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Reliability of Five Rapid D-Dimer Assays Compared to ELISA in the Exclusion of Deep Venous Thrombosis

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Summary

Studies measuring the fibrin degradation product D-Dimer (DD) using enzyme-linked immunosorbent assays (ELISA) in patients with venographically proven deep venous thrombosis (DVT) suggest that it is possible to exclude DVT when DD level is below a certain cut-off level. However, ELISA methods are time-consuming and not available in all laboratories. Different rapid latex-agglutination assays have been investigated, but their sensitivity is considerably lower.

In the present study we compared the value of four novel latex DD tests (Tinaquant[®], Minutex[®], Ortho[®] and SimpliRed[®]) and one rapid ELISA (VIDAS[®]) to a classical ELISA DD assay (Organon Mab Y18[®]) in 132 patients suspected of DVT.

The VIDAS[®], a new quantitative automated ELISA, had a sensitivity of 100% and a negative predictive value of 100% for both proximal and distal DVT at a cut-off level of 500 ng/ml. The Tinaquant[®] assay, a new quantitative latex method, had a sensitivity of 99% and a negative predictive value of 93% for both proximal and distal DVT at a cut-off level of 500 ng/ml. For proximal DVT only, both assays had a sensitivity and negative predictive value of 100%. VIDAS[®] and Tinaquant[®] correlated well with ELISA (correlation of $r = 0.96$ and $r = 0.98$ respectively). Sensitivities of the semi-quantitative latex assays Minutex[®], Ortho[®] and SimpliRed[®] were considerably lower (77%, 51% and 61% respectively).

These results suggest that VIDAS[®] and Tinaquant[®] may be used instead of ELISA DD in the exclusion of DVT. Tinaquant[®] can be performed within 20 min and VIDAS[®] within 35 min. Both assays might be used as a routine screening test and should be evaluated in large clinical management studies.

Introduction

In order to prevent the morbidity and mortality associated with untreated deep venous thrombosis (DVT) a reliable diagnosis and therapy is required. The clinical diagnosis of DVT is unsatisfactory. Over 50% of patients with symptoms suggesting DVT do not have thrombosis (1-3). The definitive test for the diagnosis of DVT is still classical ascending venography. Because this technique is invasive several non-invasive tests have been developed over the past three

decades (4, 5). None of these has been able to achieve the absolute degree of anatomical definition that is possible with contrast venography, but some of these tests have a diagnostic threshold that permits safe clinical management. Serial use of ultrasonography has recently been shown to be a safe method of managing outpatients who are suspected of having lower limb DVT (6). However, a simple and reliable blood test that could exclude DVT would have practical and economic advantages.

Increased levels of plasma D-Dimer (DD) – a cross-linked fibrin degradation product reflecting fibrin formation and subsequent dissolution – measured by enzyme-linked immunosorbent assay (ELISA), have been reported in patients with proven DVT by venography (7-10). One of the major problems of blood tests for DVT is the lack of specificity of the markers. Elevated levels of DD are also found in a wide variety of other clinical conditions (e. g. myocardial infarction, inflammation, malignancy and liver disease) (11). As a consequence studies measuring DD in patients with venographically proven DVT suggest that it should only be possible to exclude this condition.

A series of different assays have been evaluated for the presence or absence of DVT. ELISA assays have been proven to be sensitive enough, but this technique is rather time-consuming (it takes about 2 h) and not suited for routine measurements and thus has little clinical utility as a routine screening test (7, 12). DD latex agglutination assays are performed more rapidly, but their sensitivity is considerably lower (9, 10, 13-19). Recently DD latex assays have regained interest as an emergency diagnostic, because newly available assays display higher sensitivity. In the present study the value of five novel, rapid DD assays in the exclusion of DVT in outpatients with suspected DVT was assessed and compared to ELISA.

Patients and Methods

Patients

In two clinical centers (University Hospital Nijmegen and Canisius Wilhelmina Hospital) patients referred to the outpatients department or emergency unit because of clinically suspected DVT were enrolled in the study after giving informed consent. Only ambulant outpatients were included. No other exclusion criteria were applied. At the time of inclusion age, gender, duration of symptoms and risk factors (immobilization, previous DVT, surgery, malignancy, pregnancy, oral contraceptives) were recorded.

Vascular Testing

After blood was drawn patients were submitted to compression ultrasound (C-US) of the affected extremity. The deep venous system from the popliteal to the iliac vein was examined (4, 20, 21). Lack of full compressibility was the sole criteria for DVT. In case of a positive result, patients were administered for

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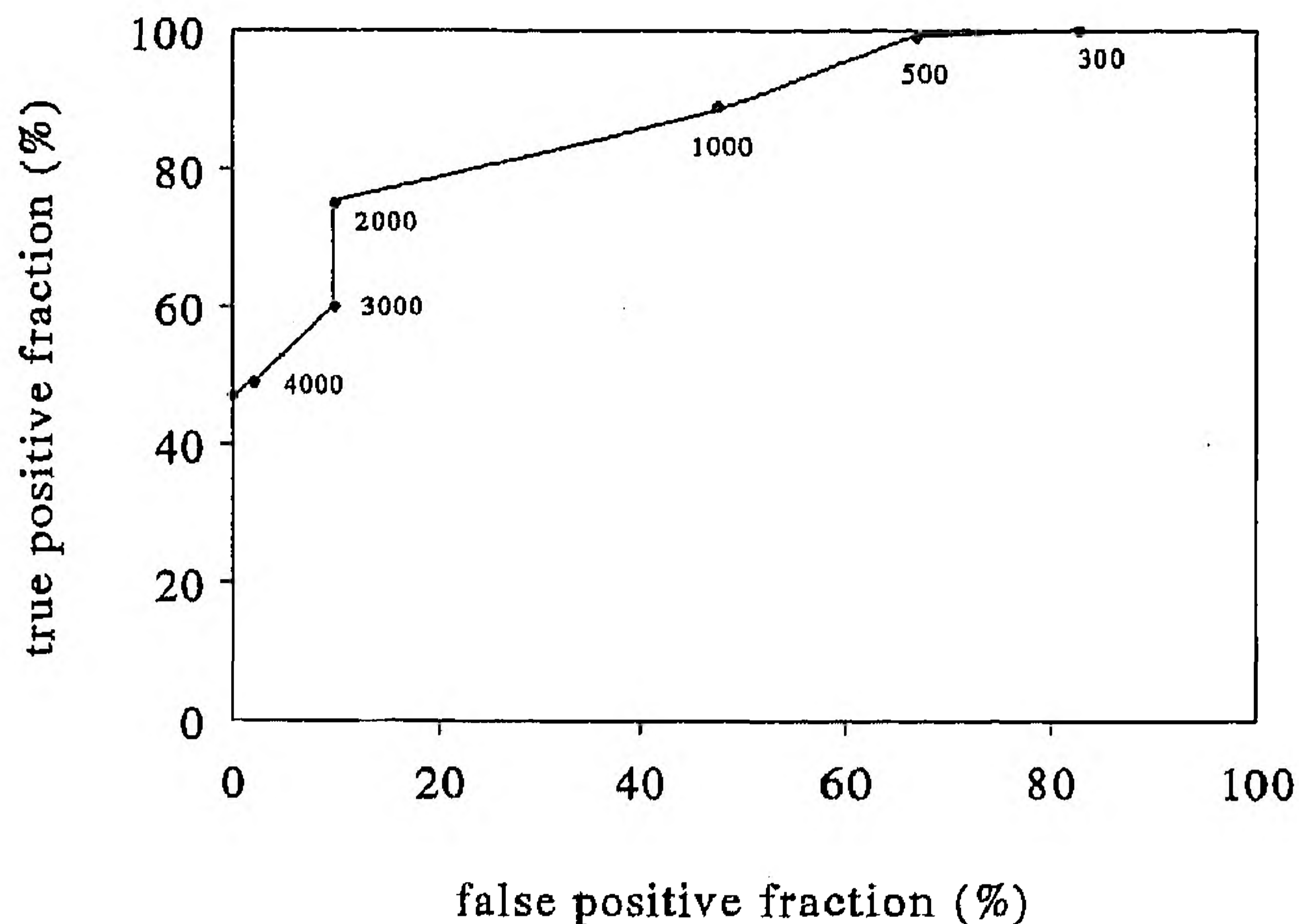


Fig. 1 ROC curve analysis of the accuracy of Tinaquant[®] plasma concentrations for DVT detected by compression ultrasound or venography. D-Dimer values (ng/ml) corresponding to sensitivities of 49%, 60%, 75%, 89% and 99% are shown

Table 1 Sensitivity, specificity, positive predictive value and negative predictive value of the different DD-assays

| Assay | sens (%) CI | spec (%) CI | NPV (%) CI | PPV (%) CI | Duration of the test (minutes) |
|--------------------------|----------------|----------------|---------------|---------------|-----------------------------------|
| ELISA (500 ng/ml) | 98 95-100 | 36 21-51 | 88 80-96 | 77 73-81 | 120 |
| VIDAS (500 ng/ml) | 100 97-100 | 19 7-30 | 100 69-100 | 72 64-80 | 35 |
| Tinaquant (500 ng/ml) | 99 97-100 | 33 18-48 | 93 80-100 | 76 68-84 | 20 |
| SimpliRed | 61 51-71 | 90 81-99 | 52 29-75 | 93 86-100 | 5 |
| Minutex | 77 68-86 | 64 49-79 | 56 27-85 | 82 74-90 | 5 |
| Ortho | 51 29-73 | 47 32-62 | 47 25-69 | 94 87-100 | 5 |

sens = sensitivity

spec = specificity

NPV = negative predictive value

PPV = positive predictive value

CI = 95% confidence interval

standard anticoagulant therapy. Whenever the C-US was inconclusive, that means in case the deep veins could not be adequately visualized, venography was performed and regarded as the definitive test. Also each time distal DVT was suspected venography was performed. If negative C-US results were obtained, C-US was repeated between 3 and 7 days after the initial testing if clinical symptoms of DVT persisted.

Laboratory Testing

At the time of initial presentation from all patients 4.5 ml of blood was drawn into a vacutainer tube containing 0.5 ml 3.8% sodium citrate. Platelet poor plasma was prepared by centrifugation at 4000 × g for 10 min. Plasma

Table 2 Sensitivity and negative predictive values of the different assays for proximal DVT

| Assay | sens overall (%) | sens prox (%) | NPV overall (%) | NPV prox (%) |
|-----------------------|---------------------|------------------|--------------------|-----------------|
| ELISA (500 ng/ml) | 98 | 99 | 88 | 94 |
| VIDAS (500 ng/ml) | 100 | 100 | 100 | 100 |
| Tinaquant (500 ng/ml) | 99 | 100 | 93 | 100 |
| SimpliRed | 61 | 63 | 52 | 56 |
| Minutex | 77 | 78 | 56 | 60 |
| Ortho | 51 | 54 | 47 | 51 |

sens = sensitivity

NPV = negative predictive value

overall = proximal + distal

prox = proximal

was snap-frozen in aliquots and stored at -70° C until assayed. DD assays were performed using commercially available kits. The results of the tests were not made known to the person performing the vascular testing and the results of the vascular testing were not known to the laboratory technician. Because some of the assays are subject to inter-observer variability each assay was performed at the same time by the same laboratory technician. The following DD-assays were performed: (1) ELISA: Mab Y18[®] (Organon Teknika, Belgium) and (2) VIDAS[®] (Biomérieux, France), a quantitative ELISA method automated on VIDAS immunoanalyzer. Latex: (3) Ortho[®] Dimertest (Ortho diagnostic systems Inc, Belgium), (4) Minutex[®] (Biopool, Sweden), (5) SimpliRed[®] (Agen diagnostics limited, Australia) and (6) Tinaquant[®] (Boehringer Mannheim, Germany). Ortho[®] and Minutex[®] D-Dimer are semi-quantitative assays; agglutination taking place in undiluted plasma was regarded as a positive test. SimpliRed[®] is an autologous red cell agglutination assay. This assay can be performed immediately in whole blood. For practical reasons we centrifugated the blood and stored the plasma until assayed. At the time of the assay erythrocytes were added ("add-back" procedure). Tinaquant[®] is a new quantitative latex assay. It was performed on a Hitachi 911 clinical chemistry analyzer. It has been standardized before against the ELISA test Asserachrom[®] D-Dimer and the correlation between the two assays was 0.998 (22).

Analysis

The presence or absence of DVT was determined on the basis of the vascular testing. The sensitivity, specificity and positive and negative predictive values with 95% confidence limits (CI) of each assay were calculated according to the presence of DVT on ultrasonography or venography using two by two tables. For ELISA, VIDAS[®] and Tinaquant[®] Receiver Operator Characteristics (ROC) curves were constructed by plotting the sensitivity (true positive fraction) versus 1-specificity (false positive fraction) (23). Cut-off values were chosen based on optimal sensitivity and negative predictive value. Correlation was calculated according to Spearman.

Results

During the study period (March-December 1995) 132 patients (83 females and 49 males) were enrolled. The mean age was 59 years (range 22 to 89 years). In 78 patients proximal DVT was detected by C-US. In 26 patients venography was performed; in 11 patients distal DVT was detected and in 15 patients venography was negative. In 28 patients C-US was negative. Only one patient with an initially negative

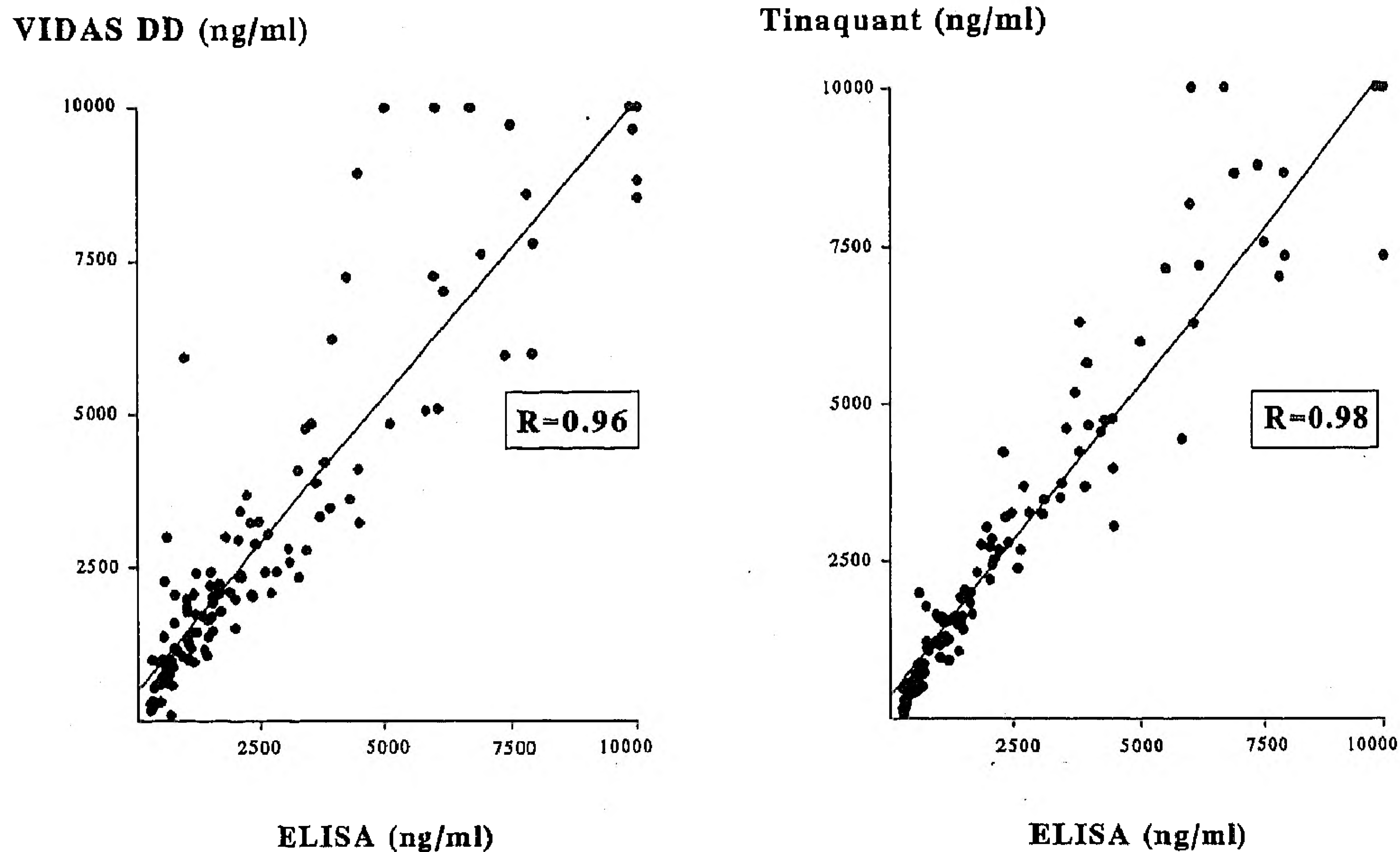


Fig. 2 Correlation between the ELISA and VIDAS[®] ($r = 0.96$) and Tinaquant[®] assay ($r = 0.98$)

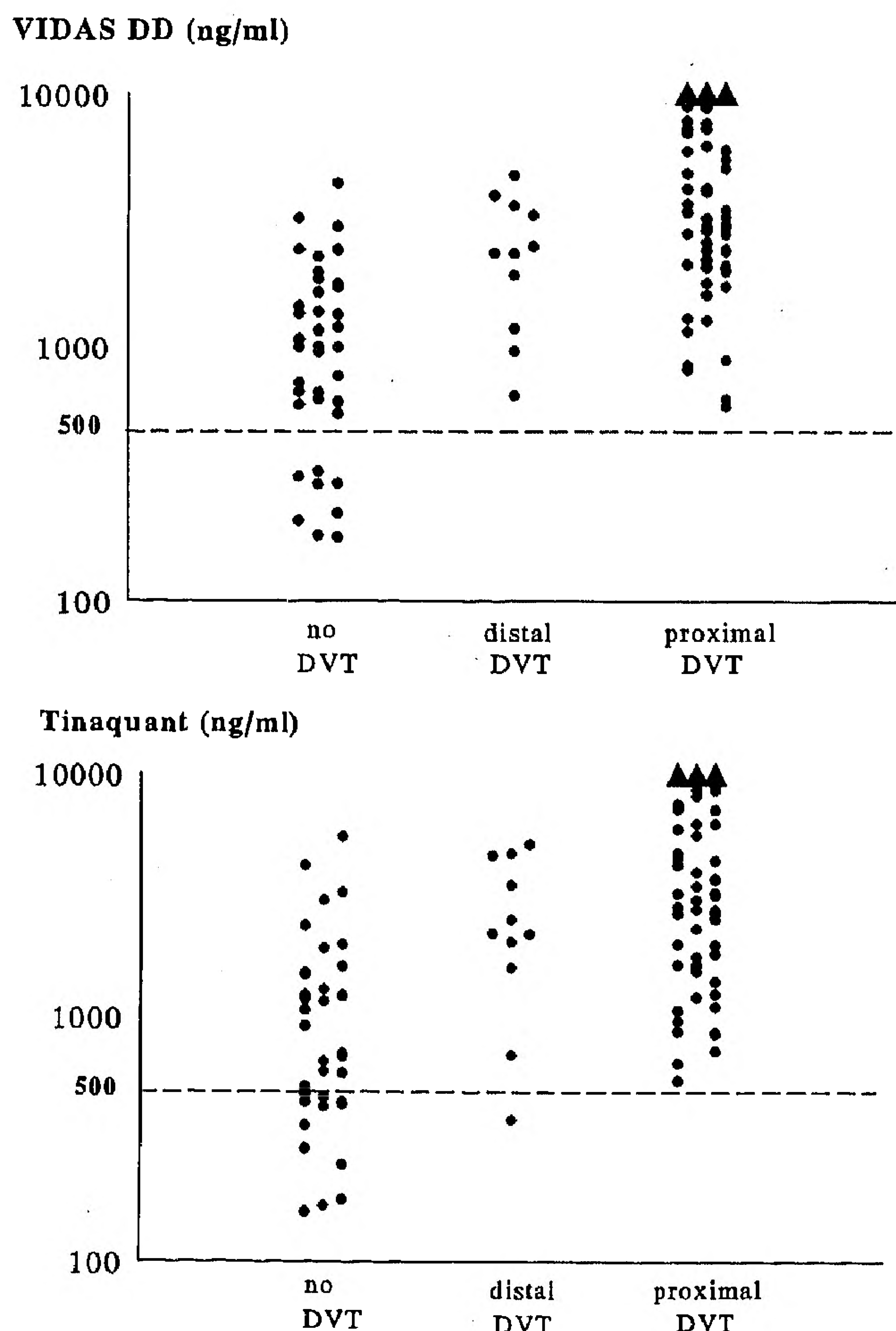


Fig. 3 Values of the VIDAS[®] and Tinaquant[®] assays in three different groups of patients; no DVT, distal DVT and proximal DVT. All patients exceeding DD 10,000 ng/ml got the value 10,000 in the figure. DVT = deep venous thrombosis

C-US developed persisting symptoms and was found to have vein incompressibility at follow-up. In summary proximal DVT was detected in 59% and distal DVT in 8% (overall prevalence 67%). Risk factors for DVT were found in 66 patients (73%) in the group with DVT (14 immobilization, 20 malignancy, 13 previous DVT, 2 previous surgery, 6 recent pregnancy, 11 oral anticonceptives) and in 21 patients (50%) in the group without DVT (2 immobilization, 3 malignancy, 5 previous DVT, 7 recent surgery, 4 oral anticonceptives).

The ROC curve of the accuracy of Tinaquant[®] for all detected DVT is shown in Fig. 1. The ROC curve for ELISA and VIDAS[®] was determined in the same way. For both assays sensitivity and specificity were optimal at a cut-off level of 500 ng/ml. Table 1 shows the overall sensitivity, specificity, positive and negative predictive values of the different assays. At a cut-off level of 500 ng/ml overall sensitivities for ELISA, VIDAS[®] and Tinaquant[®] were 98%, 100% and 99% respectively. Negative predictive values were 88%, 100% and 93% respectively. The sensitivities of the three other latex tests were considerably lower (respectively 61%, 77% and 51% for Simplired[®], Minutex[®] and Ortho[®]). Table 2 shows the sensitivity and negative predictive value for proximal DVT only; sensitivity and negative predictive value for VIDAS[®] and Tinaquant[®] were both 100%. Fig. 2 shows that there was a good correlation between ELISA and VIDAS[®] ($r = 0.96$) and between ELISA and Tinaquant[®] ($r = 0.98$). Fig. 3 shows the results of the VIDAS[®] and Tinaquant[®] tests for three categories of patients: (1) absence of DVT, presence of (2) distal and (3) proximal DVT. Values exceeding 10,000 ng/ml were exclusively found in patients with a proven DVT. Fig. 3 shows that the results of both assays are very similar and that according to both assays in all patients with DVT (except for one patient with distal DVT) the DD exceeded 500 ng/ml.

Discussion

The rationale for performing this study was to see if it was feasible to use a rapid screening test to exclude the presence of DVT in outpatients. We excluded inpatients because DD is elevated in many other

pathological/comorbid conditions (e. g. malignancy, infection, myocardial infarction, liver disease) (11).

Most hospitals are using non-invasive tests to evaluate diagnosis of DVT. C-US has proven to be the most reliable diagnostic test (6). Serial testing is necessary to detect ascending distal DVT. We compared the diagnostic efficacy of five rapid DD assays with classical ELISA in clinically suspected DVT. The diagnosis of DVT was made by C-US. Venography, considered as "gold standard" was not performed routinely. However, if C-US was inconclusive or if distal DVT was suspected, venography was performed. If clinical symptoms persisted serial C-US was performed. In only one patient vein incompressibility developed at follow-up.

Because many patients with symptoms suggestive of DVT appear not to have DVT by objective testing (1, 2), a simple screening test to rule out DVT reliably, would avoid unnecessary further diagnostic procedures. Ideally such a test must have a high sensitivity in order to rule out the diagnosis DVT correctly as these patients would be undertreated.

Classical ELISA assays have proven to be sensitive (7, 12). The disadvantage of ELISA tests is that they are time-consuming and not suitable for routine measurement. In view of the fact that ELISA tests last at least 120 min and that latex agglutination methods are extremely simple and rapid to perform (5-20 min), the availability of a reliable latex test is most interesting. The studies reported using latex tests showed discordant results (10, 12, 14). Sensitivities of most of the currently available latex assays are not sufficiently high, except for the recently developed SimpliRed® assay (24); Wells et al. demonstrated a sensitivity of 93% and a negative predictive value of 98% for this assay.

More rapid ELISA and more sensitive latex DD assays have been developed recently. We investigated the accuracy of a rapid ELISA (VIDAS®) and 4 latex assays (Tinaquant®, Minutex®, Ortho® and SimpliRed®). The results of VIDAS® and Tinaquant® were equal to those of previous reports about ELISA. We also found a good correlation with the classical ELISA ($r = 0.96$ and $r = 0.98$ for VIDAS® and Tinaquant® respectively). For VIDAS® these results are comparable to former studies (25, 26). Both assays have 100% sensitivity (CI 97-100%) and 100% negative predictive value (CI 80-100%) for proximal DVT. The measurement of plasma DD in symptomatic patients with clinically suspected DVT allows exclusion of proximal DVT when negative (<500 ng/ml); the negative predictive value of the test being 100% (CI 80-100%). The specificity of VIDAS® and Tinaquant® was relatively low (respectively 19% and 33%). As it means that according Tinaquant® one third of the patients without DVT could be sent home without further investigations it may be concluded that it might be a useful test as first screening in outpatients. The specificity of VIDAS® is lower than previously reported (25, 26). In this study only 8 out of 42 patients without DVT could have been sent home without further investigations. Another disadvantage of VIDAS® is that this ELISA can only be performed on a dedicated analyzer, while Tinaquant® can be performed on routine clinical chemistry analyzers such as the Hitachi, which is used in this study.

The remaining three latex tests had a lower sensitivity and negative predictive value when compared to standard non-invasive tests. These cannot be regarded as adequate. This lack of sensitivity has been observed in other studies. The results of the SimpliRed® assay were different from the sensitivity and negative predictive value reported by Wells et al. (24). However, they performed the DD assay immediately in whole blood, whereas for practical reasons we centrifugated the blood and stored the plasma until assayed. At the time of the assay erythrocytes were added ("add-back" procedure). This might be the

reason of the different results. Both alternatives of performing the SimpliRed® assay should be compared to assure this explanation.

The prevalence of DVT in our group of patients was relatively high compared to other studies. In these studies prevalence varies between 30 and 50%. A reason for this might be the strict referral pattern of the general practitioners to our hospital reflected in the relatively high number of patients with risk factors for DVT. Another reason might be that during the night hours we missed some samples of patients with a negative C-US.

In summary the VIDAS® and Tinaquant® assays used in this study appeared to be suitable in the exclusion of DVT. The problem of the other semi-quantitative latex tests was insufficient sensitivity. Although the tests have a high negative predictive value (100%, CI 80-100%), the safety of withholding anticoagulant therapy in patients with clinically suspected DVT and negative DD needs confirmation in a prospective trial of a large number of consecutive outpatients. Based on these results management studies should be performed to examine the safety and cost-effects of this approach.

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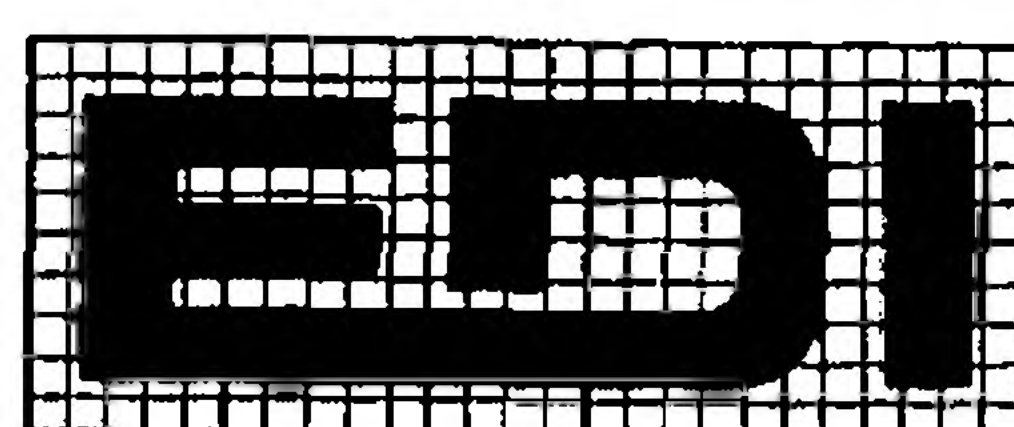
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