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DOCTORAL THESIS

The public defense of this thesis for the degree Doctor of Philosophy in Medicine will, with due permission from Lund University, Faculty of Medicine, take place in Medicinaulan ingång 35, SUS Malmö, Sweden
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Faculty opponent: Docent Hans Johnsson
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Abstract

The combination of pre-test clinical probability assessment and D-dimer test is now widely applied in the diagnostic process of DVT. The general objective of the present investigation was to validate these results in a Swedish routine emergency setting were the prevalence of the disease is high and were the clinical probability assessment was handled by many junior physicians. Furthermore, our aims were to evaluate our D-dimer method and to make comparisons with other D-dimer methods as with a new marker of coagulation, the APC-PCI complex. In addition, a cost effectiveness analysis was made of this diagnostic strategy.

Material and methods: 357 outpatients with clinical suspicion of DVT were included in the clinical management study. The diagnostic workup included estimation of pre-test probability, D-dimer determination, objective imaging as well as 3 month clinical follow up of negative patients (Paper I). 350 plasma samples from the management study was used for comparison between two well established D-dimer methods and the APC-PCI complex (Paper II) and 311 plasma samples for the evaluation of two new D-dimer methods (Paper III). Direct and indirect costs were calculated for the tested diagnostic strategy and for two hypothetical strategies. A decision analysis was performed (Paper IV).

Results and conclusions: One out of 110 patients categorized as having a low clinical probability in combination with a negative D-dimer test was diagnosed with DVT during follow up. About 30% of the patients do not need further investigation for DVT. The APC-PCI complex perform inferior to the D-dimer methods for the exclusion of DVT but slightly superior when indicating its presence.

The AxSYM® and Innovance™ D-dimer assays perform well and in good agreement with the two well established assays with NPV´s of > 98% in the low clinical probability estimate (CP). Objective imaging in all patients was the least cost effective (€581) strategy, D-dimer screening of all patients before CP (€421) and CP in combination with D-Dimer testing only in patients with low CP (€406). Conclusion: the investigated diagnostic strategy is safe, result in more convenient and cost-effective care for patients.

Key words: Venous thrombosis; D-Dimer; Clinical probability; Diagnosis; APC-PCI complex

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”EN GÖR SÅ GÔTT EN KAN.”

Ralf Edström 1978

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List of papers

(I) Elf JL, Strandberg K, Nilsson C, Svensson PJ
Clinical probability assessment and D-dimer determination in patients with suspected deep vein thrombosis, a prospective multicenter management study.
Thrombosis Research 2009; 123: 612-6.

(II) Elf JL, Strandberg K, Svensson PJ
The diagnostic performance of APC-PCI complex determination compared to D-dimer in the diagnosis of deep vein thrombosis.
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(III) Elf JL, Strandberg K, Svensson PJ
Performance of two relatively new quantitative D-dimer assays (Innovance D-dimer and AxSYM D-dimer) for the exclusion of deep vein thrombosis.
Thrombosis Research 2009; 124: 701-705.

(IV) Norlin JM, Elf JL, Svensson PJ, Steen-Carlsson K
A cost-effectiveness analysis of diagnostic algorithms of deep vein thrombosis at the emergency department.
Submitted to *Thrombosis Research*, February 12th 2010.

List of abbreviations

ADP	adenosine diphosphate
APC-PCI	activated protein C – protein C inhibitor complex
APTT	activated thromboplastin time
CI	confidence interval
CP	clinical probability
CUS	compression ultrasound
DD	D-dimer
DVT	deep vein thrombosis
ELISA	enzyme-linked immunosorbent assay
FEU	fibrinogen equivalent units
LR-	negative likelihood ratio
LR+	positive likelihood ratio
mg	milligram
mL	milliliter
ng	nanogram
NPV	negative predictive value
PDGF	platelet derived growth factor
PE	pulmonary embolism
POC	point of care
PPV	positive predictive value
TF	tissue factor
TXA2	thromboxane A2
VTE	venous thromboembolism
vWF	von Willebrand factor

Introduction

Background

The transformation of shed blood into a solid has fascinated observers for millennia. A Chinese physician named Huan-Ti described how blod clots could affect blood circulation 2600 years B.C. (1). Aristotle, in *Meteorology*, and Hippocrates, in *De Carnibus* both postulated that the phenomenon was due to the cooling of blood (2). Modern understanding of the pathophysiology of venous thrombosis is usually attributed to Rudolf Virchow in the mid 19th century (3). "Virchow`s triad" illustrates the three most important categories contributing to venous thrombosis which are changes in the:

1) vessel wall
2) blood flow
3) constitution of the blood it self.

Deep vein thrombosis (DVT) refers to the formation of one or more blood clots (a blood clot is also known as a "thrombus") in one of the body's large veins, most commonly in the lower limbs. The clot(s) can cause partial or complete blocking of circulation in the vein, which can lead to pain, swelling, tenderness, discolouration of the affected area, and the skin can be warm to touch with prominent superficial veins. Venous thromboembolism (VTE) comprises DVT with or without symptomatic pulmonary embolism (PE). PE occurs when a portion of the blood clot breaks loose and travels in the bloodstream to the lungs (embolization). A PE can be a life-threatening complication with signs and symptoms that include: shortness of breath, chest pain, cough and, more rarely, fainting due to low systemic blood pressure caused by vascular obstruction in the lungs. Modern objective diagnosis and treatment of VTE started as late as the 1930s with the first attempts to visualize DVT by contrast venography and by initiating anticoagulant treatment (4-7).

The true incidence of VTE is somewhat elusive but estimated to be about 100-200/100 000 per year (8, 9) with a per-person lifetime incidence of 5% (10). About 10-15 000 patients/year are estimated to be affected in Sweden each year (11). At least 50% of patients, who present with symptomatic DVT, have asymptomatic PE and conversely, a majority presenting with symptomatic PE have asymptomatic DVT (12). Often, interaction between several inherited and acquired riskfactors can be assignable to the development of VTE. The most common risk factors found in patients with VTE are: surgery, trauma, fractures, malignancy, pregnancy, thrombophilia and estrogen intake. However, in up to 50% of the patients, no risk factor or triggering event can be identified (idiopathic VTE) and importantly, the incidence of the disease increases markedly with age (13). VTE is recognized as a major health problem for society and is estimated to cause more then 100 000 deaths/year in the US (14) . Signs and symptoms of VTE are non-specific and the majority of patients presenting with swelling or pain in the leg do not have DVT and some studies suggest that the majority of patients with PE are undiagnosed and identified first at autopsy (15-18). Missed DVT can lead to PE with a short term mortality of 10-25%. About ≈ 5% of patients with PE will suffer from chronic thromboembolic pulmonary hypertension (CTEPH) caused by residual obstruction of the pulmonary arteries thus giving shortness of breath as the main symptom (19-21) . Postthrombotic syndrome (PTS) of the leg is the result of insufficiency of the venous system caused by inadequate clot lysis and damaged venous valves. The risk of developing PTS after an episode of DVT may be as high as 60% (22-24). The main signs and symptoms of PTS are: chronic swelling, pain, heaviness, hyperpigmentation and skin changes. PTS, especially severe PTS (5%) with venous stasis leg ulcers, has been found to cause a significant reduction in the quality of life, similar to the impact caused by chronic congestive heart disease, chronic lung disease and rheumatoid arthritis. In addition to causing a low quality of life, PTS inflicts large costs for society (25). Treatment with anticoagulants decreases the risk of recurrent VTE, PTS and CTEPH but also increases the risk of major hemorrhage (26-28). Roughly 30 percent of those who have a DVT in a given year will suffer from a recurrent VTE sometime in the next 5-10 years after discontinuation of anticoagulant treatment. Recurrence is more likely if the initial episode was idiopathic (29).

Since suspected DVT is common in emergency clinical settings and un/over treatment can be fatal, diagnostic strategies must be developed to safely rule out DVT

in the vast majority who do not have the disease and to correctly diagnose those who do have the disease (30, 31). One way to approach this problem would be to perform objective imaging in all patients with suspected DVT. This would be expensive, inefficient and cause a number of complications (32, 33). Therefore, a simple, fast and non-invasive test, allowing the clinician to exclude the disease without further objective imaging in a substantial portion of patients is of utmost interest. The diagnostic strategy for patients suspected of DVT has undergone major advances over recent years. Notably the development and validation of clinical prediction rules to categorize patients pretest probability has simplified the diagnostic process. In combination with a low clinical pretest probability (CP), determination of D-Dimer (DD) fragments (or in the future other markers of coagulation), can help the clinician to refrain from objective imaging and exclude VTE (34, 35).

Haemostasis in brief

The haemostasis system contributes to several essential body defence systems. It impedes both the loss of blood and the disturbance of blood flow, as well as providing repair of injured vessels and tissue. Perfect haemostasis means: no bleeding and no thrombosis, three main stages can be identified (36):
1) Primary haemostasis (platelet adhesion and aggregation)
2) Secondary haemostasis (plasma coagulation)
3) Fibrinolysis

During *primary haemostasis*, interaction between the damaged vascular wall, platelets and adhesive proteins leads to the formation of a platelet plug (37). Immediately after vessel injury occurs, local vasoconstriction slows blood flow allowing platelets to adhere to the vessel wall. Subendothelial thrombogenic components are exposed and anchoring of platelets occurs within seconds through binding of platelet receptors (GP1b) to the exposed collagen and to collagen-bound von Willebrand factor (vWF). The platelets then undergo morphological modifications leading to the secretion of active substances. Neighbouring platelets are recruited, activated and aggregate (minutes) by fibrinogen cross-linking through binding between newly expressed fibrinogen receptors (GPIIb/IIIa) (**Fig. 1**).

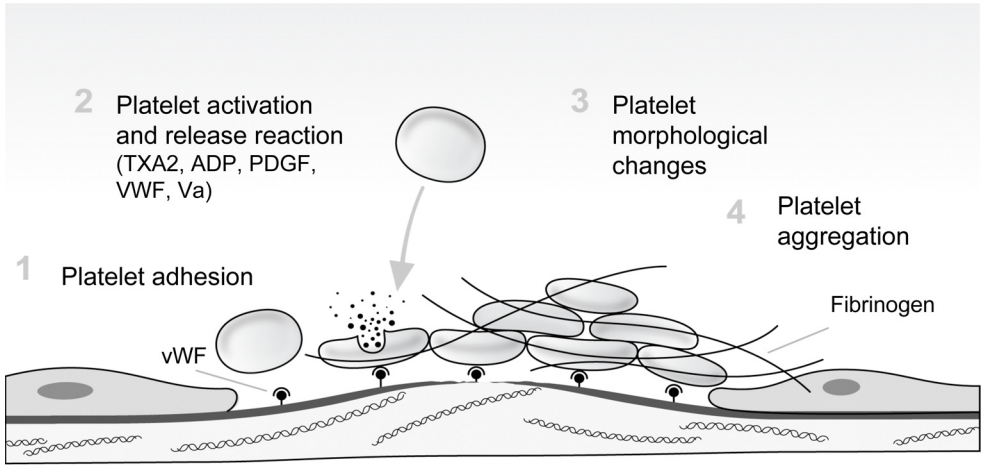

Fig. 1: Primary Haemostasis at site of vessel damage. TXA2 = Thromboxane A2, ADP = Adenosine diphosphate, PDGF = Platelet derived growth factor, vWF = von Willebrand factor. From Casper Asmussen with permission.

Vessel injury leads not only to the rapid binding of platelets to the subendothelium but also to activation of the coagulation cascade which occurs concomitantly (minutes) (37).

Traditionally two separate pathways have been described in the *secondary haemostasis*, the extrinsic and the intrinsic pathway.

These pathways meet at the level of Factor X and the residual steps, resulting in thrombin formation, are common to both pathways. Deficiencies of any of the coagulation active proteins in the pathways would prolong the time of the coagulation assay *in vitro* (prothrombin time (PT) for the extrinsic pathway and activated partial thromboplastin time (APTT) for the intrinsic pathway). It is now appreciated that coagulation does not occur as a consequence of linear sequential enzyme activation pathways but rather via a network of simultaneous interactions with regulation and modulation of these interactions during the thrombin generation process itself (38). The physiological activation of blood coagulation however, is mainly mediated by exposed subendothelial tissue factor (TF). Circulating, small amounts of activated factor VII (FVIIa) bind to TF and form the tenase complex which activates FX and FIX. FXa then binds to FVa forming a thrombin-activating complex. Probably less important *in vivo*, the intrinsic (contact) pathway is initiated by the contact factors

FXII, HMW kinogen and prekallikrein which activate FXI. FXI then activates FIX which together with its cofactor (FVIII) can activate FX. Finally FXa then act on prothrombin, in the presence of phospholipids and calcium ions, to form thrombin (**Fig. 2**).

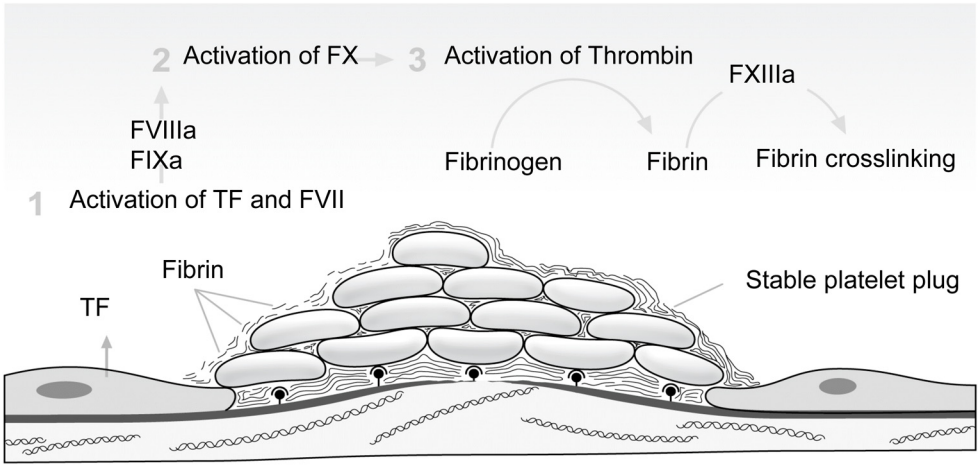

Fig. 2: Secondary Haemostasis (plasma coagulation). TF = Tissue factor. From Casper Asmussen with permission.

Uncontrolled coagulation throughout the vasculature is avoided by inhibitors of active coagulation most important tissue factor pathway inhibitor (TFPI), antithrombin and the protein C system (39). Disorders of this physiological anticoagulant system entail deficiencies of antithrombin (AT), protein C and S systems and they are all well-established, often congenital, causes of thrombophilia and especially commonly associated with VTE is the FV Leiden mutation, the principal cause of APC resistance (40).

Fibrinolysis and D-Dimer formation

The central purpose of the coagulation process is to generate a stable fibrin plug/ thrombus which is the natural seal of a vascular injury. In the coagulation process, adequate concentrations of thrombin are eventually generated and are able to cleave fibrinogen, which was first isolated by Prosper Sylvain Denis in 1856 (2). Fibrinogen is converted into fibrin by enzymatic cleavage of the fibrinopeptides A and B by thrombin, producing the soluble fibrin monomer. These monomers are then assembled with end-to-end and side-to-side association to form fibrin polymers.

After aggregation, linkage of these monomers by F XIIIa results in the dimerization of adjacent D-domains and an insoluble cross-linked fibrin clot (**Fig. 2**).The generation of a thrombus helps to solve an acute issue, but on the longer term, there is of course the risk of obstructing blood flow causing ischemia and necrosis in the affected areas. Therefore, once a fibrin clot has formed *in vivo*, it is modified by the fibrinolytic system, which constitutes the enzymatic process that leads to solubilisation of the clot by plasmin originating from tightly fibrin-bound plasminogen. Endothelial cells release tissue plasminogen activator (t-PA) into the blood stream as a result of blood flow stasis and fibrin formation. Fibrin serves as a cofactor for the activation of plasminogen by a proteolytic cleavage mediated by t-PA. Plasmin-mediated degradation of cross-linked fibrin generates fibrin degradation products of different molecular sizes, the smallest ones being dimeric fragments of the D-domain with a molecular weight of ~180kDa **(Fig. 3)** (41-44). These circulating degradation products can serve as diagnostic markers of thrombin and / or Factor XIIIa plus plasmin action that reflect prior clot formation and ongoing fibrinolysis (45). D-dimers (DD) represent only a minor fraction of what the monoclonal antibodies, in DD assays, recognize as an antigen (**Fig. 4**)(46, 47).

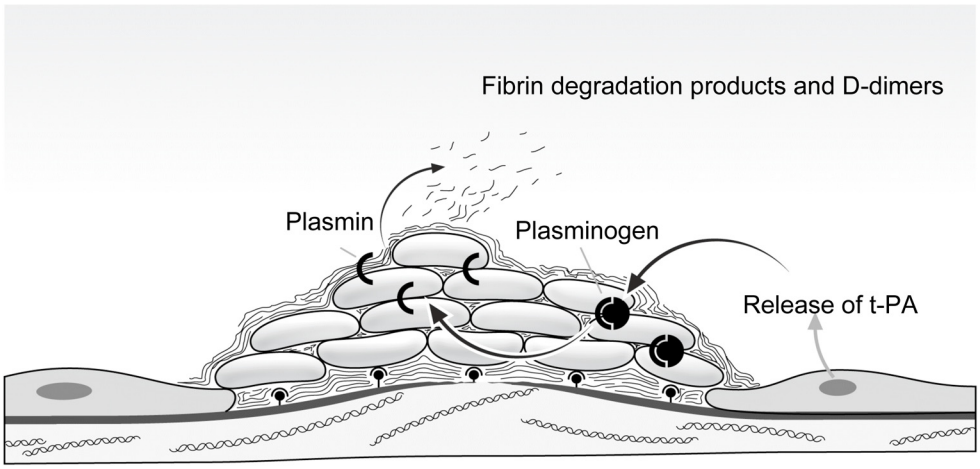

Fig. 3: Fibrinolysis. t-PA = tissue plasminogen activator. From Casper Asmussen with permission.

Small amounts of products containing DD are detectable in the plasma of healthy individuals since 2-3% of plasma fibrinogen is physiologically converted to cross-linked fibrin and then degraded. The concentration is increased in all conditions associated with enhanced fibrin formation and subsequent degradation by plasmin.

Examples are: VTE, disseminated intravascular coagulation (48), infection/ inflammation (49), cancer, old age (50, 51), pregnancy (52, 53) and trauma (54). The converse is also true, DD concentration decreases in response to anticoagulant therapy and the resolution of VTE symptoms. The half-life of DD is approximately 8 hours and clearance occurs mainly via the kidneys and reticulo-endothelial system (55).

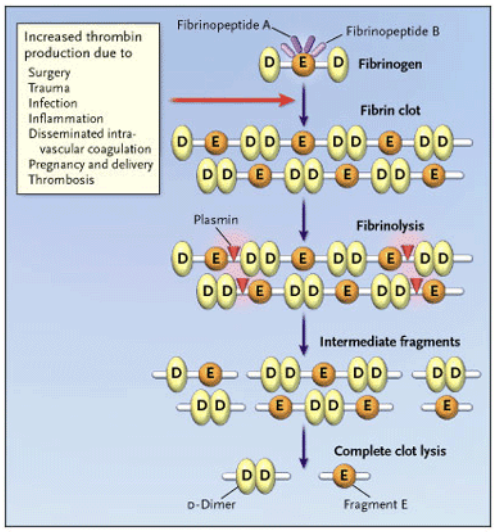

Fig. 4: D-dimer formation. Fibrinogen consisting of two D domains separated by a central E domain. Thrombin cleavage of the fibrinopeptides result in the end-to-end association of D domains into a fibrin clot. During fibrinolysis, plasmin cleaves the cross-linked fibrin into fibrin degradation products of which the D-Dimer is one of the resulting products. With permission from Massachusetts Medical Society. Copyright © All rights reserved. Bockenstedt P N Engl J of Med 2003;349:1203-04

D-Dimer assays

Monoclonal antibodies, towards the DD epitope, are generated by immunization with purified DD and have enabled measurement of the DD level in plasma/whole-blood. The first clinical assays were developed in the late 1980s (56). The resulting complexes can be detected by different biochemical methods. The results can be quantitative or qualitative. The numeric results of D-dimer assays are reported either as D-dimer concentration (assays that use purified fibrin fragment D-dimer as the calibrator) or fibrinogen-equivalent units (FEUs) (assays that use purified fibrinogen for preparation of a fibrin clot and degradation by plasmin as the calibrator). Laboratory analysts can transform D-dimer concentration to FEU based on the concept that one unit of FEU is approximately twice the mass of one unit of D-dimer (2FEU=1 D-dimer). Therefore, multiplying the D-dimer concentration by 2 converts the mass to the approximate FEU concentration.

Mainly three DD formats are available:

1) Enzyme-linked immunosorbent assays (ELISA).
2) Latex agglutination assays
3) Whole-blood agglutination assays

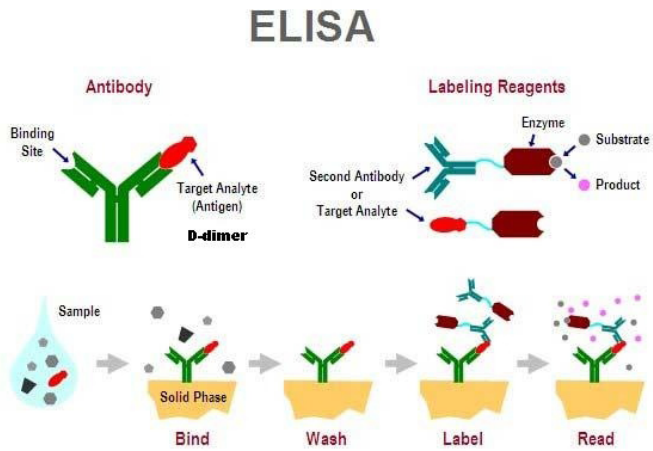

Fig. 5: Schematic picture of the ELISA-method. Modified from The Future of Things 2007

ELISA

The classic microplate ELISA technique was earlier considered the gold standard but the technique is labour intensive and not appropriate for single-test analysis which makes it ill- suited for routine emergency use (**Fig. 5**). The technique was recently evaluated in a meta-analysis by de Nisio et al giving a median sensitivity and specificity, in the diagnosis of DVT, of 94% and 53% respectively (35).

Fast-ELISA combines the ELISA technique with a final detection of fluorescence (ELFA) and can be fully automated, providing results within 15-35 min and it can be used for single sample testing (57, 58). Vidas® DD (bioMérieux) is one of the most validated ELFA-tests and this technique, has a sensitivity of 96% and a specificity of 46% in the diagnosis of DVT (31, 35). Their main limitation is the requirement of a dedicated immunoanalyzer.

Latex agglutination

These techniques work by agglutination of latex beads coated with monoclonal antibodies against the epitope of the analyte. They can be divided into qualitative,

semi-quantitative and quantitative. The qualitative and semi-quantitative assays can be used as bedside tests. However, as reading is most often visual, interobserver variability in the estimation of agglutination is unavoidable. These techniques usually have lower sensitivity (69-85%) and higher specificity (68-99%) compared to the ELISA-based or quantitative latex techniques.

The quantitative latex techniques have, compared to ELISA/ELFA, a comparable overall diagnostic performance but with a slight tendency to have lower sensitivity (93%) but higher specificity (53%). The quantitative latex techniques use photometric (turbidimetric) methods and have the advantage over the ELISA/ELFA methods of being able to run on standard biochemical/coagulation analyzers. Lately semi-quantitative and quantitative, latex agglutination-based systems have been developed as point of care devices with fully automated bedside testing and with minimal turn-around times.

Whole-blood assays

These assays use a hybrid antibody, which reacts with DD and human erytrocytes. In the presence of DD epitope in the sample, hemagglutination will occur. As reading is visual and qualitative interobserver-variability can be a problem. These test are developed as beside tests providing a result after <10minutes. Generally whole-blood assays are usually considered having a high-intermediate sensitivity (83%) and intermediate specificity (71%), however a recent meta analysis showed results comparable with laboratory based latex tests with a sensitivity of 85-96%, the quantitative tests scoring most favorably (59).

Limitations of D-Dimer tests in the diagnosis of VTE

The main confusion in the area of DD testing is due to the fact that DD is not a defined analyte but rather a name for a group of cross-linked fibrin degradation products of various molecular sizes (60-62). Due to the expected heterogeneity of the fibrin degradation products in patient samples, the differing specificities of the antibodies, assay designs, and calibrator materials used, the numerical results obtained with one assay are not always comparable with those of other assays. Therefore, optimally the performance of each assay for the exclusion of VTE should be evaluated by comparison with a clinically accepted gold standard. Then, the cut-off value below which an event can be ruled out must be determined. Finally, that cut-off value

should ultimately be confirmed in prospective outcome studies or at least compared with stored plasma from such studies (63). Attempts to harmonize and standardize have failed so far (64, 65). The different formats described above also differ in sensitivity and specificity but the difference between the two most used formats (ELISA-based and quantitative latex) is considered negligible especially after adjusting for the well known trade-off between sensitivity and specificity (35). The main limitation of the diagnostic usefulness is the reduced specificity seen in all patient categories where increased fibrin formation and the subsequent plasmin degradation is seen (inpatients, elderly, patients with cancer, during pregnancy and postoperative patients) (31). Attributes of an ideal DD assay are shown in **table 1**. However, no matter which method is used an evidence-based pretest probability assessment is vital for the interpretation of DD assay results.

Table 1: Attributes of an ideal D-dimer assay. CV = Coefficient of variation

Performance	**Goal**
Analytical	Accurate results around the cut-off -Low inter-observer variability for qualitative assays -Low CV (<5%) for quantitative assays -No interactions (e.g hemolysis, lipemia, bilirubin)
Operational	Easy to use and available 24 h a day, 7 days a week Rapid turnaround, automated and bedside analysis
Clinical	High sensitivity Reasonable specificity Validated in clinical studies -Accuracy study vs reference method to determine the optimal cut-off level -Prospective clinical outcome study to demonstrate the safety of VTE exclusion during follow up in negative patients

Diagnostic strategies for deep vein thrombosis

Clinical probability assessment

The index of clinical suspicion of VTE has increased during the last decades and, as a consequence, the prevalence of the disease among suspected individuals has dramatically decreased, with a median prevalence of 20% in a recent meta-analysis, falling as low as 10% in some studies (66-68). This is probably a result of improved access to diagnostic testing and a generally declining threshold for diagnostic uncertainty.

The generally acceptable failure rate for strategies in the exclusion of DVT is usually set at 1-(2) % which is obtained with the reference methods (gold standard), extended venous ultrasound of the leg or contrast venography (15, 69). Although depending on which DD method is used and the prevalence of disease in the studied population, using DD determination as the only diagnostic instrument is usually not recommended. According to Bayes theorem, the probability that a patient has the disease following diagnostic testing is determined by the estimated probability prior to the test (pretest probability; PTP) and the accuracy of the test (70, 71). Thus in most studied populations the negative likelihood ratio (LR-) for DD assays is not low enough to safely exclude VTE without incorporation of a low CP estimate (72).

Signs and symptoms of DVT are non-specific. Early studies in this area showed sensitivities and specificities between 60-88% and 30-72% respectively for empirical assessment of DVT (73). A major drawback of the implicit or empirical clinical assessment methods is that comparably fewer patients can be classified as having a low clinical probability and thereby be withheld from additional imaging testing (74-76). Also, the inter-observer agreement in the empirical assessment has been, at best, moderate. Several explicit clinical prediction rules (scores) have now been developed to simplify and standardize the assessment of DVT (77). These clinical prediction rules combine different patient characteristics into a score that should estimate the probability of DVT presence. The best-known prediction score is that of Wells et al and its iterations (**Table 2**) (78-80)

Table 2: Pretest clinical probability, Wells`score

– Active cancer	1
– Paresis, paralysis or recent plaster or immobilization of lower limb	1
– Bedridden >3days or major surgery < 4 weeks	1
– Localized tenderness	1
– Entire leg swollen	1
– Calf swelling > 3 cm compared with asymtomatic leg	1
– Pitting edema	1
– Collateral superficial veins	1
– Alternative diagnosis at least as likely as DVT	-2

High probability: 3 or more points
Intermediate probability: 1-2 points
Low probability: 0 or less points

This clinical prediction score (CP) is the most extensively validated and has shown strong predictive power to exclude DVT, with a LR- of 0.18 (81). In a meta- analysis involving more than 8 000 patients, the prevalence of DVT in the low probability group was only 5% (95% CI, 4-8%) and accounting for up to half of all patients studied (72). According to Bayes` theorem, the posttest odds of a disease equal the pretest odds times the likelihood ratio. Hence, DD assays reaching a negative likelihood ratio of 0.2 or less would be sufficient to safely exclude DVT when used in combination with a low CP (Pretest odds = 0.05/0.95 =0.052. Next posttest odds = 0.052 x 0.2 = 0.01. Converted to posttest probability by 0.01/1.01 ≈ 1%). However there are reports of a much higher proportion of DVT patients (12%) in the low probability estimate (82). This can be seen if the baseline prevalence in a test population is relatively high, thereby giving a higher probability of DVT also in the low probability estimate. This would affect the posttest probability to a level where DVT cannot be safely excluded. There is also a discussion on the safety and efficiency of the CPs in several clinically relevant subgroups e.g. inpatients, elderly, patients with cancer and in pregnancy (83, 84). Another limitation of the Wells` score is the uncertainty about the effect of clinical experience on the predictive power of the score. The Wells` score is not completely objective since it includes the clinician's judgement whether an alternative diagnosis is more likely than DVT, or whether localized tenderness along the course of deep vein is present, thus, this score cannot be standardized. It is possible that less experienced users would not recognize certain clinical features of alternative diagnosis, which could underestimate the score and thereby account for the high prevalence of DVT in the low CP estimate seen in some studies. Inter-observer variability has not been widely evaluated, but the reported studies involved many different physicians with a wide range of clinical experience, including junior residents (85).

Imaging techniques

Objective imaging of DVT was first available in the 1930s by **contrast venography** (5, 86). Contrast venography essentially works by assessment of the filling or non-filling of venous segments by a contrast medium, exposing films at the correct time. The method has developed into the "gold standard" for the diagnosis of DVT (15).

The **125 I Fibrinogen test** was introduced into clinical practise in 1965 (87, 88). The concept of this test is the injection of fibrinogen, labelled by radioactive iodine, into the circulation. The fibrinogen will then produce a radioactive thrombus which

can be detected. The test had a high sensitivity as long as the thrombus is still forming but low specificity for DVT and most countries have now prohibited the use of this fibrinogen preparation due to risk of virus contamination.

The possibility of using **ultrasound** for the diagnosis of DVT was demonstrated 1967 (89) and is now shown to be as accurate as contrast venography with the advantage of being a non-invasive test (69, 90).

Impedance pletysmography was developed in the 1960s. An inflatable tight pressure cuff is used to trap venous blood and allow measurement of the maximum rate of venous return by changes in electrical impedance. Although non-invasive and feasible, the method is considered to have a diagnostic performance too low for the exclusion of DVT (91).

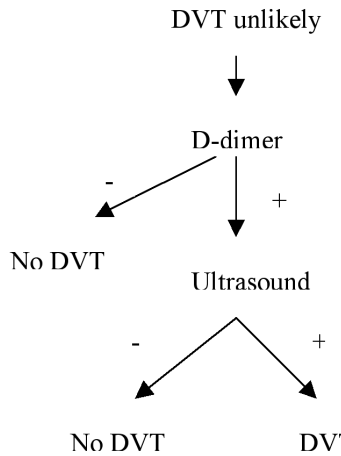

Fig. 6: Diagnostic algorithm in case of low clinical probability

Diagnostic strategies involving D-dimer determination

In combination with a low pretest CP of the disease a negative DD test can safely rule out DVT in 30-50% of outpatients with suspected DVT (80) (92-95). In the case of a low probability estimate and positive D-dimer result, ultrasound is performed (which can be delayed 12-24 hours without anticoagulant treatment) but limited to the proximal veins from the groin to the trifurcation region. If the test is positive, the diagnosis is confirmed and if it is negative, DVT is considered excluded (**Fig. 6**) (79). Patients with moderate/high clinical probability should all proceed to objective

imaging, preferably ultrasound investigation. Imaging can be delayed to the following day after giving the patients an injection of low-molecular-weight heparin. In patients with an intermediate/high probability estimate, negative DD and a normal ultrasound, a serial ultrasound investigation is not necessary (80, 85, 93, 95). Positive DD test in the intermediate/high probability estimate usually needs serial ultrasound imaging after an initially normal ultrasound or the adding of contrast venography/ extended ultrasound including distal vein segments to the diagnostic algorithm (**Fig. 7**) (85).

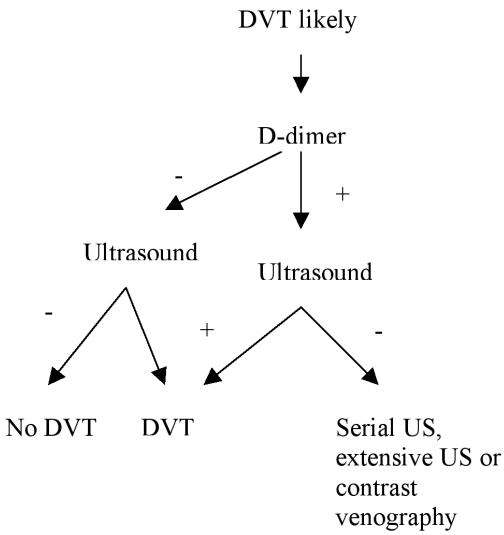

Fig. 7: Diagnostic algorithm in case of non-low clinical probability. Us = ultrasound

High D-dimer levels

Due to the generally low specificity and positive likelihood ratios of the DD assays, positive DD results are usually not considered useful to "rule in" DVT. A recent study though, found that strongly elevated DD levels substantially increase the likelihood of pulmonary embolism irrespective of pre-test probability score (96). Whether this should translate into more intensive diagnostic measures remains to be studied.

APC-PCI complex determination in the diagnosis of venous thromboembolism

Plasma concentrations of a complex between activated protein C (APC) and its inhibitor (PCI) increase in hypercoagulative states as DVT and PE (97, 98). The APC-PCI complex measured with a sensitive immunofluorometric sandwich assay in plasma, have shown promising performance in a case-control study in patients with DVT compared to controls (34). The assay can be fully automated, is sensitive and seems to meet the perquisite of a good marker of DVT by showing receiver operating characteristics (ROC) curves similar to that of the DD methods used for comparison (34, 99). The assay has recently become commercially available as an ELISA assay (APC-PCI ELISA kit, Bio Porto Diagnostics A/S, Gentofte, Denmark). In contrast to the D-dimer methods, the APC-PCI complex is a well defined analyte and therefore possible to standardize (100). Earlier studies also indicated that APC-PCI performed better than DD at high specificities and showed no correlation with c-reactive protein (CRP) concentration, suggesting that in contrast to the DD level, activation of the coagulation system as measured by the APC-PCI concentration is not influenced to the same extent by inflammatory activity (34, 49). In addition, it has been suggested that just like DD, the APC-PCI complex can be used as a marker of an increased risk of future VTE (101).

Cost-effectiveness of clinical probability assessment and D-Dimer determination in the diagnosis of DVT

Despite the wealth of published data concerning safety and feasibility in a diagnostic strategy combining CP assessment and DD determination in outpatients, cost-effectiveness comparisons with the traditional strategy (accurate but expensive objective imaging), are scarce. Furthermore, there is a risk that this new strategy is implemented in an incorrect way, leading to a wider patient selection and thereby influencing the cost-effectiveness (102). The Swedish Board of Health and Welfare states that: not enough research is done to certify which diagnostic strategy is the most cost-effective (11). Nevertheless, the new algorithm does imply large potential cost savings of the health care budget. Savings that are increasingly important as the

Swedish health care system is facing higher expenses due to the development of new and more expensive technologies and an aging population.

Aims of the study

– **Paper I:** To examine whether a combined diagnostic strategy of a pretest clinical probability score, followed by a local D-dimer test, was safe for the exclusion of deep vein thrombosis. We also wanted to address the question of whether D-dimer methods used locally were as reliable as the same method used in batch analysis under optimal circumstances with reduced variability from inter-assay differences.

– **Paper II:** To evaluate the performance of the APC-PCI complex in comparison to well established D-dimer assays in the diagnosis of deep vein thrombosis.

– **Paper III:** To evaluate the performance of two new quantitative D-dimer assays (AxSYM® D-dimer and Innovance™ D-dimer) for the exclusion of suspected deep vein thrombosis.

– **Paper IV:** To evaluate the cost effectiveness of introducing the diagnostic strategy used in Paper I, into clinical practise. For comparison the traditional strategy (diagnostic imaging of all patients) and a reversed strategy (D-dimer testing, as a screening test, on all patients prior to clinical examination) was used.

Patiens and methods

This study was performed between December 2003 and December 2005. Adult patients with a suspected first episode of DVT were potentially eligible for inclusion. Seven centres in southern Sweden, serving approximately 1 million residents, participated in the study. A total of 491 outpatients were consecutively evaluated for the study over the 2-year recruitment period of whom 357 were enrolled. The most common exclusion criteria were previous VTE or a duration of symptoms > 10 days. Patients were either self-referred, referred from primary care physicians or to a lesser extent sent in from other clinics. The patients were enrolled immediately on their arrival at the hospital emergency department. Enrollment was possible 24h/day, 7d/week. Site-specific investigators were responsible for collecting data on each site and the central database was compiled by the authors of Paper I.

The regional center research ethics board in Lund approved the study, and all patients provided written informed consent before enrollment.

All patients were evaluated by the emergency physician on duty, who was briefly introduced to the Wells score. Pretest probability for DVT according to Wells' nine item score (79) was determined. Patients were categorized as having low, intermediate or high pretest probability. Patients with a low pretest probability underwent immediate, local, DD testing. Patients with low CP and a negative result on the DD test had no further diagnostic testing for DVT and received no anticoagulant therapy. These patients were followed for VTE events and were either contacted by telephone or seen at an outpatient clinic after 3 months. Patients who could not be reached by telephone or did not return to the outpatient clinic, were checked for VTE events in the medical charts and diagnostic imaging databases at each local hospital. Patients who presented again with symptoms consistent with DVT or PE underwent objective testing with contrast venography and / or compression ultrasonography (CUS) of

the leg and ventilation/perfusion scintigraphy and / or computed tomography of the lungs respectively.

Patients with intermediate / high CP or positive DD test were further investigated with contrast venography and / or CUS. DVT was diagnosed if an intraluminal filling defect was present in two views or if noncompressibility was present in the common femoral, superficial femoral or popliteal vein respectively. If the CUS was negative, further evaluation with comprehensive ultrasound (including calf veins) or contrast venography was performed.

For D-dimer analysis, venous blood was collected. The plasma was aliquoted and one aliquot was used for the local D-dimer analysis. The rest were frozen for later analysis in batch with Innovance™ D-Dimer, AxSYM D-dimer, Vidas® D-Dimer Exclusion™ and Autodimer®. The APC-PCI complex was measured with an in-house developed sensitive immunofluorometric sandwich assay in plasma (103).

From the 357 patients included, 350 plasma samples for the comparison between APC-PCI complex and the reference assays (Autodimer® and Vidas®) were available (Paper II). In 311 patients enough plasma was collected to analyze and compare the clinical performance of all the DD assays in Paper III. However, for the regression analysis, the results are calculated on all patients having the assays performed with an exact value (n=304) for AxSYM vs Vidas/Autodimer correlation and (n=340) for Innovance vs Vidas/Autodimer correlation.
Laboratory personnel performing the D-dimer assays and APC-PCI complex analysis were not aware of the patients' CP estimate.

Further details about the collection and analysis of the clinical and laboratory data are reported in detail in Papers I-III.

In Paper IV, a decision analysis model was developed to evaluate the cost-effectiveness and compare three different diagnostic algorithms (**Fig. 8**). This evaluation was based on the results of the management study performed (Paper I), regional and national data (11, 104). For indirect costs of the time spent at the hospital, loss of productivity was estimated by using gross average wage in Sweden 2007. Lost leisure time for patients aged 65 and older was estimated by assuming a 35% value of the gross

average wage. In the decisions analysis we considered all strategies to have the same diagnostic accuracy. Diagnostic strategies evaluated were:
1) CP ± DD ± contrast venography ± CUS (Paper I)
2) Contrast venography (CV) ± CUS or CUS alone, assumption; 50% CV alone
3) DD-screening ± CP ± contrast venography ± CUS

Sensitivity analysis was performed to evaluate how robust our results were when changing the prevalence of disease (number of patients having low CP or negative DD), direct and indirect costs.

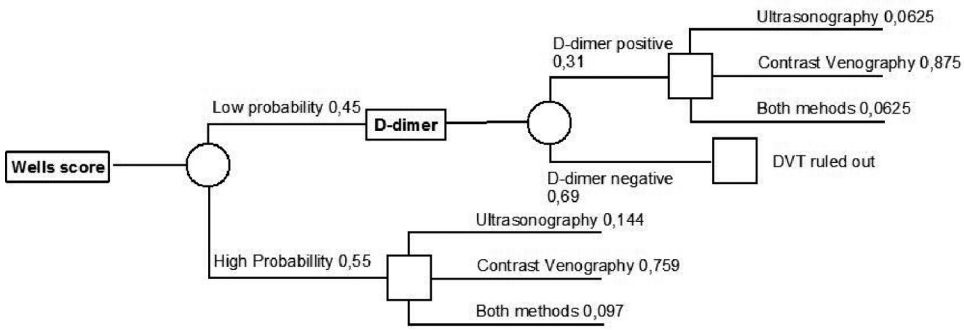

Fig. 8: Decision analysis based on the results of the SCORE-study (Paper I)

Comments on specific statistical methods

Bayes'theorem states that the probability of disease is determined not only by the accuracy (sensitivity and specificity) of the test but also by the estimated probability prior to the test (prevalence). In case of a positive result, the post-probability is identical with the positive predictive value (PPV) and in case of a negative result with the negative predictive value (NPV). Since the predictive values strongly depend on the probability prior to the test, calculating likelihood ratios (LRs) is compelling since LR x pretest odds = posttest odds. A likelihood ratio is derived from the sensitivity and specificity of the test. Positive likelihood ratio (LR+) = sensitivity / (1 – specificity) and the negative likelihood ratio (LR-) = (1 - sensitivity / specificity). Pretest odds derived from pretest probability are as follows: Pretest odds = pretest

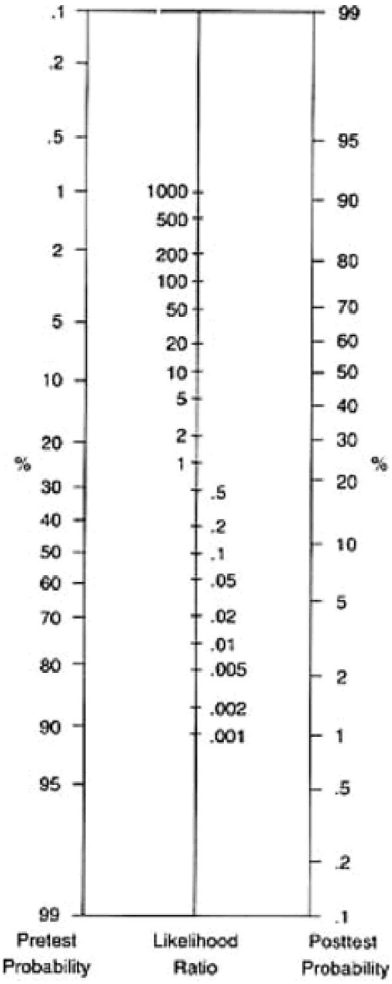

Fig. 9: Fagan´s nomogram to estimate posttest probability by extending a line drawn from the known pretest probability and likelihood ratio. Adapted from Fagan, TJ. Nomogram for Bayes Theorem.N Engl J Med 1975; 293:257

probability/ (1 - pretest probability) and similar posttest probability = posttest odds / (1 + posttest odds). An LR is telling us how many times more common, a positive result is among those who have the disease (LR+) and inversely for the negative likelihood ratio (LR-) (70). The utility of LRs is probably best exemplified by the use of a nomogram (**Fig. 9**).

In Papers II and III, the following statistical methods need further comment:

McNemar`s test: although a superficial resemblance to a test of categorical association, as might be performed by a 2x2 chi-square test or a 2x2 Fischer exact probability test, mathematically it does something quite different. This test examines if there is a significant difference between *proportions* that derives from the marginal sums of the 2x2 table i.e. what is the probability that we obtain a result this large or larger in the discordant pairs, if the null hypothesis is true? The choice to mainly use the McNemar test, instead of chi-square or Fischer`s exact test, in order to test for significant differences between descriptive statistics, was based on the fact that our proportions were paired (chi-square was not appropriate) and that we in most contingency tables had sample sizes above a total of 20 and the smallest cell numbers > than 5 (the Fischer`s exact test was less appropriate).

Pearson`s correlation: to investigate the degree of linear association between two variables when the variables are normally distributed and continuous (parametric). This association is measured by the correlation coefficient, (r).

Results and discussion

Paper I: Clinical probability assessment and D-dimer determination in patients with suspected deep vein thrombosis, a prospective multicenter management study

Three hundred and fifty- seven patients were enrolled during a 2-year period in a population of approximately 1 million residents, of whom 84 patients were diagnosed with DVT. This should be put into a perspective of the results from a recent epidemiological study of DVT incidence in Malmö, showing an incidence of 51/100 000 (105). Although inclusion of patients was supposed to be consecutive, the low number of included patients probably reflects the fact that the inclusion of patients was integrated in daily clinical practise and handled by the emergency physician on duty. Another explanation for the low inclusion rate was probably that during the study the diagnostic algorithm tested was implemented as clinical routine as a result of the guidelines from the Swedish National Board of Health and Welfare on the diagnosis of VTE established in 2004. Nonetheless, the demographic data support that the study population is representative of patients with suspected DVT since the data are similar to other studies in this area (35) and our results are in good agreement with the other studies in a recent meta-analysis, where Paper I was included (106).

The prevalence of DVT (23.5%) was lower than expected and probably reflects the international tendency to lower the index of suspicion in this patient category and the impact of study design (31). Although lower than expected, compared to many studies from the US/Canada the prevalence is rather high (72). This was one of the apprehensions we had about implementing this new algorithm i.e. that the prevalence of DVT was higher in our clinical settings than in the study populations reported and thus resulting in an unacceptable high failure rate of this algorithm.

Of the 357 patients, entering the study, 45% (n = 159) were categorized as having a low CP of whom 8.8% had DVT as final diagnosis. In total 31% of the patients had the combination of low CP and a negative DD result (**Fig. 10**).

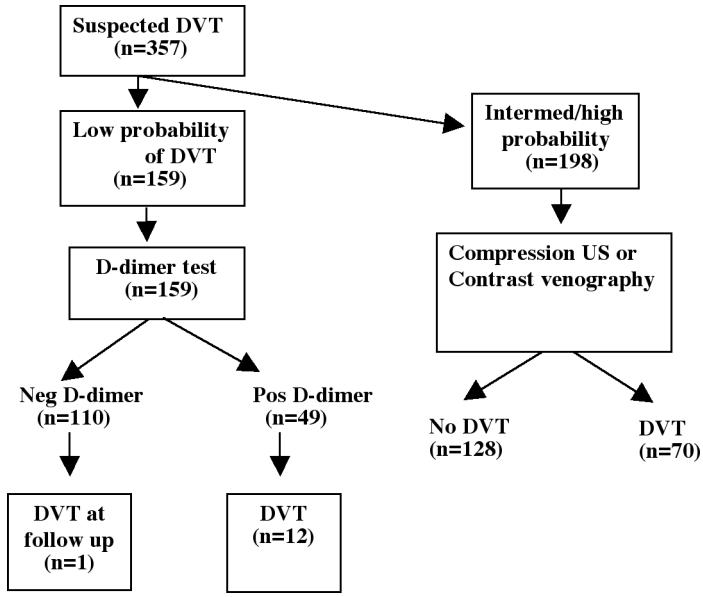

Fig. 10: Strategy for diagnosis of DVT and number of patients in each group

Over the 3-month follow up period (median 5.5 months), 1 patient (0.9%, [95% CI, 0.02-4.96]) subsequently developed distal DVT. For the real time local DD method, sensitivity, specificity, NPV and negative likelihood ratio were 86%, 74%, 98% and 0.19 respectively. These results should be put in perspective with the generally accepted failure rate of 1-(2)%. No significant difference in diagnostic performance was seen between the real time local DD analysis and the post-hoc batch analysis, thus in support for that analytical results obtained over an extended period of time under routine emergency test conditions does not affect the diagnostic performance.
An interesting observation in our study was that although the guidelines from the Swedish National Board of Health and Welfare on the diagnosis of VTE was presented at the time for inclusion and the study protocol was approved by the head of the ward at all hospitals, eleven patients in the low CP-negative DD group was further investigated with objective imaging. In one of these patients a distal DVT was revealed by compression ultrasound. There are many interesting aspects of this observation:

1) This distal DVT could be a chronic thrombus, not in need for anticoagulant treatment.
2) If, in fact, this DVT was acute, this supports the observation that the sensitivity for distal DVT, of this diagnostic algorithm, is inferior to that of proximal DVT (107, 108).
3) The good outcome in the present and other recent management studies probably indicates that a negative D-dimer test does not exclude all distal DVTs, but excludes DVT that require treatment (108, 109). Furthermore, most diagnostic algorithms used, involve compression ultrasound limited to the proximal veins and the value of diagnosing distal DVT is debatable. Studies using complete compression ultrasound or contrast venography as reference tests would probably show inferior sensitivity of the D-dimer assays compared to evaluations in outcome studies (57, 110).
4) Leaving open the possibility to override the clinical prediction score and use empirical judgement can be a better approach than strict adherence to the diagnostic algorithm (111).

Finally, the effect of clinical experience on the predictive power of the wells score in the diagnosis of DVT is still a matter of debate although the reported studies in the area involved many different physicians with a wide range of clinical experience (71, 85, 112). In our study, a post hoc analysis of this subject was done (Lundahl M, examensarbete, läkarutbildningen, Faculty of Medicine, Lund University 2009). In short, 352 Wells score examinations were identified in which we were able to identify the physician (n = 157) and correctly match him/her with his/her formal clinical experience in the national register of the Swedish National Board of Health and Welfare. Sub internship, interns and junior residents constituted about 1/3 of the included physicians, indicating that, despite a large portion of physicians with low clinical experience, the overall outcome was acceptable and further support the generalizability of our results. One interesting observation in this sub study was that, compared to more experienced colleagues, junior physicians tend to overestimate the probability of DVT, most likely because of a more hesitant use of the alternative diagnosis criteria of the Wells score.

Paper II: The diagnostic performance of APC-PCI complex determination compared to D-dimer in the diagnosis of deep vein thrombosis

Ruling DVT out

D-dimer testing is widely applied for the exclusion of DVT and PE. Due to the mixture of analytes measured, as well as the differences in antibodies, assay types and calibrators used, the numerical results obtained with one assay are not readily comparable with those of other assays (60, 68). Hence, a new and well-defined analyte with comparable diagnostic performance would simplify comparisons of future clinical trials. The ideal test should also be fast, fully automated, quantitative and observer independent. The APC-PCI complex seems to have the ability to meet these perquisites.

The study population in this paper is based on the patients included in the SCORE study, Paper I. Plasma samples for analysis of the APC-PCI complex and both DD assays, were available in 350/357 patients.

In the overall cohort, compared to the APC-PCI complex, the D-dimer assays show significantly better performance for the exclusion of DVT (sensitivity and NPV) but a significantly inferior ability to exclude DVT in the vast majority of the patients who do not have DVT and reduces the number of patients requiring additional imaging tests (exclusion rate, specificity) (**table 3**). In the low CP cohort though, no significant differences was seen concerning sensitivity and NPV.

Table 3: Diagnostic performance by clinical probability score (CP) in percentage and (95% CI)

APC-PCI (<0.26 ng/mL)	All patients (n=350)	Low CP (n=155)	Intermed/high CP (n=195)
Sensitivity	74 (63-83)	69 (39-90)	75 (63-84)
Specificity	80 (74-84)	80 (72-86)	80 (71-86)
NPV	91 (86-94)	97 (91-99)	86 (78-91)
PPV	52 (43-62)	24 (12-41)	66 (54-76)
LR-	0.32	0.39	0.31
LR+	3.6	3.4	3.7
AUTODIMER (<250 ng/mL)			
Sensitivity	93 (84-97)	85 (54-97)	94 (85-98)
Specificity	60 (54-66)	68 (59-75)	52 (43-61)
NPV	96 (92-99)	98 (92-100)	94 (85-98)
PPV	59 (51-66)	81 (68-90)	51 (42-60)
LR-	0.12	0.23	0.11
LR+	2.3	2.6	2
VIDAS (<500 ng/mL)			
Sensitivity	98 (91-100)	100 (72-100)	97 (89-99)
Specificity	40 (34-46)	47 (39-56)	31 (24-40)
NPV	98 (93-100)	100 (93-100)	95 (83-99)
PPV	33 (27-39)	15 (8-24)	43 (35-51)
LR-	0.06	0	0.09
LR+	1.6	1.9	1.4

An interesting observation in this study was that unlike the D-dimers, the APC-PCI complex does not show the usual decline in specificity with higher clinical probability estimates. Indeed, this was also indicated in an earlier study by Strandberg et al (34) and could be explained by the fact that the APC-PCI complex is less influenced by unspecific coagulation activation due to e.g. inflammation (co-morbidity) found in the intermediate/high probability estimates. However, this interpretation could also just reflect the different half lives and that different DD fragments are measured during the days after clot formation. The area under the curve in the ROC analysis showed a significantly inferior ability of the APC-PCI complex to differentiate between DVT and no DVT compared to the DD assays (**fig. 11**).

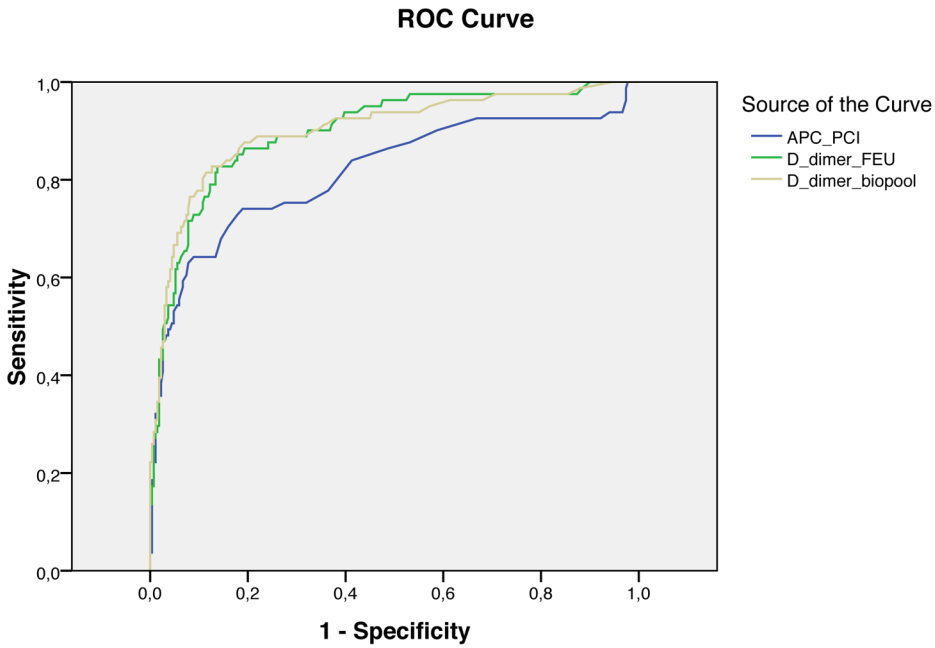

Fig. 11: ROC = receiver operating characteristics curve for each method

The cut-off value used for the APC-PCI complex is set at the upper limit of 95 % CI of healthy lab personnel. Our results indicate that the cut-off value for the APC-PCI complex, used in diagnostic strategies for acute VTE, should be lower. Indeed, on lowering the cut-off to 200 ng/mL we obtained results comparable with many latex-agglutination/whole blood assays with a LR- of 0.25 and thus have the theoretical ability to safely exclude DVT in a low CP estimate (72).

Ruling DVT in

Although generally considered high sensitive-low specific markers of VTE, very high DD levels are known to improve the positive predictive value and it has been suggested that the combination of a high CP and very high DD levels might be sufficient to establish the disease (96, 113, 114). Since the results of earlier papers and the present paper indicated that the APC-PCI is affected by co-morbidity to a lesser extent, we tried to address this question as well. At the cut-off values used for the exclusion of DVT, the APC-PCI complex shows a significantly higher positive

predictive value compared to the DD assays (66% vs 51% for Autodimer and 43% for Vidas). As indicated by the ROC curves and calculations at the 75th and 90th percentile though, there are no significant differences between the assays with regard to the positive predictive value (PPV). However, there is a tendency to achieve a higher inclusion rate for the APC-PCI complex. No additive effect on performance was seen when using APC-PCI complex in combination with the DD assays, probably due to similar performance characteristics (**Fig.11**). Compared to the results in a recent study by Tick et al (96) who showed a PPV of 65% in the PE likely-Vidas assay >4000 ng/mL group, our results with the APC-PCI complex reach a PPV of 66% in the DVT likely-standard cut-off (> 0.26 ng/mL) group. This is in spite of the fact that their study had a higher prevalence of VTE 43% in the likely group compared to ours (35%). It is important though to realize that specificity is usually lower in PE studies compared to DVT studies, indicating that for the purpose of ruling VTE in, D-dimers, and probably the APC-PCI complex, would have given a better performance in DVT studies. Clinical scenarios where addition of high APC-PCI or DD levels could be useful and perhaps initiating anticoagulant treatment considered are:

1) High CP estimate in combination with a normal proximal CUS.
2) High CP estimate and only indirect or non-conclusive imaging results.
3) Low CP and positive CUS in a patient with previous ipsilateral DVT.

Paper III: Performance of two relatively new quantitative D-dimer assays for the exclusion of deep vein thrombosis

Retrospectively, we evaluated the performance of two new DD assays on 311 plasma samples from the SCORE study (Paper I), and for comparison two well-established DD assays were used.

No significant differences were seen in sensitivity and negative predictive values between Innovance, AxSYM and the reference assays (**table 4**). The area under the ROC curve was slightly lower for the AxSYM assay (0.85 vs 0.89-0.9, p-values around 0.05) and the correlation to the reference assays was only moderate ($r < 0.8$) whereas the agreement with the Vidas assay was near excellent ($\kappa = 0.8$). The Innovance assay

reached the highest AUC, showed a strong correlation with the reference assays ($r \geq 0.9$) and a good agreement with the Vidas assay ($\kappa = 0.76$). In combination with a low pre-test probability score the Innovance assay reached a NPV of 100% (95% CI, 92-100) and the AxSYM assay 98% (95% CI, 87-100).

Table 4: Results in the overall cohort with sensitivities, specificities, NPV and LR- at a cut-off value of 250ng/mL for the Autodimer and 500ng/mL for the other assays

	Sensitivity (95% CI)	**Specificity** (95% CI)	**NPV** (95% CI)	**LR-**
Innovance	95.8 (87-99)	37.7 (32-44)	96.8 (90-99)	0.11
AxSYM	94.4 (86-98)	32.2 (26-39)	95.1 (87-98)	0.17
Vidas	97.2 (89-100)	38.5 (32-45)	97.9(92-100)	0.07
Autodimer	91.7 (82-97)	60.3 (54-66)	96.0 (91-98)	0.14

The AxSYM D-dimer assay was previously only evaluated in patients with pulmonary embolism where Ghanima and Reber et al found sensitivity and NPV of 100% (39, 45). Our results obtained on DVT patients are lower: 92.3% and 97.9% respectively. The observed differences could be explained by the observation that the sensitivity of the DD method generally, is higher in PE patients. Also, since the sample size in our study was limited, the two patients that were negative with all the assays contribute significantly to the results and they could actually represent chronic thrombosis. Lowering the cut-off values of the AxSYM assay show the usual trade off between sensitivity and specificity seen in DD evaluations and would give a specificity that make the assay less clinically useful.

The Innovance D-dimer assay was previously evaluated in a large, mixed VTE study showing excellent performance and very good correlation and agreement with the Vidas assay (115). Our evaluation supports these findings in a population of exclusively DVT patients. As for the AxSYM comparison, our data show slightly inferior performance compared to the earlier studies, but in the low-probability estimate the Innovance assay reached 100% NPV.

Paper IV: A cost effectiveness analysis of diagnostic algorithms of deep vein thrombosis at the emergency department

The most effective strategy was the use of Wells` score in combination with DD limited to those with a low CP at €406. A traditional strategy using objective imaging for all patients was much more expensive at €581(**table 5**). Screening all patients with symptoms suspicious of DVT, with DD, before clinical examination, is more cost-effective (€421) than objective imaging but attention must be made to the risk that, by implementing this strategy, a wider population could fall into the category of "suspected DVT". Indiscriminate use of DD as a screening test will, due to the low specificity, result in many unnecessary objective imaging tests and thereby an increase of costs. Indeed, this is shown in the sensitivity analysis where lower CP and negative DD patients give higher costs (**table 5**).

Table 5: Direct and indirect costs (€ 2008). Total costs of alternative algorithms in bold. PTP = Pretest probability

			Sensitivity Analysis					
			Direct cost		Indirect cost		Negative D-dimer 80%	Low PTP 35%
			Decrease 50%	Increase 50%	Decrease 50%	Increase 50%		
PTP and D-dimer	Direct cost	€ 311	€ 156	€ 467	€ 311	€ 311	€ 289	€ 338
	Indirect cost	€95	€ 95	€ 95	€ 48	€ 143	€ 94	€ 100
	Total cost	**€ 406**	**€ 251**	**€ 562**	**€ 359**	**€ 454**	**€ 383**	**€ 438**
CV/CUS	Direct cost	€ 471	€ 235	€ 706	€ 471	€ 471		
	Indirect cost	€ 110	€ 110	€ 110	€ 55	€ 165		
	Total cost	**€ 581**	**€ 345**	**€ 816**	**€ 526**	**€ 636**		
Reversed order	Direct cost	€ 332	€ 167	€ 500	€ 332	€ 332	€ 313	€ 438
	Indirect cost	€ 89	€ 89	€ 89	€ 45	€ 134	€ 80	€ 99
	Total cost	**€ 421**	**€ 256**	**€ 589**	**€ 377**	**€ 466**	**€ 393**	**€ 537**

The results seem to be robust since the CP± DD strategies are more cost-effective, compared to the imaging strategy, over a wide range of scenarios. Furthermore, 1/3 of the patients in the CP±DD strategy have the benefit of the patient's preference for an immediate diagnosis and the benefit of avoiding contrast venography, an invasive method associated with a small but significant risk of complications.

Our results are in good agreement with other cost-effective studies (102, 116).

Although considered robust, the observed differences between our algorithms should be interpreted carefully when enlarged to the regional and national level. Depending on the true incidence of DVT a decrease in expenditures with € 0.4-1.4 million per year could be achieved in the region of Scania in the southern Sweden, moving from the traditional algorithm to the CP±DD based algorithm. On the national level, savings of € 3.3-11 million per year could be achieved.

General discussion and future considerations

Over the last decades, there has been considerable research into the diagnostic process of VTE. As technical development advances, new and more non-invasive imaging techniques have evolved. It is now widely accepted that venous ultrasound is an accurate test for the diagnosis of DVT replacing contrast venography in most cases. For PE, multidetector computer tomographic pulmonary angiography (CTPA) is rapidly evolving thereby replacing ventilation perfusion lung scanning and pulmonary angiography.

Despite these advances, the accuracy of these imaging tests is still highly dependent on the level of clinical suspicion before testing. This is demonstrated in clinical studies of venous ultrasound, where the posttest probability of DVT in patients with a high pretest probability is unacceptably high though negative ultrasound. In the case of CTPA, the posttest probability will be unacceptably low for patients, suspicious of PE, with a low PTP in combination with positive CTPA results (78, 110).

Recent and future research in the area of clinical probability assessment is mainly concerned with simplifying and constructing clinical prediction scores based entirely on clinical variables, independent from physicians` implicit judgement. As long as the scores contain items that are not completely objective, the scores cannot be entirely standardized and thereby are possibly affected by the physicians' clinical experience (117, 118). Of course, constructing scores that are both simple and completely based on objective clinical variables will be difficult and probably, leaving open the possibility to override the score, is a better approach than strict adherence to the diagnostic algorithm (111). Furthermore, further validation is necessary in clinically-relevant patient subgroups. Possibly CPs will develop that are designed to be used in different clinical conditions/patient subgroups.

In the area of biochemical markers of thrombosis, research will probably deal with the evaluation of defined new markers of thrombosis that are possible to standardize. It is the capacity to generate thrombin, and the enzymatic work that thrombin does, that determines blood coagulability. Therefore, the measurement of thrombin generation provides a method for quantifying the composite effect of the multiple factors that determine coagulation capacity and the influence of the environment on these factors (38). DD and APC-PCI complex are two methods to measure thrombin generation but others will be evaluated. DD research will also try to find clinical algorithms which increase the usefulness in specific patient categories such as in elderly patients, cancer patients, patients with previous VTE, pregnant women, patients on anticoagulant treatment and research will also further evaluate the DD`s predictive power of recurrence of VTE. Furthermore, in many countries, primary care physicians are faced with the initial presentation of VTE and as a consequence the need of evaluating the diagnostic performance of newly introduced point of care DD-tests will be mandated (59).

Sweden is quite well-known for its generous, publicly-financed health care system. Citizens get health care based of need - not based on ability to pay.
Nevertheless, resources are scarce and priorities have to be made. This is where economic evaluation becomes important, and increasingly so. How much resourses are we, as a society, willing to devote to improve health gains? And how much are we willing to pay to always make an accurate diagnosis? As mentioned earlier a number of different diagnostic approaches are possible when diagnosing DVT.
The gap between what the health care institutions can provide technically and what they can provide financially is increasing. New expensive technology is being developed. Meanwhile the population in Sweden and Europe is aging, and there are less working people to finance the health care of a greater share of older people. Therefore, health care economics and economic evaluation is increasing in importance in today's public sector.

In our cost-effectiveness analysis (Paper IV) we could show that combining simple diagnostic tests provide an acceptable way to reduce the need for expensive, invasive and time consuming tests. Based on the results from Paper II concerning the possibility to rule DVT in, it would also be interesting to analyze the cost-effectiveness of objective imaging in patients with a high CP in combination with

a high DD or APC-PCI concentration. However, further studies are needed in this area and a discussion on whether initiating anticoagulant treatment without objective imaging is reasonable at all.

We believe that the results from our findings further support that this diagnostic strategy could be safely implemented in Swedish hospitals as recommended by the national and regional guidelines concerning VTE and would also be cost saving for the health sector and for patients (11, 119).

Conclusions

The main conclusions that can be drawn from the present studies are as follows:

– We can safely exclude DVT in 1/3 of outpatients by using clinical probability assessment (CP) and D-Dimer determination. The diagnostic performance of the Autodimer® assay is not significantly affected by realtime (local) vs batchwise (in a coagulation laboratory) analysis.

– The APC-PCI complex performs inferior to the D-Dimer assays when exclusion of DVT is of concern. Very high levels of APC-PCI complex or D-Dimer in combination with a non-low CP of DVT is very indicative for the presence of DVT.

– The AxSYM® and Innovance™ assays perform well and in good agreement with two well established D-Dimer assays. In combination with low CP of DVT, a negative D-Dimer result safely excludes clinically relevant DVT.

– A diagnostic algorithm involving the combination of CP assessment and D-Dimer determination is much more cost-effective than objective imaging of all patients. If implemented in the correct way, this diagnostic strategy implies great savings potential for the health care sector as well as for the society as a whole.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Övergripande syfte

Syftet med avhandlingen var att utvärdera hur en förenklad metod för diagnostisering av blodproppar i benets djupa ådror (djup ventrombos (DVT)) fungerar i svensk rutinsjukvård. Den förenklade metoden innebär användning av en strukturerad klinisk sannolikhetsbedömning tillsammans med ett blodprov (D-dimer test). Vi jämförde också vår D-dimermetod med andra D-dimermetoder samt utvärderade en ny blodproppsmarkör (APC-PCI komplexet) som vi hoppades ha förutsättningar för att bli en bättre markör för blodpropp än D-dimermetoden. Vidare ville vi se om den nya diagnostiska metoden är kostnadseffektiv jämfört med 1) den gamla metoden och 2) en felaktig användning av den nya metoden.

Allmän bakgrund

Andelen människor som årligen insjuknar i DVT ca 100/100 000 innevånare. Endast 10-25% av patienter som söker på grund av misstänkt DVT har sjukdomen. Antalet insjuknade per år är lika för män och kvinnor. Graviditet är en riskfaktor för DVT och den kliniska bedömningen är svår då bensvullnad och vidgade blodådror kan vara ett normaltillstånd framför allt i sen graviditet. De vanligaste orsakerna till att man börjar misstänka DVT är bensmärta och/eller svullnad. Eftersom symtom och undersökningsfynd är väldigt ospecifika har man historiskt låtit alla patienter med misstänkt ventrombos genomgå kontrast eller ultraljudsundersökning av de djupa blodådrorna. Ett sådant förfarande har varit kostsamt, tidskrävande och inneburit vissa medicinska risker för patienterna (kontrastmedels allergi, njurskada mm). Flera studier har sedan slutet av 90-talet påvisat att det går att förenkla diagnostiken genom

att införa en metod som bygger på att man på ett strukturerat sätt försöker skatta sannolikheten för DVT (poänggraderat diagnosstöd) och därefter använda sig av olika enkla icke-invasiva test. Kombinationen av en låg klinisk sannolikhet och ett normalt blodprovsresultat avseende nedbrytningsprodukter av en blodpropp (D-dimer), har visats sig kunna utesluta DVT hos en betydande del av patienterna. Invändningarna mot att införa denna nya diagnostiska modell har varit:

1) Andelen patienter med faktisk blodpropp kan vara högre på våra akutmottagningar än vad som var fallet i de tidigare studierna, detta skulle öka risken för att felaktigt utesluta DVT (missa sjukdomen).

2) Våra D-dimer metoder skulle kunna skilja sig åt avseende känslighet och specificitet jämfört med dem som använts i de tidigare studierna och därigenom också öka risken för att missa DVT.

3) Det poänggraderade diagnosstödet innehåller två punkter som inte är helt objektiva;
i) ömhet över djupa kärlsträngen och
ii) annan diagnos minst lika trolig som DVT.
Poänggraderingen kan därför t.ex. påverkas av om doktorn har en lång klinisk erfarenhet eller inte. Detta kan vara problematiskt då svenska läkare arbetar förhållandevis självständigt redan tidigt i karriären.

Vi har under åren 2003-2006 inkluderat 357 patienter med misstänkt DVT, i en multicenterstudie. Alla patienter har poänggraderats med hjälp av en standardiserad klinisk sannolikhetsbedömning. D-dimer är analyserat lokalt (respektive sjukhus) på de patienter som poänggraderats till en låg klinisk sannolikhet för DVT. Patienter med normal D-dimer nivå har inte erhållit någon blodförtunnande behandling eller genomgått ytterligare diagnostik avseende DVT. Dessa patienter och har sedan följts upp avseende blodproppsinsjuknande under 3 månader. Övriga patienter (förhöjd D-dimer nivå eller hög klinisk sannolikhet för DVT) har genomgått sedvanlig objektiv diagnostik med kontraströntgen av blodådrorna eller en ultraljudsundersökning. Utöver lokalt analyserat D-dimer har vi också analyserat och jämfört flera andra D-dimer metoders diagnostiska prestanda och APC-PCI komplexet i relation till kliniskt utfall.

Sammanfattning av avhandlingens studier

Arbete I: I detta arbete kunde vi visa att klinisk sannolikhetsbedömning enligt Wells och medarbetare, tillsammans med vår D-dimer metod fungerade i rutinsjukvård. Av de 110 patienter som av läkaren bedömdes ha en låg klinisk sannolikhet och där D-dimernivån var normal kom endast 1 patient tillbaka under uppföljningen med blodpropp i benet. Denna andel missade patienter (falskt negativa) är densamma som referensmetoderna (kontraströntgen och ultraljud) har. En dryg tredjedel av patienterna kan slippa kontraströntgen eller ultraljudsundersökning av de djupa blodådrorna. Vi kunde även visa att mätsäkerheten av D-dimer metoden var densamma oavsett om proverna analyseras på ett koagulationslaboratorium eller lokalt på respektive sjukhus.

Arbete II: Baseras på ovan material och syftar till att utvärdera APC-PCI komplexets prestanda i relation till kliniskt utfall och att jämföra resultatet med två etablerade D-dimer metoder. Slutsatsen i detta arbete är att APC-PCI komplexet, jämfört med D-dimer metoderna, har en något sämre förmåga att hjälpa till med att utesluta DVT men en möjlig fördel när det gäller att hjälpa till med att påvisa förekomst av DVT.

Arbete III: Två, på marknaden, nya D-dimer metoder testas och jämförs med två väletablerade metoder. Slutsatsen i detta arbete är ett de nya D-dimer metoderna är jämförbara med de etablerade och kan användas tillsammans med klinisk sannolikhetsbedömning för att utesluta DVT.

Arbete IV: Hälsoekonomisk kostnad-effektivitets analys
Två jämförelser vidtogs: 1) Den nya diagnostiska modellen jämförs med den gamla (alla patienter genomgår bilddiagnostik) och 2) en ”felaktigt” använd ny modell (alla patienter testas med D-dimer innan läkarbedömning). Utfallet av denna analys visar att det blir billigare att använda den nya modellen jämfört med den gamla, men också att en felaktigt använd ny modell innebär att de potentiella besparingarna minskar.

Diskussion och slutsatser

Vi kan genom att använda den nya diagnostiska modellen, med bibehållen säkerhet och till en lägre kostnad, utesluta behandlingskrävande DVT hos

nästan 1/3 av alla patienter som söker på våra akutmottagningar. I Skåne kan vi minska utgifterna för diagnostik av DVT med upp mot 10 miljoner SEK per år genom att införa vår diagnostiska algoritm. Studierna visar också att valet av testmetod och vald beslutsgräns (tex mängd D-dimer i blodet) för sjuk/inte sjuk är viktig. Hög diagnostisk känslighet innebär att man fångar de flesta som är sjuka men också att många friska kommer att falla ut som sjuka (falskt positiva). De falskt positiva patienterna kommer att behöva genomgå kontraströntgen eller ultraljudsundersökning i onödan. En mindre känslig testmetod riskerar att missa några sjuka patienter (falskt negativ) men innebär också att färre friska blir felaktigt klassade som sjuka. Val av testmetod och beslutsgräns bör därför helst prövas i sin egen miljö i prospektiva utfallsstudier likt vår. APC-PCI komplexets roll i diagnostiken av DVT är fortfarande inte helt klar och ytterligare studier behövs på detta område.

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Paper I

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REGULAR ARTICLE

Clinical probability assessment and D-dimer determination in patients with suspected deep vein thrombosis, a prospective multicenter management study

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KEYWORDS
Venous thrombosis;
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Abstract

Objectives: To investigate the reliability of a combined strategy of clinical assessment score followed by a local D-dimer test to exclude deep vein thrombosis. For comparison D-dimer was analysed post hoc and batchwise at a coagulation laboratory.
Design: Prospective multicenter management study.
Setting: Seven hospitals in southern Sweden.
Subjects: 357 patients with a suspected first episode of deep vein thrombosis (DVT) were prospectively recruited and pre-test probability score (Wells score) was estimated by the emergency physician. If categorized as low pre-test probability, D-dimer was analysed and if negative, DVT was considered to be ruled out. The primary outcome was recurrent venous thromboembolism (VTE) during 3 months of follow up.
Results: Prevalence of DVT was 23.5% (84/357). A low pre-test probability and a negative D-dimer result at inclusion was found in 31% (110/357) of the patients of whom one (0.9%, [95% CI 0.02–4.96]) had a VTE at follow up. Sensitivity, specificity, negative predictive value and negative likelihood ratio for our local D-dimer test in the low probability group were 85.7%, 74.5%, 98.2%, and 0,19 respectively compared to 85.6%, 67,6%, 97.9% and 0,23 using batchwise analysis at a coagulation laboratory.
Conclusion: Pre-test probability score and D-dimer safely rule out DVT in about 30% of outpatients with a suspected first episode of DVT. One out of 110 patients was

Abbreviations: DVT, deep vein thrombosis; VTE, venous thromboembolism; CUS, compression ultrasonography.

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diagnosed with DVT during follow up. No significant difference in diagnostic performance was seen between local D-dimer test and the post hoc batch analysis with the same reagent in the low probability group.

Introduction

Although patients with suspected deep vein thrombosis are common in hospital emergency departments, relatively few actually have deep vein thrombosis (DVT) [1,2]. In recent years, new diagnostic methods involving assessment of clinical probability and the use of D-dimer analysis have proven safe and have simplified the diagnostic strategy of these patients [3,4]. Recent studies have shown that low clinical probability and a negative D-dimer result exclude DVT in 30—50% of outpatients with suspected deep vein thrombosis and safely obviate the need for further diagnostic testing [5,6].

According to Bayes' theorem the probability that a patient has the disease following diagnostic testing is determined by the estimated probability prior to the test (pretest probability) and the accuracy of the test [7]. In Scandinavia several studies indicate a higher prevalence (30—50%) of confirmed DVT in outpatients than observed in many other countries [8—10]. This would of course affect the PTP and increase the risk for false negative results and decrease the diagnostic exclusion rate. Furthermore, since D-dimer assays are not standardized and actually measure different products of the fibrin degradation the performance varies substantially between assays and populations [11,12]. Because of these limitations many clinicians hesitate to implement this diagnostic strategy.

The purpose of this study was to examine whether a combined strategy of a clinical assessment score done in the emergency ward followed by a local D-dimer test was safe for the patients in a clinical setting where the prevalence of DVT in outpatients was high. We also wanted to address the question of whether D-dimer methods used locally were as reliable as the same method used in batch analysis under optimal circumstances with reduced variability from inter-assay differences.

Methods

Study design and patients

This study was performed between December 2003 and December 2005. Adult patients with a suspected first episode of DVT were potentially eligible for inclusion. Seven centres in southern Sweden, serving approximately 1 million residents, participated in the study. A total of 491 outpatients were consecutively evaluated for the study over the 2-year recruitment period. Patients were either self-referred, referred from primary care physicians or to a lesser extent sent in from other clinics. The patients were enrolled immediately on their arrival to the emergency dept. Enrollment was possible 24 h/day, 7 d/week. One hundred and seven patients were excluded due to one of the following exclusion criteria: previous VTE (n=54), duration of symptoms>10 days (n=37), inability or unwillingness to provide informed consent (n=9), symptoms suspicious for pulmonary embolism (n=3), pregnancy (n=1), ongoing anticoagulation (n=1), contraindication to contrast media (n=1) and co morbid condition likely to shorten survival to less than 3 months (n=1). Another 27 patients were excluded due to inadequate or missing case report forms, written informed consent or lost blood samples. Site-specific investigators (see acknowledgement) were responsible for collecting data on each site and the central database was compiled by the authors of this paper.

The regional center research ethics board in Lund approved the study, and all patients provided written informed consent before enrollment.

All patients were evaluated by the emergency physician on duty, who was briefly introduced to the Wells score. Pre-test probability for DVT according to Wells' nine item score [3] was determined (Table 1). Patients were categorized as having low, intermediate or high pre-test probability. Patients with a low pre-test probability underwent immediate, local, D-dimer testing. Patients with low clinical probability and negative result on the D-dimer test had no further diagnostic testing for DVT and received no anticoagulant therapy. These patients were followed for VTE events and were either contacted by telephone or seen at an outpatient clinic after 3 months. Patients who couldn't be reached by telephone or didn't return to the outpatient clinic, were checked for VTE events in the medical charts and diagnostic imaging databases at each local hospital. Patients who presented again with symptoms consistent with DVT or pulmonary embolism underwent

Table 1 Pretest clinical probability (Wells' score)

Pretest clinical probability, (Wells' score) (1)	
-Active cancer	1
-Paresis, paralysis or recent plaster or immobilization of lower limb	1
-Bedridden>3days or major surgery<4 weeks	1
-Localized tenderness	1
-Entire leg swollen	1
-Calf swelling>3 cm compared with asymtomatic leg	1
-Pittingoedema	1
-Collateral superficial veins	1
-Alternative diagnosis as likely or greater than DVT	−2

Simplified clinical model for assessment of DVT.
High probability: 3 or more points.
Intermediate probability: 1—2 points.
Low probability: 0 or less points.

objective testing with contrast venography and / or compression ultrasonography (CUS) of the leg and ventilation/perfusion scintigraphy and / or computed tomography of the lungs respectively.

Patients with intermediate / high clinical probability or positive D-dimer test were further investigated with contrast venography and / or CUS. DVT was diagnosed if an intraluminal filling defect was present in two views or if noncompressibility was present in the common femoral, superficial femoral or popliteal vein respectively. If the CUS was negative, further evaluation with comprehensive ultrasound (including calf veins) or contrast venography was performed.

For D-dimer analysis, venous blood was collected in 5 ml vacuum tubes (Becton-Dickinson, Franklin Lakes, USA) containing sodium-citrate (3.8%), and centrifuged at 3600 ×*g* for 10 minutes at 4 C within 30 minutes of collection. The plasma was aliquoted and one aliquot was used for the local D-dimer analysis. The others were frozen at −70 C for later analysis in batch with Auto Dimer® (Biopool® International Umeå, Sweden) on the BCS™ Coagulation Analyser (Dade-Behring, Marburg, Germany). The cut off value used for the post-hoc batch analyses, was set to 0.25 mg L^{-1}, according to manufacturers' recommendation.

The local D-dimer tests used were Auto Dimer® in 92% of the patients, measured on Thrombolyser™ Compact XR (Behnk Electronic, Norderstedt, Germany) or Sysmex CA 1500 and 7000 coagulation analysers (Dade-Behring, Marburg, Germany), Nycocard® D-Dimer assay(Axis-Shield PoC AS. Oslo, Norway) with the Nycocard® READER was used in 6% and, STA- LIA® D-dimer in 2%, measured on STA-R®, Diagnostica Stago, Asnieres, France). All tests were performed according to manufacturers' instructions. Lab personnel performing the D-dimer assay were not aware of the patients pre-test probability assessment.

Statistical methods

Our primary outcome was the proportion of patients who had a venous thromboembolic event during 3-month follow-up among those for whom the diagnosis of DVT had been excluded by a low clinical probability and a negative D-dimer test. On the basis of previous studies in Scandinavia showing prevalence rates of DVT ranging from about 30–50% in outpatients with suspected DVT [8–10], we estimated that approximately 40% of the patients would be categorized as having low pre-test probability and that the prevalence of DVT in this group would be about 10%. The generally accepted requirement for safely ruling out DVT and withholding anticoagulant therapy is a false negative rate of less than 2% during follow up which is achieved by a negative contrast venography or a comprehensive compression ultrasonography [13,14]. Based on the sensitivity and specificity of the Autodimer, 85% and 46% respectively [15,16], we estimated that the Auto Dimer® assay would have a negative predictive value of at least 98% in the low probability group. We calculated that the sample size needed to reach a power of 80% (2.5% risk level, one-sided test for the lower boundary of a CI of 95%, reaching a NPV of 95%) was 140 patients in the low probability, negative D-dimer cohort. The 2-sided 95% CI was calculated by using the exact method for obtaining the confidence interval for a binominal proportion except for likelihood ratios (LR) where calculations were based on a method described by Bolboaca et al. [17].

Results

Three hundred and fifty- seven patients were considered eligible and entered the study. The prevalence of DVT (final diagnosis) was 23.5% (84/357) of which 52 (63%) were considered proximal DVTs (proximal thrombosis if the thrombus was located in the popliteal or more proximal veins). The median age was 62 years and 138 (39%) were men. Other patient characteristics are shown in Table 2. Of the 357 patients entering the study, 159 (45%) were categorized as having a low, 141 (39%) intermediate, and 57 (16%) as having a high pre-test probability for DVT.

Low probability cohort

Of the 159 patients with low probability, 110 (69%) had a negative D-dimer and were not targeted to go through further diagnostic testing for DVT. In this category however, 11 patients underwent diagnostic imaging (venography (n=4), CUS (n=6) or both (n=1)) based on the physicians clinical judgement, overriding the low probability of the Wells' score or, when ultrasonography was used, because suspicion of differential diagnoses to DVT, and where the ultrasound examiner investigated the veins as well. One of these patients was diagnosed with a distal DVT (CUS). This patient was considered as "missed" DVT in the outcome analysis. In one patient with positive D-dimer, DVTwas ruled out by clinical judgement. Over

Table 2 Demographic data, results of categorization of patients according to the pretest clinical probability score and of D-dimer testing

	All patients (n=357)	Patients with DVT (n=84)	Patients without DVT (n=273)	p-values *
Low probability	159 (45%)	14 (17%)	145 (53%)	<0.001
Intermediate	141 (39%)	37 (44%)	104 (38%)	0.28
High probability	57 (16%)	33 (39%)	24 (9%)	<0.001
Age (median)	62 (33, 82) **	67 (32, 83)	60 (33, 81)	0.62
Men	138 (39%)	34 (40%)	104 (38%)	0.72
Heredity	62 (17%)	16 (19%)	46 (17%)	0.48
Smoking	66 (18%)	12 (14%)	54 (20%)	0.46
BMI	26 (21,33) ** (n=188)	26 (20, 33) (n=62)	26 (22, 33) (n=126)	0.52
D-dimer local (neg)	110 (31%)	2 (2%)	125 (46%)	<0.001
D-dimer batch (mg/L)	0.26 (0.06, 1.88) ** (n=350)	1.40 (0.31, 6.92) (n=80)	0.18 (0.05, 0.64) (n=270)	<0.001

* Mann-Whitney U test was used for comparison between patients with and without DVT. Chi-squared test was used for nominal variables.

** Median (10th, 90th percentiles).

the 3 month follow up period, 1 patient (0.9%, [95% CI, 0.02–4.96%]) subsequently developed distal DVT confirmed by venography on day 9 after initial presentation. This patient was at inclusion diagnosed for superficial thrombophlebitis and had a recent history of a long distance flight, she was initially sent home with elastic stockings and Hirudoid® ointment. Five patients returned and underwent diagnostic testing for VTE during follow up, (CUS (n=1), venograpy (n=3), V/P-scintigraphy (n=1)). In all these patients VTE was ruled out and anticoagulant treatment was withheld. Two patients were admitted to the hospital due to acute coronary syndrome and received low molecular heparin (enoxaparin 1 mg/kg b.i.d) for 1 and 3 days respectively and one patient received warfarin treatment from day 53. These patients were not excluded. No patient was lost to follow up, Fig. 1, flowchart.

In patients categorized as having low clinical probability our local D-dimer methods had a sensitivity and specificity of 85.7% (95% CI, 57 to 98%) and 74.4% (95% CI, 67 to 81%) respectively with a negative predictive value of 98.2% (95% CI, 94 to 100%) and a negative likelihood ratio (LR-) of 0.19 (95% CI, 0.06 to 0.67). This gives an exclusion rate of about 30%. Post-hoc batch analysis with the Auto Dimer® gives a sensitivity, specificity, negative predictive value and LR- of 84.6% (95% CI, 55 to 98%), 67.6% (95% CI, 59 to 75%), 97.9% (95% CI, 93 to 100%) and 0.23 (95% CI, 0.07 to 0.79) respectively. The concordance between the local D-dimer assay and the batch was 89% with a kappa value (κ) of $\kappa = 0.76$.

Intermediate/high probability cohort

The prevalence of DVT was 26% in the intermediate and 58% in the high probability group. The frequency of negative D-dimer (post-hoc batch analysis) was 44% and 14% respectively giving a sensitivity, specificity, NPV and PPV of 94.1% (95% CI, 86 to 98%), 51.9% (95% CI, 43 to 61%), 94.3% (95% CI, 86 to 98%) and 51.2% (95% CI, 42 to 60%). Contrast venography was the main diagnostic method used in this cohort (78% of the patients) and in 10% of the patients both CUS and venography was performed. Two patients in the intermediate group were not examined with CUS / venography, DVT was ruled out by clinical judgement of the responsible physician. One patient in the intermediate group was diagnosed as having DVT although venography was considered normal.

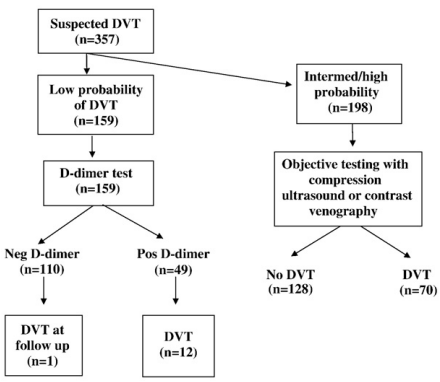

Figure 1 Strategy for diagnosis of DVT and number of patients in each group.

Discussion

Assessment of clinical pre-probability scores and the use of D-dimer testing has simplified the diagnostic strategies for DVT and reduced the need for diagnostic imaging. Implementing these strategies into the diagnostic workup lowers costs [18], reduces inconvenient for the patients and is time-saving for both staff and patients at the emergency departments.

In this study we demonstrate that anticoagulation therapy can be safely withheld in almost 1/3 of outpatients with suspected DVT by using a non invasive diagnostic strategy including clinical assessment and D-dimer. The safety of this approach was demonstrated, since only one of 110 patients who had DVT ruled out at inclusion was diagnosed with a DVT (distal). However, due to the limited sample size, the upper limit of the 95% CI reached 4.96%.

To our knowledge no other published prospective management study has used a combination of a moderate sensitive D-dimer [11] and clinical probability assessment to rule out DVT without objective imaging testing, in a clinical setting with a high prevalence of DVT and where the assessment was made by physicians with relatively low clinical experience, including many junior residents.

In the low probability cohort, comparison between locally used D-dimer assays (mainly Auto Dimer®, 92%) and post-hoc batch analysis with the Auto Dimer® method demonstrates an acceptable 89% concordance. The observed difference can probably be explained by the fact that in 8% of the patients a different D-dimer assay was used, analyses were made on different coagulation instruments and reagent batches.

The present study has strengths and limitations. Identification and inclusion of patients was done entirely by the physician at the emergency unit. This could have affected the inclusion rate of patients with intermediate/high clinical probability, since including these patients into the study would slow down the diagnostic workup. This would explain why the prevalence of DVT in the study population was lower than expected. The fact that the physicians chose to refer eleven of the patients in the low probability-negative D-dimer group to diagnostic imaging and in one of these patients a distal DVT was revealed, is worth considering, but consistent with other studies which indicate that the sensitivity of the D-dimer test is lower for distal thrombosis [10]. Indeed the good outcome in the

present and other recent management studies probably indicate that a negative D-dimer test does not exclude all distal DVTs, but excludes DVT that require treatment. We also believe that this is a strength of this study, showing that the strategy was used in the daily clinical practise, leaving open the possibility to override the clinical prediction score and use empirical clinical judgment, a better approach than strict adherence to the diagnostic algorithm [19].

The decision to stop inclusion before we reached the calculated 140 patients (low probability, negative D-dimer) needed to reach a power of 80% was mainly based on the fact that the inclusion rate successively decreased during the study as the diagnostic algorithm studied became implemented as clinical routine, probably a result of the guidelines from the Swedish National Board of Health and Welfare on diagnosis of VTE established in 2004. Continued inclusion of patients at this low rate would have increased the risk for inclusion bias.

The effect of clinical experience on the predictive power of the Wells' score in the diagnosis of DVT is a matter of debate [19,20]. In our study, the diagnostic strategy was handled by physicians only briefly introduced to the Wells clinical assessment score. No specialized research staff or vascular experts were involved in the diagnostic work up of these patients. In spite of this only one out of 110 patients was diagnosed with a DVT during follow up. These results are comparable with the results from other clinical management trials [5,21] here in a routine emergency setting.

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Paper II

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The diagnostic performance of APC–PCI complex determination compared to D-dimer in the diagnosis of deep vein thrombosis

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Abstract D-dimer testing is widely used as part of the diagnostic algorithm for the exclusion of deep vein thrombosis (DVT) but is considered of limited in value for ruling DVT in. Since D-dimers are poorly defined, there is no standardization of the assays and this makes reliable comparisons between clinical studies difficult. We report on a performance evaluation of a new marker of activated coagulation (Activated Protein-C in complex with Protein-C inhibitor, APC–PCI complex) compared to two quantitative D-dimer assays (Vidas® D-dimer Exclusion™and Autodimer®). The post-hoc comparison was made on 350 frozen plasma samples from consecutive outpatients suspected of DVT in a multicenter management study including clinical probability score, D-dimer testing, venous ultrasound and contrast venography as part of the diagnostic algorithm. *Results*: The APC–PCI complex performed inferior to the D-dimer assays in terms of sensitivity: 74 vs. >93%, negative predictive value: 91 vs. >96% and area under the curve: 0.82 vs. 0.9, but showed a significantly higher specificity: 80 vs. 40–60%. Specificity for the APC–PCI complex did not decrease with higher clinical probability score and the positive predictive value was significantly higher than that of the D-dimer assays in the intermediate/high probability cohort (66 vs. <52%). In this probability cohort, high levels of the APC–PCI complex and to a lesser extent, D-dimers, can give positive predictive values of >90% in up to 20% of the patients which indicates important clinical implications. However, for the exclusion of DVT at the pre-specified cut-off level, the APC–PCI complex perform inferior to the D-dimer assays in this study.

Keywords Venous thrombosis · Diagnosis · D-dimer · APC–PCI complex

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Introduction

Deep vein thrombosis (DVT) occurs with an incidence of about 160/100 000 per year [1–3]. Signs and symptoms of DVT are non-specific and the majority of patients presenting with swelling or pain in the leg does not have DVT [4, 5]. Missed DVT can lead to pulmonary embolism with a mortality of 10–25% [6, 7]. Treatment with anticoagulants decrease the risk of recurrent venous thrombosis, post-thrombotic syndrome and chronic pulmonary embolism but also increases the risk of major haemorrhage [8–10]. Therefore, diagnostic strategies must be developed to safely rule out DVT in the vast majority who does not have the disease and to correctly diagnose those who have the disease [11]. Because of the non-specific signs and symptoms, one way to approach this problem would be to perform objective imaging in all patients with suspected DVT. This would be expensive, inefficient and cause a number of complications [12–14]. D-dimer assays are generally sensitive but non-specific markers of DVT. In combination with a low pretest clinical probability (CP) of the disease a negative test can safely rule out DVT in 30–50% of outpatients with suspected DVT [5]. In contrast, positive D-dimer results are not considered useful to "rule in" DVT due to their low specificity and PPV. However several

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studies have shown a substantial increase in the likelihood of VTE at highly elevated D-dimer levels [15–18].

Since D-dimers are poorly defined, different D-dimer assays actually measure different fibrin degradation products. There is a wide variety of quantitative and qualitative D-dimer assays available with a wide variation of sensitivity, specificity, normal reference ranges, and cut-off values among different assays [19, 20]. Hence, a new and well defined analyte with comparable diagnostic performance is therefore in demand and would simplify comparisons of future clinical trials.

Plasma concentrations of a complex between activated protein C (APC) and its inhibitor (PCI) increase in hypercoagulative states as DVT and pulmonary embolism [21, 22]. The APC–PCI complex measured with a sensitive immunofluorometric sandwich assay in plasma, have shown promising performance in a case-control study in patients with DVT compared to controls [23]. The assay can be fully automated, is sensitive and seems to meet the perquisite of a good marker of DVT by showing receiver operating characteristics (ROC) curves similar to that of the D-dimer method used for comparison (Nycocard). The assay is now commercially available as an ELISA assay (APC–PCI ELISA kit, Bio Porto Diagnostics A/S, Gentofte, Denmark). In contrast to the D-dimer methods, the APC–PCI complex is a well defined analyte and therefore possible to standardize [24]. Earlier studies also indicated that APC–PCI performed better than D-dimer at high specificities and showed no correlation with c-reactive protein (CRP) concentration, suggesting that in contrast to the D-dimer level, activation of the coagulation system as measured by the APC–PCI concentration is not influenced to the same extent by inflammatory activity [23, 25].

This is the first evaluation of the APC–PCI complex method, in a clinical study including patients with suspected DVT, compared to a moderate and a high sensitive D-dimer assay, Autodimer® and VIDAS® D-dimer Exclusion™.

Methods

We have earlier reported on a study where 357 consecutive patients with a suspected first episode of DVT were prospectively recruited and pretest CP (according to Wells et al. [26]) was estimated [27]. If categorized as low CP, real time D-dimer (Autodimer®, Biopool, Umeå, Sweden, cut-off <250 ng/ml) was analysed at the central laboratory of each hospital and if negative, DVT was considered ruled out. These patients received no anticoagulant treatment and recurrent VTE was followed for 5.5 months (median). Suspicion of recurrent VTE was evaluated by contrast venography ($n = 3$), comprehensive ultrasound (=1) and V/P-scintigraphy ($n = 1$). Patients with intermediate/high CP or positive D-dimer test were further investigated with contrast venography and/or compression ultrasound. If a negative result was obtained in this cohort, comprehensive ultrasound was performed. For comparative analysis between the APC–PCI complex and the D-dimer assays 350 frozen plasma samples were available for analysis. In seven patients there were not enough plasma left to analyse the D-dimer assays.

The regional center research ethics board in Lund approved the study and all patients provided written informed consent before enrolment.

Venous blood for immediate and post hoc batch analysis of D-dimer was collected at the emergency department in 5 ml vacuum tubes (Becton-Dickinson, Franklin Lakes, USA) containing sodium-citrate (3.8%), and centrifuged at $3600 \times g$ for 10 min at 4°C within 30 min of collection. The plasma was aliquoted and one aliquote was used for the local D-dimer analysis, which was the Autodimer® assay in 92% of the patients. The cut-off value used was 250 ng/ml, according to the manufacturer's instructions. The other aliquots were frozen at −70°C for later analysis in batch with the Autodimer® (Trinty Biotech, Bray, Ireland), Vidas® D-dimer Exclusion™ (Biomerieux, Marcy-lEtoile, France) and the APC–PCI complex methods. The Autodimer® is a quantitative latex agglutination assay with moderately high sensitivity was analysed on the Sysmex® Coagulation Analyser (Dade-Behring, Marburg, Siemens, Germany) and the Vidas D-dimer Exclusion assay was run on a miniVidas analyzer (Biomerieux, Marcy-lEtoile, France), cut-off <500 ng/ml. The APC–PCI complex concentration was determined with the immunochemical sandwich method described by Strandberg et al [24]. The concentration in healthy individuals without medication and without a previous deep vein thrombosis was 0.07–0.26 ng/ml (95% CI) in Stabilyte-plasma with a mean and median of 0.13 ng/ml ($n = 80$, median age 42 years, males/females: 20/50). The within-run coefficient of variation (CV) was 4.8% at 0.15 ng/ml and 3.2% at 0.40 ng/ml ($n = 16$) [28]. The between-run CV was 7.1% at 0.15 ng/ml and 5.8% at 0.41 ng/ml ($n = 38$). In the present evaluation, we used the upper limit of the 95% confidence interval (CI) in healthy individuals (<0.26 ng/ml) as cut-off for the APC–PCI assay.

Statistical methods

Sensitivity, specificity, negative predictive value (NPV) and likelihood ratios (LR) were calculated for each assay in relation to the clinical outcome i.e. having DVT at the initial diagnostic workup (positive compression ultrasound or positive contrast venography) or during 3 months follow

up. The 2-sided 95% CI was calculated with the exact method for binominal proportions. McNemars and Fishers exact test was used to test for significant differences between sensitivity, specificity and NPV. *P*-values < 0.05 were considered statistically significant. Receiver Operating Characteristics curves (ROC) were constructed and the area under curve (AUC) calculated to estimate the discriminative power of the assays. Kappa coefficients (κ) were calculated to estimate the concordance between the tests, a value of >0.81 represents excellent concordance, 0.80–0.61 good concordance, 0.60–0.41 moderate concordance [29].

Logistic regression analysis was performed to calculate the additive effect of the APC–PCI and D-dimer methods. The statistical analysis was performed with SPSS version16 (SPSS Inc., Chicago, IL, USA). For calculations of Kappa values and 95% confidence intervals (CI) VassarStats clinical calculator (Vassar College, Poughkeepsie, NY: http://faculty.vassar.edu/lowry/clin1.html) was used.

Results

The prevalence of DVT was 23% (81/350) with a ratio distal/proximal DVT of 1/3. The mean age was 60 years and 61% (214/350) were women. Of the 350 patients included in the study, 155 (44%) were categorized as having a low, 138 (39%) intermediate, and 57 (16%) as having a high CP for DVT. The prevalence of DVT was 8.4% (13/155) in the low CP cohort compared to 34.9% (68/195) in the intermediate/high CP cohort. One patient (0.9%,[95% CI, 0.02–4.96%]) out of 110 patients in the low probability, negative D-dimer cohort was diagnosed with a DVT (distal) during follow up and one patient was diagnosed with DVT (distal) at inclusion (protocol violation).

Six DVT patients were negative with the Autodimer in the post hoc batch analysis, of which two had low CP (false negative in the management study), five were distal and one was proximal. The Vidas D-dimer Exclusion assay was negative in two DVT patients; both categorized as intermediate/high clinical probability (1 distal; 1 proximal), these two patients were false negative in all assays. The APC–PCI complex method was negative in 21 patients with DVT of which 4 had a low CP (11 distal; 10 proximal). The results of the APC–PCI and D-dimer tests according to clinical risk category and the presence of DVT are shown in Table 1. Table 2 shows the diagnostic performance of the assays at the given cut-off values.

In the overall cohort the Vidas Exclusion and Autodimer assays show significantly higher sensitivity and NPV but inferior specificity compared to the APC–PCI assay. In patients categorized as having a low CP though, no significant difference is seen concerning sensitivity and NPV, but due to the significantly lower specificity for the Vidas Exclusion assay, the exclusion rate would be lower, 43% compared to 63% for the Autodimer. The APC–PCI assay does not reach a 98% NPV and all confidence intervals are wide due to the limited sample size. Lowering the cut off value for APC–PCI to 200 ng/ml resulted in a sensitivity of 85%, specificity of 61%, a NPV of 98% (95% CI, 91–100) and negative likelihood ratio (LR-) of 0.25. ROC analysis was performed to evaluate the overall performance of the methods ability to differentiate between patients with and without DVT (Fig. 1). The Area Under the Curve (AUC) was 0.82 (95% CI, 0.75–0.88) for the APC–PCI complex and 0.90 (95% CI, 0.86–0.94), 0.90 (95%, CI, 0.86–0.94) for the Autodimer and Vidas Exclusion assay respectively, *P*-values of 0.006 for both comparisons implicates that the D-dimer assays have a significantly better discriminative power. Concordance between APC–PCI and D-dimer assays, expressed as κ-values was (fair) 0.26 for Vidas Exclusion and (moderate) 0.42 for Autodimer and the proportion of agreement (percentage of values found concomitantly positive or negative) was 58 and 71% respectively.

Among patients with abnormal results of APC–PCI, Autodimer or Vidas Exclusion tests in combination with intermediate/ high CP, the positive predictive values (PPV) were 66, 51 and 43% respectively. Using a cut-off at the 75th or 90th percentile though, there are no significant differences between the assays with regard to specificity and sensitivity. To test for any additive effect on performance when used in combination, ie APC–PCI complex in addition to Vidas Exclusion or Autodimer, logistic regression analysis was performed but this resulted in a negligible increment of the AUC's compared to the D-dimer assays alone (0.899–0.902 for Vidas Exclusion and 0.901–0.904 for Autodimer). In the intermediate/high probability cohort, increasing cut-off values can give very high PPV′s and positive likelihood ratios (LR+). Indeed, counting in patients with a final diagnosis of superficial thrombophlebitis as true positive, 93% (41/44) patients with a value $\geq$500 ng/ml for APC–PCI are ruled in. To reach the same magnitude of post-test probability for a positive result with Autodimer (37/40) and Vidas Exclusion (31/33), cut-off values of $\geq$1500 and $\geq$4000 ng/ml respectively would be needed. In Table 3 the results are given from changing the cut-off values of the assays to demonstrate the performance in proving the presence of DVT.

Discussion

In this study we evaluated a new marker for venous thrombosis (the APC–PCI complex) which has the advantage over D-dimer tests by being a defined analyte.

Table 1 APC–PCI complex, autodimer and vidas exclusion in patients with (DVT) and without (−) DVT by clinical probability score (CP)

	APC–PCI $n = 350$				Autodimer $n = 350$				Vidas $n = 350$			
	<0.26 ng/ml $n = 235$		≥0.26 ng/ml $n = 115$		<250 ng/ml $n = 168$		≥250 ng/ml $n = 182$		<500 ng/ml $n = 109$		500 ng/ml $n = 241$	
	DVT $n = 21$	− $n = 214$	DVT $n = 60$	− $n = 55$	DVT $n = 6$	− $n = 162$	DVT $n = 75$	− $n = 107$	DVT $n = 2$	− $n = 107$	DVT $n = 79$	− $n = 162$
CP												
Low	4	113	9	29	2	96	11	46	0	67	13	75
Im	6	84	29	19	2	60	33	43	1	37	34	66
High	11	17	22	7	2	6	31	18	1	3	32	21

Im intermediate

Table 2 Diagnostic performance at given cut-off values by clinical probability (CP) score in percentage and (95% CI)

APC–PCI (<0.26 ng/ml)	All patients ($n = 350$)	Low CP ($n = 155$)	Intermed/high CP ($n = 195$)
Sensitivity	74 (63–83)	69 (39–90)	75 (63–84)
Specificity	80 (74–84)	80 (72–86)	80 (71–86)
NPV	91 (86–94)	97 (91–99)	86 (78–91)
PPV	52 (43–62)	24 (12–41)	66 (54–76)
LR−	0.32	0.39	0.31
LR+	3.6	3.4	3.7
Autodimer (<250 ng/ml)			
Sensitivity	93 (84–97)	85 (54–97)	94 (85–98)
Specificity	60 (54–66)	68 (59–75)	52 (43–61)
NPV	96 (92–99)	98 (92–100)	94 (85–98)
PPV	59 (51–66)	81 (68–90)	51 (42–60)
LR−	0.12	0.23	0.11
LR+	2.3	2.6	2
Vidas (<500 ng/ml)			
Sensitivity	98 (91–100)	100 (72–100)	97 (89–99)
Specificity	40 (34–46)	47 (39–56)	31 (24–40)
NPV	98 (93–100)	100 (93–100)	95 (83–99)
PPV	33 (27–39)	15 (8–24)	43 (35–51)
LR−	0.06	0	0.09
LR+	1.6	1.9	1.4

The method is thereby possible to standardize. Earlier studies indicated that this marker could have advantages over D-dimer methods by having a higher specificity and not the usual trade off between sensitivity and specificity seen in D-dimer assays [19, 20, 23].

In the present analysis of a prospective multicenter management study, we compared the diagnostic performance of a recently developed APC–PCI complex method to a moderate sensitive (Autodimer) and a high sensitive (Vidas D-dimer Exclusion) D-dimer assay. For the exclusion of DVT, the results in our study show that the D-dimer assays have a significant better overall diagnostic performance compared to the APC–PCI assay. Used in combination with a low CP, the APC–PCI complex can not be used for safely ruling out DVT (NPV 96.5%, LR-0.39) at the 0.26 ng/ml cut-off level but at 0.2 ng/ml a better performance is obtained. One explanation for the inferior performance at high sensitivities seen for the APC–PCI complex could be explained by a shorter half-life ($t1/2 = 20$ min) compared to 4–8 h for DD [30]. In our study we used a duration of symptoms >10 days as an exclusion criterion whereas for the APC–PCI assay caution must be used when duration of symptoms is >5 days [23]. However, it is important not only to focus on sensitivity but also on the specificity. The specificity of the APC–PCI assay in the low probability estimate was 80% compared with 47–68% for the D-dimer tests. High specificity tests usually indicate that less invasive, time consuming and expensive additional diagnostic procedures are needed. If the APC–PCI complex method could be used safely and effectively in a population with a lower prevalence of DVT or in combination with proximal compression ultrasound of the leg as part of the diagnostic algorithm needs to be prospectively evaluated.

D-dimer tests are generally used to help rule DVT out, although very high values in quantitative tests can have a high positive predictive value for DVT. However, increasing cut-off values would inevitable reduce sensitivity and thereby the NPV to a point where DVT cannot be safely ruled out (1–2% failure rate) [31].

The present study indicate that high levels of D-dimers and APC–PCI complexes can be useful in the diagnostic algorithm to help rule DVT in (implicate that DVT is present). D-dimer tests generally have a significantly lower specificity 36–66% among patients with intermediate/high pretest probability estimate compared to 58–78% in the low probability patients [5]. In contrast, the APC–PCI complex maintain a very high 80% specificity and give a PPV of 66% in the intermediate/high CP cohort. This could be explained by the fact that the APC–PCI complex is less influenced by unspecific coagulation activation due to eg. inflammatory activity (co-morbidity) found in the intermediate/high probability estimate.

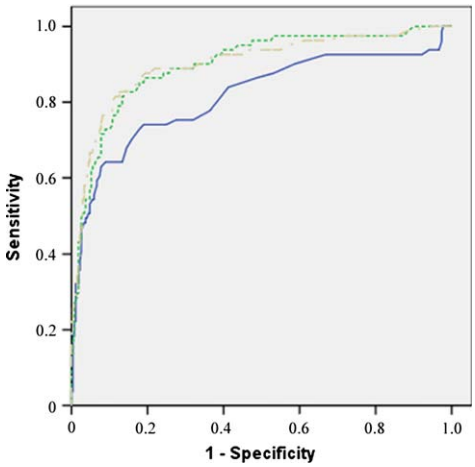

Fig. 1 ROC curves showing the results of the APC–PCI complex (*Straight line*), Vidas (*dotted line*) and Autodimer (*dash dotted line*)

Incorporating the high values of APC–PCI complex or D-dimer into the diagnostic algorithm in patients with intermediate/high probability of DVT would be helpful in clinical management of these patients if post-test probabilities that would lead to initiation of anticoagulant treatment were to be reached. In our study, the prevalence of DVT increased from 35%, in the overall intermediate/high CP cohort, to a post-test probability of around 90% when combined with APC–PCI complex> 0.75, Autodimer levels > 2000 or Vidas Exclusion levels > 4000 ng/ml, with very high positive likelihood ratios (Table 3). We believe that our results are important and show superior performance in proving presence of DVT, compared to a recent study on pulmonary embolism [18]. This study reached a PPV of 65% in patients with a pulmonary embolism-likely estimate in combination with a Vidas Exclusion level > 4000 ng/ml whereas the same assay and level in our DVT-likely group reached a PPV of 88% and a similar PPV with the cut-off level 0.26 ng/ml for the APC–PCI assay (66%).

Attention though must be paid to the prevalence in the studied population, since this influence the predictive values.

Clinical scenarios where addition of high APC–PCI or D-dimer levels could be useful and initiation of anticoagulant treatment considered should be further evaluated.

Our results are in good agreement with the first accuracy study with the APC–PCI complex in the diagnosis of DVT [23]. This study used contrast venography as reference test, and when a non filling segment was identified, CUS was added to further evaluate that segment. Although the cut-off value used in this study was based on a different tubing system for sample collection, the results from this study show approximately the same sensitivity (73%) compared to the present study (74%), although outcome studies usually show better performance, as it comes to sensitivity, when compared to accuracy studies using a reference test [32]. The specificity was slightly higher 89 vs. 80% as well as the AUC 85 vs. 81%.

In conclusion the APC–PCI complex show inferior performance compared to the two D-dimer assays for the exclusion of DVT. This result and the optimal diagnostic cut-off level for the exclusion of DVT need to be further evaluated. Unlike D-dimer methods the APC–PCI complex method has the advantage of being a well defined analyte and the method is now commercially available. High levels of D-dimers and APC–PCI complex can help to rule DVT in. The clinical importance of this needs further prospective evaluation in management studies.

Table 3 Clinical performance of the assays at increasing cut-off values, expressed as percentage and 95% CI, in patients with intermediate/high CP

Assay (n = 195)	Cut-off value (ng/ml)	Sensitivity	Specificity	PPV	LR+	Positive test
APC–PCI	0.5	56	95	86 (72–94)	11.2	23
	0.75	50	99	97 (83–100)	63.5	18
	1	35	99	96 (78–100)	44.8	13
Autodimer	500	85	84	74 (63–83)	5.4	40
	1000	69	92	82 (70–91)	8.8	29
	1500	51	96	88 (72–95)	13	21
	2000	37	98	89 (71–97)	15.6	14
	2500	26	100	100 (78–100)	Infin	9
Vidas	1000	91	64	57 (48–67)	2.5	55
	1500	85	80	70 (59–79)	4.3	43
	2000	79	84	73 (61–82)	5	38
	3000	59	94	85 (71–93)	10.7	24
	4000	43	97	88 (71–96)	13.5	17

Positive test = percentage of patients with positive test result

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Paper III

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Regular Article

Performance of two relatively new quantitative D-dimer assays (Innovance D-dimer and AxSYM D-dimer) for the exclusion of deep vein thrombosis

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ABSTRACT

Introduction: D-dimer assays are now widely used as the first-line test in the diagnostic algorithm of suspected deep vein thrombosis (DVT). The aim of this study was to evaluate the performance of two relatively new quantitative D-Dimer assays (Innovance™ and AxSYM®) by comparison with a clinical gold standard.
Patients and methods: 311 samples from outpatients with clinical suspicion of DVT, included in a prospective management study, was analysed (prevalence of DVT 23%). The diagnostic workup included estimation of pre-test probability, D-dimer determination, objective imaging as well as 3 month clinical follow up of negative patients.
Results: No significant differences were seen in sensitivity and negative predictive values between Innovance, AxSYM and the reference assays. The area under the ROC curve was slightly lower for the AxSYM assay and the correlation to the reference assays was only moderate ($r<0.8$) whereas the agreement with the Vidas assay was near excellent ($\kappa=0.8$). The Innovance assay reached the highest AUC, showed a strong correlation with the reference assays ($r\geq0.9$) and a good agreement with the Vidas assay ($\kappa=0.76$). In combination with a low pre-test probability score the Innovance assay reached a NPV of 100% (95% CI, 92-100) and the AxSYM assay 98% (95% CI, 87-100).
Conclusion: The Innovance and AxSYM assays show an overall good and comparable performance for the exclusion of DVT when compared to the established assays. Our results for the AxSYM assay indicate that the optimal cut-off value needs to be further evaluated.

Introduction

Tests for D-dimer to help exclude venous thromboembolic disease have been available since the 1980s [1–3]. D-dimers together with implicit or explicit models for pre-probability estimation of venous thromboembolism (VTE) are widely used to rule out VTE when the pretest clinical probability for VTE is assessed as low and the D-Dimer is below a certain value (cut-off value) [4–7]. Several D-dimer assays are commercially available. Although generally considered sensitive but non-specific markers of VTE, D-dimer assays differ in sensitivity, specificity and methodology [3,8]. As a result of the lack of standardisation, direct comparison between clinical trials using different D-dimer assays is not possible and therefore each assay must be validated and compared to a clinical gold standard and the established cut-off value should be confirmed in management studies [9–11].

The Innovance™ D-dimer assay is a particle-enhanced, immunoturbidometric assay for the quantitative determination of D-dimer (Dade Behring, Marburg, A Siemens Company, Germany) and the AxSYM® D-dimer assay (Axis-Shield Diagnostics, Dundee, UK) is based on a quantitative fully automated microparticle enzyme immunoassay (MEIA technology), run on an Abbot AxSYM analyzer. They were both recently commercially introduced and are under further evaluation [12–14]. The purpose of the present study was to evaluate the performance of these assays by comparing them with a clinical gold standard and two well established D-dimer assays on 311 frozen plasma samples from a prospective multicenter management study on outpatients with suspected deep vein thrombosis (DVT). For comparison we used the Vidas® D-dimer Exclusion™ which is categorized as a fast-ELISA or ELFA (enzyme-linked immunoflourescence assay) method and the Auto-dimer® which is a latex enhanced immunoturbidometric assay. The Vidas D-dimer is today one of the most validated assays available [8,9]. In contrast to Vidas and the AxSYM assay which require dedicated immunoanalysers, the turbidimetric assays can be performed on routine clinical chemistry analyzers.

Patients and methods

Patients

As reported earlier 357 consecutive outpatients with a suspected first episode of deep vein thrombosis (DVT) were prospectively

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Table 1
Clinical performance of the D-dimer assays at different cut-off values.

D-dimer assay	Median (range)	Cut-off value	Sensitivity	Specificity	NPV	LR-
AxSYM	DVT+ 2769	750	90 (42-67)	55 (42-67)	95 (90-98)	0.18
	(229-9000)	**500**	**94 (86-98)**	**32 (26-39)**	**95 (87-98)**	**0.17**
		400	97 (89-100)	23 (18-29)	96 (87-99)	0.12
	DVT- 651	300	97 (89-100)	19 (15-25)	96 (85-99)	0.14
	(5-9000)	200	100 (94-100)	13 (9-18)	100 (86-100)	0
Innovance	DVT+ 4520	750	93 (84-97)	59 (52-65)	97 (92-99)	0.12
	(150-37800)	**500**	**96 (87-99)**	**38 (32-44)**	**97 (90-99)**	**0.11**
		400	97 (89-100)	29 (23-35)	97 (89-100)	0.1
	DVT- 590	300	97 (89-100)	18 (13-24)	96 (84-99)	0.15
	(70-9640)	200	99 (91-100)	10 (7-15)	96 (78-100)	0.14
Autodimer	DVT+ 1400	500	82 (71-90)	86 (81-90)	94 (90-97)	0.21
	(50-22400)	**250**	**92 (82-97)**	**60 (54-66)**	**96 (91-98)**	**0.14**
		200	93 (84-97)	52 (45-58)	96 (91-99)	0.13
	DVT- 180	150	94 (87-98)	42 (36-48)	96 (90-99)	0.13
	(20-2690)	100	97 (89-100)	23 (18-29)	97 (87-99)	0.12
Vidas	DVT+ 3450	750	93 (84-97)	59 (52-65)	97 (92-99)	0.12
	(210-16300)	**500**	**97 (89-100)**	**38 (32-45)**	**98 (92-100)**	**0.07**
		400	97 (89-100)	27 (21-33)	97 (89-99)	0.1
	DVT- 620	300	97 (89-100)	19 (15-25)	96 (85-99)	0.14
	(80-7100)	200	100 (94-100)	10 (6-14)	100 (82-100)	0

Median and cut-off values in ng/mL. Percentage and (95% CI). Standard cut-off values in bold. NPV = Negative predictive value. LR- = Negative likelihood ratio.

recruited and pre-test probability score (according to Wells et al. [4]) was estimated [15]. Patients were either self-referred, referred from primary care physicians or to a lesser extent sent in from other clinics. Enrollment was possible 24 h/day, 7 d/week and occurred immediately on arrival to the emergency department by the emergency physician on duty. If categorized as having a low pre-test probability, real time D-dimer (Auto Dimer® (Biopool® International Umeå, Sweden), cut off 250 ng/mL) was analysed and if negative, DVT was considered ruled out. The remaining patients proceeded to contrast venography and/or compression ultrasound (CUS). The primary outcome was recurrent VTE during 3 months follow up. One out of 110 patients, in the low probability-negative D-dimer cohort, developed distal DVT during follow up.

In 311 patients enough plasma was collected in the management study to analyze D-dimer with all four assays and the clinical performance is calculated on these data. However, for the regression analysis, the results are calculated on all patients having the assays performed with an exact value. Exact values for the AxSYM assay were not available in 17 patients (results >9000 ng/mL) giving (n = 304) for AxSYM vs Vidas and Autodimer correlation and (n = 340) for Innovance vs Vidas and Autodimer correlation.

The regional center research board in Lund approved the study, and all patients provided written informed consent before enrolment.

Methods

For D-dimer analysis, venous blood was collected in 5 ml vacuum tubes (Becton-Dickinson, Franklin Lakes, USA) containing sodium-citrate (3.8%), and centrifuged at 3600 ×*g* for 10 minutes at 4 °C within 30 minutes of collection. The plasma was aliquoted and one aliquot was used for the real time local D-dimer analysis. The other tubes were frozen at -70 °C for later analysis in batch with, Innovance™ D-Dimer on a Sysmex® CA-7000 Coagulation Analyser (Dade-Behring, Marburg, a Siemens Company, Germany), AxSYM D-dimer on an AxSYM analyser (Abbott, Abott Park, IL, USA), Vidas® D-Dimer Exclusion™ (BioMerieux, Marcy l'Etoile, France) on an mini Vidas (BioMerieux) analyser and Autodimer® (Trinity Biotech, Bray, Ireland) on an Sysmex® CA-7000 Analyser. Instruments for the AxSYM and Vidas assays were provided by the manufacturer as were reagents for all assays but the Autodimer. Cut off was set to <500 ng/mL for Innovance, AxSYM and Vidas assays whereas <250 ng/mL was used for the Autodimer according to the manufacturers recommendations.

Lab personnel performing the D-dimer assay were not aware of the patient's clinical outcomes.

Table 2
False negative patients with all the assays.

	False negative 1	False negative 2
Clinical description	Distal DVT	Proximal DVT
Clinical probability	Intermed	High
Assay (ng/mL)		
AxSYM	229	268
Innovance	150	270
Autodimer	50	60
Vidas	210	220

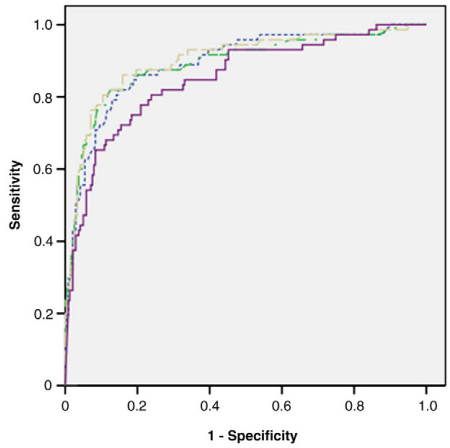


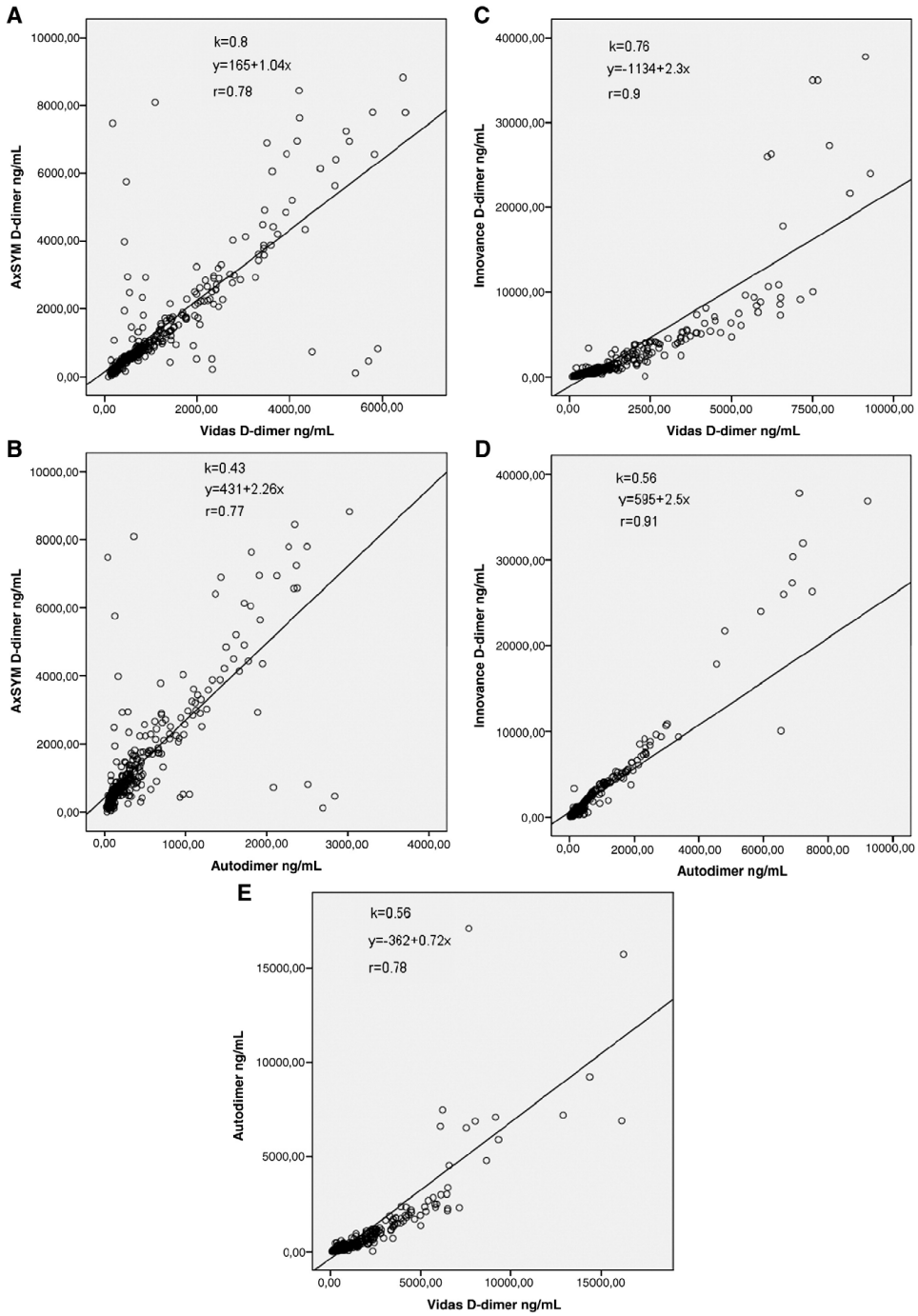
Fig. 1. Reciever Operating Curves for the four assays. (·····) VIDAS Area Under the Curve = 0.89 (95% CI, 0.85-0.94); (– –) Autodimer = 0.89 (0.84-0.94); (– –) Innovance = 0.9 (0.86-0.95); (——) AxSYM = 0.85 (0.8-0.9).

Statistical methods

Sensitivity, specificity and negative predictive value (NPV) were calculated for each assay in relation to the clinical outcome i.e. having DVT at the initial diagnostic workup (positive compression ultrasound or positive contrast venography) or during 3 months follow up. Mc Nemars and Fishers exact test was used for testing significant differences between sensitivity, specificity and NPV. P-values<0.05 were considered statistically significant. Receiver Operating Characteristics curves (ROC) were constructed and the area under curve (AUC) calculated to estimate the discriminative power of the assays. Pearson's correlation coefficient was calculated to measure the strength of association between the assays. Kappa coefficients (κ) were calculated to estimate the concordance between the tests, a value of >0.81 represents excellent concordance, 0.80-0.61 good concordance, 0.60-0.41 moderate concordance [16].

The statistical analysis and graphs was performed with SPSS version16 (SPSS Inc., Chicago, IL, USA). For calculations of Kappa values

Fig. 2. Scatterplots with regression lines for method comparison between AxSYM (n = 304) and Innovance (n = 340) versus the reference methods. The kappa value, regression equation and the coefficient of regression are inserted in each graph. (A) AxSYM versus Vidas. (B) AxSYM versus Autodimer. (C) Innovance versus Vidas. (D) Innovance versus Autodimer. (E) Autodimer versus Vidas.


A
k=0.8
y=165+1.04x
r=0.78
AxSYM D-dimer ng/mL
Vidas D-dimer ng/mL
B
k=0.43
y=431+2.26x
r=0.77
AxSYM D-dimer ng/mL
Autodimer ng/mL
C
k=0.76
y=-1134+2.3x
r=0.9
Innovance D-dimer ng/mL
Vidas D-dimer ng/mL
D
k=0.56
y=595+2.5x
r=0.91
Innovance D-dimer ng/mL
Autodimer ng/mL
E
k=0.56
y=-362+0.72x
r=0.78
Autodimer ng/mL
Vidas D-dimer ng/mL

and 95% confidence intervals (CI) VassarStats clinical calculator (Vassar College, Poughkeepsie, NY: http://faculty.vassar.edu/lowry/clin1.html) was used.

Results

Prevalence of DVT was 72/311 (23%) of which 47 (65%) where considered proximal DVTs. The median age was 60 (range16-95) years and 189 (61%) of the patients were females. The highest cut-off values to reach 100% sensitivity were well under the cut-off values recommended by the manufacturers for all the assays. Given the cut-off value as recommended by the manufacturers, all D-dimer assays had sensitivities of 92% or higher and a NPV of 95% or higher, representing the assays safety in exclusion of VTE. Median results and range for DVT+and DVT-patients, sensitivity, specificity, NPV and negative likelihood ratios (LR-) are given in Table 1.

The highest NPV (97.9%) was seen with the Vidas Exclusion assay, though 95% confidence intervals (CI) were wide and overlapping. The Vidas exclusion assay was false negative in two DVT patients, both categorized as intermediate/high clinical probability, these two patients were false negative in all assays Table 2. There was no significant difference in sensitivity and NPV between reference and AxSYM/Innovance assays. The Autodimer showed significantly higher specificity, over 60% vs less than 40%, compared to the other assays. This observation did not change by lowering the cut-off value of the Autodimer by 20% to <200 ng/mL, indicating that compared to the other assays, the Autodimer will give a higher exclusion rate and a higher clinical usefulness from an economical point of view. Indeed less than 30% of the patients had negative D-Dimer results using Innovance, AxSYM and the Vidas assays compared to 48% for the Autodimer.

Among patients estimated to have a low pre-test probability of DVT (n = 138), 13 (9.4%) patients had DVT. About 18% of the patients had a low pre-test probability in combination with a negative Innovance (55/311) or Vidas (57/311) D-dimer, none of these patients had a VTE during the diagnostic workup, thus giving a NPV of 100% (95% CI, 91.9-100% and 92.1-100% respectively). The AxSYM and the Autodimer assays were false negative in about 2% (1/47 and 2/87) of the patients in the low probability cohort giving NPV's of 97.9 (95% CI, 87.3-99.9%) and 97.7 (95% CI, 91.2-99.6%) respectively. Improvements of the NPV, in the overall cohort, were seen for the AxSYM and Vidas assays by lowering the cut-off values, but also led to a significant reduction in specificity (Table 1). Fig. 1 shows the ROC curves for all assays. The areas under the ROC curve (AUC) were similar for the Innovance and the reference assays ($p > 0.2$) but the AxSYM assay performed slightly inferior with a p-value of 0.032 for the Vidas comparison and 0.055 for the Autodimer comparison. In the regression analysis, the AxSYM assay showed a moderate correlation to the Vidas ($r = 0.78$, Fig. 2A) and Autodimer ($r = 0.77$, Fig. 2B) assay. The Innovance assay showed a strong correlation to the Vidas ($r = 0.9$, Fig. 2C) and the Autodimer ($r = 0.91$, Fig. 2D) assay. The correlation between the reference assays were moderate ($r = 0.78$). The concordance between the tests expressed as kappa values ranged from moderate (Autodimer vs the other assays) and near excellent (Vidas vs AxSYM), kappa values are inserted into the graphs Fig. 2. Proportion of agreement in samples' classification (as below or above the cut-off value) ranged from 72% (Autodimer vs AxSYM) to 92% (Vidas vs AxSYM).

Discussion

This study aims to further evaluate the diagnostic performance of two relatively new D-dimer assays for exclusion of DVT. Previous clinical studies of these assays were based on outpatients with suspected VTE or PE [12–14]. In contrast this study only consider patients with suspected DVT and this could affect the outcome since DVT patients, especially those with symptoms isolated to the calf can have small thrombi thus affecting the D-Dimer concentration and thus the relation to the chosen cut-off value for detection of thrombus [17,18]. Indeed higher sensitivities are generally seen in studies on PE patients compared to studies only including DVT patients [8]. Our results indicate that the Innovance and the AxSYM D-dimer assays have comparable overall performance to the Autodimer and the Vidas Exclusion assay. However some interesting differences need to be commented on.

First; Concerning the AxSYM assay, previous reports on PE patients found excellent performance for ruling out PE with sensitivity and NPV of 100% [12,14]. Ghanima et al found that the highest cut-off value that yielded 100% sensitivity was 765 ng/mL whereas our results indicate that even the 500 ng/mL cut-off value recommended by the manufacturer could be too high (NPV of 95.1% and a wide 95% CI of 87.2-98.4%). The ROC curve analysis showed a significantly lower AUC for the AxSYM compared to the Vidas assay but we also found a strong correlation and good concordance between these assays.

Second; The Innovance assay has been evaluated by de Moerloose et al in DVT/PE patients showing good agreement with the Vidas assay and a sensitivity and NPV of >99% [13]. In comparison to that study our values for sensitivity and NPV is lower but likewise showing a good general agreement with the Vidas assay. The Innovance assay showed the highest AUC value but slightly inferior sensitivity and NPV compared to the Vidas assay, however these differences were not statistically significant. When using standard cut-off values none of the tested assays reached a NPV>98% in the overall cohort. Lowering the cut-off values stepwise by 100 ng/mL (50 ng/mL for Autodimer) we can achieve an NPV>98% for the Vidas and AxSYM assays but at cut-off levels way below the recommended and the subsequent decrease in specificity makes the assays less clinically useful (Table 1). Indeed, at these cut-off levels, the assays would give false positive rates of over 70% with less than 20% negative tests which would require further diagnostic workup in the vast majority of the patients. Although our results indicate that the studied assays are unable to safely exclude DVT as a stand-alone test in the overall cohort, when used in combination with a low pre-test probability score from our management study, a negative D-dimer result gives a NPV of 100% for the Vidas and Innovance, 97.7% for the Autodimer and 97.9% for the AxSYM assay. These results should be set in relation to the generally acceptable failure rate for strategies in the exclusion of DVT at 1-(2) % [19,20] and due to the limited sample in our study the lower bound of the 95% CI is unacceptably low (87-92%).

The Autodimer performs somewhat differently than the other assays in regard to the remarkably high specificity (60%), compared to <40% for the other assays. In combination with a low probability score a diagnostic cut-off value of <200 ng/mL gives a NPV>98% without affecting the specificity significantly. Furthermore a much lower false positive fraction and thereby a significantly higher exclusion rate of 54% vs 34-41% for the other assays, is obtained.

The D-Dimer assays in our study all showed generally high sensitivity and NPV with small but not significant differences. Compared to previous studies on these assays our results show lower sensitivities and NPV's. One explanation for this could be that contrast venography was the main diagnostic tool used (80%) in the intermediate/high pretest probability or positive D-dimer cohort, thereby revealing more calf-vein DVT's than strategies using proximal and serial CUS and hence lowering the sensitivity of the assays. Indeed, distal DVT was found in 10/15 of the false negative tests. To answer the question whether two cut-off levels should be used, one for distal and one for proximal DVT's, we also analysed our data after splitting the patients into distal and proximal DVT. Our results (data not shown) indicate that there are no significant differences concerning the NPV's obtained but, it has to be stressed that only eight patients where false negative with either assay (five distal and three proximal) thereby giving wide 95% CI's. Another explanation for the inferior performance in our study could be the observation that, two false negative patients were negative with all assays, both had the diagnosis confirmed by contrast venography and one DVT was proximal. These patients could actually have had chronic thrombosis.

Our study has some limitations: First, plasma samples for comparison between the four assays were only available in 311 out of 357 patients included in the management study. The main reason for this was lack of plasma, but we don't think this could have lead to any selection bias. Second, due to the limited sample size, the lower limit of the 95% CI's for the NPV's reached down to around 90% which is unacceptably low for the exclusion of DVT.

In conclusion, our results indicate that AxSYM and Innovance assays have comparable diagnostic performance to the more well established Vidas and Autodimer assays and seems to be safe in the diagnostic work-up for ruling out DVT in outpatients with low pre-test probability score. This study also shows the importance of high specificity in terms of reducing additional imaging tests and brings down costs. The optimal cut-off value for the AxSYM assay in DVT patients needs further evaluation but should probably not be increased.

Conflict of interest statement

None

Acknowledgements

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Paper IV

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Manuscript Draft

Manuscript Number:

Title: A Cost-effectiveness Analysis of Diagnostic Algorithms of Deep Vein Thrombosis at the Emergency Department

Article Type: Regular Article

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Abstract: Introduction: Suspected cases of deep vein thrombosis are common at emergency departments and they often require extensive and costly diagnostic testing. The objective of this study was to evaluate whether a diagnostic algorithm based upon pre-test probability and D-dimer in diagnosing deep vein thrombosis may be cost-effective from a societal perspective in a Swedish setting.
Material and Methods: The cost-effectiveness of two alternative diagnostic algorithms were calculated using decision analysis. An algorithm which out ruled deep vein thrombosis among low probability patients with negative D-dimer was compared to a traditional algorithm including compression ultrasonography and/or contrast venography for all patients. For sensitivity analysis, a third reversed algorithm, where D-dimer was followed by pre-test probability, was analyzed. Estimates of probabilities were obtained from a prospective management study, including 357 outpatients with clinical suspicion of deep vein thrombosis. Direct costs were estimated using prices from Scania, Sweden. Indirect costs were estimated using time spent at the local emergency department and gross average wages in Sweden.
Results: The total cost of the pre-test probability and D-dimer algorithm was estimated to €406 per patient and the traditional algorithm was estimated to €581 per patient. Reversing the order of the score and test resulted in an estimate of €421 per patient.
Conclusion: At no significant difference in diagnostic efficacy the algorithm based upon pre-test probability and D-dimer was cost-effective, while the reversed algorithm and diagnostic imaging for all patients were not.

A Cost-effectiveness Analysis of Diagnostic Algorithms of Deep Vein Thrombosis at the Emergency Department

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Keywords

D-dimer; Deep Vein Thrombosis; Cost-effectiveness

Abbreviations

Contrast venography (CV)

Compression Ultrasonography (CUS)

Deep Vein Thrombosis (DVT)

Pre-Test Probability (PTP)

Pulmonary Embolism (PE)

Venous Thromboembolism (VTE)

Abstract

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Conclusion: At no significant difference in diagnostic efficacy the algorithm based upon pre-test probability and D-dimer was cost-effective, while the reversed algorithm and diagnostic imaging for all patients were not.

Introduction

Suspected cases of deep vein thrombosis (DVT) are common at emergency departments and they often require extensive and costly diagnostic testing [1-3]. Clinical studies and meta analyses show that algorithms based upon Pre-Test Probability (PTP) assessment and D-dimer safely rule out venous thromboembolism (VTE) when the PTP for the disease is assessed as low and D-dimer is negative [1-3]. By these means DVT is ruled out in 30–50% of outpatients with suspected DVT and safely obviates the need for further diagnostic testing [4,5].

At times of economic constraint, the demand for effective use of scarce resources in the health sector may be more present than ever. New technologies often involve increased benefits to patients, but at a higher cost. Other new technologies are instead developed to achieve the same goal, but at a lower cost. Since few of patients with suspected DVT actually have the disease [6,7] the need for compression ultrasonography (CUS) and/or contrast venography (CV) could be obviated among a large number of patients. The implementation of an algorithm based upon PTP and D-dimer thus implies great cost savings at emergency departments. Earlier studies evaluating algorithms based upon PTP and D-dimer have mainly considered health care costs [8-11]. This study also includes the cost of waiting time at the emergency department, which is an important part of the societal costs from the patient's perspective.

The objective of this study was to evaluate whether a diagnostic algorithm based upon PTP and D-dimer in diagnosing DVT may be cost-effective compared to a traditional algorithm including CUS and/or CV for all patients, using Swedish data. The evaluation was made from a societal perspective.

Methods

Study Design

A decision analysis model was applied to evaluate the cost-effectiveness of two alternative diagnostic algorithms for DVT. The first algorithm is based upon PTP and D-dimer. Figure 1a illustrates the different possible pathways in the algorithm. DVT was excluded for patients with low PTP and a negative D-dimer in the PTP±D-dimer algorithm, while patients with high PTP or positive D-dimer continued with CUS and/or CV. The second algorithm (figure

1b), which has been used traditionally, involved diagnostic imaging for all patients. The mutually exclusive pathways were results of prior decisions and probabilities of different events. The expected cost for the algorithm was determined by the sum of the costs weighted by the probabilities of events for the particular pathway. Total costs were the sum of direct health care costs and indirect costs measured by patient time spent in the emergency department. To see the potential total cost to society, these costs were enlarged to the regional and the national level. For sensitivity analysis, a third algorithm was analyzed (Figure 1c), where the order of D-dimer and pre-test probability were reversed compared to the first algorithm.

Data

The probabilities used in the analysis were derived from a clinical management study [12] where 357 outpatients with a suspected first episode of DVT were prospectively recruited. PTP was estimated by the emergency physician using Wells score [13,14]. Enrollment was possible 24h/day 7d /week and occurred immediately on arrival to the emergency department by the emergency physician on duty. If categorized as having a low pre-test probability, real time D-dimer (Auto Dimer ® (Biopool ® International Umeå, Sweden), cut off 250 ng/mL) was analyzed and if negative, DVT was considered ruled out. The remaining patients proceeded to CV and/or CUS. The primary outcome was recurrent VTE during 3 month follow up. One out of 110 patients, in the low probability-negative D-dimer cohort, developed DVT (distal) during follow up. PTP followed by D-dimer safely ruled out DVT in about 30% of patients with a suspected first episode of DVT. As recently shown in a meta-analysis [2], this outcome was consistent with other similar clinical studies. Table 1 shows patients characteristics.

Estimates of time patients spent at the hospital were based on estimates from the emergency department at the Lund university hospital (low probability patients with negative D-dimer, 3h 50 min, high probability patients or positive D-dimer, 8h, and CV/ CUS alone without D-dimer determination, 7h) [Personal communication Dr J L Elf].

Distributions between the different methods of diagnostic imaging in the CUS/CV alone algorithm were estimates about hospital practice of diagnosing DVT before PTP and D-dimer was an available option [15]. These estimates were in accordance with previous research [16].

Previous studies have shown that the incidence of DVT is between 48/100.000 and 160/100.000 in the population [17-20]. The prevalence of patients with actual DVT in the group of suspected cases of DVT at the emergency department was 23.5% [12]. The number of suspected cases of DVT in the county Scania, with 1.2 million inhabitants, was thus estimated to reach 2400 - 8200 cases each year. In Sweden, with 9.2 million inhabitants, the number of suspected cases of DVT was estimated to reach 19.000 – 63.000 cases each year. A previous study have reported 40.000 suspected cases of DVT in Sweden in a year [16].

Valuation of costs

All costs are in 2008 Euros (€1= SEK 9,6055). Direct costs were estimated using the pricelist from Southern Regional Health Care Committee [21] (D-dimer €16, CUS €157, CV €461). To ensure that prices used reflected full costs of the algorithms, prices between health care regions were used. Indirect costs occurred when patients spent time at the hospital instead of working, or as a loss of leisure time. We used the human capital approach to value time as loss of production [22]. For patients in working age, productivity loss was estimated by using gross average wage including payroll tax (38.8% [23]) in Sweden 2007. For patients assumed to be retired (aged 65 and older) lost leisure time was estimated by assuming a 35% value of the gross average wage, following previous research [24, 25]. We used age specific probabilities when estimating the indirect cost, as suspected DVT is more likely among elderly patients.

Outcome

Based on previous results [26] we assumed that the alternative diagnostic algorithms did not differ significantly in terms of diagnostic efficacy. In this analysis all cases of DVT were assumed to be detected. As a consequence, risks and costs associated with a false negative diagnosis, such as DVT developing to pulmonary embolism (PE) or post-phlebitic syndrome, or a false positive diagnosis, such as the cost due to side effects of over-treatment of anticoagulation therapy, were not included.

Sensitivity analysis

It has been reported from clinical practice that D-dimer is commonly analysed before the PTP assessment, for both low and high probability patients. A sensitivity analysis was therefore performed in which the order of D-dimer and PTP was reversed (Figure 1c). This reversed algorithm resulted in D-dimer tests for both high and low risk patients. The time spent at the

hospital was assumed to be one hour shorter for the reversed algorithm, as a nurse could perform the D-dimer test and get the result before the patient meets with the physician.

Because of parameter uncertainty, D-dimer analysis and proportion of low and high probability patient groups were varied in one-way sensitivity analyses based on assumptions made in a previous study [15]. Prices were varied from a decrease of 50% to an increase of 50%. Time spent at the hospital was varied likewise, due to differences in procedures between hospitals and over time.

Results

The expected total cost for using the PTP±D-dimer algorithm was €406 per patient, where the direct and indirect costs were €311 and €95 respectively. The CV/CUS algorithm was estimated to €581 per patient, where the direct and indirect costs were €471 and €110 respectively. The PTP±D-dimer algorithm is therefore cost-effective. These results are presented in Table 2.

The potential regional cost of the algorithm which involves a PTP±D-dimer was estimated to a total cost of €1- €3.3 million for the county of Scania based on our estimate of 2400-8200 cases per year. The CV/CUS algorithm was estimated to €1.4-4.7 million.

The national potential expenditure of the PTP±D-dimer algorithm in Sweden was estimated to €7.7 –25.6 million per year based on our estimates of 19.000-63.000 cases per year, using cost in Scania as approximates for local costs. The CV/CUS algorithm was estimated to €11 – 36.6 million at the national level.

Sensitivity analysis

If the order of PTP and D-dimer was reversed, D-dimer followed by PTP, the total average cost was estimated to €421 per patient, where the direct and indirect costs were €332 and €89 respectively.

Table 2 shows how sensitive the direct costs are to an increase or decrease of the direct costs with 50% and how sensitive indirect cost are to an increase or decrease of 50% in time spent

at hospital. The PTP±D-dimer algorithm remains cost-effective, but the difference to the reversed algorithm decreases as direct cost decreases.

The cost of the PTP±D-dimer algorithm was sensitive to the number of patients in the population who has negative D-dimer as well as low probability. Table 2 shows how the result was affected if 80% of the patients had negative D-dimer, instead of 69% and if the number of patients with low probability would be 35% instead of 45%. The PTP±D-dimer algorithm remains cost-effective compared to the reversed algorithm and the CV/CUS algorithm, which is not affected by the share of patients with negative D-dimer or low probability.

Discussion

The expected cost per patient of using the PTP±D-dimer algorithm was €406 and the cost of the traditional CV/CUS algorithm was 43 % higher. The PTP±D-dimer algorithm is thus cost saving for the health care sector and for patients. At no differences in diagnostic efficacy [26], the PTP±D-dimer algorithm may be considered a dominant strategy, i.e. giving the same result at a lower price. The reduction in cost is mainly due to the possibility to avoid costly and time-consuming CV and/or CUS among patients with low probability and negative D-dimer. Furthermore, the cost-effective algorithm contains the benefit of patient's preference of an immediate diagnosis and of avoiding the inconvenience of CV, an invasive method associated with a small but significant risk of complications, among low probability patients.

This cost-effectiveness analysis was based on a management study made in clinical practice [12] and available published data. Sensitivity analysis was performed for important variables because of uncertainty. The main result did not change although these variables were varied in different scenarios; the algorithm including PTP followed by D-dimer remains the cost-effective algorithm.

Goodacre and colleagues [8] analyzed various different strategies in an UK setting, which in accordance with our study, showed that diagnostic imaging for all patients is not cost-effective. Humphreys and colleagues [10] performed a similar analysis in the case of acute PE comparing two algorithms, with the result that PTP score and D-dimer was less costly than a

standard algorithm involving diagnostic imaging. Ten Cate- Hoek and colleagues [11] recently showed in a cost-effectiveness analysis that an algorithm based on PTP D-dimer was cost-effective in a primary care setting. Hence, previous research is consistent with our results that the PTP±D-dimer algorithm is both safe and cost-effective.

Our model entails the simplifying assumption that all cases of DVT are detected even though one patient in the low probability-negative D-dimer cohort developed DVT (distal) during follow up in the clinical management study [12]. Previous research suggests that the algorithms do not differ substantially in efficacy [26].

Waiting time was clearly an important component in the cost of the alternative algorithms. By including productivity loss, one assumes that the community loses employed labor. Indirect costs may have been overestimated if loss of production was compensated by unemployed labor, colleagues or by the patient at a later point [27] or underestimated as informal care (e.g. the time family members spend accompanying a patient) was not included in the analysis. We used information from estimates of expected waiting time and we carried out a sensitivity analysis of these estimates. The estimates used here were assumed to reflect current practice in Swedish emergency departments.

Differences between algorithms become more evident, even though they should be interpreted carefully, when enlarged to the regional and national level. Our estimates of suspected cases of DVT were based on literature [12, 17-20] and it was in agreement with a survey on the extent of diagnostic imaging of DVT made in Sweden from 2002 [16]. Based upon our estimates the county of Scania would decrease expenditures with €0.4 – 1.4 million per year depending on the incidence rate, moving from the traditional algorithm to the PTP±D-dimer based algorithm. On the national level, savings were estimated to € 3.3 - 11 million per year, by implementing the PTP±D-dimer algorithm instead of the traditional algorithm.

The limitations of this study are that direct costs and waiting times at the emergency departments can vary in different settings and over time. Furthermore, the incidence of DVT can vary between countries [17-20] and cohorts of patients. Our analysis is based on available published research, which explains the wide interval of potential cost on the regional and national level.

In the short run, the PTP±D-dimer algorithm may not be considered as cost-saving for hospitals that currently have excess capacity of diagnostic imaging. The excess capacity will favor patients who are waiting for CV or CUS in the short run, since the demand for such diagnostic methods have decreased. However, in the longer term the reduction of investment costs in equipment and education of staff, associated with diagnostic imaging, is likely to make the PTP±D-dimer algorithm cost-effective.

The reversed algorithm was shown to be suboptimal as the direct costs increased when D-dimer is used for all patients; not only low probability patients. Indirect costs savings made by allowing a nurse to take the D-dimer test before the patient meets with the physician, was not enough to compensate for the increase in direct cost.

D-dimer is only to be applied if the physician is convinced that DVT is a diagnostic possibility. If the D-dimer test is used as a screening test, the reversed algorithm increases the risk of physicians to suspect more patients for DVT, as a positive D-dimer can depend on many other factors then DVT, and the positive predictive value is low. Indiscriminate use of D-dimer may result in many unnecessary diagnostic imaging tests and thus at a higher costs.

Conclusion

The findings of this economic evaluation support the implementation of an algorithm which involves PTP followed by a D-dimer test. If implemented in the this way, the diagnostic algorithm implies better use of resources for the health care sector as well as the society as a whole, compared to a traditional algorithm which involves diagnostic imaging for all patients.

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Conflict of interest statement

There are no conflicts of interest declared from the authors.

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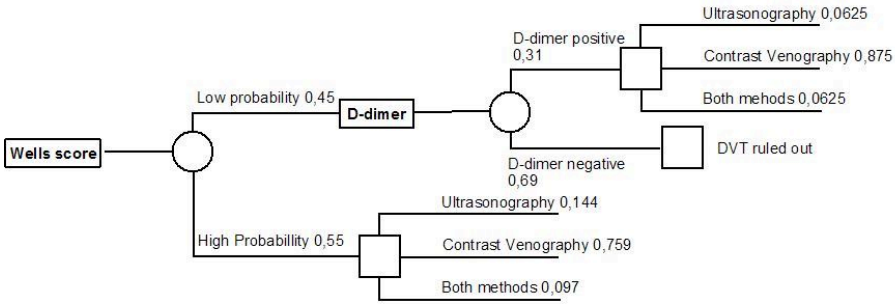
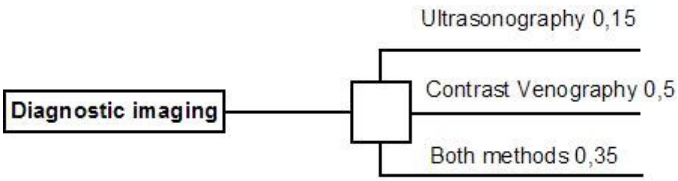
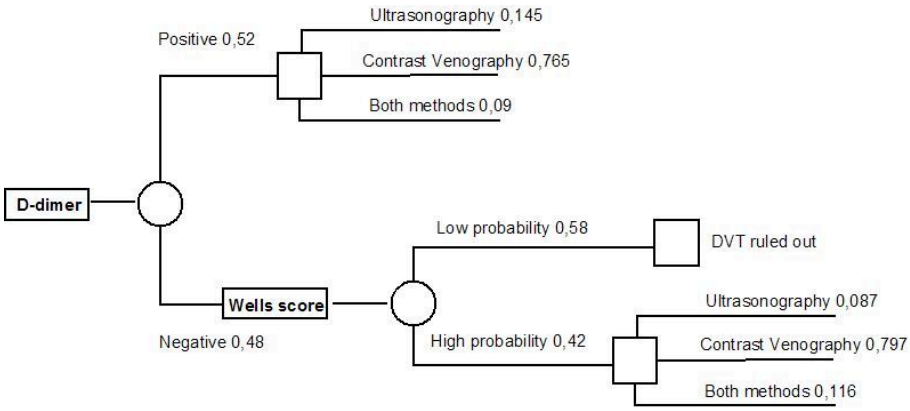
Figure


Ultrasonography 0,0625
D-dimer positive
0,31
Contrast Venography 0,875
Low probability 0,45
D-dimer
Both mehods 0,0625
Wells score
DVT ruled out
D-dimer negative
0,69
Ultrasonography 0,144
High Probabillity 0,55
Contrast Venography 0,759
Both methods 0,097

Figure


Diagnostic imaging
Ultrasonography 0,15
Contrast Venography 0,5
Both methods 0,35

Figure


Ultrasonography 0,145
Positive 0,52
Contrast Venography 0,765
Both methods 0,09
D-dimer
Low probability 0,58
DVT ruled out
Wells score
Ultrasonography 0,087
Negative 0,48
High probability 0,42
Contrast Venography 0,797
Both methods 0,116

Table

	All patients (n=357)	Patients with DVT (n=84)	Patients without DVT (n=273)
Low probability	159 (45%)	14 (17%)	145 (53%)
Intermed/high probability	198 (55%)	70 (83%)	128 (47%)
Age (median)	62 (33, 82)*	67 (32, 83)	60 (33, 81)
Men	138 (39%)	34 (40%)	104 (38%)
Heredity	62 (17%)	16 (19%)	46 (17%)
Smoking	66 (18%)	12 (14%)	54 (20%)
BMI	26 (21,33)*	26 (20, 33)	26 (22, 33)
* Median (10th, 90th percentiles).			

Table

Main Results			**Sensitivity Analysis**					
			Direct cost Decrease 50%	Indirect cost Increase 50%	Indirect cost Decrease 50%	Indirect cost Increase 50%	Negative D-dimer 80%	Low PTP 35%
PTP and D-dimer	Direct cost	€311	€156	€467	€311	€311	€289	€338
	Indirect cost	€95	€95	€95	€48	€143	€94	€100
	Total cost	**€406**	**€251**	**€562**	**€359**	**€454**	**€383**	**€438**
CV/CUS	Direct cost	€471	€235	€706	€471	€471		
	Indirect cost	€110	€110	€110	€55	€165		
	Total cost	**€581**	**€345**	**€816**	**€526**	**€636**		
Reversed order	Direct cost	€332	€167	€500	€332	€332	€313	€438
	Indirect cost	€89	€89	€89	€45	€134	€80	€99
	Total cost	**€421**	**€256**	**€589**	**€377**	**€466**	**€393**	**€537**