thrombosis and cancer : advances in diagnosis and prognosis

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THROMBOSIS AND CANCER: ADVANCES IN DIAGNOSIS AND PROGNOSIS

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Contents

1

Introduction

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The relationship between cancer and venous thrombosis has been recognised since a long time, and can be dated back to the early 19th century with the first observations by Bouillard (1823). Despite the considerable and ever growing amount of epidemiological and clinical data which have been accumulating since then, the pathophysiology of such association remains poorly understood. The malignant disease per se and its treatments produce an hypercoagulable state which might lead to thrombosis. [1] In fact, cancer patients have a higher risk of developing first and recurrent venous thrombotic events relative to non-cancer patients. [1-2] In addition, an unprovoked venous thromboembolism can be the presenting manifestation of an occult cancer. [1] A relatively new emerging hypothesis is that the association between thrombosis and cancer works bidirectionally, i.e. not only cancer favours the development of thrombosis, but also the coagulation system might promote tumour development and progression. In this setting, the use of anticoagulants could possibly influence the natural history of patients with cancer.

Diagnosis of Venous Thromboembolism in Cancer Patients

The diagnostic work-up of patients with clinically suspected venous thromboembolism has been significantly improved in recent years by the introduction of diagnostic algorithms which incorporate methods such as the D-dimer test and clinical probability scores. [3-4] These tools have the great advantage to limit the number of patients requiring further evaluation with costly and invasive imaging techniques. With a high negative predictive value, the D-dimer test represents an excellent screening tool in patients with suspected venous thromboembolism. When combined with a low or unlikely pre-test clinical probability of disease, a normal D-dimer result can safely exclude the diagnosis of venous thromboembolism. [3] The value of both the D-dimer test and clinical probability scores has been evaluated in large population based studies which

included a variable proportion of cancer patients. However, due to the relatively small size of the cancer subgroup, it has not been possible to separately assess the accuracy of the diagnostic algorithms in patients with malignancy. Indeed, patients with cancer have unique characteristics which might limit the usefulness of the D-dimer test or the clinical scores. D-dimer concentrations are generally increased in the presence of cancer and can be further raised by cancer treatments such as chemotherapy. On the other hand, concomitant clinical conditions such as oedema from lymphatic compression or dyspnoea from lung metastases may generate a clinical picture which could mimic venous thrombosis or pulmonary embolism.

Thus, the use and effectiveness of the D-dimer test and clinical probability scores in cancer patients deserves special attention in light of the high percentage of patients who might undergo unnecessary further testing or anticoagulant treatment with the associated risk of bleeding.

Impact of low-molecular-weight heparin on cancer prognosis

A few years ago, meta-analyses on anticoagulant treatment in patients with venous thromboembolism generated the intriguing hypothesis that anticoagulants, and in particular low-molecular-weight heparin, could improve cancer survival. [5-6] Since then, several experimental and clinical studies have provided data in support of this fascinating theory.

The study by Lee and colleagues, for instance, suggested that a 6-month course of dalteparin may improve, relative to vitamin-K antagonists, the 12-month survival of cancer patients without metastasis at the time of their venous thromboembolic event. [7] For the whole cancer population as for the subgroup of cancer patients with metastatic disease, the overall survival was comparable between the two study-groups.

Three other randomized studies have recently shown an effect of low-molecular-weight heparin on the survival of cancer patients, especially those with a

relatively good prognosis at inclusion. [8-10] In the FAMOUS trial, cancer patients without venous thromboembolism were randomized to 1-year prophylactic dalteparin or placebo. [8] As compared to placebo, the use of dalteparin was associated with a statistically significantly longer survival in the subgroup of patients with a better prognosis at baseline and who were alive 17 months after randomization. No survival difference was observed for the entire study cohort. In the MALT study, patients with advanced malignancy without venous thromboembolism were randomized to a 6-week course of nadroparin or placebo. [9] Median survival was prolonged in the group receiving nadroparin, with a major advantage in those with a life expectancy of at least 6 months at enrolment. An improvement in survival and disease progression by low-molecular-weight heparin was also seen in a clinical trial on small-cell lung carcinoma patients randomized to standard chemotherapy with or without prophylactic dalteparin. [10] The increase in median survival associated with low-molecular-weight heparin was again particularly marked in cancer patients with limited disease.

Thus, the existing evidence suggests an anticancer activity of low-molecular-weight heparin which may depend on the tumor burden, with greater benefits for the earlier cancer stages. However at present, the large heterogeneity across the studies does not allow the identification of a subgroup, if any exists, which may benefit more from low-molecular-weight heparin treatment. The diversity in the low-molecular-weight heparin schedules evaluated, the multiplicity of the definitions used for patient with better prognosis, and the variety of the types and stages of malignancies included, leave still perplexities over the possible use of low-molecular-weight heparin in this setting.

Central Theme of the Thesis

The central themes of this thesis are two fold. Firstly, we evaluated the usefulness of the commonly used diagnostic approaches for venous

thromboembolism in patients with as compared to those without cancer. Secondly, the role of low-molecular-weight heparin on cancer prognosis as well as the capability of circulating markers to predict outcomes and response to anticoagulant treatment were evaluated.

Outline of the Thesis

The diagnostic work-up for suspected venous thromboembolism in cancer patients follows the same criteria as for the general population. However, simply applying diagnostic algorithms to the subgroup with cancer might be improper due to clinical and laboratory differences between patients with and without malignancy.

In Chapter 2, entitled *"Diagnostic accuracy of D-dimer test for exclusion of venous thromboembolism: A systematic review"*, we performed a thorough systematic review and meta-analysis to obtain precise summary estimates of diagnostic accuracy of a large number of D-dimer test categories in the exclusion of deep venous thrombosis or pulmonary embolism, adjusting for known sources of bias and variability.

The predictive value of the D-dimer test in cancer patients referred for clinically suspected pulmonary embolism was evaluated in Chapter 3, *"D-dimer test in cancer patients with suspected acute pulmonary embolism*". This study retrospectively determined the predictive value of D-dimer test in patients referred for clinically suspected pulmonary embolism and compared the performance of the D-dimer test in patients with and without cancer. Subsequently, the accuracy of the D-dimer test was validated in a prospective cohort of patients with suspected pulmonary embolism.

In Chapter 4, *"Combined use of clinical pre-test probability and D-dimer test in cancer patients with clinically suspected deep venous thrombosis"*, we attempted to determine the value of the D-dimer test with and without the pre-test

clinical probability score in patients with cancer. The aim of this analysis was to assess the distribution of the various pre-test clinical probability categories in patients with cancer as well as the occurrence of deep venous thrombosis in each of these groups. Secondly, we evaluated the diagnostic accuracy of the D-dimer test in combination with a low, a moderate, and with a low-moderate pre-test clinical probability in cancer patients as compared to non-cancer patients.

Patients with an unprovoked venous thromboembolism might have occult cancer which could be detected by adequate screening tests. The value of such screening strategies is discussed in Chapter 5, *"Decision analysis for cancer screening in idiopathic venous thromboembolism"*. Available data from the SOMIT trial [11] were used to determine, for each of the evaluated screening strategies, the number of patients needed to screen to detect one additional case of cancer and the number of patients harmed. Finally, the costs of the various strategies were calculated and a cost-effectiveness analysis was performed.

The thesis continues with Chapter 6, *"Antithrombotic therapy and cancer"*, which introduces the association between thrombosis, cancer, and the possible impact of anticoagulants on cancer survival. The aim of this review was to assess the evidence from clinical studies investigating antithrombotic agents for the prophylaxis and treatment of venous thromboembolism in cancer patients and for the effects of these agents on cancer progression.

Chapter 7, *"Prevention of catheter-related venous thrombosis with nadroparin in patients receiving high dose chemotherapy for hematological malignancies, a randomized placebo controlled study"*, is a clinical study in which patients with haematological malignancies were randomised to low-molecular-weight heparin or placebo.

In Chapter 8, entitled *"The prognostic value of the D-dimer test in cancer patients treated with and without low-molecular-weight heparin"*, we evaluated the prognostic value of D-dimer plasma levels in patients with incurable cancer both at

study entry and after 6-week treatment with low-molecular-weight heparin. Moreover, the correlation between changes in D-dimer plasma levels and survival was analysed.

The aim of Chapter 9, *"Plasma cytokines and P-selectin levels in advanced malignancy: prognostic value and impact of low-molecular-weight heparin administration"*, was to evaluate whether the plasma levels of P-selectin, Interleukin-6, Interleukin-10, and Interferon-gamma predicted survival in patients with advanced stage cancer. Secondly, we assessed whether the levels of these markers responded to low-molecular-weight heparin treatment.

Chapter 10 *"The hematocrit target in Polycythemia vera"*, assessed the predictive value of haematocrit and platelet count for thrombotic and/or haematological complications in patients with polycythemia vera.

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2

Diagnostic accuracy of d-dimer test for exclusion of venous thrombo-embolism: a systematic review

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Abstract

Background: The reported diagnostic accuracy of D-dimer test for exclusion of deep-venous thrombosis (DVT) and pulmonary embolism (PE) varies. It is unknown to what extent this is due to differences in study design or patient groups, or to genuine differences between D-dimer assays.

Methods: Studies evaluating the diagnostic accuracy of D-dimer in the diagnosis of venous thromboembolism were systematically searched in MEDLINE and EMBASE databases up to March 2005. Reference lists of all included studies and of reviews related to the topic of the present meta-analysis were manually searched for other additional potentially eligible studies. Two reviewers independently extracted study characteristics using standardized forms.

Results: A total of 217 D-dimer test evaluations for DVT and 111 for PE could be analyzed. Several study design characteristics were associated with systematic differences in diagnostic accuracy. After adjustment for these features, the sensitivities of the ELFA D-dimer (DVT 96%; PE 97%), ELISA microplate (DVT 94%; PE 95%), and latex quantitative (DVT 93%; PE 95%) assays were superior to those of whole-blood D-dimer (DVT 83%; PE 87%), latex semiquantitative (DVT 85%; PE 88%) and latex qualitative (DVT 69%; PE 75%). Latex qualitative and whole-blood D-dimer assays had the highest specificities (DVT 99%, 71%; PE 99%, 69%).

Conclusions: Compared to other D-dimer assays, ELFA, microplate ELISA, and latex quantitative have a higher sensitivity but a lower specificity, resulting in a more confident exclusion of the disease at the expense of more additional imaging testing. These conclusions are based on the most up-to-date and extensive systematic review of the topic area including 184 articles, with 328 D-dimer test evaluations.

Introduction

A prompt recognition of venous thromboembolism is mandatory, but only about 25% of the suspected episodes are confirmed by objective testing. [1;2] To avoid unnecessary anticoagulant treatment and the associated risk of bleeding, it is crucial to accurately identify the 75% of patients with symptoms prompting a suspicion of venous thromboembolism who do not have the disease.[3]

With its high negative predictive value, the D-dimer test represents an excellent non-invasive triage test in patients with suspected venous thromboembolism. Combined with a low pre-test clinical probability of disease, a negative D-dimer result can safely exclude venous thromboembolism and limit the number of patients requiring further evaluation with imaging techniques.[2;4-7]

A large variety of D-dimer assays has been evaluated and their characteristics have been extensively described by others.[8] In general, enzyme-linked-fluorescent immunoassays (ELISAs) and microplate enzyme-linked immunosorbernt assay (ELFA) methods seem to dominate the comparative ranking among D-dimer assays for sensitivity, at the expense of only moderate specificity. Latex quantitative, latex semi-quantitative, and whole blood assays might represent valid alternatives for the exclusion of venous thromboembolism and remain widely used tests both among general practitioners and specialists.

Results on D-dimer diagnostic accuracy have been discordant, possibly because of the differences in the design and conduct of the studies.[9;10] Despite the extensive literature on the topic, which now also includes a number of systematic reviews, the dilemma of whether ELISAs and ELFA D-dimer tests outperform the others, or if two or more D-dimer methods can have a similar accuracy remains.[5;8;11-13] Previous reviews have summarized data using summary estimates of the respective diagnostic odds ratio [8;11;12], or detection rates [5], which may impede clinical interpretation. Only a subset of these reviews has incorporated the correlation between sensitivity and specificity.[11-13] In these

previous reviews, the inclusion of studies was restricted to a limited number of assays [11;12], and limited to studies that fulfilled a number of pre-specified design criteria. [8;11-13] Such a restriction to presumably optimal studies is but one method to handle design differences and may be precarious, as empirical evidence has shown that the biasing effect of design differences may vary in direction and size. [9;10]

The aim of this systematic review and meta-analysis was to obtain precise summary estimates of the sensitivity and specificity of a large number of D-dimer test categories in the exclusion of deep venous thrombosis or pulmonary embolism, adjusting for known sources of bias and variability without resorting to restrictive inclusion criteria.

Methods

Study Identification

A systematic search of the MEDLINE and EMBASE databases up to March 2005 was performed to identify studies reporting on the diagnostic accuracy of a D-dimer test in patients suspected for deep venous thrombosis of the lower extremities, or pulmonary embolism. The following search terms (MeSH and textwords) were used for the MEDLINE search: d dimer, fibrin fibrinogen degradation products, vein thrombosis, venous thrombosis, thrombosis, lung embolism, pulmonary embolism, thromboembolism, venous thromboembolism, sensitivity-and-specificity, predict$, diagnos$, di.fs, du.fs, accura$, and for the EMBASE database search: d-dimer, fibrin degradation product, fibrinogen degradation product, vein thrombosis, venous thrombosis, thrombosis, lung embolism, pulmonary embolism, thromboembolism, venous thromboembolism, sensitiv$, detect$, accura$, specific$, reliab$, positive diagnos$, negative diagnos$, di.fs.

Reference lists of all included studies and of reviews related to the topic of the present meta-analysis were manually searched for other additional potentially eligible studies.

Study Eligibility

Two investigators (MDN and AS) independently reviewed titles and abstracts from the initial search to determine whether the inclusion criteria were satisfied. According to pre-specified selection criteria, any article evaluating the diagnostic performance of D-dimer in the diagnosis of venous thromboembolism was eligible if it allowed the calculation of a 2x2 table for deep venous thrombosis and/or pulmonary embolism. Decisions regarding inclusion were made independently, results were compared, and any disagreement was solved through discussion. Where necessary, the authors were contacted for additional information. Case-reports and editorials were excluded. Articles were excluded if data could not be extracted to calculate a 2x2 table, or if the 2x2 table could not be calculated separately for deep venous thrombosis and pulmonary embolism in case both conditions were investigated in the same study. In addition, we excluded articles which included only very unstable cases, such as critically ill or post-trauma patients, since they would likely have introduced a major source of variability in the accuracy estimates of the tests, reducing applicability. No language restrictions were applied. Any disagreements about eligibility were solved by involving a third reviewer (PB).

Data Extraction

Two reviewers (MDN with AS or AR) independently extracted study characteristics using standardized forms that were accompanied by a background document. All assessors attended a training session to become familiar with the use of these forms. Study characteristics had been identified based on their potential for

bias and variability, as listed in the STARD statement, and in a recent systematic review. [9;14;15] The study design characteristics extracted and considered for the analysis are listed in Table 1.

Any disagreements on the extracted data were solved by consensus and, if necessary, by involving a third reviewer (PB). No attempts to mask for authorship, journal name or institution were made here or in any other step of the review process.

D-dimer Methods Evaluated

We classified D-dimer assays using seven categories: ELFA, microplate ELISA, membrane ELISA, Latex quantitative, Latex semi-quantitative, Latex qualitative, Whole-blood assays as described previously. [8] ELFA, ELISA, and latex quantitative are all quantitative methods, with reproducible results that are hardly prone to observer variability. Microplate ELISA takes several hours for the results whereas latex quantitative tests can generate results within 15 minutes. In addition, microplate ELISA is labor-intensive and has to be run in batches rather than on single samples. ELFA produces results within 35 minutes and has the advantage over microplate ELISA that it can be run on single samples. Membrane ELISA is also a rapid, but not quantitative method. latex qualitative, latex semi-quantitative and whole-blood D-dimer assays are rapid and easy to perform, but they are qualitative tests, observer-dependent, and limited in their ability to detect minimally increased D-dimer concentrations, which can result in lower sensitivities.

Statistical Analysis

We used a bivariate random effects regression approach to obtain summary estimates of both sensitivity and specificity of the respective D-dimer tests while adjusting for sources of bias and variability. This model assumes that the logit

Table 1a. Design Characteristics of the Included Studies

Item No.	Label of Design Characteristic, with Categories	No. of Studies
1	Study design	
	Cohort design	315
	Case–control design using healthy controls	1
	Case–control design using other controls or nested case control design	13
2	Data collection	
	Prospective	203
	Retrospective	34
	Timing data collection not reported	92
3	Sampling method*	
	Consecutive series	161
	Random sample	7
	Not consecutive nor random	119
	Sampling method not reported	42
4	Interpretation of index tests results	
	Blinded for reference standard results	142
	Index test not blinded or blinding not described	187
5	Interpretation of reference standard results	
	Blinded for index test results	165
	Reference standard not blinded or blinding not described	164
6	Availability of clinical information	
	Clinical information available while interpreting D–dimer test results	5
	Clinical information not available while interpreting D–dimer test results	34
	Availability of clinical information not described.	290
7	Availability of clinical information	
	Clinical information available while interpreting reference standard results	70
	Clinical information not available while interpreting reference standard results	10
	Availability of clinical information not described.	248

Table 1a–Continued

Item No.	Label of Design Characteristic, with Categories	No. of Studies
8	Type of reference standard*	
	For deep venous thrombosis:	
	Venography	70
	Compression ultrasonography, or phletysmography	47
	Diagnostic strategy, using follow–up in D–dimer negative results only	9
	Other diagnostic strategies	91
	For pulmonary embolism:	
	Pulmonary angiography	21
	Ventilation–perfusion scan or computed tomography scan	6
	Diagnostic strategy, using follow–up in D–dimer negative results only	8
	Other diagnostic strategies	76
9	Completeness of verification*	
	Complete	284
	Partial	44
	Completeness of verification not described	1
10	Time lag (between execution of D–dimer test and reference standard)*	
	Maximally 24 hours for pulmonary embolism and 48 hours for deep venous thrombosis	164
	Time lag too long	17
	Time lag not reported	148
11	Drop–outs	
	Drop out more than 10% reported	98
	Drop–out not described	231
12	Cut–off definition*	
	Standard or pre–defined	216
	Cut–off from ROC curves	46
	Cut–off selected for maximal sensitivity	21
	Not reported	45

* denotes variables that are selected for the multivariable approach

Table 1b. Patient Group Characteristics of the Included Studies

Item No.	Label of Patient Group Characteristic, with Categories	No. of Studies
1	Age*	
	Mean age below 60 years	130
	Mean age above 60 years	107
	Mean age not reported	92
2	Gender*	
	Patient group included predominantly females (>50%)	236
	Patient group included predominantly males	50
	Gender distribution not reported	42
3	Outpatients or Inpatients *	
	Outpatients	176
	Inpatients or mixture of in– and outpatients	109
	Type of patients not reported	44
4	Type of selection criteria*	
	Selection of the patients based on:	
	Clinical suspicion only	234
	Previous test	23
	Referral to reference standard or on reference standard results	72
5	Treatment	
	No treatment given in the time window between application of D–dimer test and reference standard	79
	Treatment received before both D–dimer test and reference standard were applied	34
	No information concerning treatment reported	212

* denotes variables that are selected for the multivariable approach

transformed sensitivities and specificities of the included D-dimer studies follow a bivariate normal distribution around a common mean of logit-transformed sensitivity and specificity, incorporating any correlation that might exist between logit sensitivity and logit specificity. [16] The number of patients testing positive

among the diseased in a particular study is assumed to follow a binomial distribution, as well as the number of patients testing negative among the non-diseased.

Bivariate Analysis with Covariates

To adjust for potential sources of bias and variation and the resulting heterogeneity in study results, 12 design and 5 patient group characteristics (Table 1) were analyzed in the bivariate random-effects model. We used a three-stage approach.

In the first stage, 17 bivariate models were used to evaluate the effect of the respective design characteristics on the estimates of sensitivity and specificity in deep venous thrombosis and pulmonary embolism. An effect was considered statistically significant if $p<0.1$.

In the second stage, a multivariable bivariate regression analysis was performed, adjusting for all study features identified in the first stage. This final model included indicators for type of venous thromboembolism (deep venous thrombosis or pulmonary embolism), type of D-dimer method, design and patient group characteristics.

In the third stage, the sensitivity and specificity of commercial D-dimer kits were calculated within each D-dimer method. Only those patient- and design characteristics that were significantly associated with the diagnostic accuracy in the second stage were incorporated in the per commercial kit analysis. To allow corrections for differences in patient- and design characteristics, only commercial D-dimer kits evaluated in at least 10 studies were included in this stage.

Stage 1 analyses were performed with the PROC NLMIXED module in SAS statistical software, version 9.1 (SAS Institute). The multivariable models were fitted with WINBUGS, version 1.4, using non-informative priors and posterior distributions obtained using Markov Chain Monte Carlo methods.

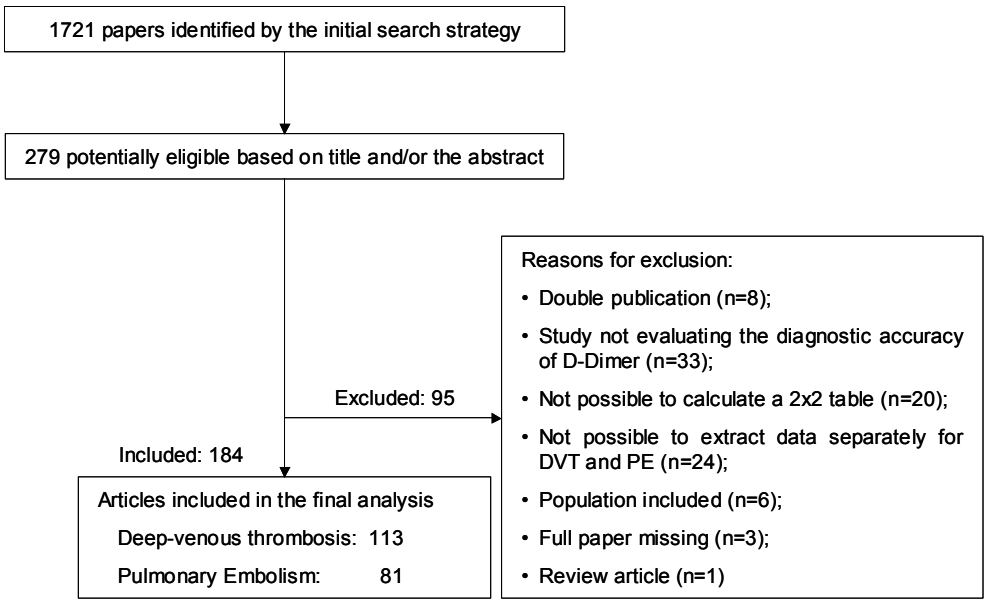
Parameter estimates are the medians of the posterior distributions. WINBUGS, or Bayesian statistics in general, do not provide p-values. Therefore, the range from the 2.5% to the 97.5% percentiles is used to quantify the uncertainty in the parameter estimates. This range can be loosely interpreted as a 95% confidence interval. If this interval excluded the null value, the corresponding effect is seen as statistically significant. Differences in D-dimer assays were evaluated similarly, evaluating medians and ranges of the posterior distributions for both sensitivity and specificity.

Results

Of 1721 papers identified with the initial search strategy, 279 were considered potentially eligible based on the title and/or abstract. Of these papers, 33 did not address test accuracy, in 24 it was not possible to extract data separately for deep venous thrombosis and pulmonary embolism, in 20 we were unable to make a 2 by 2 table, 8 were double publications, 6 included critically-ill or trauma patients only, 3 full length articles could not be retrieved, and one was a review paper. After excluding these 95 articles not meeting the pre-specified inclusion criteria, a total of 184 articles and letters, with 328 D-dimer test evaluations, was included in the final analysis (Figure 1, references available online).

We observed significant effects on sensitivity for the following design characteristics: outpatients or inpatients, age, gender, type of reference standard, and cut-off definition. A significant effect on specificity was found for outpatients or inpatients, age, type of selection criteria, sampling method, type of reference standard, completeness of verification, and time lag between the D-dimer and the reference test. One outlier study used a case-control design with healthy controls and reported very high estimates of specificity.[17] The results of this study were not used in the present analysis.

Figure 1. Process of selecting and assessing primary studies of the accuracy of D-dimer tests



Deep Venous Thrombosis

For 113 studies with 217 D-dimer test evaluations on deep venous thrombosis it was possible to calculate a 2x2 contingency table for the D-dimer test versus a reference standard. Design and patient group characteristics of the included studies test evaluations are given in Tables 1A and Table 1B. Table 2 describes the median prevalence, sensitivity and specificity across D-dimer methods. Some ELISA, latex and other assays could not be assigned to any of the D-dimer categories, due to incomplete descriptions. For completeness, descriptions of these groups of assays are given in Table 2 as well.

The prevalence of venous thrombosis in the included studies ranged widely, from 1% to 78% (Table 2). Reported sensitivity also varied, whereas

Table 2. Median Prevalence, Sensitivity and Specificity with Range for D–dimer Methods

Type of D–dimer	**Deep Venous Thrombosis**			
	Studies (n)	Median prevalence of DVT (range)	Median sensitivity (range)	Median specificity (range)
ELISA				
Microplate	35	42 (13-72)	95 (71-100)	47 (21-82)
Membrane	31	43 (1-75)	94 (50-100)	52 (12-94)
ELFA	23	35 (20-67)	97 (88-100)	42 (5-82)
LATEX				
Quantitative	45	39 (1-72)	96 (57-100)	48 (26-97)
Semiquantitative	22	40 (23-67)	84 (61-100)	63 (22-92)
Qualitative	2	54 (40-68)	82 (77-87)	100 (100-100)
Whole–blood assay	34	26 (3-72)	86 (53-100)	66 (20-94)
Undefined methods				
Elisa	9	40 (36-78)	95 (80-100)	48 (29-80)
Latex	14	46 (19-78)	78 (48-100)	81 (43-100)
Other	2	3 (2-5)	94 (88-100)	59 (46-72)

DVT: deep venous thrombosis; PE: pulmonary embolism; ELFA: enzyme-linked fluorescent immunoassay; ELISA: enzyme-linked immunosorbent assay

reported specificity even ranged from 5% to 100%. The multivariable analysis, incorporating indicator effects for study features, showed that several study characteristics were significantly related to the D-dimer test performance. Studies

that used single or serial compression ultrasound or phletysmography as the sole reference standard (45, 1, and 1 test evaluations, respectively) had significantly lower sensitivities, in comparison to studies that used a combination of these tests within a diagnostic strategy. The use of follow-up to verify D-dimer test negative results led to a small increase of sensitivity and decrease of specificity, but these effects were not significant. Studies that used venography as the sole reference standard had considerably higher specificities than studies that used a combination of tests within a diagnostic strategy. The inclusion of inpatients, exclusively or combined with outpatients, was associated with lower specificities, as compared to studies including outpatients only.

Studies that based patient selection on previous test results had considerably higher specificities in comparison to studies that selected patients on clinical suspicion only.

Corrected for the 9 study design characteristics identified in the previous stage, we obtained corrected estimates of sensitivity and specificity. The ranking of the D-dimer methods on sensitivity was not affected by these corrections. The sensitivity of ELFA (96%), ELISA microplate (94%), and latex quantitative (93%) were significantly higher than for the other D-dimer tests (Table 3).

Membrane ELISA had a lower sensitivity (89%) than latex quantitative, but this difference was not significant.

Specificity of latex qualitative was superior to that of other methods, but also the specificities of whole-blood D-dimer tests and latex semi-quantitative were higher than those of latex quantitative, ELISA methods and ELFA. Figure 2 visualizes accuracy indexes of the D-dimer methods in the ROC space, illustrating the negative association between sensitivity and specificity in patients with suspected deep venous thrombosis.

Table 4 shows the sensitivity and specificity of commercial D-dimer kits for which at least 10 studies were available. After adjusting for differences in

Table 3. Summary Estimates of Sensitivity and Specificity of D–dimer Methods

Type of D–dimer	Deep Venous Thrombosis		Pulmonary Embolism	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
ELISA				
Microplate	94 (86-97)	53 (38-68)	95 (84-99)	50 (29-71)
Membrane	89 (76-95)	53 (37-68)	91 (73-98)	50 (29-72)
ELFA	96 (89-98)	46 (31-61)	97 (88-99)	43 (23-65)
LATEX				
quantitative	93 (89-95)	53 (46-61)	95 (88-98)	50 (36-64)
semiquantitative	85 (68-93)	68 (53-81)	88 (66-97)	66 (43-83)
qualitative	69 (27-93)	99 (94-100)	75 (25-96)	99 (92-100)
Whole–blood assay	83 (67-93)	71 (57-82)	87 (64-96)	69 (48-84)

Estimates derived from the bivariate multivariable model adjusting for differences in study design. CI: confidence Intervals; ELFA: enzyme-linked fluorescent immunoassay; ELISA: enzyme-linked immunosorbent assay

patient and design characteristics, the sensitivity and specificity of the Asserachrome, Tinaquant, STA-liatest, were not significantly different from VIDAS. The sensitivity of Instantia, Nycocard, and SimpliRED were significantly lower than VIDAS. The specificity of SimpliRED and Instantia were higher than VIDAS. Average age above 60 years was associated with a higher sensitivity and reduced specificity. The inclusion of inpatients alone or together with outpatients correlated to a decrease in specificity.

Pulmonary Embolism

Eighty-one studies with 111 D-dimer test evaluations in pulmonary embolism were included. Sensitivity and specificity of D-dimer tests varied largely across studies (Table 2). The prevalence of venous thromboembolism in the included studies ranged widely, from 3% to 69%, with similarly wide ranges for sensitivity (from 40% to 100%), and even wider ranges for specificity (from 7% to 100%).

Table 4. Summary Estimates of Sensitivity and Specificity for D-Dimer kits

Type of D–dimer (studies, n)	**Deep Venous Thrombosis**		**Pulmonary Embolism**	
	Median sensitivity (range)	Median specificity (range)	Median sensitivity (range)	Median specificity (range)
Microplate ELISA:				
Asserachrome (24)	94 (83-98)	47 (29-65)	96 (80-99)	44 (21-69)
Membrane ELISA:				
Instantia (13)	86 (59-96)	65 (43-81)	89 (54-98)	62 (33-84)
Nycocard (23)	88 (68-96)	50 (31-68)	91 (64-98)	47 (23-72)
Latex quantitative:				
Tinaquant (12)	92 (75-98)	53 (32-73)	94 (71-99)	50 (23-76)
STA-lia test (25)	94 (83-98)	46 (28-64)	96 (80-99)	43 (20-68)
ELFA:				
VIDAS (40)	96 (93-98)	44 (36-52)	97 (91-99)	41 (26-57)
Whole-blood assay:				
SimpliRed (40)	82 (59-93)	72 (56-84)	86 (43-97)	70 (44-87)

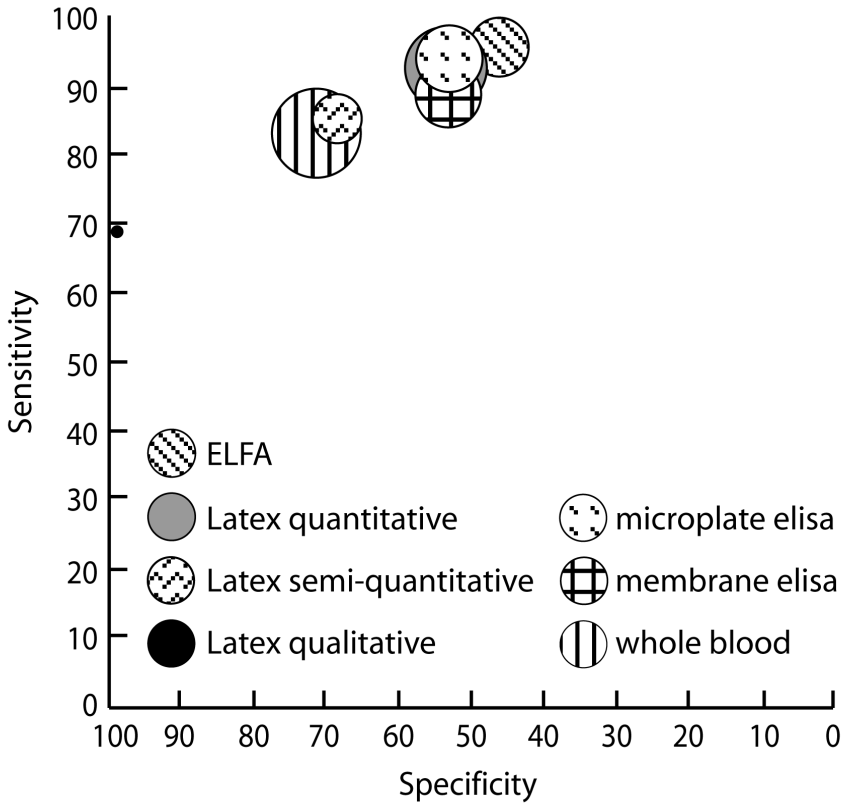
ELFA: enzyme-linked fluorescent immunoassay; ELISA: enzyme-linked immunosorbent assay

As for deep venous thrombosis, the multivariable analysis showed that differences in design were associated with differences in the estimated sensitivity and specificity. Significantly higher sensitivities but lower specificities were observed in studies that included more elderly patients (mean age above 60 years) while significantly lower estimates of specificity were found in studies using pulmonary angiography as the sole reference standard. The inclusion of inpatients, exclusively or with outpatients, was also associated with lower specificities compared to studies including outpatients only. Studies that based patient selection on previous test results had higher specificities in comparison to studies that selected patients on clinical suspicion only.

After adjusting for study design features, ELFA, microplate ELISA, and latex quantitative had a sensitivity that was significantly higher (97%, 95% and 95%, respectively) than the other D-dimer methods, although the absolute difference between latex quantitative and membrane ELISA was not statistically significant (Table 3). As in deep venous thrombosis, the specificities of latex qualitative (99%), whole-blood (69%), and latex semi-quantitative (66%) tests were statistically superior to those of the other D-dimer assays. The relation between sensitivity and specificity for the various D-dimer tests in pulmonary embolism is represented in Figure 3.

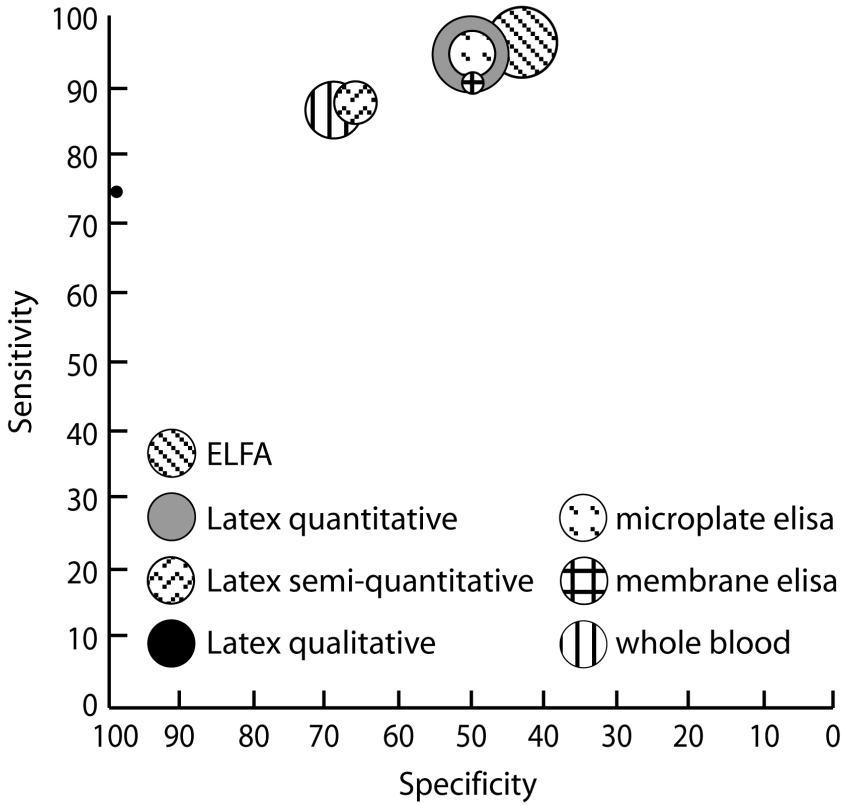
Similar results as for deep venous thrombosis were observed in the per kit analysis (Table 4).

Figure 2. Adjusted Summary Estimates in ROC–Space for Deep Venous Thrombosis*



*Summary estimates derived from the bivariate multivariable model adjusting for differences in study design; circles denote D–dimer assays, proportional to number of patients included

Figure 3: Adjusted Summary Estimates in ROC–Space for Pulmonary Embolism*



*Summary estimates derived from the bivariate multivariable model adjusting for differences in study design; circles denote D–dimer assays, proportional to number of patients included

Discussion

D-dimer is increasingly used in the work-up of patients with suspected venous thromboembolism, both by general practitioners and by specialists. In this study, we have obtained summary estimates of sensitivity and specificity of seven different D-dimer methods after an extensive and comprehensive systematic search of the literature. We found systematic differences between studies on the same test due to differences in study design and patients included. Adjusting for these differences, we found a trade-off between sensitivity and specificity between the various methods, without obvious evidence of any method being inferior to others on both sensitivity and specificity. ELFA, microplate ELISA, and latex quantitative were found to have a comparably high sensitivity but a lower specificity than whole-blood D-dimer, latex semiquantitative and latex qualitative. We feel this is an important result, correcting some misunderstandings about the different D-dimer methods that have appeared in the literature. We base this result on the findings of 184 articles and letters, with 328 D-dimer test evaluations in the final analysis, which also makes this the largest diagnostic accuracy systematic review ever made.

Despite the overall large number of studies included, results on latex qualitative tests have to be taken with caution due to extremely limited amount of evidence available. Our analysis was not designed to evaluate whether kits within each of the respective D-dimer test categories have an identical diagnostic accuracy. While the general principles for the development and execution of the test may be similar within each category, differences in the reagents and conditions could change the final test performance.

Some spectrum effects, as for example the value of the D-dimer in cancer patients, could not be evaluated given the small number of subgroup analyses for specific subgroups of patients. Recent studies have suggested that the sensitivity and negative predictive value of D-dimer may be lower in cancer patients [18],

while others have advocated that the negative predictive value is comparable to non cancer patients. [19;20]

Our summary estimates for microplate ELISA, ELFA and latex quantitative are in line with those reported by Brown and colleagues [11;12], but diverge from those of a previous review by Stein and colleagues, which claimed the superiority of the ELISA and ELFA assays over other D-dimer tests, including latex quantitative assays. [13] Although our summary estimates for the ELISA microplate and ELFA closely resemble those of Stein, the estimates for latex quantitative are quite different. For the latter method, Stein and colleagues reported a sensitivity and specificity of 85% and 66% for deep venous thrombosis, and 89% and 45% for pulmonary embolism, respectively. In our meta-analysis the estimates for latex quantitative are, on the contrary, close to those of microplate ELISA and ELFA.

Several points may help to explain this discrepancy between this and the previous review. The number of studies included in the meta-analysis of Stein et al. was far lower than the total considered in the current analysis. This difference may be partly due to new studies, published after that review was completed, but also to the selection criteria. Stein and colleagues used rather strict inclusion criteria, excluding, among others, all studies with differential verification. We did not exclude these studies, in which the method of verification depended on the D-dimer test result, but accommodated any resulting differences in the estimated accuracy in our statistical analysis. Stein and colleagues also used a multivariable regression model but their the approach to handling differences in design and patient groups differed. Stein initially pooled high-quality studies only, using 3 quality indicators to explain remaining variation in results. We used a larger number of indicators to adjust for design and patient group variability in our multivariable regression model. We feel these differences illustrate the importance

of adjusting for multiple design characteristics in assessing the overall accuracy of diagnostic methods.

The analysis on commercial D-dimer kits seemed to support the concept of comparable accuracy of latex quantitative methods, ELFA and ELISA methods. SimpliRED and Instantia D-dimer tests showed lower sensitivity, but higher specificity than VIDAS. The findings of the per kit analysis should however be interpreted with caution as for some of the kits only a relatively small number of studies was available.

The best design to compare the performance of the D-dimer methods would be a full comparative design, where included patients either receive all D-dimer methods or are randomly allocated to one of them, whereupon all test results are verified. In our study we included a number of comparative studies, but none of them studied all available methods.

In general, systematic reviews of diagnostic accuracy studies are challenged by the variability in design characteristics of the primary studies and by the poor quality of reporting. Our results confirm the finding of previous evaluations which showed that the type of reference standard [8] and age [11] significantly affect the estimated accuracy. It is not easy to denote which reference standard or strategy classifies venous thromboembolism more correctly, but such differences should be taken into account when comparing or pooling study results. Heim and colleagues reported a non-significant 40% lower diagnostic odds ratio in studies using a mixture of in- and outpatients compared to studies that used only outpatients. [8] We found that studies with a mixture of in- and outpatients had a significantly lower specificity but this did not affect sensitivity. It is generally acknowledged that the use of the D-dimer test is more reliable in outpatients, since raised D-dimer concentrations can also be observed in other disease states such as myocardial infarction, pneumonia, or cancer. [21] We also found that selection based on previous test results (pre-test clinical probability and negative ultrasound)

significantly increased specificity. Other reviews have excluded these studies. [11-13]

Our summary estimates can be used to calculate negative and positive likelihood ratios that could be used with Bayes' theorem to calculate post-test probabilities from pre-test probabilities. [22] Negative likelihood ratios lower than 0.1 and positive likelihood ratios larger than 10 generate large and often conclusive changes from pre- to post-test probabilities.[22] Only ELFA, ELISA microplate, and latex quantitative had low negative likelihood ratios for deep venous thrombosis (0.09, 0.11, and 0.13, respectively) and pulmonary embolism (0.07, 0.10, and 0.10, respectively). Latex qualitative was the only method with a positive likelihood ratio greater than 3.

As the exclusion of venous thromboembolism is the main goal of the D-dimer test, a high sensitivity of the assay is required. Yet the specificity of the test directs the number of further imaging procedures required. Our analysis showed the typical inverse relation between sensitivity and specificity: D-dimer methods with a high true positive fraction also have a higher false positive fraction. As a consequence, a larger number of patients with a positive result will be referred to additional imaging tests if D-dimer methods with high sensitivity, such as the ELFA D-dimer, are used. This problem can be partially circumvented by incorporating D-dimer assays with a higher specificity but lower sensitivity in diagnostic algorithms in which they are used in combination with a probability score. [23-25] Only patients with a low or low to moderate pre-clinical probability and a negative D-dimer receive no additional imaging.

In summary, the sensitivities of ELFA, ELISA microplate and latex quantitative were found to be comparable and higher than those of the other D-dimer assays, although their specificities are lower. Before recommending the ELFA, the ELISA microplate, or the latex quantitative D-dimer assays as the best available options, more large direct comparisons of these assays are warranted

within diagnostic algorithms in which the D-dimer test is combined with the pre-test clinical probability score. In addition, the cost-effectiveness of these approaches should be weighted against venous thromboembolism diagnostic work-ups using D-dimer assays with a higher specificity.

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3

D-dimer test in cancer patients with suspected acute pulmonary embolism

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Abstract

Background: The safety of a D-dimer (DD) measurement in cancer patients with clinically suspected pulmonary embolism (PE) is unclear.

Objectives: The aim of this study was to assess the accuracy of the DD test in consecutive patients with clinically suspected PE with and without cancer.

Methods: The diagnostic accuracy of DD (Tinaquant D-dimer) was first retrospectively assessed in an unselected group of patients referred for suspected PE (n=350). Subsequently, the predictive value of the DD was validated in a group of consecutive inpatients and outpatients with clinically suspected PE prospectively enrolled in a management study (n=519). The results of the DD test in cancer patients were assessed according to the final diagnosis of PE and the 3-month clinical follow-up.

Results: In the first study group, DD showed a sensitivity and a negative predictive value (NPV) of 100% and 100% in patients with cancer and 97% and 98% in those without malignancy, respectively. In the validation cohort, the sensitivity and NPV of DD were both 100% (95% CI 82%–100% and 72%–100%, respectively), whereas in patients without malignancy, the corresponding estimates were 93% (95% CI 87%–98%) and 97% (95% CI, 95%–99%), respectively. The specificity of DD was low in patients with (21%) and without cancer (53%).

Conclusions: A negative DD result safely excludes the diagnosis of PE in patients with cancer. Because of the low specificity, when testing 100 patients with suspected PE, a normal DD concentration safely excludes PE in 15 patients with cancer and in 43 patients without cancer.

Introduction

Pulmonary embolism (PE) might be the first manifestation of an underlying occult malignancy or represent a complication of a known malignancy [1]. As the majority of preventable deaths associated with PE can be ascribed to a missed diagnosis and anticoagulation is associated with a risk of bleeding, it is crucial to exclude or confirm the diagnosis of PE to avoid unnecessary anticoagulation or promptly start such treatment if appropriate [2,3].

Only 25% of the patients suspected for PE have a diagnosis confirmed by objective testing [4]. For this reason, several non-invasive diagnostic tests, such as D-dimer (DD), have been developed to limit the number of patients requiring an invasive and costly test [5]. The use of DD aims at safely excluding rather than confirming the presence of PE as elevated DD concentrations are not specific for PE and are observed in many other circumstances, including advanced age, pregnancy, trauma, inflammatory states, and cancer [6–8]. As a consequence, false-positive results are common in hospital inpatients, particularly in patients with infections and cancer. Although the DD test has been investigated in various algorithms to exclude PE [5], the safety and diagnostic accuracy of DD in cancer patients has not been established. Both cancer and its treatments can reduce the accuracy because of more frequent abnormal results than in patients without cancer [9]. A safe exclusion of PE in patients with overt malignancy is extremely important, as in these patients PE is associated with a high mortality and anticoagulant therapy greatly increases the risk of major bleeding [10].

Recently, two studies investigated the diagnostic accuracy of the DD test in cancer patients with clinically suspected deep venous thrombosis (DVT) reaching divergent conclusions on the predictive value and clinical utility of DD in this setting [11,12].

The aim of our study was first to retrospectively determine the predictive value of DD in patients referred for clinically suspected PE and compare the

performance of the DD test in patients with and those without cancer. Subsequently, the accuracy of the DD test was validated in a prospective cohort of patients with suspected PE.

Methods

Patients

The initial group consisted of an unselected sample of inpatients and outpatients referred to the thrombosis unit for clinically suspected acute PE. Only data from patients whose initial DD test results and final PE diagnosis were recorded in the initial database were included. Patients whose cancer status was not confirmed on chart review were excluded from the analysis. The diagnosis of PE was excluded in case of: (i) normal spiral computed tomography (CT) scan and normal ultrasonography, (ii) alternative diagnosis made by spiral CT, (iii) normal pulmonary angiography, or (iv) normal ventilation–perfusion (V/Q) lung scan. In addition to these tests, another requirement was that no episode of venous thromboembolism (VTE) during a 3-month clinical follow-up had occurred.

To validate the results, the predictive value of DD was further evaluated in a cohort of consecutive inpatients and outpatients evaluated at the thrombosis units of three teaching hospitals in The Netherlands for clinically suspected acute PE [13]. Exclusion criteria were any objective testing for a clinically suspected episode of VTE in the previous 7 days, age <18 years, pregnancy, treatment with vitamin K antagonists or therapeutic doses of heparin for >24 h before inclusion, indication for thrombolysis, follow-up not possible or if written informed consent could not be obtained. Cancer status was recorded at presentation. Patients were considered to have active cancer if they were receiving treatment for cancer or if they had received treatment for cancer in the past 6 months. Patients in whom the diagnosis

of cancer was made after study enrolment were not considered to have active cancer at presentation.

The cut-off value for the DD test (Tinaquant D-dimer; Roche Diagnostica, Mannheim, Germany) was 0.5 μg mL^{-1}, with DD values below or equal considered normal and value above the cut-off abnormal.

In the validation group, the diagnosis of PE was excluded if: (i) clinical probability estimate <20% combined with a normal DD, (ii) normal perfusion scintigraphy, (iii) non high probability V/Q scan in combination with a normal result on serial leg ultrasonography on days 1, 3 or 4 and 7 or normal pulmonary angiography [13]. All patients were followed up for 3 months for possible subsequent thromboembolic events with objective testing performed in all suspected cases. All deaths were classified by the adjudication committee using clinical reports of treating and/or family physicians and, if available, autopsy reports. Death was attributed to PE (i.e. confirmed by objective testing as well as in those cases in which PE could not be ruled out as the possible contributing factor), cardiovascular disease, malignancy, or other causes.

Statistical analysis

To compare the performance of the DD assay in patients with cancer and those without cancer, the sensitivity, specificity, the positive and negative predictive values, and the negative likelihood ratio were determined separately in the two patient groups. The number of patients to be tested with DD to exclude a diagnosis of PE was also determined. The 95% confidence interval for the negative likelihood ratio was calculated using the profile maximum-likelihood method.

Results

In the first study population, the diagnosis of PE was confirmed in 85 (24%) of the 350 inpatients and outpatients with an initial suspicion of PE. A diagnosis of active cancer was made in 35 patients and all the 12 PE cases had an abnormal DD result, while the test gave 21 false-positive results in the 23 cancer patients for whom PE was excluded. The sensitivity and the NPV among cancer patients were therefore 100% [95% confidence interval (CI) 74%–100%) and 100% (95% CI 16%–100%), respectively], whereas the specificity and positive predictive value (PPV) were lower (9%; 95% CI 0%–20% and 36%; 95% CI 20%–53%, respectively). In the group of patients without cancer, the sensitivity, specificity, NPV, and PPV of the DD were 97% (95% CI 94%–100%), 44% (95% CI 38%–50%), 98% (95% CI 96%–100%), and 34% (95% CI 28%–41%), respectively.

The validation cohort

From the original study group of 631 patients, a DD test was performed at presentation in 519 patients (82%) who represent the validation set [13]. A diagnosis of PE was confirmed in 102 patients (20%). The baseline characteristics and the distribution of the PE risk factors in patients with and without cancer are presented in Table 1. A total of 72 patients were diagnosed with cancer including tumors of the lungs and respiratory tract (10), gastrointestinal system (19), breast cancer (8), urinary and reproductive systems (18), or other tumor types (17). When compared with patients without cancer, those with active cancer were older, more frequently males, more likely to have undergone surgery in the previous 3 months, and they used more often hormone therapy, but less oral contraceptives. A positive family history of VTE was more common among patients without cancer. The median DD level in patients with and without cancer was 1.70 μg mL^{-1} (0.12 to 36.6 μg mL^{-1}) and 0.63 μg mL^{-1} (0.01 to 95.0 μg mL^{-1}), respectively ($P < 0.001$).

Table 1. Baseline characteristics of patients with and without cancer in the validation cohort

Characteristic	**Patients with cancer (N=72)**	**Patients without cancer (N=447)**
Age (years, median)	60	48**
Males (%)	56	40*
Outpatients, n (%)	33 (46)	317 (71)
History of Arterial disease[§] (%)	6.9%	13.9%
Concomitant Symptoms of DVT (%)	15.3	11.7
Use of oral contraceptives (%)	1.4	13.5**
Hormone therapy (%)	5.6	1.6**
History of VTE (%)	8.3	11.7
Surgery in the past 3 months (%)	30.6	15.5**
Trauma past 3 months (%)	-	3.6
Family history of VTE (%)	2.8	9.0*
Patients with PE, n (%)	19 (26)	83 (19)
D-dimer, μg/mL (range)	1.70 (0.12-36.6)	0.63 (0.01-95.0)**

*p<0.05 **p<0.01 [§]Cardiovascular disease, cerebral vascular disease, peripheral arterial disease. DVT=deep venous thrombosis; PE=pulmonary embolism; VTE=venous thromboembolism

Table 2. Diagnostic accuracy of DD test in patients with and without cancer

	Sensitivity (%)	**Specificity (%)**	**Negative Predictive Value (%)**	**Positive Predictive value (%)**	**Negative Likelihood Ratios**
Patients with cancer	100 (82-100)	21 (10-32)	100 (72-100)	31 (20-43)	0 (0-0.5)
Patients without cancer	93 (87-98)	53 (48-58)	97 (95-99)	31 (25-37)	0.14 (0.06-1.34)

Data in parentheses are the 95% confidence interval.

Of the 72 patients with active cancer, PE was diagnosed in 19 patients (26%) and excluded in 53. There were no false-negative DD results among cancer patients with PE, whereas the test was negative in only 11 of the 53 patients without PE. The sensitivity, the NPV, and the negative likelihood ratio were 100% (95% CI 82%–100%), 100% (95% CI 72%–100%), and 0 (95% CI 0–0.5), respectively, whereas the specificity and PPV were 21% (95% CI, 10%–32% and 31% (95% CI, 20%– 43%), respectively (Table 2).

Of the 447 patients without malignancy, 83 (19%) had a diagnosis of PE and 77 of these patients had an abnormal DD result. Among the remaining 364 patients in this group, DD result was normal in 193. The sensitivity (93%; 95% CI 87%–98%) and NPV (97%; 95% CI, 95%–99%]) were lower than in the group with cancer, but comparable with the values reported in the literature [6] (Table 2).

In cancer patients with suspected PE, six patients have to be tested with DD to find one true negative result whereas in patients without cancer and with suspected PE one in every two patients will have a true negative DD result. In other words, when 100 patients are tested with DD, a normal DD result can safely rule out the presence of PE in 15 patients with active cancer and in 43 patients without cancer. During the 3-month follow-up, there were six (8.3%) and nine (2.0%) deaths among patients with and without cancer, respectively. Among the six cases in the cancer group, the diagnosis of PE was excluded in five patients and confirmed in one by the initial diagnostic work-up. The DD was abnormal in all these six patients. In the group of patients without malignancy, PE was excluded in six (four abnormal and two normal DD results) and confirmed in three (all abnormal DD results).

Discussion

While plasma DD measurement is increasingly accepted as a first-line test in patients with clinically suspected PE, the accuracy of this test in cancer patients is limited. The results of the present study suggest that a negative DD is useful in the diagnostic work-up for the exclusion of PE in this high-risk group of patients given a NPV of 100% (95% CI, 72%–100%) and a sensitivity of 100% (95% CI, 82%–100%). The specificity and PPV of DD were low both in cancer and non cancer patients. The clinical utility of the DD to confirm PE is limited because of the non-specificity of a positive DD result.

Patients with cancer who develop VTE have a reduced life expectancy and the mortality risk after an acute PE is four-to eightfold higher when compared with patients without cancer [1,14]. This might be due to a more aggressive course of malignancies associated with VTE [14,15]. With anticoagulant treatment, the rates of recurrent PE and death can be decreased from 26% to 2–9% over 3–6 months [2]. The implementation of non-invasive tools such as the DD test could help to avoid invasive and costly examinations in the diagnostic work-up of suspected PE.

Recently, two studies evaluated the diagnostic accuracy of DD in cancer patients with suspected DVT [11,12] with conflicting results regarding the predictive value of DD in this context. In the first study, the value of the SimpliRED DD assay was retrospectively assessed in 1068 consecutive outpatients with suspected DVT included in three prospective studies [11]. When compared with patients without cancer, the NPV of the DD test was significantly lower in cancer patients (78.9% vs. 96.5%, p=0.008) and the authors concluded that a normal DD result could not safely exclude the diagnosis of DVT in patients with concomitant malignancy. In the second study, 1739 consecutive outpatients with suspected DVT were evaluated with a diagnostic strategy including the SimpliRED DD test and compression ultrasonography [12]. The NPV of the DD test was found to be 97% in both cancer and non-cancer patients. Moreover, the combination of a

normal DD test and ultrasonogram results at referral could safely exclude the diagnosis of DVT and safely withhold anticoagulant therapy in patients with malignancy [12]. The discrepancy in the findings of these two studies might be partially explained by the different reference tests used, differences in the populations included as well as in the design characteristics of the studies. The evidence on the role of DD for the diagnosis of DVT in cancer patients remains scarce and unclear.

To our knowledge, this is the first study that investigated the use of DD for the diagnosis of PE in patients with malignancy. Given the morbidity and mortality associated with PE, a test with a high NPV and a low number of false-negative results is mandatory. The 100% NPV of DD in the present study, suggests that this assay can be used to safely exclude the presence of PE in cancer patients, although these results need to be confirmed in a larger sample. Moreover, the validation group represented the 82% of the original study population for whom a DD test result was available. Almost all of the patients (109/112) in whom a DD result was not obtained had a clinical probability of >20% and the attending physician decided not to perform the DD test in these cases as the DD result would have not influenced the management decisions. The exclusion of part of the patients with a high clinical probability could have resulted in selection bias, as inclusion of only patients with a low to moderate clinical probability may lead to an overestimation of the NPV of the DD. In clinical practice, however, the most often used algorithm in the diagnostic workup to exclude PE involves the combination of a normal DD test in combination with a low or low–moderate clinical probability score. Our results support the usefulness of a normal DD result only in this group of patients. The safety of excluding PE in cancer patients with a high clinical probability has still to be established.

When screening 100 patients with clinically suspected PE, the number of patients in whom PE could be excluded based on a negative DD test result was 15

in the presence of cancer when compared with 43 patients without a malignancy. This difference was due to the lower specificity of the assay in cancer patients (21%) in comparison with non-cancer patients (53%) which led to the higher number of false-positive DD results in the presence of cancer.

In conclusion, a negative DD result safely excludes the diagnosis of PE in patients with cancer. Whether the combination of DD with other imaging techniques, such as the CT scan or serial leg ultrasonography, might improve the diagnostic work-up warrants further investigation.

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4

Combined use of clinical pretest probability and D-dimer test in cancer patients with clinically suspected deep venous thrombosis

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Abstract

Background: The value of the D-dimer (DD) test in combination with the clinical pre-test probability (PTP) has not been evaluated in cancer patients with suspected deep vein thrombosis (DVT), whereas this group of patients usually accounts for 10–25% of clinically suspected DVT.

Methods: A cohort of 2066 consecutive patients with clinically suspected DVT was investigated. Patients were judged to be positive or negative for DVT according to the outcomes of serial compression ultrasound and a 3-month follow-up period with imaging test verification of the symptomatic cases. Diagnostic accuracy indices of the DD test according to the PTP score were assessed in patients with and without cancer.

Results: Of the cohort, 244 (11%) were known to have cancer at presentation. A venous thromboembolic event was diagnosed in 41% of the patients with cancer and in 22% of the patients without malignancy. Among the cancer patients, 17% were considered to have a low PTP, 35% a moderate and 41% a high PTP. The negative predictive value (NPV) of the DD test was 100% (95%CI, 85–100) and 97% (95% CI, 88–99) among cancer patients with low PTP or low-moderate PTP. In the absence of malignancy, the corresponding NPV were 98% and 97%, respectively. The specificity of the DD test progressively decreased moving from the low to the higher PTP.

Conclusions: In cancer patients with clinically suspected DVT, a negative DD might be useful in excluding the diagnosis within the low or low-moderate PTP groups. More studies are warranted to confirm these findings.

Introduction

Deep venous thrombosis (DVT) often complicates the clinical course of patients with malignancy [1] and a fourfold risk increase of DVT in patients with cancer compared with non cancer patients has been reported [1,2]. Furthermore, the failure of anticoagulant therapy occurs more frequently in cancer patients, resulting in an increased risk of recurrent thrombotic events and bleeding compared with patients without cancer [3–7]. While a prompt recognition of DVT is mandatory, only approximately 30% of the suspected DVT episodes is subsequently confirmed [8]. Thus, to avoid unnecessary anticoagulant treatment, it is crucial to identify the 70% of the patients with DVT symptoms who do not have the disease. Currently, the 'gold standards' for the diagnosis of DVT of the legs are compression ultrasound (CUS), which if negative at baseline is repeated after 1 week (serial CUS); or ascending contrast venography. However, the routine use of venography is limited by the associated invasiveness, the complication rate, the costs, and the high inter-observer variability, while serial CUS has the disadvantage to be expensive and time-consuming.

Given the shortcomings of the currently available reference standards for DVT, several non-invasive diagnostic tests, such as the D-dimer (DD) test, have been developed to limit the number of patients requiring (further) imaging testing [8,9]. With a high negative predictive value (NPV), the DD represents an excellent screening test to exclude the presence of DVT [9]. In cancer patients; however, both the cancer and its treatments may reduce the DD accuracy because of the more frequently elevated DD concentrations than in patients without malignancy [10]. In this respect, the diagnostic accuracy of the DD test in cancer patients with clinically suspected DVT remains unclear with limited data from small subgroup analyses and two retrospective studies which reached different conclusions regarding the clinical utility of DD in this setting [11–17].

The clinical pre-test probability (PTP) is another useful triage tool to select patients who have to be further evaluated for DVT by imaging tests. The PTP score developed by Wells et al. [18] uses explicit medical history and physical examination criteria to stratify patients into low, moderate, and high risk of DVT. While cancer patients can present with the classical DVT symptoms of leg pain and swelling, the clinical diagnosis alone could misclassify over half of these patients as many of the clinical findings can derive from other causes than an underlying DVT such as for instance a lymphatic obstruction by the tumor, an external tumor compression of the veins, or a concomitant superficial or deep venous infection. In the Wells' clinical model, the presence of malignancy with an ongoing (palliative) treatment, or a treatment stopped within the last 6 months is included with a score of one point. As a result, many patients with cancer would be classified in the highest PTP categories according to this model, and additional image testing would be still necessary for these patients. While there would be still room to lower this score in case of an alternative diagnosis, it has to be acknowledged that the Wells' model was not developed specifically for cancer patients and the evidence to apply this tool to this population is limited. Recently, Wells et al. [19] have proposed a dichotomization of the Wells' score into 'likely' and 'unlikely' DVT, in an attempt to achieve a more efficient clinical rule to exclude DVT.

Furthermore, while the combination of PTP and DD results can safely exclude the diagnosis of DVT in patients without cancer [9,20,21], there is no evidence in support of this strategy in cancer patients with suspected DVT [13].

The aim of the present analysis was to determine the distribution of the various PTP categories in patients with cancer as well as the occurrence of DVT in each of these groups. Secondly, we evaluated the diagnostic accuracy of the DD test in combination with a low PTP, a moderate PTP, and with a low-moderate PTP in cancer patients vs. non-cancer patients.

Patients and methods

The study population consisted of consecutive outpatients referred for clinically suspected DVT from November 1995 to December 2004. Part of this cohort (n=1739) was included in a previous reported multicenter study on the diagnosis of venous thromboembolism (VTE) [11], while the remaining group consisted of consecutive patients referred for a suspicion of DVT to the thrombosis unit of the Academic Medical Center, Amsterdam, The Netherlands from February 1999 to December 2004. Cancer status was recorded at presentation and patients were considered to have active cancer if they were receiving treatment for malignancy, if treatment for cancer was stopped within the last 6 months, or if they were receiving palliative treatment for cancer. Prior to diagnostic testing, patients were assigned a PTP score of DVT using a validated model including an assessment of clinical symptoms and signs, risk factors for DVT, and alternative diagnoses (Table 1) [18]. Serial CUS was performed in all patients without knowledge of the cancer status and the DD results. In case of an initial normal CUS, serial testing was performed 1 week later and if CUS was still negative, patients were followed up for 3 months for the occurrence of symptomatic VTE. When VTE [DVT or pulmonary embolism (PE)] was suspected during the follow-up, the presence of DVT was further investigated by means of CUS and/or venography and the presence of PE by ventilation-perfusion scanning and/or pulmonary angiography.

Outcome measures

Patients were classified as DVT positive or negative according to the results of serial CUS and the 3-month follow-up. Patients were considered as DVT positive in the absence of compressibility of the common femoral vein and/or popliteal vein on CUS examination or in case of a symptomatic VTE event during the 3-month follow-up verified by imaging test.

Table 1. Clinical model for pretest clinical probability for deep venous thrombosis[18]

Major Criteria
• Malignancy with ongoing treatment, treatment stopped within last 6 months, palliatively treated only • Paralysis, paresis, or recent plaster immobilization of the lower extremities • Recent bedridden more than 3 days and/or major surgery within 4 weeks • Localized tenderness along the distribution of the deep venous system • Thigh (measured 10 cm above the patella base) and calf (measured 10 cm below the tibial tuberosity) swollen • Calf swelling 3 cm or more compared to symptomless side (measured 10 cm below the tibial tuberosity) • Strong family history of deep venous thrombosis (2 or more first degree relatives with history of deep venous thrombosis)
Minor Criteria
• History of recent trauma (within last 6 days) to the symptomatic leg • Pitting edema of symptomatic leg only • Dilated superficial veins (non-varicose) in symptomatic leg • Hospitalization within previous 6 months • Erythema
Alternative diagnosis
Scoring method -High probability: no alternative diagnosis and 3 or more major criteria; no alternative diagnosis and 2 or more major criteria and 2 or more minor criteria -Low probability: no alternative diagnosis and 1 major criterion and 1 or more minor criteria; no alternative diagnosis and 0 major criteria and 2 or more minor criteria; alternative diagnosis and 1 major criterion and 2 or more minor criteria; alternative diagnosis and 0 major criteria and 3 or more minor points -Moderate probability: all other combinations

Diagnostic tests

The SimpliRED DD test (Agen Biomedical Ltd, Brisbane, Australia) was used. This whole-blood cell agglutination assay uses a monoclonal antibody specific to human DD linked to a monoclonal antibody that binds to the surface of

human red blood cells. Agglutination occurs at DD concentrations >200 mg $L)^{-1}$ within 2 min and the outcomes of the test are categorized as positive in case of agglutination and negative otherwise. The technicians performing and interpreting the DD assays were unaware of the results of the diagnostic tests for DVT as well as of the cancer status.

Compression ultrasound was performed assessing the compressibility on the transverse plane of the common femoral vein and the popliteal vein down to the trifurcation of the calf veins. CUS was considered positive in case of non-compressibility, negative otherwise.

Analysis and statistical methods

Baseline characteristics in cancer vs. non-cancer patients were compared with chi square tests for proportions, and T-tests for comparison of continuous variables with a significance level of 0.05 (SPSS for Windows version 11.0; SPSS Inc., Chicago, IL, USA). The accuracy indices (sensitivity, specificity and NPV) for the DD test and the prevalence of VTE were determined in the groups at low, moderate, low-moderate, and high PTP and compared in patients with and without cancer. The decision to evaluate the diagnostic accuracy of DD for the PTP groups separately and for the low/moderate PTP group combined was made a priori. All accuracy indices and differences in the indices between cancer and non-cancer patients were calculated with CIA software version 2.0.0 (Gardner MJ, Altman D. Confidence interval analysis (CIA). Statistics with confidence. London: BMJ Publishing Group, 1989). The number needed to test (NNT) represents the number of DD tests needed to rule out one DVT and is calculated as the inverse of the ratio between the number of true-negative DD results and the total number of patients in the considered cohort. Confidence intervals were calculated with the Wilson calculation method, using a two-sided detection limit of 0.05.

Table 2. Baseline clinical characteristics of the 2066 patients with clinically suspected deep venous thrombosis

	Patients with cancer (n=244)	**Patients without cancer (n=1822)**
Age, years mean (±SD)	64 (14)	58 (18)*
Male/female, n	79/165	685/1137
Current use of oral contraceptives, n (%)	4 (2)	124 (7) †
Paralysis, paresis or recent plaster immobilization of the lower extremities, n (%)	12 (5)	126 (6)
Recently bedridden for more than 3 days and/or major surgery in the past 4 weeks, n (%)	46 (19)	176 (10)‡
Previous VTE, n (%)	19 (8)	240 (13)
Positive family history for VTE, n (%)	12 (5)	81 (4)
History of recent trauma (within last 60 days) of the symptomatic leg, n (%)	16 (6)	282 (15)‡
Hospitalization within the previous 6 months, n (%)	130 (53)	320 (17)‡

* =T-test statistic, $p<0.05$ † =Chi Square statistic, $p<0.05$; ‡ =Chi Square statistic, $p<0.01$; VTE=venous thromboembolism

Results

The baseline clinical characteristics of the study population are reported in Table 2. Of the 2066 patients enrolled in the study, 244 (11%) had a diagnosis of cancer at the time of presentation. Patients with and without malignancy were comparable with respect to sex, history of previous VTE, and family history of VTE. Cancer patients were older, more often recently bedridden or had undergone major surgery in the past 4 weeks and were more frequently hospitalized within the previous 6 months. Current use of oral contraceptives and a history of recent

trauma (within last 60 days) of the symptomatic leg occurred more often among patients without malignancy.

In patients with cancer, the incidence of VTE was 41% (100/ 244) with the diagnosis confirmed in 10% of the patients with low PTP, in 27% of those with moderate PTP, and in 68% of those with high PTP (Table 3A). The distribution of the cancer patients according to PTP was significantly different than in patients without cancer (Table 3A and B). In the low PTP group, there were 17% of the patients with cancer vs. 58% of those without cancer (difference 0.41; 95% CI, 0.35–0.46); in the moderate PTP 42% vs. 26% (difference 0.16; 95% CI, 0.10–0.23); and for high PTP 41% vs. 16% (difference 0.25; 95% CI, 0.19–0.31), respectively. In the group without malignancy, the incidence of VTE was half (22%) that of cancer patients (41%), whereas the prevalence of VTE in each of the PTP groups was similar to that in patients with malignancy (Table 3A and B).

Table 3 presents the results and accuracy indices of the DD test for the various PTP groups in patients with (Table 3A) and without cancer (Table 3B). In cancer patients with low PTP the sensitivity, specificity, and NPV of the DD were 100%, 58%, and 100%, respectively. The corresponding estimates in the group without malignancy and low PTP were 86%, 66%, and 98%.

For the combined low-moderate PTP group, the NPV was comparable in patients with cancer (97%) and without cancer (97%), but the specificity of the DD was lower in the presence of malignancy (51% and 64%, respectively; difference 0.13; 95% CI, 0.03–0.22). Among those with moderate PTP, the DD test showed a NPV of 95% both in patients with and without cancer whereas the specificity was smaller in the former group (47% vs. 57%). As expected, the combination of a negative DD was not clinically useful in the exclusion of DVT within the group at high PTP (Table 3).

The decrease in NPV and specificity observed from the low to the higher PTPs was associated with larger rates of DD false positive results and higher NNT.

Table 3A. D-dimer results and accuracy indexes according to clinical pre-test probability in patients with cancer

	VTE+	VTE-	Total	Accuracy Indexes (95% Confidence Interval)
Low PTP (n=42, 17%)				
				Sensitivity: 100 (51-100)
DD +	4	16	20	Specificity: 58 (42-72)
DD-	0	22	22	NPV: 100 (85-100)
				VTE prevalence: 4/42=10%
Moderate PTP (n=102, 42%)				
				Sensitivity: 93 (77-98)
DD+	26	39	65	Specificity: 47 (36-59)
DD-	2	35	37	NPV: 95 (82-99)
				VTE prevalence: 28/102=27%
Low-Moderate PTP (n=144, 59%)				
				Sensitivity: 94 (80-98)
DD+	30	55	85	Specificity: 51 (42-60)
DD-	2	57	59	NPV: 97 (88-99)
				VTE prevalence: 32/144=22%
High PTP (n=100, 41%)				
				Sensitivity: 97 (90-99)
DD+	66	20	86	Specificity: 38 (23-55)
DD-	2	12	14	NPV: 86 (60-96)
				VTE prevalence: 68/100=68%

When screening 100 cancer patients with low PTP or low-moderate PTP, a negative DD could adequately exclude a diagnosis of DVT in 52 and 39 patients, respectively. The corresponding numbers in patients without malignancy are estimated to be 60 and 54. In other words, the NNT increased from the low PTP

Table 3B. D-dimer results and accuracy indexes according to clinical pre-test probability in patients without cancer

	VTE+	VTE-	Total	Accuracy Indexes (95% Confidence Interval)
Low PTP (n=1060, 58%)*				
DD +	81	328	409	Sensitivity: 86 (78-92)* Specificity: 66 (63-69)
DD-	13	638	651	NPV: 98 (97-99)* VTE prevalence: 94/1060=9%
Moderate PTP (n=470, 26%)*				
DD+	118	148	266	Sensitivity: 92 (86-96) Specificity: 57 (51-62)
DD-	10	194	204	NPV: 95 (91-97) VTE prevalence: 128/470=27%
Low-moderate PTP (n=1530,84%)				
DD+	199	476	675	Sensitivity: 90 (85-93) Specificity: 64 (61-66)*
DD-	23	832	855	NPV: 97 (96-98) VTE prevalence: 222/1530=14%
High PTP (n=292, 16%)*				
DD+	176	58	234	Sensitivity: 96 (92-98) Specificity: 47 (38-56)
DD-	7	51	58	NPV: 88 (77-94) VTE prevalence: 183/292=63%

*Statistically significant different ($p<0.05$) between patients with and without cancer. DD+/DD-=d-dimer positive/negative result; NPV=negative predictive value; PTP=pretest clinical probability; VTE=venous thromboembolism

[1.9 (95% CI, 1.5–2.8)] to the low-moderate PTP group [2.5 (95% CI, 2.1–3.1)] among cancer patients as well as in those without malignancy [1.7 (95% CI, 1.6–1.7) and 1.8 (95% CI, 1.7–1.9), respectively].

Discussion

The current data indicate that the distribution of the PTP categories differs significantly between patients with and without cancer. While most of the patients without malignancy could be classified as low or moderate PTP, in those with cancer the low PTP and moderate PTP represented a relatively smaller fraction, with 41% of the patients included in the high PTP group. The present findings also suggest that the combination of a low or low-moderate PTP score and a negative DD may be a valid tool to exclude the diagnosis in cancer patients presenting with clinically suspected DVT.

The diagnosis of malignancy is a part of the Wells score that therefore weighs against low PTP classification and this could result in larger proportion of cancer patients classified in the higher PTP groups. In the present study, the low PTP category included 17% of the cancer patients vs. 58% of the patients without malignancy (difference 0.41; 95% CI, 0.35–0.46). When compared with low PTP, the low-moderate PTP category would allow to enlarge the group of screened cancer patients in whom a diagnosis could be subsequently excluded based on a negative DD result. Indeed, 52% of the cancer cohort in the present study was classified in the low-moderate PTP category. Of relevance, the ability of the DD test to safely exclude DVT in the combined low-moderate PTP was comparable among patients with or without malignancy (NPV of 97% in both groups) at the expense of a slightly higher NNT in the presence of active cancer (2.5 vs. 1.9). Thus, the usefulness of low PTP plus a negative DD result appears to be limited by the low percentage of cancer patients within this category, whereas the low-

moderate PTP combined with DD could represent a more powerful diagnostic strategy. This finding is in agreement with the latest version of the Wells' score that dichotomizes patients with clinically suspected DVT as 'likely' or 'unlikely' [19].

The evidence about the safety and clinical utility of the DD test in cancer patients with suspected DVT is limited, with conflicting data in the literature [11–17]. Lee et al. [12], retrospectively assessed the value of the SimpliRED DD assay in 1068 consecutive outpatients with suspected DVT included in three prospective studies which used miscellaneous reference standards to objectively confirmed the diagnosis. While the sensitivity of the DD was comparable in patients with and without malignancy, the NPV of the DD test was found significantly lower in cancer patients (79% vs. 96%, p=0.008). In a previous investigation, we evaluated consecutive outpatients (n=1739) with suspected DVT with a diagnostic strategy including the SimpliRED DD test and CUS [11]. The NPV of the DD test was found high both in cancer and noncancer patients [97% (95% CI, 89–100) and 97% (95% CI, 96–98), respectively]. It was argued that in cancer patients the decreasing effect of a higher DVT prevalence on the NPV of the DD test was counterbalanced by a higher sensitivity of the test resulting in a NPV comparable to the NPV obtained in patients without malignancy. The discrepancy in the findings of the previous two studies may be partially explained by the different reference tests used, differences in the populations included as well as in the design characteristics of the studies.

In the current analysis, the NPV of the DD in both groups at low and low-moderate PTP appeared comparable to that one of the sole DD test in our previous study [11]. However, within the group of patients with high PTP, the NPV of the DD test was only 86% with the lower bound of the 95% confidence interval as low as 60%. Thus, applying a general NPV of 97% throughout the whole cancer

population would lead to a relatively high percentage of false negative results among cancer patients with high PTP who represented a large part of this group.

None of the previous studies evaluated the combination of PTP and DD test and the only available evidence is a small subgroup analysis from a cohort of patients suspected of DVT or PE [13]. In agreement with the present findings, that study suggested that the DD test may have a high NPV and a high sensitivity in cancer patients with low-moderate PTP while it has scarce utility within the high PTP group.

Our findings show an increase in DVT prevalence moving from the low to the high PTP group which was paralleled by a decrease in specificity and by a small increase in NNT both in patients with and without malignancy. The relatively high rate of false positive results limit the clinical utility of DD test in ruling in DVT in cancer patients, in agreement with existing literature [10–12].

In the current analysis, the potential for bias was eliminated by the blinding of the clinicians performing objective tests both for the DD results and the cancer status of the patient as well as the blinding of the technologists who performed the DD assay for the clinical status of the patient and the results of the objective testing. Serial CUS, a widely accepted surrogate for venography in the diagnosis of DVT [8], was used to verify the presence or absence of the disease with the conclusions strengthen by a long-term clinical monitoring for VTE occurrence. It cannot be excluded, however, that a small proportion of patients with distal DVT was misclassified as DVT negative. As the study population included consecutive outpatients, it is unlikely that a selection bias might have occurred and, indeed, the high VTE prevalence in cancer patients (41%) was similar to those previously observed [11–13].

In conclusion, the distribution of the PTP categories in cancer patients appears significantly dissimilar than in patients without cancer. Given the low percentage of low PTP in cancer patients, a combination of low PTP with moderate

PTP, or the recently proposed 'unlikely' category [19] would probably be more useful in clinical practice. This is further supported by the fact that a negative DD results seems effective in excluding the diagnosis of DVT in combination with both a low or a low-moderate PTP. As in patients without malignancy, this approach might be able to limit the number of further unnecessary image testing. However, further studies with more patients in the low PTP category as well as management or outcome studies are warranted to demonstrate the safety of the combination of DD and PTP in cancer patients.

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Decision analysis for cancer screening in idiopathic venous thromboembolism

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Abstract

Background: The SOMIT trial randomized patients with idiopathic venous thromboembolism (IVTE) and without signs of cancer at routine medical examination, to extensive screening for cancer plus 2 years of follow-up or to just 2-year follow-up.

Methods: The data of the SOMIT trial were used to perform a decision analysis. The screening tests were divided in several possible strategies. The number of detected cancer patients and the number of patients investigated further for an eventually benign condition were calculated for each strategy. The total costs for the screening strategy and for each detected cancer patient were determined. Based on the tumor type, stage, age and gender of the individual cancer patient, the difference in live years gained (LYG) was calculated between the two study groups.

Results: Computed tomography (CT) of the abdomen combined with sputum cytology and mammography detected 12 of the 14 patients with cancer and had one false-positive result. In general, screening strategies including abdominal/pelvic ultrasonography (US) or tumor markers yielded a higher number of patients needed to screen in comparison with those using abdominal/pelvic CT. Furthermore, the strategies which included colonoscopy, tumor markers, and abdominal/pelvic US were significantly more costly, had inferior LYG and higher costs per LYG, when compared with strategies using abdominal/pelvic CT.

Conclusions: Despite the limitations of this analysis, the screening for cancer with a strategy including abdominal/pelvic CT with or without mammography and/or sputum cytology appears potentially useful for cancer screening in patients with IVTE. The cost-effectiveness analysis of this strategy needs confirmation in a large trial.

Introduction

The first report about the relationship between venous thromboembolism (VTE) and occult cancer was published in 1935 [1]. Only recently, however, the incidence of malignancy in the 2–3 years after the first VTE event has been determined and was shown to be 7.5% in patients with idiopathic VTE (IVTE) vs 1.6% in patients with a secondary VTE [2–10]. When the doubling time of cancer cells and the minimal tumor volume necessary for detection are taken into account, it is likely that the cancers were indeed occult at the time IVTE was diagnosed [11]. The usefulness and the extension of the screening for cancer in patients with IVTE have been long debated. Several investigators advise only a basic screening by means of a thorough clinical history, physical examination, simple laboratory tests and a chest X-ray [3,12,13]. Others advocate a more extensive screening with computed tomography (CT) scans, ultrasound (US) and the determination of circulating tumor markers [5,6,10,14]. Recently, the results of the Subsequent diagnosis Of Malignancy in patients presenting with Idiopathic venous Thromboembolism (SOMIT) trial comparing an extensive screening procedures with a basic screening in patients with IVTE have become available [15]. Unfortunately, the SOMIT study was terminated prematurely because of slow recruitment and logistic problems. In spite of this, the results remain useful.

In the present analysis, the available data from the SOMIT trial were used to determine, for each of the evaluated screening strategies, the number of patients needed to screen (NNTS) to detect one additional case of cancer and the number of patients harmed. Finally, the costs of the various strategies were calculated and a cost-effectiveness analysis was performed.

Methods

Study population

The analyses are based on the data from the SOMIT study [15]. Only patients over the age of 25 years, with a first IVTE event were included. VTE was defined as idiopathic if it occurred in the absence of known malignant disease, trauma of the leg, surgical procedures or immobilization within 6 months prior to presentation, confirmed spontaneous VTE in a first degree relative, thrombocytosis of more than 600 $x10^9$ L^{-1}, circulating lupus anticoagulant, pregnancy, childbirth, or deficiency of antithrombin, protein C or S. The basic screening for malignant disease had to be completed and without abnormal results. When allocated to the extensive screening group patients were offered to undergo US of the abdomen and the pelvis, followed by CT scan of these areas, gastroscopy or double contrast X-ray of the stomach, colonoscopy or sigmoidoscopy followed by barium enema X-ray of the colon, fecal occult blood tests (FOBT), sputum cytology and tumor markers including carcinoembryonic antigen (CEA), a-fetoprotein (-FP), and cancer antigen (CA)-125. In addition, mammography and PAP-smear were performed in women and trans-abdominal US of the prostate and prostate specific antigen (PSA) plasma levels in men. The cut-off values for all tumor markers were twice the upper limit of the normal range. All screening procedures were performed on an out-patient basis. The patients in the control group were not additionally investigated, but were just followed up for 2 years, as in the screening group. During the follow-up visits, scheduled at 3, 12 and 24 months after the IVTE, special attention was paid to the recent medical history by means of a standardized form. Tumors were staged according to the system of the American Joint Committee for Cancer and comparison for tumor stage was performed with the Fischer exact test [16].

Decision Model

A decision model was developed representing 19 possible strategies for the diagnostic work-up of patients with IVTE (Table 1). There were 8 diagnostic strategies with abdominal/ pelvic CT, 8 similar strategies using abdominal/pelvic US instead of CT, and 3 strategies with tumor markers. Since a normal basic evaluation was the prerequisite for inclusion, this standard basic assessment was left out of the decision model. Sigmoidoscopy followed by barium enema X-ray of the colon was not considered in any model, because this combination is more expensive than colonoscopy and it lacks the possibility of taking biopsies proximal from the sigmoid.

A common sense approach was used to determine the strategy for further evaluation after an abnormal test result. An elevated CEA led to colonoscopy and abdominal/pelvic CT, an elevated CA-125 to a consultation of a gynecologist and abdominal/pelvic CT, and an elevated a-FP to abdominal/ pelvic CT. An elevated PSA was followed by a US and a biopsy of the prostate, a positive FOBT by colonoscopy and if negative also by gastroscopy. The detection of a mass resulted in a biopsy, unless the suspicion of cancer was high enough to perform surgery (i.e. in the patients with renal or ovarian cancer).

Number needed to screen analysis

All cancers detected by the extensive screening were considered as new cases. The NNTS to detect one additional case of cancer was calculated by dividing the total number of patients in the extensively screened group, by the number of detected cases (ND). This calculation was performed for each of the diagnostic strategies. When a diagnostic test had an abnormal result, the patient was evaluated further. If cancer was suspected by more than one diagnostic procedure within a specific strategy, it was counted once. The number of patients evaluated further,

Table 1. The number needed to screen, number of detected patients and number harmed per strategy

Strategy	NNTS	ND	NH
CT abdomen/pelvis	9.9	10	1
CT abdomen/pelvis + mammography	9.0	11	1
CT abdomen/pelvis + sputum cytology	9.0	11	1
CT abdomen/pelvis + markers	9.0	10	26
CT abdomen/pelvis + mammography + sputum cytology	8.3	12	1
CT abdomen/pelvis + mammogr. + sputum cytol. + markers	7.6	13	26
CT abdomen/pelvis + FOBT	9.0	11	15
CT abdomen/pelvis + Colonoscopy	9.0	11	4
US abdomen/pelvis	19.8	5	1
US abdomen/pelvis + mammography	16.5	6	1
US abdomen/pelvis + sputum cytology	16.5	6	1
US abdomen/pelvis + markers	12.4	8	26
US abdomen/pelvis + mammography + sputum cytology	14.1	7	1
US abdomen/pelvis + mammogr. + sputum cytol. + markers	10.0	9	26
US abdomen/pelvis + FOBT	16.5	6	15
US abdomen/pelvis + Colonoscopy	16.5	6	4
CEA. CA-125. α-FP. PSA and FOBT	16.5	6	43
CEA. CA-125. α-FP. PSA	16.5	6	29
CEA. CA-125. α-FP	19.8	5	23

CT=Computed Tomography; CA=cancer antigen; CEA=carcinoembryonic antigen; FOBT=Fecal occult blood tests; α –FP=α-foetoprotein; NNTS=Number needed to screen to detect one patient with cancer; ND=Total number of cancer patients detected by extensive screening in the cohort; NH=Total number of patients evaluated further because of an eventually, benign condition in the cohort; PSA=prostate specific antigen; US=Ultrasonography. The data in table are based on the study cohort of 99 patients

eventually because of a benign condition, was calculated for each strategy and was defined as the "number harmed" (NH).

Costs

Costs were calculated from the perspective of the health insurance system and were determined according to the Committee Tariffs Healthcare in 2001 (This Committee regulates the maximum fees to be charged by healthcare workers/institutes in the Netherlands). To calculate the costs of the screening strategies, the costs of the initial treatment of VTE, of the basic screening, and of follow-up were not taken into account as these were considered necessary and standard for every patient with VTE. Moreover, the costs related to the treatment of the cancer patients were excluded from the calculations, as these were inevitable for any cancer patient and not part of the screening procedure. Thus, the analyses focused on the incremental costs of screening for cancer in patients with IVTE.

The costs of the screening tests and the subsequent evaluations were added up to calculate the total costs for each strategy, and were multiplied by the number of patients who underwent that particular (set of) investigation(s). Patients with an abnormal test result were evaluated further. If a test was used that already had been part of the initial strategy, the cost of that test was counted only once. The costs to detect or to rule out a diagnosis of malignancy were calculated dividing the total cost of the screening procedure by the number of patients with cancer detected by that specific strategy. All costs were calculated in Euro.

Cost-effectiveness

Overall life expectancy was estimated, adjusted for age and sex, using the figures of the Dutch Bureau of Statistics [17]. For all cancer patients, the likelihood of curative treatment was determined, based on the type of tumor and its stage, according to current cancer treatment [18]. Based on the data of life expectancy in

stage IV cancers, patients with incurable malignancies were assumed to live (median) for another year [18]. Life expectancy of patients with a potentially curable cancer was calculated by multiplying the overall life expectancy with the chance of a curative treatment. If the time between the index IVTE and the detection of the cancer was more than 1 month, it was added up to the life expectancy. The Live Years Lost (LYL) for the screened and control patients were determined by the subtraction of the life expectancy with malignancy from the life expectancy without malignancy. If in a certain strategy a cancer was not detected, the mean LYL of the control group was assigned to this case. In case a life expectancy was less than the mean LYL of the control group, the latter was conservatively considered to be the LYL. The mean LYL per strategy was calculated by dividing the total LYL by the number of patients in the group (14 and 10 for the screened and control group, respectively). The Live Years Gained (LYG) per strategy were calculated subtracting the LYL of the screened group from the LYL of the control group. Total LYG were then calculated for each strategy by multiplying the LYG per the ND of the specific strategy. Furthermore, the costs per LYG and incremental costs (the additional cost per additional LYG) were calculated for all strategies. Sensitivity analysis was performed by varying the costs (50% increase) and LYG (50% decrease) of the most attractive strategies. Scenario analysis was performed to evaluate the potential role of the chest CT.

Results

The median age of the 99 patients in the extensive screening group was 66 years and 45 were women. Within this group, the screening detected 13 of the 14 patients who had a histological confirmed malignancy within 2 years from the IVTE. In the control group, the median age was 67 years and 56 were women. The mean time between the IVTE and the detection of the cancer in this group was 370

days (120–628 days). In the extensive screening group, five patients were diagnosed with stage I and seven with stage II tumors whereas in the control group no stage I and four stage II tumors were detected. All other malignancies (two in extensive screening group and six in control group) were more advanced at the time of detection. In total, nine out of 14 patients with cancer in the extensive screening group vs. two of the 10 patients in the control group had T1–T2 disease without signs of local or distant metastasis. (p=0.047) [15].

Number needed to screen analysis

The results of the NNTS and NH analysis are summarized in Table 1. As most cancers were detected by means of abdominal/pelvic CT with a low number of false-positive results, abdominal/pelvic CT was combined with most of the other diagnostic tests. The lowest NNTS (7.6) was achieved by the combination of abdominal/pelvic CT, mammography, sputum cytology and tumor markers, but for every cancer patient detected two patients were evaluated further for an, eventually, benign condition. This problem was observed in all strategies that included the determination of circulating tumor markers. The NNTS of the combination abdominal/pelvic CT and colonoscopy was 9.0 with a NH of 4.

As the addition of abdominal/pelvic US or a gastroscopy to abdominal/pelvic CT yielded no additional detected cases, these test combinations were not evaluated. However, because of the radiation exposure associated to the CT, an abdominal/ pelvic US may be preferred over an abdominal/pelvic CT. For this reason, the abdominal/pelvic US was assessed in combination with other tests. The strategies that used abdominal/ pelvic US, as well as those using only tumor markers, yielded a higher NNTS in comparison with those using abdominal/ pelvic CT. However, the NH by the strategies involving tumor markers were 2–6.6 times the ND of cancer patients.

Costs analysis

The costs per strategy varied between €10547 for tumor markers without PSA and €61607 for abdominal/pelvic US combined with colonoscopy (Table 2). The lowest costs per detected cancer patient was achieved with the abdominal/pelvic CT (€1974), whereas the highest costs were reached by the combination of abdominal/pelvic US with colonoscopy (€10268). These high costs were due to the low number of detected cancer patients and the high costs of the investigations. The costs per detected cancer patient for strategies that included abdominal/pelvic US were at least €4262. All strategies using FOBT showed a substantial increase in costs due to the high rate of false-positive results and the subsequent (expensive) investigations. The strategies that included colonoscopy were substantially more costly, whereas those including abdominal/pelvic CT instead of US were substantially less costly per ND.

Abdominal/pelvic CT combined low costs with a low NH and the addition of mammography or sputum cytology did not result in a substantial increase of the costs per ND (€2085 and €2258, respectively).

Cost-effectiveness analysis

The life expectancy of all cancer patients would have been 214 years (15.3 years per patient) and 112 years (11.2 years per patient) in the absence of cancer, in the extensively screened and in the control group, respectively. Due to the malignant disease and taking into account the chance of curative treatment, the life expectancy declined to 128 years (9.1 per patient) in the extensively screened group and to 40.6 (4.1 per patient) in the control group. The main determinants of the life expectancy were the age and the stage of the cancer. To bypass the effect of age, the life expectancy was determined for all patients to be equal by dividing the life expectancies of the two groups by 24. The LYL per cancer patient for the various strategies in the extensively screened group varied between 5.7 and 7.8

Table 2. Costs (total, per detected cancer patient, incremental), life years gained, and false positive test results per strategy

Strategy	Costs/ Strategy	ND	Costs/ ND	LYG	Costs/ LYG	Incremental Costs/LYG*	NFP
CEA, CA-125, α-FP	10547	5	2109	4,0	2617	2637	23
US a/p	25488	5	5098	5,2	4942		1
US a/p + sput cyt	30586	6	5098	6,2	4942	-	1
US a/p + mammo + sput cyt	33788	7	4827	7,2	4679	-	1
CEA, CA-125, α-FP, PSA, FOBT	19123	6	3187	8,0	2398	-	43
CEA, CA-125, α-FP, PSA	12017	6	2003	8,0	1507	367	29
US a/p + mammo	28690	6	4782	8,7	3284	-	1
US a/p + Colonoscopy	61607	6	10268	9,3	6603	-	4
US a/p + FOBT	35733	6	5955	9,3	3830	-	15
US a/p + markers	34099	8	4262	15,4	2209	-	26
US a/p + mammo + sput cyt + markers	41285	9	4587	17,4	2377	-	26
CT a/p	19737	10	1974	19,7	1000	643	1
CT a/p + sput cyt	24835	11	2258	21,7	1144	-	1
CT a/p + mammo	22939	11	2085	26,4	870	534	1
CT a/p + Coloscopy	56126	11	5102	27,5	2044	-	4

Table 2. Continued

Strategy	Costs/ Strategy	ND	Costs/ ND	LYG	Costs/ LYG	Incremental Costs/LYG*	NFP
CT a/p + FOBT	29982	11	2726	27,5	1092	-	15
CT a/p + markers (+ PSA)	27643	11	2513	27,5	1006	4703	26
CT a/p + mammo + sput cyt	28037	12	2336	28,8	974	197	1
CT a/p + mammo+ sput cyt + markers + FOBT	44933	13	3456	38,0	1183	-	40
CT a/p + mammo +sput cyt + markers	35534	13	2733	38,0	936	833	26
CT Thorax a/p #	41285	12	3440	23,7	1743		1
CT Thorax a/p + mammo #	44487	13	3422	31,2	1427		1

a/p = abdomen/pelvic; CT=Computed Tomography; CA=cancer antigen; CEA=carcinoembryonic antigen; FOBT=Fecal occult blood tests; α –FP=α-foetoprotein; LYG = Live years gained; mammo = mammography; ND = Total number of cancer patients detected by extensive screening in the cohort of 99 patients.; NFP = Number of false positive patients; PSA=prostate specific antigen; sput cyt = sputum cytology; US=Ultrasonography. *: Indicates that the strategy is dominated by a strategy that resulted in an equal or higher LYG, but is less costly. # : These strategies were only included in the additional analysis because they were not used in the SOMIT study.

compared with 8.6 in the control group. The LYG varied for the different strategies between 4 and 38 years. The strategies that included abdominal/pelvic CT yielded the highest LYG (mean 28, range 20–38) whereas the LYG was generally lower for strategies in which US was used (LYG mean 9.6, range 5–17) (Table 2). The costs per LYG varied between €870 (abdominal/pelvic CT plus mammography) and €6603 (abdominal/pelvic US plus colonoscopy). Strategies that included US, FOBT and/or colonoscopy were inferior in terms of LYG and costs per LYG when

compared with strategies using the abdominal/pelvic CT and thus were dominated by them. The determination of tumor markers appeared not to be cost-effective and was accompanied by a high rate of false-positive results (Tables 1 and 2). The most attractive strategies seemed to be the abdominal/pelvic CT-mammography combination with or without the sputum cytology given the associated costs per LYG (€974 and €870, respectively) and the low NH (Table 1).

Additional analysis

For these two latter strategies, an additional analysis was performed. Hypothesizing a 50% increase in the total costs because of false-positive test results, the cost-effectiveness ratio (costs per LYG) would have been €1450 and €1323, respectively. Furthermore, a 50% decrease in LYG due to a lower incidence of cancer in the population, to a lower sensitivity of the screening test used or due to co-morbidities caused by false-positive test results, or any combination of these, would lead to a cost-effectiveness ratio of €1934 and €1764, respectively. As can be appreciated from Table 2, strategies that included US, FOBT and/or colonoscopy were always dominated by those including abdominal/pelvic CT. The determination of tumor markers did not seem to be cost-effective and was accompanied by a high rate of false-positive results. Replacing the sputum cytology by CT chest in the strategy combining abdominal/ pelvic CT plus mammography would increase the costs-effectiveness ratio from €974 to €1427.

Discussion

The current decision analysis suggests that extensive screening for cancer in patients with IVTE has the potential to be cost effective. In particular, the abdominal/pelvic CT, with or without mammography and/or sputum cytology, seems an attractive combination. All other screening strategies, and especially those including US, FOBT or colonoscopy performed less because of higher NNTS

and/or NH. The costs for abdominal/pelvic CT plus mammography with or without the sputum cytology would be €974 and €870, respectively, in order to achieve one LYG in this population. Compared with the costs per LYG in other cancer screening programs (median approximately $15 000=€19500), these costs seem very reasonable [19–21].

The addition of the CT of the chest to abdominal/pelvic CT and mammography could be an alternative to the sputum cytology. Indeed, the CT-chest is likely to be more sensitive and specific for the detection of lung cancers than sputum cytology, although this has not been adequately investigated [22]. Moreover, the CT of the chest has the potential advantage of detecting other malignancies than lung cancer in the area of the thorax. The obvious disadvantage will be the increasing number of false positive (i.e. due to lymphadenopathy). Assuming that CT-chest would have the same sensitivity and specificity as the sputum cytology, the costs per LYG would be €1427.

Some important limitations of the present analysis have to be acknowledged. Firstly, the SOMIT study was stopped prematurely and included only a total of 201 patients. Although there was a difference in mortality in favor of the screening group, this difference was not statistically significant. Secondly, the sensitivity of the screening method(s) was based on a limited number of patients with detected cancer. Hence, the true sensitivity for any of the evaluated strategies remains uncertain. However, even with a decrease of sensitivity to 50% and an increase of the costs of 50%, the cost-effectiveness ratio of the abdominal/pelvic CT combined plus mammography with or without sputum cytology remains within acceptable margins (€1934 and €1764).

The estimates of LYGs used for the analysis were based on chances of curative treatment and life expectancy without cancer that were longer than the time-frame of the SOMIT study (2 years). Moreover, several additional assumptions were made in the analysis. Based on recent data from the literature,

patients with stage IV cancer were assumed to live another year. The life expectancy of patients in the extensive screening group whose cancer was not detected was considered to be the mean life expectancy of those in the control group. Therefore, the results of this decision analysis should be considered exploratory. However, the results of the 2-year follow-up of the SOMIT study are in line with those of the LYG per patient in the present analyses, especially for patients with a high chance (>75%) of curative treatment. It has been suggested that the life expectancy of cancer patients with a concomitant VTE may be shorter than in cancer patients without VTE [23]. However, the overall life expectancy of cancer patients with IVTE remains unknown, so adjusted estimates could not be used for the present analysis.

Finally, in this analysis the costs to diagnose a malignancy in the cancer patients and in the control group were not assessed. The use of diagnostic resources was based on clinical practice, whereas the costs were calculated based on standard fees rather than real-life costs. However, using real-life costs rather than standard fees is unlikely to change the conclusion on the cost analysis.

In summary, this decision analysis indicates that extensive screening for cancer in patients with IVTE, in particular strategies combining CT and mammography, results in a high yield of detected cancer patients with a low number of patients harmed, at acceptable cost per life-years gained. However, the cost-effectiveness and the possible impact on survival of the strategy including abdomen/pelvis CT plus mammography warrant further evaluation in a large trial. Considering the difficulties because of the randomized design of the SOMIT study, the study would have to be a large prospective, cohort follow-up project.

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6

Antithrombotic therapy and cancer

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Purpose of the review

To assess the current evidence from recent clinical trials investigating antithrombotic agents for the prophylaxis and treatment of venous thromboembolism in cancer patients and for the effects of these agents on cancer progression.

Recent findings

A growing body of evidence supports the preventive use of antithrombotic strategies in subgroups of cancer patients. Moreover, in the long-term management of deep venous thrombosis in cancer patients, low-molecular-weight heparin seems to represent a valid alternative to vitamin K antagonists. Finally, several studies have claimed a direct anticancer activity and a positive impact on prognosis of some antithrombotic agents, e.g. aspirin and low-molecular-weight heparin.

Summary

Although recent evidence suggests low-molecular-weight heparin as a possible option in the management and prevention of venous thromboembolism in cancer patients, more evidence from large randomized, prospective, controlled trials is needed to determine the exact the magnitude of the risk-benefit ratio associated with its use. The promising results on the effects of antithrombotic agents in the prognosis of cancer patients deserve further evaluation to estimate the potential and the feasibility of this approach.

Introduction

Cancer is a chronic hypercoagulable state increasing the risk of venous thromboembolism (VTE) [1–3]. Moreover, other concomitant factors such as surgery, chemotherapy, radiotherapy, immobilization, and the use of central venous catheters can further increase the risk of VTE. An important matter of debate is in which situations patients with cancer should receive thromboprophylaxis. It also remains controversial which anticoagulant treatment strategy should be implemented once a VTE has been diagnosed in a patient with active cancer. New regimens with known drugs have been tested, but new drugs have also been developed that may have important implications in the treatment of VTE in patients with malignancy. Finally, there have been indications that antithrombotic agents may have an influence on cancer progression and survival. Recent studies have further evaluated this both in the presence and in the absence of VTE. The current evidence from recent clinical studies evaluating the use of antithrombotic agents for the prophylaxis and treatment of VTE and for possible effects on cancer progression is reviewed here.

Venous thromboembolism prophylaxis

While routine prophylaxis with antithrombotic therapy in patients undergoing surgery for malignancy is strongly recommended [4], controversy still exists about the efficacy and safety of this approach for non surgical cancer patients [1,2].The benefits of VTE prophylaxis must be balanced against the risk of bleeding associated with antithrombotic agents, which is notably higher in the cancer population [5]. Recent questionnaire surveys showed that only about 50% of clinicians routinely use thromboprophylaxis in surgical cancer patients and that the vast majority use none in non surgical cancer patients. When prophylaxis was used, the strategies differed substantially [6–8].

Recently, a beneficial effect of low-molecular-weight heparin (LMWH) prophylaxis in medical cancer patients was claimed in a post hoc analysis of the MEDENOX study [9], in which in the 72 cancer patients studied, 40 mg enoxaparin once daily as compared with placebo reduced the rate of VTE from 20% to 10% (non significant). No data about bleeding complications were reported for this subgroup of patients, however.

For the subpopulation of cancer patients with a central venous catheter, still no firm recommendations are available. Two recent reviews [10,11] concluded that some form of prophylaxis might be indicated in this population, but due to various shortcomings in study design, the differences in the reference standard tests used to confirm the diagnosis of VTE, and the scarce reporting of major bleeding incidences in the available studies, no definite conclusions can be drawn.

Treatment of venous thromboembolism

An intriguing problem in the treatment of cancer patients with VTE is their increased risk of recurrence and bleeding despite the current standard treatment [12].A reason may be the difficulty in maintaining the international normalized ratio (INR) within the therapeutic range in this group of patients due to multiple drug interactions, gastrointestinal upset, liver dysfunction, and poor venous access. Because the therapeutic effects of LMWH would not be significantly influenced by any of these variables, a long-term LMWH administration has been tested in several studies as a possible alternative to vitamin K antagonists (VKAs) [13].

Two recent studies that have investigated this will be described here. Meyer et al.[14] randomly assigned 138 consecutive patients with a diagnosis of cancer and VTE to subcutaneous enoxaparin sodium (1.5 mg/kg once a day) or to warfarin given for 3 months in a open-label study. The main combined outcome was major bleeding or recurrent VTE within 3 months. During this period, 21.1%

of the warfarin patients experienced such an event, as compared with 10.5% of the enoxaparin recipients (relative risk [RR], 2.02; 95% CI, 0.88–4.65).

A similar benefit of LMWH over VKAs has also been also observed in the CLOT study. This was a large multicenter, randomized open-label study that compared the efficacy and safety of long-term dalteparin with that of long-term VKAs in the secondary prophylaxis of VTE in cancer patients [15••].Of the 672 patients randomized, 9% of the dalteparin recipients had a recurrence during the 6-month follow-up vs 17% in the coumarin group (p=0.002). The rates of major bleeding were 6% and 4% (p=0.27) and of any bleeding 14% and 19% (p=0.09), respectively. In agreement with previous studies that underlined the difficulty in the INR control in cancer patients, in CLOT the INR was in the therapeutic range only 46% of the time in the VKA group. The INR was below the range in 30% and above in 24% of the time, with 50% of the major bleedings occurring with an INR greater than 3.0.

The results of these two studies suggest that long-term LMWH, compared with VKA, could provide greater benefits together with a better safety profile in cancer patients. Nonetheless, before recommending this strategy more data are needed on the associated costs and incidence of rare complications, such as thrombocytopenia and osteoporosis [16,17]. Moreover, new antithrombotic agents have been developed that could improve the management of VTE in terms of efficacy and safety or in simplification of the treatment.

One of these, fondaparinux, is a synthetic pentasaccharide with specific anti–factor Xa activity. Its pharmacokinetic properties allow a fixed-dose, once-daily regimen of subcutaneous injection, without the need for monitoring. Its use in the initial treatment of pulmonary embolism as compared with unfractionated heparin was investigated in the MATISSE-PE trial [18]. Approximately 10% of the 2213 enrolled patients had active cancer, defined as a cancer that had been treated within the previous 6 months or was not cured. In the fondaparinux group, 8.9%

had recurrent VTE vs 17.2% in the unfractionated heparin group (non significant). Bleeding rates were similar in the two groups.

Antithrombotic agents as an anticancer treatment: effects on cancer progression and survival

Although in the past various antithrombotic agents have been investigated for their effects on cancer progression and survival, recent studies have predominantly focused on aspirin and heparins.

Aspirin

A growing body of evidence from observational studies suggests that aspirin has a role as a chemopreventive agent, although the underlying mechanisms are still a matter of debate [19–25]. The most persuasive data are on colorectal cancer prevention, where the use of aspirin is tantalizing because aspirin is cheap and readily available and because colorectal cancer is the second most common cause of cancer-related death in the Western world [26••,27••].

In a large prospective study on the primary prevention of colorectal adenoma, aspirin was associated with an RR of 0.75 (95% CI, 0.66–0.84), as compared with non aspirin use [28••]. A dose-response effect was observed with maximal benefits associated with the use of 14 or more aspirin tablets/wk (RR =0.49; 95% CI, 0.36–0.65). This dose-response relation is in contrast to data from most observational studies, where the anticarcinogenic effects of aspirin are not strongly dependent on the dose. The benefit of aspirin was also demonstrated in three recently published randomized trials about the chemopreventive effects of aspirin on the recurrence of sporadic polyps in patients with a history of colorectal adenomas or colorectal cancer (secondary prevention) [26••,27••,29]. Although extensive data from observational studies suggest that 10 to 20 years of treatment are required to lower the risk of colorectal cancer, the data from the secondary

prevention studies suggest that a few years of aspirin can reduce adenoma recurrence.

It is well known that the risk of hemorrhage complications is proportional to the aspirin dose. Consistently, Chan et al.[28••] found a twofold higher risk of subarachnoid hemorrhage in participants taking more than two tablets per day. The risk-benefit ratio could be improved through several strategies, such as lowering the aspirin doses [26••], use of cyclooxygenase-2-selective inhibitors [30], or selection of high-risk populations, e.g. patients with familial polyposis or patients with a history of colon cancer [27••,31]. Patients with increased inflammation markers may also prove an interesting target, given the recently suggested correlation between these markers and the risk of subsequent colorectal cancer [32]. Another way to increase the benefit is by selecting patients who are especially sensitive to the protective action of aspirin. Interestingly, the aspirin chemopreventive activity was enhanced by the presence of a polymorphism in the ornithine decarboxylase gene, a gene involved in the tumorigenesis process of the colon [33]. Aspirin users homozygous for the A-allele were about 10-fold less likely to have an adenoma recurrence as compared with nonaspirin users homozygous for the major G-allele.

Besides colorectal cancer, reduced risk estimates have been found for esophageal cancer and breast cancer [34,35], whereas aspirin use does not seem to have a chemopreventive activity in lung cancer [36] or ovarian carcinoma [37]. Moreover, an increase in risk has been claimed in the case of non-Hodgkin lymphoma [38] and pancreatic cancer [39]. In the latter, a dose-dependent rise in the risk was evidenced, with the worse effect associated with more than 14 tablets/wk (RR =1.86; 95% CI, 1.03–3.35).

Low-molecular-weight heparin

Several post hoc studies have found a beneficial effect of LMWH on survival in patients with cancer and VTE [40,41].The mechanisms behind these findings are still incompletely understood but may be related to the effects of LMWH on coagulation, angiogenesis, and metastasis [42–44]. A relevant finding in this perspective is that structural and chain length variations in LMWHs may result in differential anticancer activity [45–47] and in turn explain some discrepancies encountered in literature on the anticancer effects of heparins [46,47].

A subanalysis of the previously mentioned CLOT study found no benefit in 1-year mortality associated with 6-month-dalteparin, compared with long-term VKA [48]. However, in the subgroup of patients with non metastatic disease (n=150), LMWH significantly prolonged survival (hazard ratio =0.50; 95% CI, 0.27–0.95), as compared with VKA [48].

In the aforementioned study by Meyer et al.[14], total mortality and cancer progression were secondary outcomes and did not differ significantly among the treatment groups (p=0.07 and p=0.25, respectively), although a trend for reduced mortality with LMWH as compared with 3 and 6 months of warfarin treatment was observed.

Other studies have evaluated the effects of LMWH in cancer patients without VTE. In the FAMOUS study by Kakkar et al.[49], patients with advanced malignant disease were randomly assigned to receive LMWH in a prophylactic dose or placebo for 1 year. In the primary analysis of the whole cohort, no difference in survival was seen between the two groups. Similarly to the CLOT analysis, in the FAMOUS study only the subgroup of cancer patients with a good prognosis was claimed to benefit in terms of prolonged survival from 1-year dalteparin, as compared with placebo.

Recently, in a double-blind, placebo-controlled study, patients with a diagnosis of incurable solid malignant tumors were randomly assigned to receive 6 weeks of LMWH or placebo [50••]. LMWH administration significantly prolonged survival as compared with placebo, with the greatest effects for the subgroup of patients with a better prognosis at baseline. The overall hazard ratio for mortality was 0.75 (95% CI, 0.59–0.96) with a median survival of 8.0 months in the nadroparin arm vs 6.6 months in the placebo group. Major bleedings occurred in 3% and 1% of the nadroparin and placebo group, respectively (p=0.12).

Additionally, observational studies have suggested an improvement in prognosis for patients with advanced, hormone-refractory prostate cancer [51] and malignant melanoma [52] receiving LMWH. Finally, LMWH has been reported to enhance the antineoplastic effect of chemotherapy in advanced pancreatic cancer, leading to increased 1-year survival rates (47.7%), as compared with chemotherapy alone (13.5%) (p=0.029) [53].

Conclusions

There is evidence that cancer patients need a form of VTE prophylaxis in certain high-risk settings, but apart from the surgical context the evidence is as yet insufficient to formulate strong recommendations Likewise, there is cumulating proof that certain treatment strategies may be better for cancer patients than other strategies, but the downsides of the various treatment regimens must be further explored and data on the various new antithrombotic agents are awaited.

From the data that are currently available on the anticancer effects of antithrombotic agents, we may cautiously conclude that some agents may be beneficial in some settings. However, additional large, randomized controlled trials are awaited to confirm the survival benefits reported and to specify the possible subpopulations in which the various antithrombotics may be safe and effective at preventing or treating cancer.

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7

Prevention of catheter-related venous thrombosis with nadroparin in patients receiving high dose chemotherapy for hematological malignancies, a randomized placebo controlled study

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Abstract

Background

Hemato-oncology patients treated with intensive chemotherapy usually require the placement of a central venous catheter (CVC). CVCs are frequently complicated by catheter-related thrombosis (CRCVT) which has been associated with an increased risk of pulmonary embolism (PE) and catheter-related infection (CRI). We determined the efficacy and safety of thromboprophylaxis with subcutaneous low molecular weight heparin (LMWH, nadroparin^{R}) administrated once daily in a randomized placebo-controlled double blind trial in patients with hematological malignancies.

Patients and methods

Consecutive patients with hematological malignancies requiring intensive chemotherapy including autologous stem cell transplantation were eligible. The patients were randomized to nadroparin 2850 anti Xa units once daily or placebo subcutaneously for 3 weeks. Venography was performed on day 21 after CVC insertion. Secondary outcomes were bleeding and CRI.

Results

A total of 113 patients were randomized to nadroparin or placebo and 87 patients (77%) underwent venography. The frequency of venographically proven CRCVT was not significantly different between both study groups (9%; 95% CI: 0.002 to 0.16; in the placebo versus 17 %, 95% CI: 0.06 to 0.28, in the nadroparin group). In addition, no difference in the incidence of CRI or bleeding was seen between the groups.

Conclusions

Nadroparin prophylaxis does not prevent CRCVT in patients with hematological malignancies who receive intensive chemotherapy.

Introduction

In the last three decades substantial progress has been made in the treatment of patients with hematological malignancies. In particular, high dose chemotherapy including autologous stem cell transplantation and better supportive care has improved the prognosis of these patients. Nowadays, most patients undergo a central venous catheter (CVC) placement before intensive chemotherapy for the frequently combined administration of cytotoxic agents, blood products, antibiotics and parenteral nutrition.

However, these CVCs are often complicated by catheter-related thrombosis (CRCVT) and infection. Previous studies have shown that the incidence of venographically proven CRCVT in cancer patients treated with high dose chemotherapy varies considerably and ranges between 27% and 66% [1-3]. Importantly, although the majority of these cases are asymptomatic, prospective studies have shown that 15% of CRCVTs are accompanied by (silent) pulmonary embolism (PE) [1;4]. In addition, CRCVT and in particular asymptomatic CVCRT increase the risk of catheter-related infections (CRI)[5] and subsequent catheter-related sepsis (CRS) [6]. Besides the high risk of fatal outcome of CRS in neutropenic patients, infected catheters have to be often replaced since administration of parental nutrition, intravenous medication and blood products remain indicated.

It is a matter of debate whether thromboprophylaxis is indicated in cancer patients with CVC, and if so, which type of drug or regimen can be recommended. Several studies have evaluated the efficacy of low dose warfarin or heparin prophylaxis in a variety of cancer patients with CVC's [7-15], but do not allow any firm conclusion regarding the clinical effectiveness. Most of the studies were hampered by an open study design, small sample size or used different study outcome definitions. A few placebo-controlled trials in cancer patients with venographically proven CRCVT have been recently performed, although these

studies have excluded most patients with hematological malignancies, because of the high bleeding complications [11;15-17].

We therefore performed a prospective, placebo-controlled double-blind randomized trial to assess the effect of low molecular weight heparin (LMWH) on the incidence of CRCVT and CRI in a group of patients with hematological malignancies receiving high-dose chemotherapy including autologous stem cell transplantation. In addition, the bleeding risk of the prophylactic dose of LMWH was evaluated.

Patients and Methods

Patients

Consecutive patients with hematological malignancies who were going to receive a CVC for high dose chemotherapy including autologous stem cell transplantation were eligible for the study. Patients were excluded in case of: age below 17 years, allergy for intravenous contrast medium; previous CRCVT; current use or indication for anticoagulant treatment; acute promyelocytic leukemia (APL); previous CVC; evident hemorrhagic diathesis or renal failure (creatinine> 200 μmol/L).

Study design

This study was designed as a single center, prospective, randomized, placebo controlled double blind trial, to evaluate the efficacy and safety of LMWH 2850 anti Xa units once daily (nadroparin) for the prevention of CRCVT in patients with hematological malignancies. Patients were randomized to receive either once daily nadroparin or placebo injections subcutaneously. The study medication was started 2 hours before insertion of the CVC and was continued for three weeks or until the day of CVC removal, whichever came first. The CVC was inserted

according to a standard protocol under sterile conditions. The study was performed at the Department of Hematology of the Academic Medical Center in Amsterdam, the Netherlands. The study protocol was approved by the local ethics committee, and all participating patients gave written informed consent.

Outcomes

The primary outcome was venographically proven CRCVT. CRCVT was defined as either occlusive or non-occlusive thrombosis of the vein in which the CVC was placed or a contiguous vein. Patients with clinically suspected CRCVT prior to venography were investigated by duplex ultrasonography first. A positive ultrasound was also considered to be diagnostic for CRCVT. A normal ultrasound had to be followed by venography. In all other patients, venography was scheduled for day 21 after CVC insertion or earlier in case of premature removal of the CVC. Venography was performed according to standard procedures using a distal vein in the ipsilateral hand or arm to inject the contrast medium. All venograms were independently adjudicated by an expertise radiologist using *a priori* found criteria[18] without knowledge of treatment allocation.

Occlusive CRCVT was defined as complete stasis of contrast with filling of collateral veins. In case of occlusive CRCVT, the catheter was removed and therapeutic anticoagulantion was started and continued for three months. Non-occlusive CRCVT was defined as an intravascular filling defect with normal flow to the superior or inferior cava vein. Non-occlusive CRCVT was not treated, but patients were carefully followed-up.

Secondary outcomes of this study were the frequency of bleeding, the incidence of catheter colonization, CRI and CRS. Bleeding was classified as major, clinically relevant non-major or minor bleeding. Major bleeding was defined as overt bleeding with a fall in hemoglobin of 2 g/dL or more, or leading to a transfusion of 2 or more units of packed red blood cells, or bleeding in a critical

Table 1. Definitions of the different types of CVC-related infections

CVC related infections	
1. Systemic CVC-related infection	presence of fever (body temperature >38.5 C) or hypothermia (body temperature <36 C) with one or more positive peripheral drawn blood cultures (for Staphylococcus epidermidis at least two positive cultures are required) in combination with either a positive blood culture drawn from the catheter, or tip colonisation (15cfu) with the same organism, or with a purulent insertion point with the same organism in culture as the blood cultures.
2. Insertion site infection	a purulent insertion point with or without localized findings such as pain, erythema or tenderness without fever.
3. CVC colonisation:	At least more than 15 colony-forming units (cfu) found on the catheter tip through the rolling method without systemic signs of infection

organ such as intracranial, retroperitoneal or pericardial, or contributing to death. Clinically relevant non-major bleeding was defined as overt bleeding not meeting the criteria for major bleeding and included skin hematoma larger than 100 cm^2, epistaxis lasting more than 5 minutes or repetitive (i.e. two or more episodes within 24 hours) or leading to an intervention (packing, electro coagulation), macroscopic hematuria if spontaneous or lasting for more than 24 hours after instrumentation (e.g. catheter placement or surgery), or any other bleeding type that was considered to have clinical consequences for the patient. All other bleeding episodes not meeting the criteria for clinically relevant bleeding were classified as minor bleeding. Since prolonged exposure to heparins may be associated with heparin-induced thrombocytopenia (HIT) all patients underwent screening antibody screening on day 14. HIT was only diagnosed if there was a clinical suspicion and positive antibodies against the heparin-platelet factor 4. Antibody screening for

HIT was performed with the previously described PF4-heparin enzyme-linked immunosorbent assay (ELISA) [20;21].

CVC-related infection were distinguished in Systemic CVC-related infection, Insertion site infection, and CVC colonisation according to well established definitions (Table 1) [19]

Statistical analysis

The study was powered to find a reduction in CRCVT of minimally 50% with an anticipated incidence rate of 60% in those receiving placebo. It was therefore estimated that 50 patients per group were required. Patients were randomized in the placebo group or intervention group. Baseline characteristics for continuous variables were expressed depending on the distribution of the data, as mean (SD) or median (range).The primary outcome was venographically proven CRCVT and was dichotomously scored. Differences between the two treatment groups were analyzed using the X^2 test. Statistically significance was established at $p<0.05$. All analyses were performed using SPSS

Results

Patient population

A total of 202 consecutive patients were eligible. Forty-five patients were excluded for the following reasons: 3 (3%) patients had an allergy to contrast medium; 16 (18%) patients had an indication for anticoagulation; 8 (9%) patients had a previous CVC in the same vein; 1 (1%) patient had an evident hemorrhagic diathesis; 3 (2%) patients had renal failure and 14 (16%) patients could not be included because of logistic problems. Of the remaining 157 eligible patients, 44 (28 %) patients refused consent. A total of 113 patients were eventually randomized to nadroparin or placebo (Table 2).The study-treatment groups were

Table 2. Baseline characteristics of the two study groups

Characteristics	**Nadroparin (n=56)**	**Placebo (n=57)**
Age, years (mean +/- SD)	58 +/- 10	55 +/- 13
Gender, female (%)	27 (48 %)	24 (42 %)
Hematological tumors		
acute myeloid leukemia/ MDS RAEB*	23 (41%)	17 (30 %)
acute lymphoblastic leukemia	2 (4 %)	10 (18 %)
multiple myeloma	14 (25 %)	16 (28 %)
(non) Hodgkin lymphoma- relapsed	17 (30 %)	14 (24 %)
Central venous catheter location		
subclavian vein	15 (27 %)	20 (35 %)
left subclavian vein	32 (57 %)	28 (49 %)
right jugular vein	0 9 (16 %)	1 (2%)
left jugular vein	-	8 (14%)

* MDS RAEB; myelodysplastic syndromes refractory anemia and excess blasts.

well balanced with regard to gender, CVC location, and type of hematological malignancies. The majority of patients included had acute myeloid leukemia or multiple myeloma.

CRCVT and CRI

Of the 113 randomized patients, 15 patients (27%) in the nadroparin group and 11 patients (19 %) in the placebo group did not undergo venography due to prior catheter removal because of suspected serious infections (5 patients in each group), logistic problems including catheter removal during weekends (6 patients in the nadroparin group and 4 patients in the placebo group) or withdrawal of informed consent (4 patients in the nadroparin group and 2 patients in the placebo group). Eighty-seven patients (77%) underwent venography which was adequate in all (Table 3). Eleven CRCVT were diagnosed, 7 (17%) in the nadroparin group -

Table 3A. Catheter related complications during the study

Number of patients	**Nadroparin (n=41)**	**Placebo (n=46)**	**p value**
Adequate venography	41	46	
Duration of catheter placement (days)			
Median	20	19	
Quartiles (25-75%)	18 - 21	17 - 20	
Clinical suspected DVT of catheter			
Number (%)	0 (0%)	1(2%)	
Venography			
Thrombosis	7 (17 %)	4 (9 %)	0.49
occlusive	3 (7%)	2 (4 %)	
non-occlusive	4 (10 %)	2(4%)	
No thrombosis	34 (83 %)	42 (92 %)	

Table 3B. Catheter related infection

Number of patients	**Nadroparin (n=56)**	**Placebo (n=57)**	**p**
Infection			
Systemic CVC-related infection	9 (16%)	10 (18%)	0.35
Insertion site infection	0 (0%)	0 (0%)	
CVC- Colonisation	0	2 (4%)	

versus 4 (9%) in the placebo group (p=0.49). Only 1 patient (in the placebo group) had a symptomatic CRCVT which was confirmed by venography. The median number of days between CVC insertion and venography was 20 days (quartile 25-75%, 18-21 days) in the nadroparin group versus 19 days (quartile 25-75%, 17-20 days) in the placebo group.

Nineteen patients (17%) were diagnosed with systemic CRI (9 patients in the nadroparin group versus 10 patients in the placebo group; p=0.35). The micro organisms involved in systemic CRI were 18 cases of coagulase-negative

Staphylococcus infections (8 cases in nadroparin group and 10 cases in the placebo group) and 1 with *Klebsiella pneumonia* (in the nadroparin group). None of the patients had a localized infection of the insertion site. Two patients (4%) in the placebo group had colony forming units on the catheter tip with coagulase-negative *Staphylococcus* in both cases. CRCVT and CRI coincided in 2 out of 7 patients in the nadroparin group and 2 out of 4 patients in the placebo group.

Safety

There were no cases of major bleeding (Table 4). Clinically relevant non-major bleeding occurred at a similar rate in the nadroparin and placebo group. Minor bleeding was experienced by 5 patients (9%) in the nadroparin group versus 2 patients (4 %) in the placebo group. Two patients had positive serology for antibodies against platelet factor 4-heparin complexes but no clinical suspicion of HIT. One of these 2 patients developed thrombosis but had no persistent thrombocytopenia and was uneventfully treated with nadroparin in therapeutic dose.

Table 4. Safety Outcomes

Number of patients	**Nadroparin (n=56)**	**Placebo (n=57)**
Frequency of bleeding		
Major bleeding	0	0
Clinically relevant non-major bleeding	2 (4 %)	2 (4 %)
Minor bleeding	5 (9 %)	2 (4 %)
Frequency of positive HIT* serology	2 (4%)	0 (0%)

* HIT; Heparin induced thrombocytopenia

Discussion

In this study, no beneficial effect of prophylactic nadroparin on the incidence of CRCVT was demonstrated (9%; 95% CI: 0.002 to 0.16; in the placebo versus 17 %; 95% CI: 0.06 to 0.28; in the nadroparin group). In addition, no significant difference in CRI or bleeding was observed between the groups.

Previous studies in cancer patients have reported a wide range of CRCVT rate with incidences up to 66%[22]. Lower incidence rates of CRCVT have been reported in more recent studies [11;13;15] as compared to the initial observations [8;14;22;23] The frequency of CRCVT in our study is very comparable to what was found in three recent studies which demonstrated a venographycally proven thrombosis rate between 3.4% and 18%[11;15]. Moreover, two of these studies did not find any effect of LMWH thrombo-prophylaxis on the incidence rate of CVCRT whereas a non-statistically significant reduction in the rate of thrombosis was found in the third study of Verso and colleagues [15]. Since the proportion of patients with a hematological malignancy varied from 9%[13], 10% [11]and 100%[15] in these studies, the reduced incidence of CRCVT can not be explained by the fact that more hematological patients were included than in the older studies. Also no difference in the treatment period was found between the studies that could explain the difference in thrombosis. Probably other factors such as catheter type, standardized catheter care and improved supportive care may be responsible for the lower rate of CVCRT in more recent studies.

Some aspects of our study require comment. Firstly, the study has a limited sample size relatively to the low incidence of CVCRT. However, since thrombosis rates in our study appeared to be even slightly higher in the nadroparin treated group (17%) as compared to the control group (9%) it is unlikely that a potential effect of nadroparin was missed. Secondly, approximately a quarter of the randomized patients were not analyzed by venography for various reasons. Although this is a considerable proportion, the number of missed venographies was

similar in both groups. Furthermore, the study groups (with and without venography) were comparable with respect to baseline and prognostic variables. Therefore, the internal validity of the study remains high. Thirdly, although our study is a single center study, we included a representative group of patients with hematological malignancies requiring high dose chemotherapy and hence we believe that our findings can be extrapolated to these types of patients.

In conclusion, this study showed that the actual risk for CRCVT in patients with hematological malignancies is lower (approximately 13%) than suggested in earlier studies in cancer patients. Furthermore, prophylactic administration of nadroparin had no effect on the incidence rate of CRCVT. Although thromboprophylaxis with nadroparin appeared to be safe in this group of patients with a high risk of bleeding, it cannot be recommended for the prevention of catheter-related thrombosis or infection in patients with hematological malignancies.

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8

The prognostic value of the D-dimer test in cancer patients treated with and without low-molecular-weight heparin

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The activation of coagulation in cancer patients has been implicated in both tumor progression and development of metastasis [1]. In this context, plasma levels of D-dimer (DD), a marker of endogenous fibrinolysis, may have clinical and prognostic significance with higher DD values being associated with a more advanced stage of disease and shortened survival [2–5]. Given the involvement of the hemostatic system in tumor progression, the therapeutic use of anticoagulants could have a positive impact on the survival of cancer patients and several studies have indeed suggested that low-molecular weight heparin (LMWH) might prolong survival in cancer patients with [6–8] and without venous thromboembolism (VTE) [9–11]. Recently, the Malignancy and Low-molecular weight Therapy (MALT) study, randomized 302 patients with advanced malignancy without VTE to a 6-week LMWH (nadroparin) or to placebo [11]. In the intention-to-treat analysis, the mortality rate was found to be significantly lower in the LMWH group (p=0.02). The aim of this study was to retrospectively assess the prognostic value of DD plasma levels in patients with incurable cancer both at study entry and after 6-week treatment with LMWH and to evaluate the correlation between changes in DD plasma levels and survival. The study population was represented by the subgroup of the MALT study patients included in two teaching hospitals in the Netherlands (66 placebo-and 75 LMWH-treated patients). DD plasma levels were determined (MDA, bioMerieux, Durham, NC, USA) at study entry, at 1–2 weeks (during the treatment phase), and at 6 weeks (end-of-treatment phase). At study entry, DD levels were available for all the 75 nadroparin treated patients and in 64 of the 66 patients of the placebo group. The DD measurements during treatment were available for 41 patients treated with nadroparin and for 50 patients who received placebo whereas and at the end of the study treatment DD values were available for 35 and 40 patients, respectively (p=0.04). At study entry, most of the clinical characteristics were balanced between the two study groups. Breast cancer was more frequent among the nadroparin patients, whereas cervical and colorectal

cancer were seen more often in the placebo group. As compared with the placebo patients, those on nadroparin received more often radiotherapy (p=0.02), but less frequently chemotherapy (p=0.0001). There was no difference in VTE events between the two groups (eight and seven cases respectively).

In the placebo group, 55 patients died during the follow-up period (median, 6.5 months). DD plasma levels were associated with increased mortality after adjustment for other prognostic factors (p=0.001). Patients with DD plasma levels below the predefined cut-off value of 0.5 μg mL^{-1} did not have a better prognosis than patients with DD plasma levels above this value However, both the median (0.78 μg mL^{-1} and the upper-tertile (1.07 μg mL^{-1} cut-off values divided the patients into two groups with a statistically significant difference in prognosis (p=0.01 and p=0.001, respectively; Table). For instance, patients with a DD value below the upper-tertile had a median survival of 8 months whereas those with DD levels above this had a median survival of 4 months. In other words, the 6-month survival was 72% in patients with a DD plasma level below this cut-off vs. 43% in those with a value above. The 12-month survival rates were 38% and 0%, respectively. DD plasma levels during and at the end of the 6-week placebo period were related to prognosis when taken as continuous variables (both p<0.001). However the statistical significance of the various cut-off values varied considerably (Table). As compared with the placebo group, patients who received LMWH had DD levels similar at study entry, but significantly reduced at the end of treatment (0.60 μg mL^{-1} vs. 0.83 μg mL^{-1}, p=0.03). In the LMWH group, 64 of the 75 patients died during follow-up (median 8 months). When adjusted for other prognostic variables, the DD levels at study entry showed an inverse correlation with survival (p=0.06), but none of the predefined cut-off points divided the LMWH-treated patients into groups with statistically different prognosis. However, during-and end-of-treatment median DD were significantly associated with survival (Table). In both the placebo and LMWH group, DD levels varied over time. Of the

Table. DD levels and 6-month survival percentage in the placebo and LMWH group

DD cut-off value	Placebo group		LMWH group	
	Below cut-off	**Above cut-off**	**Below cut-off**	**Above cut-off**
Study entry				
Median	72 (56-87)	53 (35-70)*	76 (62-89)	60 (45-76)
Upper tertile	72 (58-85)	43 (22-64)**	73 (61-86)	58 (39-77)
During treatment-phase				
Median	77 (61-93)	62 (43-82)	88 (76-100)	40 (15-65)**
Upper tertile	77 (63-91)	53 (28-78)	77 (63-91)	33 (0-71)
End of treatment phase				
Median	83 (65-100)	68 (49-88)*	91 (80-100)	75 (51-99)*
Upper tertile	81 (66-97)	61 (35-88)**	90 (79-100)	67 (29-100)

DD median=0.78 μg/mL; DD upper-tertile=1.07 μg/mL. p-values were calculated for the overall survival difference and adjusted for other prognostic variables (WHO status, life-expectancy, type of cancer, histological type of the cancer and anti-neoplastic therapy during the study period). Ninety-five percent confidence intervals are given in parentheses. *p<0.05; **p<0.01

38 patients in the placebo group, who had data available at study entry and end-of-treatment, 13 showed a median reduction in DD plasma levels of 0.20 μg mL^{-1}, and 25 had a median rise of 0.32 μg mL^{-1}. In the 35 LMWH-treated patients who had data available at study entry and end-of-treatment, 24 showed a median fall in DD plasma levels of 0.35 μg mL^{-1}, and 11 a median rise of 0.44 μg mL^{-1} (p=0.003). In the placebo group, a rise in DD concentrations was associated with a median

survival of 8 months vs. 11 months for a reduction, with corresponding 6-month survival rates of 76% and 77% (not statistically significant difference). Similar differences in prognosis were observed in the LMWH-treated patients with median survivals of 7 months vs. 13 months for a rise and a reduction in DD, respectively (6-month survival: 73% and 92%, respectively). In the LMWH-treated group, the difference in survival between rise and reduction was statistically significant ($p=0.005$) and remained so even after adjustment for other prognostic variables.

In the present study, we confirmed the prognostic value of DD plasma levels in a cohort of cancer patients with incurable solid malignant tumors, in agreement with previous investigations [2–5]. In patients treated with LMWH, high DD levels at study entry were associated with a worse prognosis, and high DD at the end of the 6-week LMWH treatment significantly correlated with reduced survival. Consistently, a reduction in DD concentrations over time (from start to end of treatment) correlated with a better outcome as compared with a rise in DD plasma levels. Evidence is accumulating that LMWH may have antineoplastic properties [6–11] and our data suggest that DD plasma levels could become helpful in monitoring the LMWH anticancer effects in that patients who show a reduction in DD plasma levels may be those who benefit the most from LMWH administration. Moreover, the present findings suggest that the effects of LMWH are more pronounced in patients with high DD concentrations at the start of treatment. This is especially interesting as the current data show that these patients have a worse prognosis, in contrast with previous observations suggesting a greater benefit from LMWH in patients with a better prognosis [7,9,11]. Some patients in the nadroparin group did not respond and showed an increase in DD levels associated with a bad prognosis, possibly because of different sensibility of the several cancer types or cancer stages to LMWH. Given the sample size and the retrospective nature of the current study, the results should be interpreted with caution and mainly used as hypothesis generating. Moreover, these findings leave

the question of the best prognostic DD cut-off still unanswered, also because of the wide variety of cut-off levels applied [2–5].

In conclusion, our data indicates a prognostic role of the DD test in patients with advanced cancer. The value of the DD plasma levels as prognostic marker to guide anticancer therapy with LMWH deserves further clinical evaluation.

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9

Plasma cytokines and P-selectin levels in advanced malignancy: prognostic value and impact of low-molecular-weight heparin administration

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Abstract

Background. The survival benefit described in patients with cancer treated with low molecular weight heparin (LMWH) may result from a LMWH-mediated effect on the immune system or on the cross-talk between platelets and tumor cells.

Methods. Plasma levels of interleukin (IL)-10, IL-6, interferon (IFN)-γ, and P-selectin were measured in patients with advanced stage malignancy who were randomized to receive standard cancer care with or without the addition of LMWH.

Results. Patients with IL-6 levels above the median had a median survival of 6.5 months versus 8.8 months for those with values below this cut-off (p=0.02). IL-10 levels were found to be similarly correlated with survival such that IL-10 concentrations above the detection limit of the assay were associated with a doubled risk of dying in comparison to undetectable IL-10 (p=0.02). No significant association was found between survival and circulating levels of IFN-γ. For P-selectin, patients with values below the fourth quartile had a median survival of 8.8 months versus 6.5 months for patients with levels above the fourth quartile (p=0.02). In multivariate analysis, IL-10 remained an independent unfavorable prognostic factor (hazard ratio, 2.13; 95% confidence interval, 1.08–4.20). In patients treated with LMWH, the plasma levels of IL-6, IL-10, IFN-γ, and P-selectin demonstrated similar correlations with survival. However, none of the markers was associated with the beneficial survival effects observed with the administration of LMWH.

Conclusions. IL-10, IL-6, and P-selectin levels predicted a poor outcome in patients with advanced stage malignancy. The prolongation in survival observed with LMWH in patients with cancer apparently cannot be explained by a LMWH effect on these circulating markers.

Inflammation and innate immunity are considered essential in the defence against cancer.[1–4] Several studies have suggested that host responses are often defective in patients with cancer favouring, rather than opposing, the progression of the tumor.[1,2] Both tumors and innate immunity cells can produce immunomodulating agents that divert the host-protective mechanisms and suppress tumoricidal activity leading to a predominant humoral immunity, which is ineffective against the tumor.[1–4]

Accumulating data suggest that the plasma levels of some cytokines might reflect the activity of the immune system against the tumor and correlate to the extent of disease and prognosis.[5–19] Interleukin (IL)-6, for example, can stimulate cell growth and angiogenesis, and induce resistance to therapy in cancer cells [1,3,20] and high serum IL-6 levels have been found to predict a poor clinical outcome. [5–8,10–16]

In addition, animal studies have shown an impairment in the host response against the tumor due to an abnormal production of IL-10 by the malignant and host immune cells. [21,22] To our knowledge, the prognostic value of IL-10 levels in humans remains uncertain with conflicting data reported in the literature.[9,17–19,23] Similarly, the possible prognostic value of other cytokines, such as interferon (IFN)-γ, that promote a cell mediated immune response, has not been clearly established to the best of our knowledge. [24–26]

Recently, a role for low-molecular-weight heparin (LMWH) in the management of patients with cancer has been claimed after several clinical studies demonstrated a prolongation of survival in patients with cancer who were treated with LMWH in addition to standard cancer care.[27–32] The beneficial effects of LMWH on survival could be related to an effect on the host immune response, although data are limited, with discordant results published across the studies, possibly due to differences in experimental conditions. [33–38]

Another possible mechanism with which to explain the anticancer activity of LMWH could be that LMWH interferes with the cross-talk between platelets and tumor cells. Platelets have the potential to promote several steps of the tumor progression and markers of platelet activation have been correlated with a worse prognosis in patients with cancer.[39–41] It has been suggested that heparin can inhibit tumor metastasis by blocking P-selectin,[42] a marker of platelet activation that has been associated with mortality and recurrent disease risk in patients with malignancy.[43]

The aim of the current study was, first, to evaluate whether the plasma levels of P-selectin, IL-6, IL-10, and IFN-γ predict survival in patients with advanced stage cancer. Second, we assessed whether the levels of these markers respond to LMWH treatment.

Materials and Methods

Patients

Plasma samples were obtained from patients participating in the malignancy and low molecular weight therapy (MALT) trial.[28] In that study, 302 patients without signs or symptoms of venous thromboembolism and with a diagnosis of advanced stage solid malignant tumor that was not curable with the standard available treatment were randomized to receive a 6-week cycle of subcutaneous nadroparin (Sanofi-Synthelabo, Paris, France) or placebo. The characteristics of the MALT patients have been described in more detail elsewhere.[28] Briefly, patients with a life expectancy <1 month, an indication for anticoagulant treatment, a contraindication for LMWH, thrombocytopenia (defined by a platelet count of <50,000 platelets/mm^3), or who were pregnant were excluded from the study. At baseline, data were collected concerning the demographic characteristics, as well as information regarding the type, histology, stage, and

duration of cancer. Moreover, the World Health Organization (WHO) performance status and the physician's assessment of life expectancy (<6 mos vs. ≥6 mos) were determined. Patients were followed until death or until the end of the study, with a median follow-up of 12 months. In the intention-to-treat analysis, treatment with nadroparin was associated with a significantly prolonged survival compared with placebo with the greatest effects noted in the subgroup of patients with a better prognosis at baseline. The overall hazards ratio (HR) for mortality was 0.75 (95% confidence interval [CI], 0.59–0.96) in favour of LMWH.

In the current analysis, IL-6, IL-10, IFN-γ, and P-selectin levels were determined in an unselected group of 141 patients from the MALT study for whom plasma samples were available. Of these patients, 75 were randomized to receive nadroparin.

Study Objectives

We sought to evaluate 1) the prognostic value for survival of circulating levels of IL-6, IL-10, IFN-γ, and P-selectin in all the 141 patients at the time of entry into the study; 2) the association between these circulating markers and prognosis in the group of patients treated with LMWH; and 3) whether the beneficial survival effects observed in the MALT study were related to the influence of LMWH on plasma levels of soluble P-selectin or cytokines. This latter effect could be reflected by changes in immune mediators and plasma concentrations. Given the role of IL-6, IL-10, and IFN-γ in the host response and in the promotion of tumor progression, an increase in IFN-γ and a decrease in IL-6 and IL-10 was hypothesized to result from the administration of LMWH.

Blood Sampling and Sample Analysis

At the start of study treatment (Time 0) and at 6 weeks (the end of LMWH treatment phase), a blood sample was obtained and anticoagulated with sodium

citrate (0.109 M, 1/10 volume/volume). Platelet-poor plasma samples were frozen in small aliquots and stored at 70 °C until analysis. IL-6, IL-10, and IFN-γ levels were measured with the Bio-Plex cytokine assay (Bio-Rad, Veenendaal, The Netherlands). The detection range was 0.49–32.000 pg/mL. P-selectin plasma levels were measured by DuoSet enzyme-linked immunoadsorbent assay (R&D Systems, Abingdon, U.K.) with a detection limit of P-selectin concentrations of 0.21 ng/mL.

Statistical Analysis

The chi-square and the Mann–Whitney U tests were used for descriptive purposes. Survival estimates were calculated according to the Kaplan–Meier method with the analysis based on the time from randomization to death. Patients alive at the end of follow-up were censored. The Cox regression model was used to adjust for potential confounding variables: life expectancy (<6 mos vs. ≥6 mos), WHO performance status (≤1, 2, ≥3), concomitant treatment (chemotherapy, radiotherapy [RT], hormonal therapy, or other antineoplastic treatment), type of carcinoma (breast, colorectal, cervical, or other), and histology (adenosquamous carcinoma, squamous carcinoma, or other). Ninety-five percent CIs were calculated when appropriate. IL-6, IL-10, IFN-γ, and P-selectin demonstrated a non normal distribution and therefore median values were calculated. Because plasma IL-10 was detectable in only a fraction of patients, the predictive value of IL-10 was assessed with IL-10 as a dichotomous variable taking the value 0 when below the detection limit or 1 otherwise. Finally, the association between IL-6, IL-10, IFN-γ, and P-selectin levels and prognosis as well as the effects of LMWH on these circulating markers (Mann–Whitney–Wilcoxon test) were evaluated in the group of patients treated with nadroparin.

The statistical analysis was performed using the SPSS package for Windows, version 11.0 (SPSS Inc., Chicago, IL).

Results

The Entire Study Group

The characteristics of the study population are detailed in Table 1. The median levels at the time of entry into the study for IL-6, IL-10, IFN-γ, and P-selectin were, respectively, 9.4 pg/mL (range, 0.6–438.8 pg/mL), 1.2 pg/mL (range, 0.6–24.1 pg/mL), 3.6 pg/mL (range, 2.0–322.2 pg/mL), and 4.3 ng/mL (range, 0.7–11.9 pg/mL) (Table 1).

Table 1. Main baseline characteristics of the study population (n=141)

Characteristic	
Age (at inclusion), years	62.3 (38.4-85.7)
Gender (Males/females)	83/58
Weight, kg	73.2 (±11.5)
Months of follow-up	7.2 (0.6-51.1)
Months of cancer duration at baseline	16 (0-217)
Months of metastasis duration at baseline	5 (0-84)
WHO status (%)	
0 or 1	90.8
2	7.1
3 or 4	2.1
Life Expectancy (%)	
less than 6 months	48
at least 6 months	52
IL-6, pg/mL	9.4 (0.6-438.8)
IL-10, pg/mL	1.2 (0.6-24.1)
IFN-γ, pg/mL	3.6 (2.0-322.1)
P-selectin, ng/mL	4.3 (0.7-11.9)

For variables with a normal distribution, values are presented as the mean (standard deviation) while for those without a normal distribution the median (range) is used. WHO=World Health Organization; IL=interleukin; IFN=interferon.

The plasma levels of IL-6 predicted a shorter survival, with both the median and the quartiles of the IL-6 distribution dividing patients into groups with a significant difference in prognosis. The median survival for patients with IL-6 concentrations above the median was 6.5 months compared with 8.8 months for patients with IL-6 values below this cut-off (p=0.02). In other terms, the risk of dying was 56% higher in patients with IL-6 values above the median (HR=1.56; 95% CI, 1.05–to 2.30) (Fig. 1). It is noteworthy that in the group of patients who

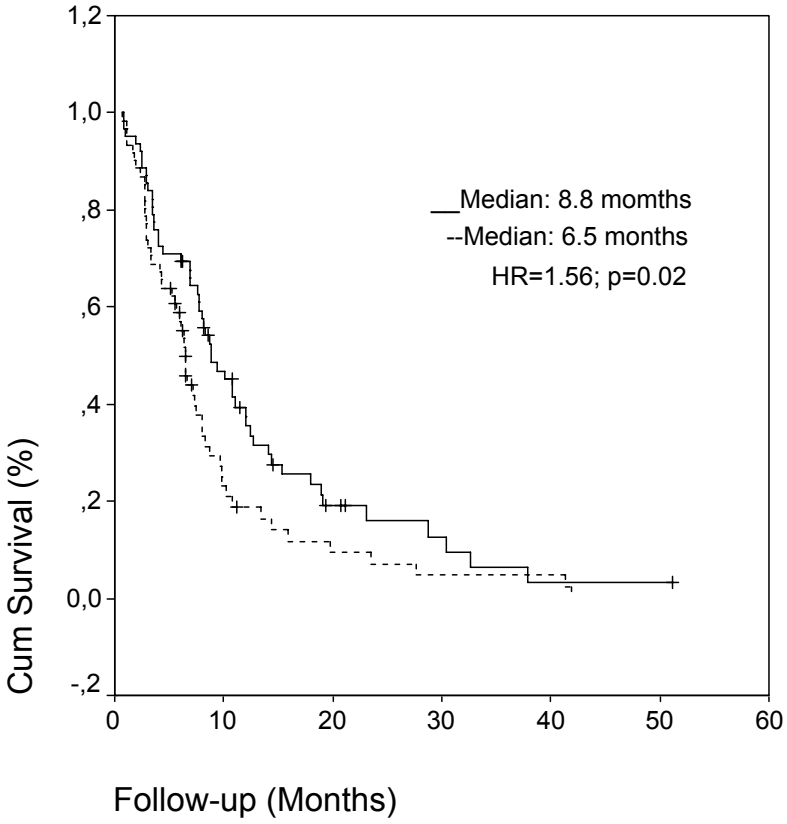

Figure 1. Association between survival and IL-6 median for the whole study group at entry into the study. The vertical axis represents the cumulative survival (%) and the horizontal axis the follow-up (months). The dotted line shows survival of patients with IL-6 values above the median (9.4 pg/mL), the continuous line of patients with values above this level.

were still alive at the end of the 6-week study treatment period (n=73), IL-6 maintained this prognostic power (8.0 months vs 11.0 months, respectively; p=0.007). The association of IL-6 with an adverse outcome was even more remarkable when the analysis included the extreme values of the IL-6 distribution. The median survivals in patients with IL-6 levels above and below the fourth quartile (24.3 pg/mL) were remarkably different (7.3 mos and 13.4 mos, respectively; p=0.018).

Circulating IL-10 was similarly correlated with a poor prognosis. In patients in whom IL-10 was detectable, the median survival was lower than in patients with IL-10 values below the detection limit of the assay (3.3 mos vs 8.2 months; p=0.02). This difference corresponded to a risk of death that was 2 times higher in patients with measurable IL-10 (HR=2.14; 95% CI, 1.10–3.89) (Fig. 2A). At the end of the study treatment period, the number of patients with IL-10 within the range of the assay was too small to assess the IL-10 predictive value (n=5).

No association with survival was evident using the median IFN-γ levels as a cut-off point (p=0.37). It is noteworthy that prognosis tended to improve with increasing IFN-γ quartiles, and IFN-γ concentrations above the fourth quartile (5.1 pg/mL) were related to a longer median survival (13.4 mos) compared with lower levels (8.4 mos) (p=0.78).

The median survival was comparable between patients with P-selectin levels above or below the median (8.0 mos vs 8.2 mos; p=0.61). Significant differences in prognosis were evident at the extremes of the P-selectin distribution. In particular, patients with P-selectin concentrations higher than the fourth quartile (5.4 ng/mL) had a shorter survival (6.5 mos) than those with lower values (8.8 mos) (HR=1.72; 95% CI, 1.1–2.7). Such association with poor prognosis was, however, not found to be statistically significant at the end of the study treatment (8.0 mos vs 10.1 mos, respectively; p=0.46).

In a univariate analysis, the study treatment (nadroparin or placebo); patient weight; a life expectancy ≥6 months; the treatment received during the study period (chemotherapy, surgery, RT, or hormone treatment); and IL-10, IL-6, and P-selectin levels were all found to be significantly associated with survival. When adjusting in a Cox multivariate model for all possible confounders, IL-10 remained an independent prognostic marker (HR=2.13; 95% CI, 1.08–4.20). After regression analysis, the predictive value of both IL-6 and P-selectin levels was maintained, but was no longer statistically significant (HR=1.44 [95% CI, 0.96–1.44] and HR=1.65 [95% CI, 0.99–2.73]).

Patients Treated with LMWH

In the group of patients randomized to receive nadroparin, a similar inverse relation with prognosis was evident for IL-6, IL-10, and P-selectin at baseline, as in the whole study group. A shorter median survival was observed for IL-6 above the median and above the fourth quartile in comparison to lower levels (6.4 mos vs 10.1 mos [p=0.10] and 3.3 mos vs. 8.8 mos [p=0.04], respectively). In the same way, IL-6 concentrations at the end of the study treatment period represented an unfavourable prognostic marker (p=0.001).

Patients with detectable IL-10 were found to have a poorer outcome than those in whom this cytokine was not detectable (median survival of 3.0 mos vs. 8.8 mos; p=0.0008) (Fig. 2B). For both IFN-γ and P-selectin, there was no difference in prognosis noted with any of the cut-offs. On multivariate analysis, IL-10 remained a predictor of poor prognosis (HR=10.8; 95% CI, 2.99–39.4). In contrast to what was expected, none of the circulating markers evaluated was affected by nadroparin and none was found to be correlated with the survival benefit that was evident in the patients randomized to receive LMWH. Surprisingly, an increase in IL-6 from 8.1 pg/mL at baseline to 10.4 pg/mL at the end of the study treatment was observed in patients who received nadroparin (p=0.03). Although IL-10 and P-

selectin concentrations were basically unchanged after the administration of nadroparin, plasma IFN-γ levels increased when compared with baseline (1.7 pg/mL vs 3.6 pg/mL; p=0.48).

Discussion

Detectable IL-10 predicted shorter survival in patients with advanced stage malignancy, and also after correction for other potentially confounding variables. These results are in keeping with previous data that suggested an involvement of IL-10 in the immune escape mechanisms of the tumor,[21,22] and a prognostic value of plasma IL-10 levels in patients with cancer.[9,17–19] The correlation between IL-10 and a worse outcome, however, has not been always consistent.[5] Differences in the spectrum of included cancers and/or in the disease severity likely explain these discrepant results. The size of the current study sample did not allow us to perform a subgroup analysis for cancer type. However, when correcting for tumor type and tumor histology in the multivariate analysis, the relation between IL-10 and survival remained unchanged, suggesting a similar prognostic role for IL-10 across different types of cancer.

Circulating IL-6 has been found to be associated with an adverse outcome in a variety of tumors.[5,6,10,12–16] In agreement with the available literature, the current analysis found shorter survivals in those patients with high IL-6 levels (Fig. 1). The progression of the malignancy could be promoted by IL-6 in several ways, such as an induction of vascular endothelial growth factor release, the activation of the coagulation system, or a modulating effect on the immune system.[1–4,38,39]

The current study results demonstrate an association between poor prognosis and high levels of IL-10, an antiinflammatory cytokine, as well as high values of IL-6, a pro-inflammatory marker. Therefore, these data appear to support

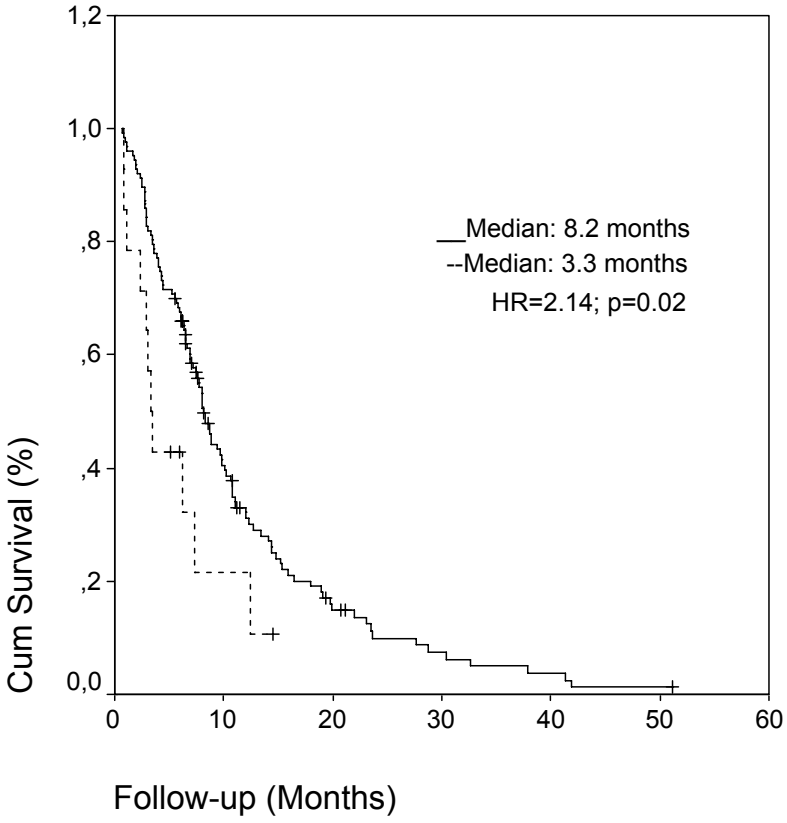

Figure 2A

the notion that despite the general activation of the immune system, host responses remain ineffective against the tumor and would indeed favour the progression of the disease.[1,2]

IFN-γ represents an important marker of the "cell-oriented" immune response.[1,2] Experimental and preliminary clinical studies suggest that IFN-γ could reverse the defective immune response induced by other cytokines such as IL-10, and favour the development of an effective response against the tumor.[24–26] To our knowledge, the current study is the first to evaluate the prognostic value for

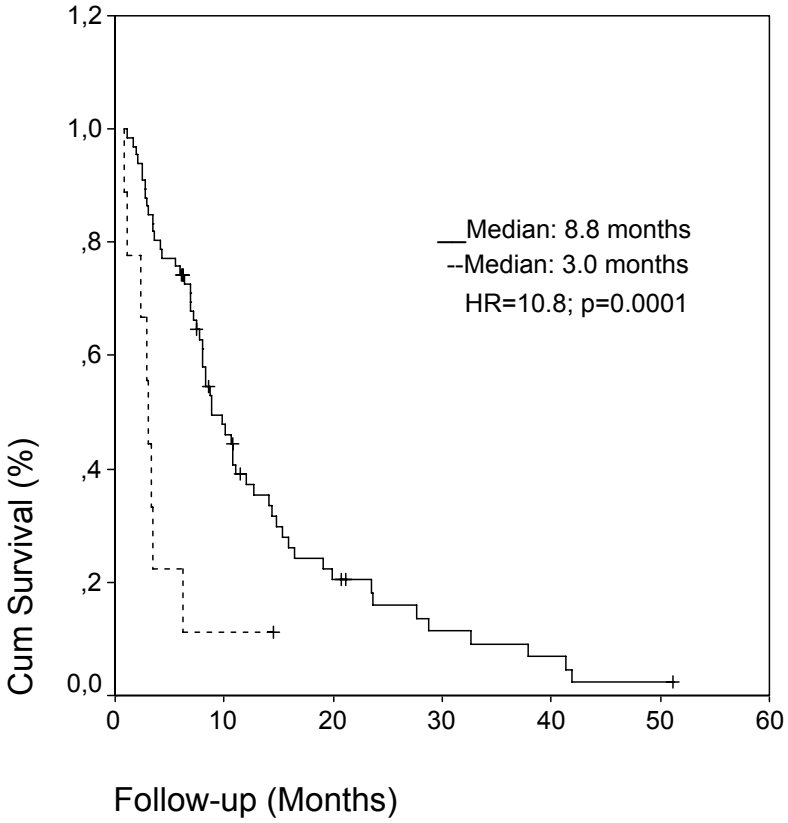

Figure 2B

Figure 2. Association between survival and IL-10 as a dichotomous variable. The vertical axis represents the cumulative survival (%) and the horizontal axis the follow-up (months). IL-10 is defined detectable when within the detection limit of the assay, not detectable otherwise. (2A) Association between survival and IL-10 in the whole group. (2B) Association between survival and IL-10 in the nadroparin group. The dotted line shows survival of patients with detectable IL-10, continuous line of patients with not detectable IL-10.

survival of circulating IFN-γ in patients with cancer. A non statistically significant prolongation in survival was found for patients with IFN-γ above the highest quartile. However, the relevance of this association needs to be evaluated further.

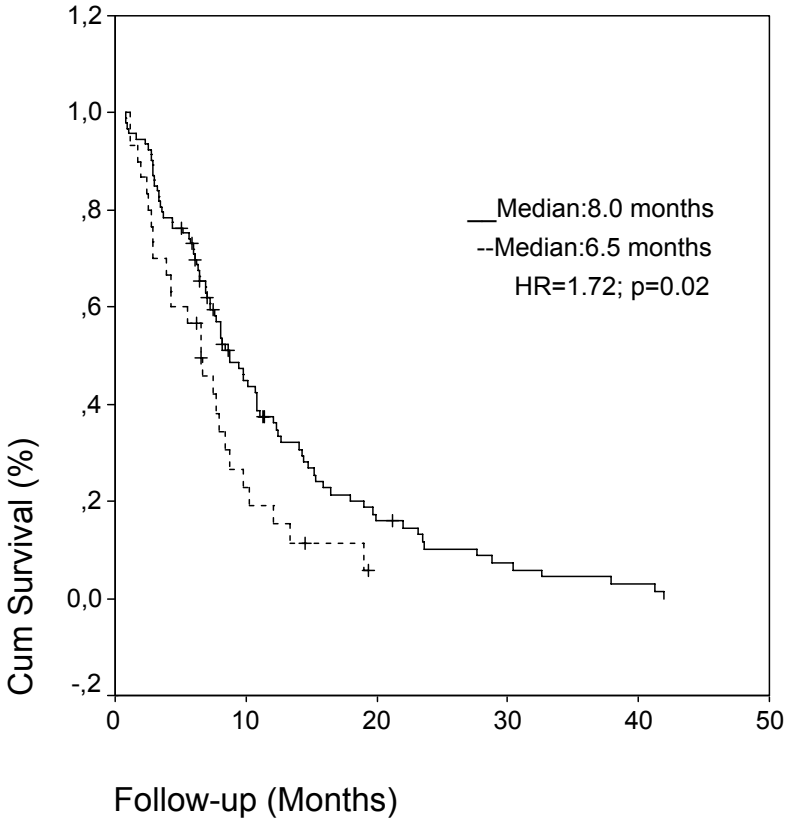

Figure 3. Association between survival and P-selectin for the whole study group at entry into the study. The vertical axis represents the cumulative survival (%) and the horizontal axis the follow-up (months). The dotted line shows survival of patients with P-selectin values above the forth quartile (5.4 ng/mL), the continuous line of patients with values above this level.

Several studies have suggested that platelets can promote tumor progression by regulating angiogenesis[40] or favouring tumor metastasis.[41] Platelet activation could lead to the release of P-selectin, which, in turn, may facilitate the attachment of tumor cells to the vascular wall.[41] Indeed, plasma levels of P-selectin

have been associated with survival and disease recurrences in patients with cancer.[43] The predictive role of P-selectin is supported by our findings (Fig. 3).

In the current analysis, the influence of LMWH on circulating levels of IL-6, IL-10, IFN-γ, and P-selectin also was assessed. Data from a growing number of clinical trials suggest that LMWH does improve the prognosis and prolongs the survival of patients with cancer.[27–32] Although the mechanisms behind the anticancer activity of LMWH remain poorly understood, these could involve an effect on the host immune response.[33–38] However, this hypothesis was not confirmed by the results of the current study, in which none of the measured cytokines was found to correlate with the beneficial survival effects of nadroparin.[28] Although a change in these cytokine concentrations would have given an indication of the general immune system activity against the tumor, the lack of such an effect cannot exclude a possible influence of LMWH on other immune markers or on immune pathways not reflected in circulating markers. In contrast to what was hypothesized initially, nadroparin treatment was associated with a modest increase in IL-6 levels, the clinical relevance of which to our knowledge remains unknown.

The survival prolongation noted with LMWH also could be explained by an effect on pathways such as angiogenesis or on P-selectin–mediated interactions between platelets and tumor cells,[42] although the current data do not support this theory.

Given the relatively small sample size, the results of the current study have to be interpreted with caution and mainly considered as hypothesis generating. Because the investigated group included a broad range of malignancies, it was not possible to determine a hypothetical cancer type-specific effect of LMWH. However, it is reasonable that a potential LMWH anticancer activity directed against general mechanisms of cancer progression, such as the immune system or on platelets, would be less dependent on the type of cancer.

Circulating levels of IL-6, IL-10, and P-selectin appear to be predictive of an adverse prognosis in patients with advanced stage malignancy. Whether IL-10 circulating levels might help in guiding therapeutic decisions in patients with cancer remains to be evaluated. These markers were not sensitive to the LMWH administration, leaving the question of how LMWH positively impacts cancer progression unanswered.

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The hematocrit target in Polycythemia Vera

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Abstract

Polycythemia vera (PV) is a chronic myeloproliferative disorder whose major morbidity and mortality are thrombohemorragic events and progression to acute leukemia or myelofibrosis. Whether hematocrit and platelet count predict such complications remains unclear. The European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) prospective study included 1.638 PV patients. A total of 164 deaths (10%), 145 (8.85%) major thrombosis, and 226 (13.8%) total thrombosis were encountered during 4,393 person-years follow-up (median 2.8 years). In time-dependent multivariable analysis, hematocrit in the evaluable range of 40-55% we encountered in the studied population was not associated with the occurrence of thrombotic events, mortality nor with hematological progression. The hematocrit of patients in the highest and lowest deciles at baseline were maintained within a narrow interval of hematocrit values ranging from 40% to 47% throughout follow-up. High platelet count was associated with a lower progression rate to acute leukemia/myelofibrosis, whereas it had no significant relationship with thrombotic events or mortality. Our findings do not suggest that the range of hematocrit (<55%) and platelet counts (<600 x 10^9/L) we encountered in our population had an impact on the outcome of PV patients treated by the current therapeutic strategies.

Introduction

Polycythemia vera (PV) is a chronic myeloproliferative disorder characterized by panmyelosis, splenomegaly, a predisposition to venous and arterial thrombosis, and a possible progression to myelofibrosis, and acute leukemia (Spivak, 2002; Schafer, 2006).[1;2]In PV, the proliferation of a multipotent hematopoietic progenitor cell leads to the accumulation of red cells, white cells, and platelets. The dominant feature of PV and the "sine qua non" for its diagnosis remains erythrocytosis which is also regarded as the main cause for PV most frequent and serious complications, namely thrombosis and hemorrhagic episodes (Spivak, 2002; Schafer, 2006).[1;2]

Current PV treatment recommendations are to keep hematocrit <45% in males, <42% in women, <36% during pregnancy, and platelet count below 400 x 10^9/L (Spivak, 2002; Schafer AI 2006)[1;2] based on some earlier evidence suggesting a proportional increase of thrombotic events with high hematocrit and platelet count (Schafer, 2006; Pearson *et al*, 1978).[2;3] An aggressive management of these hematologic variables is thus widely practiced, despite the lack of solid data backing this recommendation (Schafer, 2006; Prchal *et al*, 2003).[2;4]

The European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study was recently concluded (Landolfi *et al*, 1997; Landolfi *et al*, 2004; Marchioli *et al*, 2005; Finazzi *et al*, 2005).[5] This large, prospective multicenter project, which included 1.638 PV patients, provides an unique opportunity for a comprehensive reassessment of the prognostic value of hematocrit and platelet count relative to thrombotic events and/or progression to acute leukemia and myelofibrosis.

The aim of this analysis was to evaluate hematocrit and platelet count as possible predictors of thrombotic and/or hematological complications in patients with PV.

Patients, Materials, and Methods

Patients

The characteristics of the ECLAP study have been described previously elsewhere (Landolfi *et al*, 1997; Landolfi *et al*, 2004; Marchioli *et al*, 2005; Spivak *et al*, 2003). [5-7] Briefly, all patients with a most current or conventional diagnosis of PV made according to the criteria established by the PVSG (Berk *et al*, 1995) [8] or by Pearson et al (Pearson *et al*, 1996) [9] were included in a prospective study with no exclusion with respect to age, therapy, or duration of disease. Treatment strategies had to comply with the recommendation of maintaining the hematocrit below 45% and platelet count less than 400 $x10^9$/L. Data regarding clinical outcomes, treatments, and laboratory values during the prospective follow-up were recorded at 12, 24, 36, 48, and 60 months of follow-up. The mean duration of the disease at entry and the duration of the follow-up were 5.0 and 2.7 years, respectively.

Aims of the current study were to determine whether hematocrit and platelet count in the ECLAP study may suggest a "specific target value" to be maintained during the course of the disease or, in other words, if treatment strategies should be more or less aggressive to control the disease. We also attempted to assess if such "specific target value" (if existent) would impact on thrombotic and neoplastic events.

Outcome events

Outcome events were total mortality, major thrombosis (i.e., non-fatal myocardial infarction, stroke, deep venous thrombosis, pulmonary embolism, or cardiovascular death), total thrombosis (i.e., major thrombosis plus transient ischemic attacks, peripheral artery thrombosis, or superficial thrombophlebitis), hematological transformation (i.e. leukemia and myelodysplasia), and myelofibrosis. Myocardial infarction was defined as at least two of the following:

chest pain of typical intensity and duration; ST elevation or depression of 1 mm or more in any limb lead of the ECG, of 2 mm or more in any precordial lead, or both; and at least a doubling in cardiac enzymes. Stroke was defined as unequivocal signs or symptoms of a neurological deficit, with sudden onset and duration of more than 24 hours. Diagnosis had to be confirmed by computed tomography, magnetic resonance imaging, or by autopsy. Deep venous thrombosis was defined as a typical clinical picture with positive instrumental investigation (phlebography, ultrasonography, impedance plethysmography, and computed tomography at unusual sites). In case of a suspected recurrence in a site of previous deep venous thrombosis, the diagnosis could be accepted if the instrumental test showed extension or recurrence of thrombosis as compared with previous testing. Pulmonary embolism was defined by a positive pulmonary angiogram, a high probability ventilation-perfusion scan, or evidence of pulmonary embolism at necropsy. Cardiovascular death included: documented diagnosis of myocardial infarction or stroke in the absence of any other evident cause, sudden death, death from heart failure, and all deaths classified as being cardiovascular in nature. A transient ischemic attack was defined as the abrupt onset of unilateral motor or sensory disturbance, speech defect, homonymous hemianopsia, constructional apraxia, or transient monocular blindness that resolved completely in less than 24 hours. Diagnosis and classification of leukemia and myelodysplasia were established using the French-American-British (FAB) Cooperative Group criteria (Bennett *et al*, 1982; Bennett *et al*, 1985).[10;11] Myelofibrosis was defined as the development of leukoerytroblastic blood picture, in the presence of splenomegaly, corroborated with a bone marrow biopsy showing diffuse bone marrow fibrosis.

The validation of causes of death, as well as thrombotic and hemorrhagic events was ensured by an ad hoc committee of expert clinicians. Each event was validated independently by two evaluators, and any disagreement was solved by the chairperson of the study.

Statistical analysis

Cox proportional hazards models were used to evaluate risk, with censoring at first event, death, or last follow-up visit through December 2002. Covariates were chosen based on biologic plausibility as confounders and associations with exposure and outcome in the present population. Multivariable models were evaluated unadjusted, adjusted for age, and gender, and further adjusted for other potential confounding factors (see Table 2 footnote).

Data were explored using multivariable time-dependent analysis in order to assess whether the level of exposition to a factor that had been recorded in the last clinical visit before the outcome event of interest was associated with the probability of having that event during follow-up. Time-varying covariates were used to update information on white blood cell and platelet count, and other risk factors (see Table 2 footnote) at 1, 2, 3, 4, and 5 years. Where appropriate, the substitution of the missing data for incomplete repeated measures was done with the last value carried forward. Indicator variables were used for missing data on baseline covariates; values were otherwise carried forward for missing time-varying covariates. Some covariates (age at recruitment, gender, time from diagnosis to enrolment, thrombotic or hemorrhagic events prior to recruitment, history of hypertension, claudication, or erythromelalgia) were determined only at baseline whereas others were updated during follow-up (smoking habits, diabetes mellitus, total blood cholesterol, splenomegaly, immature cells, hematocrit, platelets and leukocyte count, therapeutical interventions such as phlebotomy, interferon, hydroxyurea, antiplatelets agents and anticoagulants; as well as myelosuppressive therapy that included 32P, busulphan, chlorambucil, and pipobroman). For the variables updated during follow-up visits the last measurement before an event was considered in the time-dependent analysis. Hematocrit and platelet count were tested as continuous variables, median values,

approximate tertiles, quintiles, and deciles depending on the number of events in each analysis (i.e. robustness of the model).

Since evolution to either myelofibrosis or leukaemia could be the cause and not be caused by modification of blood parameters collected during follow-up, a multivariable analysis using values measured at baseline was used to assess whether the level of exposition for a potential risk predictor captured at enrolment could be found to be a statistically

Table 1. Baseline characteristics of the ECLAP population according to tertiles of hematocrit and platelet count

	Hematocrit (%)			**Platelet count (x10^9/l)**			
	≤ 45 (N=556)	46-50 (N=530)	>50 (N=345)	≤ 300 (N=592)	301-500 (N=622)	>500 (N=407)	Total (N=1638)
Females	50.9	37.7	33.9	33.8	47.0	48.2	42.5
Age at diagnosis (mean±SD)	61.5 (12.8)	60.2 (13.4)	59.9 (12.9)	59.0 (13.3)	61.0 (12.8)	61.5 (13.5)	60.4 (13.2)
Age at recruitment (mean±SD)	67.3 (12.0)	65.0 (12.6)	63.3 (12.9)	64.4 (12.9)	66.2 (12.3)	65.5 (12.7)	65.4 (12.7)
Years from diagnosis of PV to enrolment (mean±SD)	5.8 (5.0)	4.8 (4.8)	3.4 (4.3)	5.4 (5.3)	5.2 (4.9)	4.0 (4.5)	5.0 (5.0)
Prior bleeding	10.1	7.6	5.8	8.3	8.8	6.9	8.1
Prior thrombosis	45.1	38.7	29.9	35.0	40.5	41.8	38.6
Erythromelalgia	5.4	5.3	6.4	3.0	4.5	9.6	5.3
Intermittent claudication	6.7	3.6	3.2	5.4	4.0	4.7	4.7
Smoke	10.3	13.6	15.9	15.4	10.6	12.8	12.8
Hypertension	42.6	40.2	39.1	40.4	38.1	40.5	39.5
Diabetes Mellitus	6.5	7.4	7.0	6.9	7.9	6.6	7.1
Splenomegaly	38.3	45.7	55.9	41.1	45.8	49.1	44.7
Immature cells (any type or number)	4.3	4.2	7.5	4.9	5.6	4.4	5.1
Packed cell volume (l/l) (mean ±SD)	0.415 (0.036)	0.476 (0.014)	0.556 (0.044)	0.467 (0.061)	0.470 (0.063)	0.480 (0.065)	0.472 (0.063)
Platelet count (x10^9/L) (mean ±SD)	384 (198)	385 (197)	444 (243)	214 (55.0)	391 (59.2)	678 (185)	398 (208)

significant marker of increased probability of myelofibrosis or hematolological transformation during follow-up.

Tests for trend were calculated by assigning the median value of each category and evaluating this as a continuous variable. Stratified analyses were used to assess for effect modification, with significance evaluated using likelihood-ratio testing and multiplicative (exposure times covariate) interaction terms. Analyses were performed with SAS 9.1 software. All probability values are 2 tailed (<0.05).

Results

The baseline characteristics of the cohort are shown in Table 1. Six hundred thirty-three (39%) patients had a prior history of thrombosis, which was made of an arterial thrombotic event in three quarters of the cases.[6] Cerebrovascular events accounted for two thirds of arterial thrombosis, while deep venous thrombosis represented approximately 40% of vein thromboses. A positive history of bleeding was present in 8.1% of patients of which 79 (4.8%) was major bleeding (gastrointestinal, 4.1%; intracranial, 0.7%). Patients with hematocrit values ≤ 45 at baseline were significantly more likely to have intermittent claudication and to have had a prior thrombotic or hemorrhagic event as compared with subjects with higher hematocrit levels.

The proportion of patients with the target hematocrit ≤45% was approximately 40% at baseline, 48% at 12 months, 49% at 24 months, 49% at 36 months, 47% at 48 months, and 46% at the end of the study period (Figure 1 Panel A). The range of hematocrit levels was maintained at relatively low values throughout the study, with only 10% of the patients having a hematocrit above 50%. With PV treatment, the absolute difference of hematocrit levels at baseline between the highest and the lowest decile groups decreased during the whole follow-up period from 24% to about 5-7% (Figure 1 Panel B). Median platelet

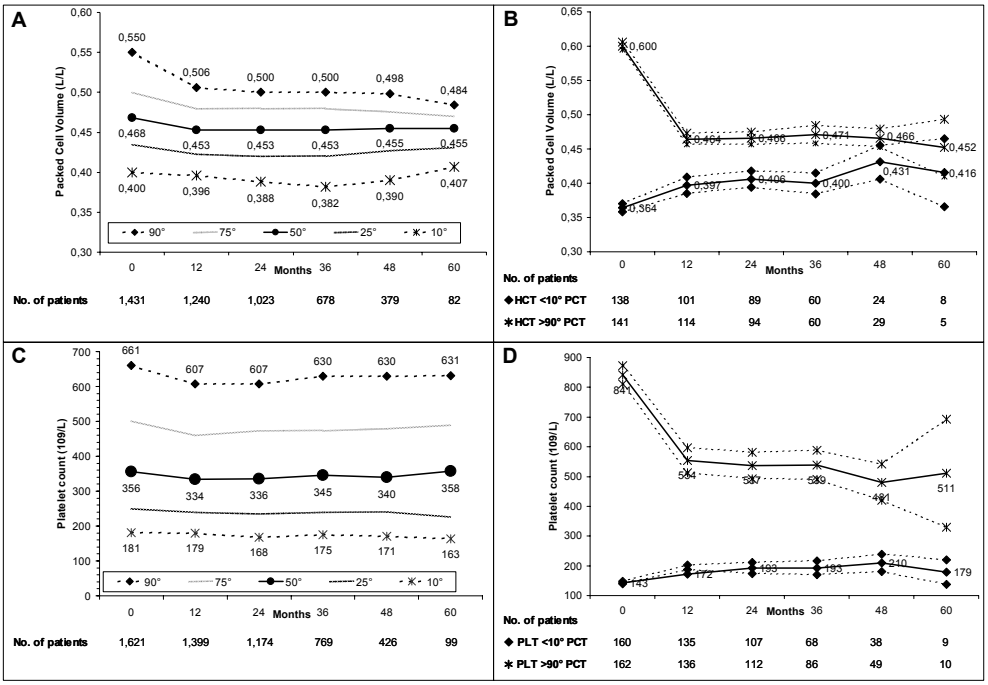

Figure 1. Distribution (percentiles) of hematocrit values and platelet count at baseline and during follow-up of 1,638 PV patients enrolled in the ECLAP study. A) Hematocrit levels distribution in the whole study population (each time point is shown independently from the other ones). B) Hematocrit control during follow-up by selecting patients in the highest and lowest deciles of hematocrit at recruitment; C) Platelet count at baseline and during follow-up in the whole study population (each time point is shown independently from the other ones); D) Platelet cont control during follow-up by selecting patients in the highest and lowest deciles of platelet count at recruitment.

count was 356 x 10^9/L at baseline. Thirty-six percent of patients had levels higher than 400 x 10^9/L at 12 months, and 62% had platelets below this cut-off at 60 months. (Figure 1 Panel C and D).

Analysis of Mortality, Major and Total Thrombosis, and Bleeding

In a 4.393 person-years follow-up (median 2.8 years), a total of 164 deaths (10%), 145 (8.9%) major thrombosis, 226 (13.8%) total thrombosis, and 35 (2.1%) major bleedings were observed. At multivariable analysis, major thrombosis was

Table 2. Time-dependent multivariable analysis on the relative risk of major thrombosis, total thrombosis, and death among 1.638 men and women with Polycythemia vera. (Reference categories: hematocrit ≤ 45%, N=556; platelet count ≤ 300 x10^9/l, N=592)

	Hematocrit (%)		Platelet count (x10^9/l)	
Hazard ratio (95% CI) P-value	46-50 (N=530)	>50 (N=345)	301-500 (N=622)	>500 (N=407)
Major thrombosis (n=145)				
(1) Unadjusted	0.80 (0.55-1.17) 0.2480	0.88 (0.53-1.48) 0.6320	0.86 (0.60-1.24) 0.4183	0.82 (0.53-1.28) 0.3794
(2) Age- and gender-adjusted	0.87 (0.60-1.28) 0.4890	0.96 (0.57-1.62) 0.8759	0.88 (0.61-1.27) 0.5028	0.85 (0.54-1.32) 0.4600
(3) + Disease duration-, prior thrombosis- and prior hemorrhage-adjusted	0.88 (0.60-1.29) 0.5043	1.04 (0.61-1.75) 0.8941	0.84 (0.58-1.21) 0.3460	0.78 (0.50-1.22) 0.2834
(4) + RF-, and comorbidity-adjusted	0.84 (0.57-1.24) 0.3796	0.98 (0.57-1.67) 0.9364	0.77 (0.53-1.13) 0.1782	0.64 (0.39-1.03) 0.0656
(5) + cytoreductive- and antithrombotic treatment-adjusted	0.89 (0.60-1.34) 0.5844	1.04 (0.61-1.78) 0.8884	0.78 (0.53-1.15) 0.2099	0.67 (0.41-1.09) 0.1099
Total thrombosis (n=226)				
(1) Unadjusted	0.86 (0.64-1.16) 0.3173	0.76 (0.50-1.16) 0.1992	0.91 (0.68-1.21) 0.5131	0.80 (0.56-1.14) 0.2199
(2) Age- and gender-adjusted	0.93 (0.68-1.25) 0.6130	0.82 (0.53-1.25) 0.3487	0.91 (0.68-1.22) 0.5264	0.81 (0.57-1.16) 0.2556
(3) + Disease duration-, prior thrombosis- and prior hemorrhage-adjusted	0.94 (0.70-1.28) 0.7030	0.90 (0.59-1.38) 0.6257	0.86 (0.64-1.16) 0.3277	0.75 (0.52-1.07) 0.1120
(4) + RF-, and comorbidity-adjusted	0.89 (0.66-1.21) 0.4699	0.84 (0.54-1.30) 0.4259	0.80 (0.59-1.09) 0.1598	0.64 (0.44-0.95) 0.0249
(5) + cytoreductive- and antithrombotic treatment-adjusted	0.98 (0.71-1.34) 0.8752	0.91 (0.59-1.42) 0.6888	0.85 (0.62-1.15) 0.2906	0.70 (0.48-1.04) 0.0801

Table 2. Continued

Hazard ratio (95% CI) P-value	Hematocrit (%)		Platelet count (x10^9/l)	
	46-50 (N=530)	>50 (N=345)	301-500 (N=622)	>500 (N=407)
Death (n=164)				
(1) Unadjusted	0.74 (0.52-1.04) 0.0858	0.54 (0.30-0.97) 0.0399	1.04 (0.74-1.47) 0.8040	0.90 (0.59-1.38) 0.6291
(2) Age- and gender-adjusted	0.85 (0.60-1.22) 0.3806	0.63 (0.35-1.14) 0.1263	1.08 (0.77-1.52) 0.6646	0.95 (0.62-1.45) 0.8042
(3) + Disease duration-, prior thrombosis- and prior hemorrhage-adjusted	0.88 (0.61-1.25) 0.4664	0.71 (0.39-1.28) 0.2531	1.03 (0.73-1.46) 0.8581	0.90 (0.59-1.38) 0.6333
(4) + RF-, and comorbidity-adjusted	0.82 (0.57-1.17) 0.2706	0.64 (0.35-1.18) 0.1509	0.90 (0.63-1.28) 0.5509	0.69 (0.44-1.09) 0.1125
(5) + cytoreductive- and antithrombotic treatment-adjusted	0.91 (0.63-1.32) 0.6258	0.68 (0.37-1.26) 0.2209	0.92 (0.64-1.32) 0.6555	0.72 (0.45-1.15) 0.1677

Risk estimates are given as hazard ratio (95% CI) p-value.
Model 1: Hematocrit or platelet count (3 categories each, in two separate analyses).
Model 2: Model 1 + age (4 categories), gender.
Model 3: Model 2 + time from PV diagnosis to recruitment (5 categories), thrombotic or hemorrhagic events prior to recruitment (yes/no).
Model 4: Model 3 + smoking (yes/no), history of diabetes (yes/no), hypertension (yes/no), claudicatio intermittens (yes/no), erythromelalgia (yes/no), splenomegaly (yes/no), circulating immature cells (yes/no), leukocyte count (tertiles), total blood cholesterol (tertiles), hematocrit or platelet count (3 categories each, in the pertinent model).
Model 5: Model 4 + phlebotomy use (yes/no), interferon use (yes/no), hydroxyurea use (yes/no), antiplatelets use (yes/no), anticoagulants use (yes/no), 32P use (yes/no), busulfan use (yes/no), chlorambucil use (yes/no), and pipobroman use (yes/no)

associated with age above 65, history of thrombosis (hazard ratio, HR, 1.74, 95% confidence interval, CI, 1.21 to 2.51, p=0.0031), arterial hypertension, and claudication (data not shown). Total thrombosis was significantly associated with age above 65, history of thrombosis, and antiplatelet therapy. Total mortality was

significantly associated with age above 65, history of thrombosis, antiplatelet therapy, diabetes, smoking, prior bleeding, and splenomegaly (data not shown).

Hematocrit was not related to any of the thrombotic outcomes nor to bleeding events in univariate and multivariable analysis. The risk for major thrombosis remained similar across hematocrit deciles (Table 2 and Figure 2-Panel A), with analogous results for total thrombosis (Table 2 and Figure 2-Panel B), and mortality (Table 2 and Figure 2-Panel C). As compared to patients with hematocrit levels ≤ 45%, those with hematocrit above 45% had a comparable risk of death (HR 0.85, 95% CI 0.60 to 1.31, p=0.3761), major thrombosis (HR 0.94, 95% CI 0.65 to 1.36, p=0.7396), or total thrombosis (HR 0.97, 95% CI 0.72 to 1.30, p=0.8171). The results of unadjusted analyses for hematocrit levels did not change meaningfully in the four, progressively adjusted, time-dependent predictive models (Table 2).

As shown in Figure 3 Panels B and C, platelet count was not significantly associated with thrombotic events nor total mortality. Major thrombotic events occurred in 8.3% of patients with platelet count at baseline above 400 x 10^9/L versus 9.3% of those with lower platelet levels (HR 0.96, 95% CI 0.66 to 1.38, p=0.8107).

Age, disease duration, and history of bleeding were the only variables significantly associated with the risk of total bleeding during follow-up. A history of previous bleeding was correlated with subsequent major bleeding (data not shown). There was no association between hematocrit or platelet count with total bleeding or major bleeding events.

Analysis of Hematological Transformation

There were 22 (1.3%) cases of acute leukemia and 38 (2.3%) myelofibrosis. Age ≥70 years and cytoreductive drugs (other than hydroxyurea and interferon) predicted the risk of leukemia, whereas a long disease duration was

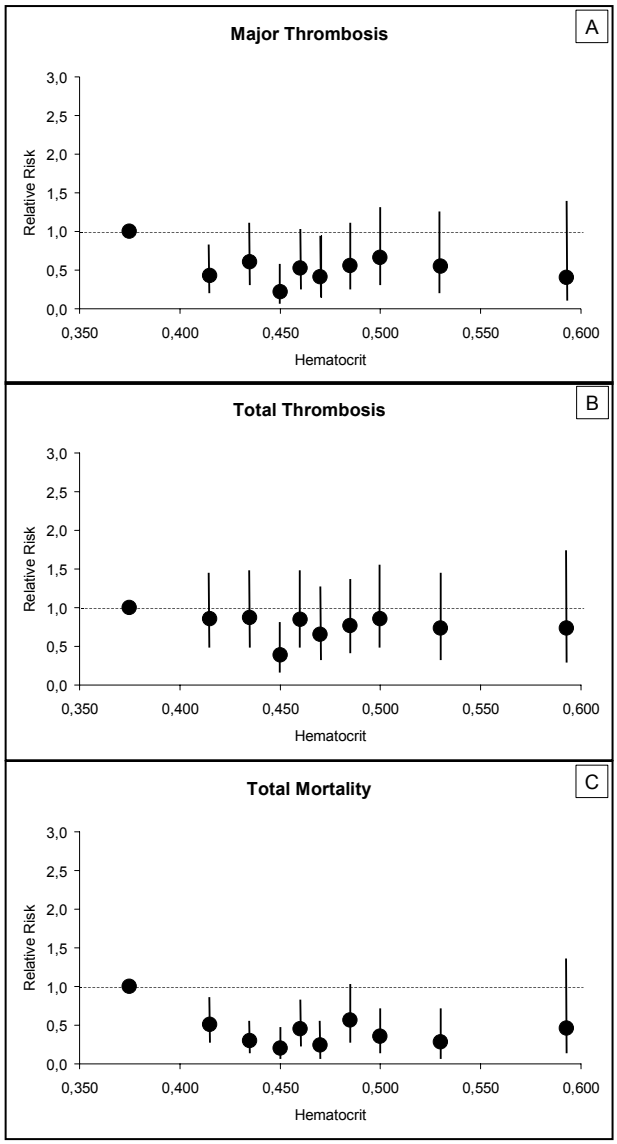

Figure 2. Time-dependent multivariable analysis for A) major thrombosis, B) total thrombosis, and C) total mortality according to deciles of hematocrit. Circles with vertical bars indicate hazard ratios along with their 95% confidence interval. Reference category = lowest decile. When the 95% confidence interval (vertical line) crosses the line of no effect (horizontal line) the results are not statistically significant.

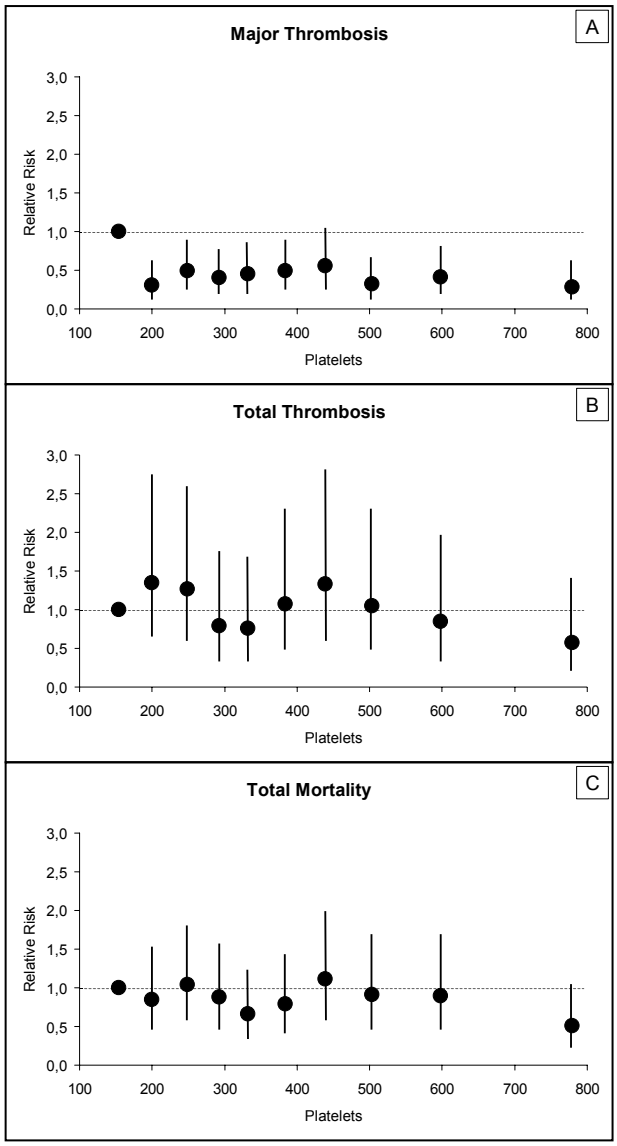

Figure 3: Time-dependent multivariable analysis for A) major thrombosis, B) total thrombosis, and C) and total mortality according to deciles of platelet count. Circles with vertical bars indicate hazard ratios along with their 95% confidence interval. Reference category = lowest decile. When the 95% confidence interval (vertical line) crosses the line of no effect (horizontal line) the results are not statistically significant.

significantly associated with an increased risk of developing myelofibrosis.[6]

High hematocrit was not associated with progression to leukemia, whereas hematocrit values above the third tertile (above 50%) seemed to predict a higher risk for myelofibrosis (HR 1.84, 95% CI 0.71 to 4.79, p=0.21) (Table 3). Patients with platelet count in the second (301-500x10^9/L) or third category (above500x10^9/L) had respectively a 54% (HR 0.46, 95% CI 0.21 to 1.02, P=0.0550) and 66% (HR 0.34, 95% CI 0.12 to 0.97, p=0.0431) lower risk of myelofibrosis as compared to those with platelets in the reference category (platelet count ≤ 300x10^9/L). However, when we assessed the prognostic role of hematocrit and platelet counts measured at baseline, no statistically significant association could be found.

Discussion

The present analysis of outcome events during follow-up with the more recent laboratory data obtained before the occurrence of that same event and its results does not support a prognostic value of hematocrit in PV complications, namely thrombosis events, hematologic progression, and myelofibrosis. While significantly higher at baseline, the hematocrit of patients in the highest decile was conservatively reduced by treatment to levels comparable to the median value of hematocrit during follow-up. The same phenomenon, though in the opposite direction, was observed for patients in the lowest decile of hematocrit at baseline. With the extremes of hematocrit distribution maintained throughout follow-up within a narrow interval around 45% (Figure 1, Panel B), high hematocrit was not found as a significant predictor of death, thrombotic events, nor hematologic progression. Our findings suggest that high platelet count might be associated with a decreased risk of hematological transformation and myelofibrosis.

Thrombosis, myelofibrosis, and acute leukemia frequently complicate the course of PV (Spivak *et al*, 2003, Spivak, 2002).[1;7] The relevance of hematocrit and platelet count in predicting any of these outcomes has not been clearly established in prospectively-conducted studies. According to a recent survey conducted among North America hematologists, there seems to be little consensus and high variability over the control of hematocrit and/or thrombocytosis in PV (Streiff *et al*, 2002).[12] Such heterogeneity in current clinical practice might possibly reflect the uncertainty over the benefit of strict hematocrit and/or thrombocytosis control.

Mortality, Major and Total Thrombosis, and Bleeding

Based on some initial observations suggesting a higher risk of thrombosis at moderately increased hematocrit levels, it has been advised that hematocrit should be maintained below 45% in males and 42% in women (Pearson *et al*, 1978; Spivak *et al*, 2003).[3;7] Hematocrit could increase the risk of thrombosis by several mechanisms such as raising blood viscosity, impacting on nitrous oxide level, or by enhancing platelet-vessel wall interactions (Spivak, 2002; Schafer, 2006).[1;2] Data in support of an association between elevated hematocrit and thrombotic events, however, have not been always concordant (Berk *et al*, 1995; Wehmeier *et al*, 1991).[8;13]

The PVSG-01, the largest prospective PV cohort together with the ECLAP, included 431 patients (Berk *et al*, 1995). [8] In the PVSG-01, no hematologic parameter measured at the closest observation prior to the thrombotic event was associated with increased risk of thrombosis. In addition, patients of the PVSG protocol developed thrombotic complications when the hematocrit was reasonably well controlled by phlebotomy or myelosuppression. Other studies suggested that hematocrit did not correlate with thrombosis in Chuvash Polycythemia (Gordeuk *et al*, 2004; Gordeuk *et al*, 2006) [14;15] while large studies of patients with polycythemia of high altitude or resulting from Eisenmenger syndrome and other

cyanotic heart diseases argue against hematocrit as the only factor causing thrombosis (Thorne *et al*, 1998; Vongpatanasin *et al*, 1998; Prchal *et al*, 2005) [16-18]. Though haematocrit levels could be not strictly correlated with red blood cell mass, which has been suggested to be the real causative factor associated with the risk of thrombosis in PV (Spivak, 2004), our findings are in agreement with these previous results and do not support a predictive value of hematocrit for death or thrombotic events in patients receiving current antithrombotic and cytoreductive treatments, the latter allowing to maintain the hematocrit level below 45% in half of PV patients and below 50% in more than 90% of PV subjects.

Several differences might explain the conflicting data between the initial observations (Pearson *et al*, 1978) [3] and later studies. First, previous studies included a limited number of patients who did not receive an adequate control of cardiovascular risk factors, antihypertensive and antiplatelet therapy, and cytoreductive therapy as in the ECLAP. Moreover, methodological limitations such as the use of univariate analysis and of not taking into account the dependency of observations might have biased some of previous conclusions (Pearson *et al*, 1978).[3]

A correlation between hematocrit and cardiovascular disease has been previously reported in patients without PV (Irace *et al*, 2005; Brown *et al*, 2001; Erikssen *et al*, 1993; Sorlie *et al*, 1981).[19-22] Differences in study populations or use of hematocrit measurements at inclusion rather multiple determinations and a time-dependent analysis, and a univariate analysis of the data may partially explain the contrasting results.

Despite the widespread belief that thrombotic tendency in PV may be related to thrombocytosis, no study to date, either prospective or retrospective, has demonstrated a significant correlation between platelet number or function and thrombosis (Spivak, 2002; Schafer *et al*, 2006).[1;2] In the PSVG study, platelet counts at the nearest times before the thrombotic events did not predict thrombosis

(Berk *et al*, 1995).[8] Accordingly, we did not find any association between platelet count and thrombotic events. The currently proposed target of 400 x 10^9/L did not predict a higher risk of thrombosis and neither any of the platelet count deciles. In our study, platelet count remained relatively high during the whole study period which seem to suggest that current PV treatment does not primarily aim at lowering platelets (Figure 3).

Platelet activation, rather than platelet number might be an important determinant of thrombotic events in PV. While no specific platelet abnormality seems to correlate to an increased thrombotic risk, platelet activation, as indicated by increased thromboxane B formation, has been described in PV (Murphy, 1995; Landolfi *et al*, 1992).[23;24] Accordingly, the trial component of the ECLAP study recently showed a significant 60% reduction of the combined endpoint of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes in PV patients assigned to aspirin, as compared to those receiving placebo (Landolfi *et al*, 2004).[25]

Recently, a point mutation (V617F) in the JAK2 kinase has been described in 70-95% of PV patients (Schafer *et al*, 2006)[2] and preliminary data suggest a pivotal role of this mutation in the PV phenotype. New biomarkers, such as JAK2 kinase, may prove useful in future but more extensive research is needed to clarify their predictive role and their utility as surrogate endpoints.

A high platelet count has been associated with a hemorrhagic diathesis in patients with PV and literature has rather consistently showed that a reduction of platelet count with myelosuppressive therapy reduces the bleeding rate (Schafer *et al*, 2006; Chien *et al*, 1995).[2;26] In PSVG-05 high platelet count tended to be associated with a higher risk of hemorrhage not thrombosis (Berk *et al*, 1995). [8] A higher bleeding risk at high platelet count could be explained by von Willebrand factor deficiency caused by an increased clearance through platelet-dependent

mechanisms. Our study does not confirm a pro-hemorrhagic tendency in patients with high platelet count.

Hematological transformation and Myelofibrosis

The prognostic value of hematocrit or platelet count for PV progression to leukemia/myelofibrosis has not been investigated. In the current analysis, we found no association between hematological progression and hematocrit whereas there was a trend for a higher risk of myelofibrosis at hematocrit levels above the highest tertile (HR 1.84;95% CI, 0.71- 4.79).

Hematological complications seemed to occur more frequently in patients with low platelet count. During follow-up, in the time-dependent analysis, patients with platelets $\leq$ 300 x 10^9/L had a more than 3-fold and 2-fold higher risk of developing acute leukemia (HR 3.69, 95% CI 1.31 to 10.45, p=0.0138) and myelofibrosis (HR 2.40, 95% CI 1.18 to 4.87, p=0.0157), respectively, than for higher platelet counts. Such association, however, could be due to the development of hematological transformation, though a non-statistically significant high rate of hematological malignancy was evident at baseline in subjects with low platelet count. The association between hematocrit or platelet count and hematological transformation remains unclear. It could be speculated that high hematocrit and/or low platelets identify a subgroup of patients with a more aggressive form of the disease who are more likely to develop hematological complications. However, the relatively low absolute number of cases of leukemia-myelofibrosis in the ECLAP study, while comparable to previous trials (Finazzi *et al*, 2005) [27], does not allow to draw firm conclusions. Thus, the present findings have to be taken with caution and need confirmation in large prospective trials.

In summary, the results of the present analysis seem not to support a prognostic importance of hematocrit and platelet count in PV and challenge the need for an aggressive control of these parameters in patients with PV for the

prevention of thrombohemorragic complications. The current findings together with current available evidence underscore the lack of specific therapeutic targets in the management of PV.

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Su

Summary

Chapter 1 provides a brief background to the role of D-dimer testing and pre-test clinical probability in the diagnosis of venous thromboembolism in patients with or without cancer. Moreover, this introductory chapter discusses the link between coagulation, thrombosis, cancer progression and the possible impact of anticoagulants on cancer survival. Chapter 1 also provides the outline of this thesis.

In **Chapter 2** we aimed at summarising the current evidence on the diagnostic accuracy of the D-dimer test in the exclusion of venous thromboembolism while adjusting for known sources of bias and variability.

Relatively to other D-dimer assays, ELFA, microplate ELISA, and latex quantitative showed a higher sensitivity but a lower specificity, resulting in a more confident exclusion of the disease at the expense of more additional imaging testing. The sensitivities of the ELFA D-dimer ranged from 93% to 97% whereas those of whole-blood D-dimer, latex semiquantitative, and latex qualitative were between 69% and 88%. Latex qualitative and whole-blood D-dimer assays had the highest specificities.

In **Chapter 3** the value of the D-dimer test for the exclusion of clinically suspected pulmonary embolism was evaluated in patients with and without cancer. We showed that, with a sensitivity and a negative predictive value of 100%, a normal D-dimer result can safely exclude the diagnosis of pulmonary embolism in patients with cancer. The low specificity was an evident limitation for the practical use of the test. When testing 100 patients with suspected pulmonary embolism, a normal D-dimer concentration could safely exclude the diagnosis in only 15 patients with cancer as compared to 43 patients without malignancy.

Chapter 4 evaluated the performance of the D-dimer test in combination with the pre-test clinical probability in cancer patients. We found that a normal D-dimer test

result could exclude the diagnosis of venous thromboembolism among cancer patients with a low or low-moderate pre-test probability, which represented 17% and 52% of the cancer population, respectively. The specificity of the D-dimer test progressively decreased moving from the low to the higher pre-test probabilities.

In **Chapter 5** we compared various screening strategies for the detection of occult cancer in patients presenting with an unprovoked venous thromboembolism. Despite the limitations of the study, the screening for cancer with a strategy including abdominal/pelvic computed tomography with or without mammography and/or sputum cytology appeared useful. Indeed, 12 of the 14 patients with cancer were detected by this strategy with one false-positive result. Other approaches such as abdominal/pelvic ultrasonography or tumor markers yielded a higher number of patients needed to screen. Strategies including colonoscopy and tumor markers were significantly more costly.

Chapter 6 summarises the available evidence about the optimal prophylactic and treatment strategies of venous thromboembolic events occurring in patients with cancer. Low-molecular-weight heparin emerged as a very good option in the management and prevention of venous thromboembolism in cancer patients. This review also discussed the promising results on the effects of antithrombotic agents, especially aspirin and low-molecular-weight heparin in improving the prognosis of cancer patients.

In **Chapter 7** we investigated in a randomized placebo-controlled double blind trial the efficacy and safety of thromboprophylaxis with subcutaneous low-molecular-weight heparin in patients with hematological malignancies who were going to receive a central venous catheter. The frequency of venographically proven catheter-related thrombosis was low in both study groups and it was not further

reduced by low-molecular-weight heparin administration. The thromboprophylaxis' regimen used appeared to be safe with no differences in catheter-related infections or bleeding events between the two study groups.

Chapter 8 assessed the prognostic value of the D-dimer test in cancer patients randomised to receive standard anticancer care with or without low-molecular-weight heparin. Our data suggested a prognostic role of the D-dimer test in that patients with a reduction in D-dimer plasma levels seemed to be those who benefited the most from low-molecular-weight heparin administration. Moreover, the effects of low-molecular-weight heparin tended to be more pronounced in patients with high D-dimer concentrations at the start of treatment. The sample size and the retrospective nature of this study did not allow for firm conclusions

In **Chapter 9** we found that increased interleukin-10, interleukin-6, and P-selectin levels predicted a poor outcome in patients with advanced stage cancer. In particular, high interleukin-10 predicted a two-fold increase in the risk of dying even after adjustment for other prognostic markers. The included population was part of a large trial which randomised cancer patients to receive low-molecular-weight heparin or placebo on top of standard care. The prolongation in survival observed with low-molecular-weight heparin was not explained by an effect of low-molecular-weight heparin on interleukin-10, interleukin-6, and P-selectin circulating levels.

In **Chapter 10** we found that nor the hematocrit nor platelet count predicted the occurrence of major thrombotic events and/or survival in a large cohort of patients with polycythemia vera. In contrast with current belief, a haematocrit in the evaluable range of 40–55% was neither associated with the occurrence of thrombotic events, nor with haematological progression or mortality in the studied

population. a high platelet count was associated with a lower progression rate to acute leukaemia/myelofibrosis, whereas it had no significant relationship with thrombotic events or mortality. Our findings are at variance with current recommendations for the treatment of polycythemia vera, which are to maintain haematocrit <45% and the platelet count below 400 $x10^9$/L.

This thesis presents some new emerging aspects in the relationship between cancer and thrombosis. For what concern the diagnosis of venous thromboembolism in cancer patients, it appears that features peculiar of the malignancy status and its management could make the diagnostic approach for venous thromboembolism different in patients with cancer as compared to those without. In particular, the chapters on diagnosis show the limited usefulness of the D-dimer test as the sole method in the diagnosis of venous thromboembolism. On the contrary, the combination of the test with a clinical probability score could reduce the number of false-positive results.

The second part of the thesis deals with prognostic factors in patients with and without cancer. Some chapters provide data reinforcing the idea of a relationship between coagulation and the development of cancer and its complications. Despite the relatively small size of some of these observations, data seem quite consistent in suggesting a link between coagulation and inflammatory circulating markers with the natural history of cancer. Several studies have recently proposed an anticancer activity of low-molecular-weight heparins. [1-4] However, the miscellany of low-molecular-weight heparins schedules being evaluated and the variety of the types and stages of malignancies included, leave still perplexities over the possible use of low-molecular-weight heparins in this setting. Some findings of the current thesis raise the hypothesis that circulating factors could help in distinguishing those who benefit most from low-molecular-weight heparins and could help to guide therapy with these agents. Certainly, the available evidence is

still too limited to consider the routine addition of low-molecular-weight heparins in the therapeutic armamentarium against cancer. New clinical trials such as the IMPACT study have been commenced to evaluate the role of low-molecular-weight heparins in cancer progression and survival. Hopefully, these investigations will shed some light to move forward in this exciting area of research.

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Sa

Samenvatting

Hoofdstuk 1 geeft een korte achtergrond over de rol van D-dimeer testen en klinische kansschattingen in de diagnostiek van veneuze tromboembolie in patiënten met en zonder een maligniteit. Daarnaast bespreekt dit inleidende hoofdstuk de relatie tussen bloedstolling, trombose, kankerprogressie en de mogelijke betekenis van anticoagulantia in kanker overleving. Hoofdstuk 1 besluit met een bespreking over de opzet van dit proefschrift.

In **hoofdstuk 2** hebben we getracht de huidige bewijsvoering voor de accuratesse van de D-dimeertest in het uitsluiten van veneuze tromboembolie samen te vatten, met inachtneming van bekende bronnen van vertekening en variabiliteit.
In vergelijking tot andere D-dimeer testen, hadden de ELFA, microplaat ELISA en de kwantitatieve latex, een grotere sensitiviteit, maar een lagere specificiteit, hetgeen resulteert in een meer zekere uitsluiting van de ziekte ten koste van meer additionele beeldvormende onderzoekingen. De sensitiviteit van de ELFA-D-dimeer varieerde van 93% tot 97%, terwijl die van de volbloed D-dimeer en de (semi) kwantitatieve latexmethoden waarden hadden tussen de 69% en 88%. De kwalitatieve D-dimeer en volbloedtesten hadden de hoogste specifiteit.

In **hoofdstuk 3** werd de waarde onderzocht van de D-dimeer test in de exclusie van longembolieën bij patiënten met een klinische verdenking hierop en met of zonder een maligniteit. We toonden aan dat met een sensitiviteit en een negatief voorspellende waarde van 100%, een normaal testresultaat veilig de diagnose longembolie kan worden verworpen in patiënten met een maligniteit. Echter de gevonden lage specificiteit was een duidelijke beperking van het nut voor de klinische praktijk. Ter illustratie: wanneer 100 patiënten met een klinische verdenking op een longembolie worden onderzocht, dan kan een normale D-dimeer testuitslag bij 15 patiënten met een maligniteit de ziekte uitsluiten, terwijl dit 43 patiënten zou betreffen zonder een maligniteit.

Hoofdstuk 4 onderzocht de betekenis van de D-dimeer test in combinatie met de klinische kansschatting in kanker patiënten. We konden aantonen, dat een normale D-dimeeruitslag in combinatie met een laag dan wel matige klinische kansschatting de diagnose veneuze tromboembolie kon uitsluiten en dat dit respectievelijk voortkwam bij 17% en 52% van de onderzochte populatie met een maligniteit. De specificiteit van de D-dimeer test daalde progressief gaande van een lage naar hogere klinische kansschatting.

In **hoofdstuk 5** vergeleken we diverse screeningsstrategieën voor het opsporen van een occulte kanker bij patiënten met een spontane veneuze tromboembolie. Ondanks de beperkingen van het studiemateriaal, bleek een strategie met een abdomen/bekken spiraal CT met of zonder mammografie en/of sputumcytologie van waarde te kunnen zijn. Met deze benadering konden 12 van de 14 patiënten met een kanker worden opgespoord met één vals positieve bevinding. Andere strategieën, zoals abdomen/bekken echografie of tumor markers resulteerden in een groter aantal patiënten die onderzocht moesten worden. Benaderingen met coloscopie en tumor markers waren duidelijk kostbaarder.

Hoofdstuk 6 vat het beschikbare bewijs samen over de meest optimale profylactische en behandelstrategieën van veneuze tromboembolische complicaties bij patiënten met een maligniteit. Laag-moleculair-gewichtsheparines zijn nadrukkelijk een goede optie voor zowel de preventie als de behandeling van veneuze trombose bij kankerpatiënten. Dit hoofdstuk bespreekt tevens de veel belovende resultaten van de effecten van antithrombotica, in het bijzonder aspirine en laag-moleculair-gewichtsheparines op het verbeteren van de overleving van kankerpatiënten.

In **hoofdstuk 7** onderzochten wij in een gerandomiseerde, placebo gecontroleerde dubbelblinde studie de effectiviteit en veiligheid van tromboprofylaxe met subcutane laag-moleculair-gewichtsheparine bij patiënten met een hematologische maligniteit, die een centraal veneuze lijn kregen voor hun behandeling. De frequentie van venografisch aangetoonde catheter gerelateerde trombose was laag in beide studie groepen en de toevoeging van laag-moleculair-gewichtsheparine had geen waarneembaar effect. De profylaxe met laag-moleculair-gewichtsheparine leek veilig en er werden geen verschillen waargenomen in de frequenties van cathetergerelateerde infecties of bloedingen.

Hoofdstuk 8 evalueerde de prognostische waarde van de D-dimeer test in kankerpatiënten, die naast hun standaardbehandeling werden gerandomiseerd naar wel of geen laag-moleculair-gewichtsheparine. Onze waarnemingen suggereerden dat patiënten die een daling van hun D-dimeer plasmawaarden vertoonden het meeste baat hadden van hun behandeling met laag-moleculair-gewichtsheparines. Verder kon aannemelijk worden gemaakt dat vooral die patiënten met een hoge D-dimeerconcentratie vóór de behandeling een meer uitgesproken effect lieten zien. De steekproefgrootte en het retrospectieve karakter van deze studie manen tot voorzichtige conclusies.

In **hoofdstuk 9** werd aangetoond dat verhoogde bloedwaarden van de interleukines 6 en 10 en p-selectine een slechtere uitkomst voorspelden in patiënten met een gevorderd stadium van hun maligniteit. In het bijzonder bleek een verhoogde interleukine 10 spiegel na correctie van andere prognostische variabelen geassocieerd te zijn met een tweevoudige toename in de kans om te sterven. De studiegroep bestond uit patiënten die naast hun standaardbehandeling participeerden in een gerandomiseerd onderzoek naar placebo of laag-moleculair-gewichtsheparine. Het gevonden effect van laag-moleculair-gewichtsheparine op

de overleving kon niet worden verklaard door een effect van de heparine op de interleukines of p-selectine.

Hoofdstuk 10 beschrijft de waarneming dat noch het hematocriet, noch het aantal bloedplaatjes het optreden van belangrijke trombotische complicaties of de overleving voorspelt in een grote groep van patiënten met polycythemiavera. In tegenstelling tot de huidige opvattingen, was een hematocriet van 40-55% niet geassocieerd met trombotische ziekten en ook niet met hematologische progressie of mortaliteit in de bestudeerde populatie. Een verhoogd aantal plaatjes was wel geassocieerd met een lagere kans op progressie naar een acute leukemie of myelofibrose. Onze waarnemingen zijn niet in overeenstemming met de algemene aanbevelingen over de behandeling van polycythemiavera, die voorschrijven om het hematocriet onder de 45% te houden en het aantal plaatjes niet boven de 400.109/L te laten stijgen.

Dit proefschrift presenteert enkele nieuwe inzichten in de associatie tussen kanker en trombotische ziekten. Op het gebied van de diagnostiek van veneuze trombose bij kankerpatiënten zouden kenmerken, typerend voor kwaardaardige kankersoorten en bijbehorende behandeling, er toe kunnen leiden dat diagnostische procedures voor veneuze trombosen verschillen bij patiënten met en zonder kanker. In het bijzonder de hoofdstukken over diagnostiek tonen aan dat d-dimeer als *stand-alone* test voor de diagnose veneuze tromboembolie weinig bruikbaar is. Daartegenover staat dat het combineren van d-dimeer testresultaten met de klinische waarschijnlijkheidscore het aantal fout-positieve testresultaten zou kunnen verlagen.

Het tweede deel van het proefschrift bespreekt prognostische factoren in patiënten met en zonder kanker. Enkele hoofdstukken presenteren data die de veronderstelling bekrachtigen dat er een relatie is tussen coagulatie en de

ontwikkeling van kanker en de complicaties daarvan. Ondanks de relatief kleine aantallen in sommige van deze observaties, lijkt de data vrij consistent te duiden op een link tussen coagulatie, circulerende ontstekingsmarkers en het natuurlijke beloop van kanker. Meerdere onderzoekingen hebben recentelijk de hypothese gelanceerd dat laag-moleculair-gewichtsheparinen anti-kankeractiviteiten zouden hebben. [1-4] Doordat studies een mengselwerk van beleid met laag-moleculair-gewichtsheparinen hebben onderzocht in uiteenlopende vormen en stageringen van kanker, blijft er echter verwarring bestaan over de waarde van laag-molecularire-gewichtsheparinen in deze setting. Enkele bevindingen in dit proefschrift genereren de hypothese dat circulerende markers zouden kunnen dienen om dié patiënten te onderscheiden, die baat zouden kunnen hebben bij behandeling met laag-moleculaire-gewichtsheparinen en tevens zouden kunnen helpen bij het bepalen van het beleid met deze medicijnen. Uiteraard is de huidige evidentie nog te summier om een routinematige additie van laag-moleculaire-gewichtsheparinen in het arsenaal van behandelmethoden voor kanker op te nemen. Klinische onderzoekingen zoals de IMPACT worden thans uitgevoerd om de rol van laag-moleculaire-gewichtsheparinen in de progressie van kanker en overleving te onderzoeken. Hopelijk leiden deze initiatieven tot verdere inzichten zodat dit fascinerende onderzoeksgebied zich verder blijft ontwikkelen.

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