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Incidence and Possible Reasons for Discordant Results between Positive FDP and Negative D-dimer Latex Assays in Clinical Specimens

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Abstract

In general, FDP and D-dimer values have a correlation in clinical conditions associated with disseminated intravascular coagulation(DIC) or coagulation activation. However, there are some patients with discordant results who demonstrate elevated FDP and negative D-dimer results by latex agglutination assays. The incidence and possible reasons for the discordance between FDP and D-dimer results were investigated through simultaneous measurements (n=763) from clinical patients with suspected DIC or coagulation activation. 24.8% (189/763) of samples with elevated FDP were negative for D-dimer assays by the latex agglutination method. Further detailed analysis on randomly-selected discordant samples (n=41) revealed that the most common reason for the discordance was the lower sensitivity of the semiquantitative latex agglutination method for D-dimer, compared with quantitative enzyme or other latex immunoassay. The other contributing factors to the discordance were accelerated fibrinogenolysis without secondary fibrinolysis, elevated soluble fibrin monomer and rheumatoid factor.

Key Words: FDP, D-dimer, discordance, fibrinogenolysis

INTRODUCTION

The laboratory findings of disseminated intravascular coagulation (DIC) may be highly variable, complex, and difficult to interpret.[1] Although the clinical usefulness of fibrinogen/fibrin degradation products (FDP) has been well established, elevated FDP may occur in some conditions other than DIC and may sometimes be negative in DIC.[2] A newer test for fibrin degradation products, D-dimer assay, is currently being used as a more specific diagnostic aid.[3] In general, FDP and D-dimer values have a correlation in clinical conditions associated with DIC or coagulation activation.[4] However, there are some patients with discordant results with elevated FDP and negative D-dimer assays. The aim of this study was to observe the incidence of these discordant findings in clinical practice and to analyze the possible reasons for the discordance between FDP and D-dimer results on the samples from patients with suspected DIC or accelerated coagulation activation with fibrin(ogen)olysis.

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MATERIALS AND METHODS

Patients

Simultaneous measurements of FDP and D-dimer by latex agglutination methods were performed in 763 serum samples from patients with clinically-suspected DIC or coagulation activation. Among all of them, 41 samples with discordant results were randomly selected. Those patients had various conditions (18 infection, 7 diabetes mellitus, 6 hematological disease, 2 trauma, 1 carcinomatosis, and 7 miscellaneous conditions), and were evaluated for the possible reasons for the discordant results between FDP and D-dimer assays by further detailed analysis, including quantitative enzyme and latex immunoassays for D-dimer, detection of soluble fibrin monomer and rheumatoid factor.

Sample preparation

Serum samples for FDP and D-dimer by latex agglutination method were prepared by collecting 2

mL of peripheral blood onto a glass tube containing trypsin inhibitor and thrombin (Murex, Dartford, England). Blood samples for further detailed analysis were collected by drawing 4.5 mL of venous blood into evacuated siliconized tubes containing 0.5 mL of 3.8% sodium citrate (Vacutainer, Becton Dickinson, Rutherford, NJ, USA). Platelet-poor plasmas for quantitative enzyme and latex immunoassays for D-dimer and soluble fibrin monomer assay were prepared by centrifuging at 2000 g for 15 min. D-dimer measurements (latex, EIA and LIA), which are not restricted to sample type (serum or plama) due to their use of the monoclonal antibody specific to D-dimer, were performed in the same samples to compare these results.

Assay methods

Serum FDP was semiquantitated by latex agglutination (Thrombo-Wellcotest, Murex, Dartford, England) using monoclonal antibodies against D and E fragments. The assay for D-dimer was performed initially by latex agglutination(Dimertest, Dade, Miami, FL, USA) and then by enzyme immunoassay (EIA) (VIDAS D-Dimer, bioMerieux, Marcy l'Etoile, France) using an automated VIDAS immunoanalyzer and by latex immunoassay (LIA) using an automated coagulyzer (STA, Diagnostica Stago, Asnieres, France). Soluble fibrin monomer was measured by hemagglutination technique (Diagnostica Stago, Asnieres, France). Normal ranges (cut-off) were < 1:5 (10 μg/mL) for FDP, 0.5 μg/mL for D-dimer, and negative for soluble fibrin monomer. Determination of serum rheumatoid factor was performed by nephelometric method (N latex RF, Behring, Germany).

RESULTS

Incidence of discordant results (positive for FDP but negative for D-dimer) according to the FDP titers

In 763 samples with positive results for FDP assay, 189 (24.8%) samples were negative for D-dimer assays by latex agglutination methods (Table 1). Frequencies of discordant results were different according to the FDP titers, but the majority of discordant results (87.3%) were found to be associated with relatively low FDP titers (<80 μg/ml).

Further detailed analysis of discordant samples

Comparison of D-dimer results between different methods: In 41 discordant samples with negative results for D-dimer latex assays, 35 (85.4%) samples were found to be positive (>0.5 μg/ml) for D-dimer by enzyme immunoassay (EIA). Twenty-one (60%) of 35 samples were found to be higher than 1 μg/ml by quantitative EIA for D-dimer (Table 2). Thirty-five samples positive for D-dimer by enzyme immunoassay (EIA) were all positive for D-dimer by latex immunoassay (LIA) while 3 samples were positive for D-dimer by LIA among 6 samples negative for D-dimer by EIA. The concordance rate between EIA and LIA was 92.7% (Table 3).

Detection of soluble fibrin and rheumatoid factor in the discordant samples: Of 41 samples with discordant results between elevated FDP and

Table 1. Frequencies of Discordance* According to the FDP Titers

FDP (μg/ml)	No. (%)	No. Discordance (%)
10−40	354 (46.4%)	144 (76.2%)
40−80	173 (22.7%)	21 (11.1%)
80−160	136 (17.8%)	16 (8.5%)
160−320	54 (7.1%)	4 (2.1%)
320−640	32 (4.2%)	1 (0.5%)
640−1280	6 (0.8%)	2 (1.1%)
1280−2560	3 (0.4%)	1 (0.5%)
2560−5120	2 (0.3%)	0 (0.0%)
>5120	3 (0.4%)	0 (0.0%)
Total	763 (100%)	189 (100%)

*Positive FDP but negative D-dimer results by latex agglutination assays.

Table 2. Comparison of FDP Titer with D-dimer Levels by EIA

FDP (μg/ml)	D-dimer (μg/ml)	n (%)
10 - <80	0.25−0.50	6 (14.6)
10 - <80	0.50−1.0	14 (34.1)
10 - <80	>1.0	19 (46.3)
80 -160	>1.0	1 (2.4)
>160	>1.0	1 (2.4)
Total	−	41 (100)

EIA, enzyme immunoassay.

negative D-dimer, 2 (4.9%) samples were positive for rheumatoid factor, one from a patient (A) with mitral stenosis and subacute bacterial endocarditis, and one from a patient (B) with autoimmune disease. The sample from patient (A) was positive for D-dimer by EIA and LIA but the sample from patient (B) was negative for D-dimer by EIA and LIA. Only one sample from a premature infant (C) with sepsis was positive for soluble fibrin monomer. The sample from patient (C) was positive for D-dimer by LIA but negative by EIA. The remaining 2 cases were the patients with hematologic malignancies on chemotherapy, one (patient D) with acute myeloid leukemia (AML) and one (patient E) with non-Hodgkin's lymphoma whose samples were repeatedly negative for D-dimer both by EIA and LIA.

Summary of the possible reasons for discordant results: Incidences and the possible reasons for discordant results are summarized in Table 4. The frequent reasons were regarded as false negative latex agglutination assay due to lower sensitivity, compared with enzyme immunoassay or latex immunoassay. Analysis of samples suggested that other possible reasons for the discordance were accelerated fibrinogenolysis without secondary fibrinolysis, elevated soluble fibrin monomer, and false positive FDP levels due to the presence of rheumatoid factor in serum samples.

Table 3. Correlations of D-dimer Results by Two Different Methods

LIA method	EIA method		
	Positive	Negative	Total
Positive	35	3	38
Negative	0	3	3
Total	35	6	41

EIA, enzyme immunoassay; LIA, latex immunoassay.

DISCUSSION

The aim of this study was to investigate the incidence of discordant results between positive FDP and negative D-dimer assays by routine latex agglutination method. Following the harvesting of monoclonal antibodies, a latex agglutination procedure using a latex particle coated with anti-DD-3B6/22 antibody has been developed into a commercially-available test kit.[1] Studies have shown that enzyme immunoassay with the monoclonal antibody is essentially equivalent to the latex agglutination assay, thus making the latex assay routinely applicable for measuring D-dimer in plasma or serum for patients with DIC or activation of coagulation.[5] However, as simultaneous measurements of both FDP and D-dimer have become popular, it has been known that there are some patients who manifest high or elevated levels of FDP and negative or relatively lower levels of D-dimer.[4] We have noted discordant results (positive for FDP and negative for D-dimer by latex agglutination assays) in about 25% of patients with suspected DIC or coagulation activation. The incidence seems to be rather high and may be related to the sensitivity of the reagents used in this study as judged by the fact that results assayed using more sensitive enzyme immunoassay (EIA) or latex immunoassay (LIA) kits showed positive results with higher values than cut-offs. This finding was in agreement with the lower sensitivity of the latex assay compared with the enzyme immunoassay, which has been observed in venous thromboembolism, including deep vein thrombosis and pulmonary embolism.[6] The variations in the results obtained with different D-dimer assays may be caused since degradation products of

Table 4. Summary of the Possible Reasons for Discordant Results

Category	n (%)	Results of analysis	Possible reason for discordance
I	36 (87.8)	false (−) D-dimer	insensitivity of latex agglutination method
II (patient A)	1 (2.4)	false (−) D-dimer	insensitivity of latex agglutination method, presence of RF
III (patient B)	1 (2.4)	false (+) FDP	presence of RF
IV (patient C)	1 (2.4)	false (−) D-dimer	insensitivity of latex agglutination method, presence of FM
V (patient D, E)	2 (4.8)	increased FDP	accelerated fibrinogenolysis

RF, rheumatoid factor; FM, fibrin monomer; EIA, enzyme immunoassay.

cross-linked fibrin occur in a wide range of molecular weights in vivo and comprise varying numbers of the D-dimer motif.[7] Moreover, differences in reactivity of the various monoclonal antibodies and calibrators may cause variations in the results obtained with different D-dimer assays.[8]

Although the clinical usefulness of fibrinogen/fibrin degradation products (FDP) has been well established,[9,10] the widely used latex agglutination assays for FDP using antibodies to fibrinogen fragments D and E cannot differentiate primary fibrinogenolysis and secondary fibrinolysis. Theoretically, elevated FDP with a negative D-dimer result is suggestive of primary fibrinogenolysis, however, the presence of fibrin monomer or unclottable fibrinogen remaining in the serum samples may cause positive results.[4] In addition the presence of rheumatoid factor in serum may rarely cause false positives in latex assay and therefore results from patients with suspected rheumatoid arthritis should be regarded with caution. In this study, two samples from one patient (A) with mitral stenosis and subacute bacterial endocarditis and one patient (B) with autoimmune disease were positive for rheumatoid factor (RF), but repeat assay for FDP after absorption of RF for confirmation could not be performed due to the lack of samples.

Interestingly in this study, two cases of hematologic malignancies (acute myeloid leukemia and non-Hodgkins lymphoma) showed elevated FDP, but normal levels of D-dimer both by EIA and LIA, which were also negative for fibrin monomer and rheumatoid factor. This finding may result from the acceleration of fibrinogenolysis in the absence of thrombin generation or false positive FDP due to other unknown factors. Non-plasmic polymorphonuclear (PMN) elastase may play some role on fibrinogenolysis, as previously reported in promyelocytic leukemia,[11] acute leukemia,[12] and chronic myelocytic leukemia.[4] It was known that leukemic cells had elastase activity and the elastase specific split product of fibrinogen resulted in the elevation of FDP level without D-dimer increase.[13] The patient with non-Hodgkin's lymphoma in this study had high WBC count (36,820/ul), which may be related with elevated PMN elastase. Besides elastase, cathepsin G, metalloproteases and human mast cell tryptase are also involved in fibrinogenolysis.[15] Kitazume et al. reported a false positive FDP test due to monoclonal IgM associated with malignant lymphoma,[16] which was not observed in our case.

In conclusion, simultaneous measurements of FDP and D-dimer are useful for the assessment of coagulation activation with accelerated fibrin(ogen)olysis, but the use of a more sensitive D-dimer assay and meticulous interpretation of discordant results needs to be done based on the pathophysiology of individual patients in clinical practice.

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