Some understanding of diagnostic tests for pulmonary embolism

Mac Gillavry, M.R.

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Clinical evaluation of a monoclonal antibody-based enzyme immunoassay for fibrin degradation products in patients with clinically suspected pulmonary embolism

Melvin R. Mac Gillavry¹, Wouter de Monyé², Jeroen G. Lijmer³, Willem Nieuwenhuizen⁴, Harry R. Büller⁵, Menno V. Huisman⁶, Dees P. M. Brandjes¹, on behalf of the ANTELOPE-Study Group

¹Department of Internal Medicine, Slotervaart Hospital, Amsterdam; ²Department of Radiology, Leiden University Medical Center, Leiden; ³Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam; ⁴Gaubius Laboratory, TNO Prevention and Health, Leiden; ⁵Department of Vascular Medicine, Academic Medical Center, Amsterdam; ⁶Department of General Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

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Summary

We prospectively evaluated the diagnostic accuracy of the Fibrinostika® FbDP assay in 304 consecutive patients with suspected pulmonary embolism and examined potentially useful cut-off points at which the disease can be excluded. The prevalence of pulmonary embolism was 31%. The assay generated an area under the Receiver Operating Characteristic curve of 0.79 (95% CI 0.73-0.84). A cut-off point of 0.05 µg/ml yielded a sensitivity, specificity, negative predictive value and an exclusion efficiency of 100% (95% CI 96-100), 5% (95% CI 2-9), 100% (95% CI 69-100) and 3% (95% CI 2-6), respectively. A clinically useful cut-off point seems to be 0.11 µg/ml which corresponded with a sensitivity, specificity, negative predictive value and an exclusion efficiency of 96% (95% CI 90-99), 27% (95% CI 24-28), 93% (95% CI 84-98) and 20% (95% CI 16-25), respectively. We conclude that the assay has potential clinical utility for the exclusion of pulmonary embolism, but it cannot be used as a sole test.
**Introduction**

Under physiological conditions the haemostatic balance can be described as the equilibrium between fibrin formation and fibrinolysis. Fibrin formation commences with the thrombin-mediated cleavage of fibrinopeptides A and B from the fibrinogen molecule to form fibrin monomers (1). At low concentrations, these monomers will be kept in solution by complexing with fibrinogen. These complexes are called soluble fibrin (2). At higher concentrations, fibrin monomers will segregate from the complexes and form a macroscopic gel in which the fibrin monomer subunits can eventually become crosslinked in the presence of calcium and activated factor XIII (1). The fibrinolytic system counteracts this fibrin formation with the digestion of fibrin by plasmin, which results in degradation products from non-crosslinked (soluble) fibrin (fragments X, Y, D and E) and crosslinked (insoluble) fibrin (X-oligomers, D-dimer and fragments E) (3, 4).

Increased plasma levels of D-dimer containing fibrin degradation products, for reasons of simplicity called here “D-dimer”, are considered to reflect the presence of pulmonary embolism (5). Therefore, it has been suggested that normal plasma levels of D-dimer can be used for the exclusion of pulmonary embolism (5-13). The use of a rapid, readily available and accurate blood test for the exclusion of pulmonary embolism could optimize the diagnostic management. It would enable to complete the diagnostic work-up within a shorter period of time, which reduces the hospitalization period and the days of exposure to heparin therapy.

The Fibrinostika® FbDP assay is a rapid monoclonal antibody-based enzyme immunoassay that measures both crosslinked and non-crosslinked fibrin degradation products (FbDP) (14). This assay gives thus a more complete reflection of fibrinolysis activation as compared to the assays measuring only crosslinked fibrin degradation products.

In the present study, we aimed to determine the diagnostic accuracy of the FbDP assay in consecutive patients with clinically suspected pulmonary embolism and to examine potentially useful cut-off points of FbDP concentration at which pulmonary embolism can be excluded.

**Patients and Methods**

**Patients**

Six teaching hospitals in The Netherlands participated in the study from June 1997 through March 1998. Consecutive in- and outpatients with clinically suspected pulmonary embolism who were referred for diagnostic work-up were eligible. Patients were excluded if they were younger than 18 years of age, were preg-
nant, had an indication for thrombolytic therapy, had already undergone objective diagnostic testing for pulmonary embolism, or if there was an expected inability to complete the diagnostic protocol within 48 hours of presentation. The study protocol was approved by the Institutional Review Boards of all hospitals and written informed consent was obtained from all participating patients.

**Diagnostic Investigations**

A six-view perfusion lung scintigraphy was performed within 24 hours of referral using 100 MBq of $^{99m}$Tc-Technetium-labelled macro-aggregates of albumin. If segmental or larger perfusion defects were seen, ventilation scintigraphy was added using $^{81m}$Krypton gas. Ventilation-perfusion scans were classified as normal (no perfusion defects), high probability for pulmonary embolism (at least one segmental or larger perfusion defect with locally normal ventilation) or non-diagnostic (ventilation-perfusion abnormalities not meeting the criteria for a high probability scan) (15, 16). Pulmonary angiography was performed using standard techniques (17, 18) in all patients with a non-diagnostic lung scan. The maximum allowed time interval between diagnostic investigations was 24 hours. All lung scans were interpreted by a panel of experienced nuclear medicine physicians and final conclusions were reached by consensus. The angiograms were interpreted independently by two radiologists and in case of disagreement, the interpretation of a third was decisive. Pulmonary embolism was considered present in case of a high probability lung scan or abnormal angiogram and absent in case of a normal lung scan or normal angiogram.

**Laboratory Intervention**

Upon study inclusion, prior to or within 24 hours of heparinization, 4.5 ml venous blood was collected into a vacutainer tube (Becton Dickinson, New Jersey; USA) containing 1 ml of 0.105 M sodium citrate solution. Plasma was immediately separated from cellular elements by centrifuging at 2500 g for 15 minutes at 4°C. The plasma was removed, aliquoted into plastic tubes, snap-frozen and stored at -80°C. Samples were thawed only once and the assay was performed batch-wise at the end of patient inclusion. The laboratory technicians who analyzed the samples were unaware of the results of the other diagnostic investigations.

Plasma levels of FbDP were quantitated using a sandwich-type enzyme immunoassay (Fibrinostika® FbDP, Organon Teknika, Boxtel, The Netherlands) according to a previously described procedure (14). This assay is based on a combination of the specificities of two monoclonal antibodies (FDP-14 and DD-13). FDP-14 is the capture antibody with its epitope in the E-domain of the fibrinogen molecule on the Bß-chain between amino acids 54-118. This epitope interacts with FDP-14 only in degraded forms of fibrinogen and fibrin. Thus, FDP-14 binds both fibrinogen degradation products (FbgDP) and FbDP, but does not
Evaluation of enzyme immunoassay for fibrin degradation products

react with intact fibrinogen or fibrin molecules. DD-13 is used as a tagging antibody conjugated with horse-radish peroxidase and detects the D-fragments of non-crosslinked fibrin and D-dimer of crosslinked fibrin, and not the D-domains of fibrinogen bound to FDP-14. Consequently, the combined specificities of FDP-14 and DD-13 make the assay specific for degradation products of crosslinked and non-crosslinked fibrin. It does not detect degradation products of fibrinogen. The assay has a time-to-result of 45 minutes.

Statistical Analysis

The Mann-Whitney-U test was used to compare the FbDP levels between different subgroups of patients. Two-tailed p-values of less than 0.05 were considered to indicate statistical significance. We used Receiver Operating Characteristic (ROC) analysis to determine the diagnostic accuracy of the FbDP assay. The non-parametric area under the ROC curve (AUC) and corresponding 95% confidence interval (CI) were calculated as measure of the diagnostic accuracy independent of the cut-off points (19). An ideal test providing a sensitivity and specificity of 100% generates an AUC of 1, while a fully non-informative test generates an AUC of 0.5.

We examined the cut-off points that were associated with sensitivities of 95% or above. We postulated that a sensitivity of at least 95% was minimally required for the assay to have a clinically relevant potential for the exclusion of pulmonary embolism. The sensitivity, specificity, negative predictive value and the exclusion efficiency, defined as the proportion of patients with a “normal” test result, were calculated at the examined cut-off points using standard methods. The corresponding 95% CIs were calculated according to the binomial distribution. Analyses were performed with SPSS statistical software (version 9.0).

Results

A total of 807 consecutive patients with clinically suspected pulmonary embolism were screened. Of these patients, 130 were excluded for the following reasons: age under 18 years (n=11), pregnancy (n=7), need for thrombolytic therapy (n=4), objective diagnostic tests for pulmonary embolism already performed prior to study entry (n=24) and an expected inability to complete the diagnostic work-up within 48 hours (n=84). Hence, a total of 677 patients were eligible for inclusion in the study of whom 440 (65%) gave informed consent. A final diagnosis regarding the presence or absence of pulmonary embolism was not reached in 70 of the 440 included patients because of withdrawal of informed consent (n=23), clear evidence for an alternative diagnosis for the presenting symptoms (pneumonia n=14; heart failure n=8; lung cancer n=6; rib fracture n=2; pneumothorax n=1; pericarditis n=1) or technical failure (n=15). In 66 other patients, plasma was not
Table 2.1 Clinical and demographic characteristics of the 304 study patients with clinically suspected pulmonary embolism

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study patients (n = 304)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>121 (40%)</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>50 (18)</td>
</tr>
<tr>
<td>Outpatients</td>
<td>245 (81%)</td>
</tr>
<tr>
<td>Median duration of symptoms, days (interquartile range)</td>
<td>3 (1-11)</td>
</tr>
<tr>
<td>Previous VTE'</td>
<td>51 (17%)</td>
</tr>
<tr>
<td>Family history of VTE</td>
<td>63 (21%)</td>
</tr>
<tr>
<td>Risk-period for VTE</td>
<td>101 (33%)</td>
</tr>
<tr>
<td>Active malignancy</td>
<td>26 (9%)</td>
</tr>
</tbody>
</table>

1 venous tromboembolism
2 period of immobilization, surgery or trauma in period of 3 months before presentation

available due to logistic reasons. The study cohort therefore consisted of 304 patients of whom the clinical and demographic characteristics are shown in Table 2.1.

Of the 304 study patients, 93 (31%) were classified as having pulmonary embolism on the basis of 78 high probability lung scans and 15 abnormal pulmonary angiograms. The diagnosis of pulmonary embolism was refuted on the basis of a normal perfusion scan in 131 patients and a normal angiogram in 80 patients.

Table 2.2 Diagnostic accuracy indices and exclusion efficiency of the FbDP assay at several examined cut-off points

<table>
<thead>
<tr>
<th>Cut-off point (µg/ml)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>NPV (95% CI)</th>
<th>Exclusion efficiency (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\leq 0.05)</td>
<td>100% (96-100)</td>
<td>5% (2-9)</td>
<td>100% (69-100)</td>
<td>3% (2-6)</td>
</tr>
<tr>
<td>(\leq 0.07)</td>
<td>98% (93-100)</td>
<td>8% (6-8)</td>
<td>89% (65-98)</td>
<td>6% (4-9)</td>
</tr>
<tr>
<td>(\leq 0.09)</td>
<td>96% (90-99)</td>
<td>18% (15-19)</td>
<td>91% (77-97)</td>
<td>14% (10-18)</td>
</tr>
<tr>
<td>(\leq 0.11)</td>
<td>96% (90-99)</td>
<td>27% (24-28)</td>
<td>93% (84-98)</td>
<td>20% (16-25)</td>
</tr>
</tbody>
</table>

NPV = negative predictive value

The median FbDP level in patients with pulmonary embolism (0.88 µg/ml, interquartile range 0.38-2.11) was significantly higher than in patients without the disease (0.17 µg/ml, interquartile range 0.11-0.43) (p<0.001). The subgroup of inpatients (n=59) showed a significantly higher median FbDP level (0.68 µg/ml,
interquartile range 0.26-1.36) as compared to the outpatients (0.22 μg/ml, interquartile range 0.12-0.64) (p<0.001). The median FbDP levels in patients who were already receiving heparin therapy at the time the blood samples were obtained (n=14) (0.42 μg/ml, interquartile range 0.15-1.95) and in patients that were not on heparin (0.28 μg/ml, interquartile range 0.12-0.83) were not significantly different.

The ROC curve of the FbDP assay had an AUC of 0.79 (95% CI 0.73-0.84) (Fig. 2.1). The accuracy indices and the exclusion efficiency of the assay at several examined cut-off points are shown in Table 2.2. The highest cut-off point with a sensitivity (and negative predictive value) of 100% was 0.05 μg/ml, but the specificity was only 5%. Using this cut-off point, pulmonary embolism could have been reliably excluded in only 10 (3%) of the 304 symptomatic patients. The highest cut-off point with a sensitivity of at least 95% was 0.11 μg/ml which yielded a sensitivity and specificity of 96% and 27%, respectively. Of all study patients, 61 (20%) showed FbDP results of ≤ 0.11 μg/ml of whom 4 had pulmonary embolism.

**Discussion**

This study in a large cohort of consecutive patients with clinically suspected pulmonary embolism demonstrates that the Fibrinostika® FbDP assay has a good diagnostic accuracy (AUC 0.79). We observed some cut-off points of FbDP concentration at which pulmonary embolism can be excluded. A cut-off point of
0.05 µg/ml yielded a sensitivity of 100%, but the corresponding specificity was then only 5%. The highest cut-off point with a sensitivity of at least 95% was 0.11 µg/ml and corresponded with a sensitivity, specificity and a negative predictive value of 96%, 27% and 93%, respectively.

The limited specificity corresponding with the cut-off point of 0.05 µg/ml would compromise the clinical utility of the assay for the exclusion of pulmonary embolism, since almost all patients without this disease then still have “abnormal” results and thus require further diagnostic testing. A cut-off point of 0.11 µg/ml seems to be more clinically useful, since the exclusion efficiency, i.e. the proportion of patients with a normal test result, increased to 20%. However, the prevalence of pulmonary embolism in this group of patients was 7%. This implies that the assay has potential clinical utility if a cut-off point of 0.11 µg/ml is used, but it cannot be used as a sole test for the exclusion of pulmonary embolism.

We further showed that the FbDP levels were higher in inpatients than in outpatients. This could be due to the fact that the inpatients more often had coexisting hypercoagulable conditions than the outpatients. These findings suggest that the cut-off points of FbDP concentration at which pulmonary embolism can be safely excluded may be dependent on the distribution of in- and outpatients in a population.

The diagnostic accuracy of the FbDP assay that we observed is not fully in agreement with the findings of an earlier prospective study in which the same assay was evaluated in 134 consecutive patients with clinically suspected pulmonary embolism (8). In that study, the prevalence of pulmonary embolism was 45% and the 95% confidence intervals of the sensitivity and the specificity were 94%-100% and 6%-22%, respectively. Although these reported accuracy indices appear similar to our results, they corresponded with a higher cut-off point (0.55 µg/ml). This discrepancy may be attributable to different lots of reagents, the relatively small sample size in that study or a difference in the patient characteristics, such as the distribution of in- and outpatients. Furthermore, a previous meta-analysis showed that several D-dimer assays with sensitivities of at least 95% had comparable specificities (6). Thus, although the FbDP assay in theory gives a more complete reflection of fibrinolysis activation than the D-dimer assays, it was not able to further improve the diagnostic accuracy in patients with clinically suspected pulmonary embolism in the present study.

A limitation of our study was that a proportion of the initially included patients had to be excluded from this analysis. It might thus be possible that selection bias has influenced our results. However, the clinical and demographic characteristics of the study cohort were comparable to those of the initially included patients and the prevalence of pulmonary embolism (31%) was in accordance with earlier studies (16, 20). Furthermore, a small proportion of the study patients (5%) already received heparin therapy at the time the blood
samples were obtained. Theoretical, heparin could have induced a decline in FbDP levels, as was described earlier for D-dimer levels in patients with pulmonary embolism (21). This could have negatively influenced the diagnostic accuracy of the assay. However, the FbDP levels in the small number of patients on heparin therapy and in the untreated patients were not different. In addition, reanalysis of the data with exclusion of the heparinized patients from our calculations (data not shown) did not change our results significantly, showing that the influence of heparin, if any, was small. A definitive conclusion regarding the potential influence of heparin on FbDP levels cannot be drawn on the basis of our study, because the number of heparinized patients was very small.

How can the safety of withholding anticoagulant therapy on the basis of FbDP results probably be further optimized? As shown, the assay has potential clinical utility for the exclusion of pulmonary embolism, but it appears not safe to use it as a sole test. It is conceivable that the negative predictive value at the cut-off point of 0.11 μg/ml may further increase, when the assay is used in a subgroup of patients with a lower prevalence of pulmonary embolism. Previous studies have shown lower prevalences of pulmonary embolism (3 to 9%) in patients who were categorized as having a low pre-test probability for the disease, using a clinical probability estimate or a standardized clinical model (22, 23). It is reasonable to hypothesize that a combination of a normal FbDP test result with a low clinical pre-test probability for pulmonary embolism, as determined by a clinical probability estimate or a standardized clinical model, might assure a safe exclusion of this disease. Further prospective studies evaluating the safety of withholding anticoagulant treatment in patients with both a normal FbDP result and a low clinical probability for pulmonary embolism are needed to prove this hypothesis.

References


