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Evaluation of the ratios of high molecular weight fibrin degradation products (HMW FDP) to D-dimer in patients' blood by the immunoassay equally recognizing D-dimer and HMW FDP

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Introduction

Fibrin clot lysis in blood results in the formation of a broad range of fibrin degradation products (FDP) of different molecular weights, including D-dimer (DD), which is the smallest one. The information regarding the ratios of high molecular weight FDP (HMW FDP) to DD in the blood of patients is limited because the commercial assays have different specificity to various forms of FDP. Therefore, we suggest that the assay with equal specificity to all FDP forms could help in evaluating the real ratios of HMW FDP to DD in the blood of patients and following this enable the clarification of its potential clinical significance.

Materials and Methods

MURINE MONOCLONAL ANTIBODIES against DD were produced by the hybridoma technique.

HIGH MOLECULAR WEIGHT fibrin degradation products (HMW FDP) and D-dimer (DD) with equal amounts of cross-linked material were prepared from human fibrinogen that was clotted by thrombin and then lysed by a streptokinase-plasminogen mixture.

A SANDWICH FLUOROIMMUNOASSAY (FIA) was used for the detection of HMW FDP and DD in the plasma samples of patients. First, the plates were coated with the capture antibody DD189. In the second step, the detection antibody DD255 conjugated with Eu^{3+} chelate was added which was then followed by the addition of tested samples.

THE GEL FILTRATION procedure of plasma samples of patients was performed using the AKTA pure system (GE Healthcare, USA) on a Superdex 200 column (GE Healthcare, USA). The D-dimer immunoreactivity in the fractions was measured via different FIA and quantified as the areas under the corresponding peaks using the OriginPro 8 program.

SEVEN PATIENTS with deep vein thrombosis, one patient with mesenteric thrombosis, fifteen patients with sepses of different etiologies, nine patients who were undergoing abdominal surgical operations and seven patients with pulmonary embolisms were enrolled in the study. In the cases of surgical operation, the blood samples were collected one day before and one day after the operation.

EDTA PLASMAS from patients and healthy volunteers were prepared by using a routine procedure and stored frozen at -70°C.

Results and Discussion

PREPARATION OF FDP AND DD CONTAINING EQUAL AMOUNTS OF CROSS-LINKED MATERIAL. To select MAbs with equal specificities to D-dimer and FDP, preparations of D-dimer and FDP with an equal amount of the cross-linked material were prepared (Fig. 1). The FDP preparation mainly contained HMW FDP and to a lesser extent DD. The DD preparation contained DD and minor (if any) amounts of HMW FDP.

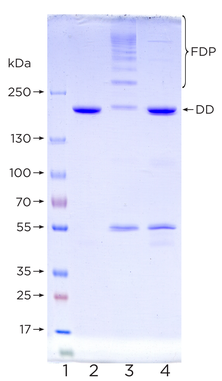

FIGURE 1. SDS PAGE of FDP and D-dimer prepared sequentially from the same fibrin clot. Electrophoresis in 3-10% gradient gel according to Laemmli under non-reducing conditions.
1: MW standard, 2: D-dimer standard, 3: HMW FDP solution, 4: D-dimer prepared from HMW FDP solution

MAB DEVELOPMENT AND ANALYSIS. Twenty-two MAbs specific to D-dimer were obtained and tested in pairs. A sandwich assay that utilized the DD189 and DD255 MAbs as the capture and detection antibodies respectively was found to give similar signals with HMW FDP and DD (Fig. 2).

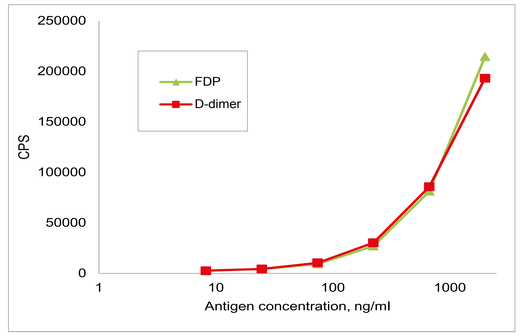

FIGURE 2: Titration curves of DD and FDP preparations in the DD189-DD255 assay.

ANALYSIS OF FDP AND DD IN PLASMA SAMPLES. Eighteen plasma samples from patients suffering from different diseases (five with thrombosis, six who had undergone surgical operations and seven with sepsis) were separated by gel filtration and the DD immunoreactivity in the fractions was analyzed with the DD189-DD255 assay. In each of the eighteen cases, the immunoactivity profiles consisted of two distinctly separated peaks that represented FDP and DD. Figure 3 shows the results from a patient with thrombosis and a patient who had a surgical operation.

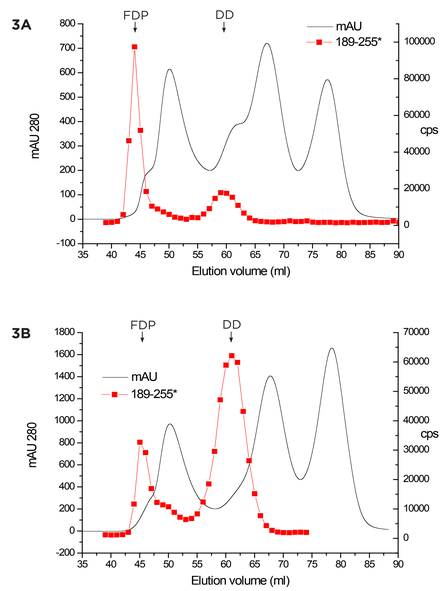

FIGURE 3. Gel filtration of plasma samples from patients with thrombosis (A) and one day after surgical operation (B). Fractions were analyzed using the DD189-DD255 pair in a sandwich immunoassay.

ANALYSIS OF THE FDP/DD RATIOS IN PLASMA SAMPLES FROM PATIENTS SUFFERING FROM DIFFERENT DISEASES . We used gel filtration results to calculate the FDP/DD ratio in each sample. The results showed that the FDP levels exceeded the DD levels in the plasma of patients suffering from thrombotic diseases by up to 3.5 times. In patients undergoing surgical operations, the FDP and DD levels measured one day after the surgical operation were comparable although DD levels usually prevailed over the HMW FDP levels. In samples from septic patients there was no clear pattern observed (Fig. 4).

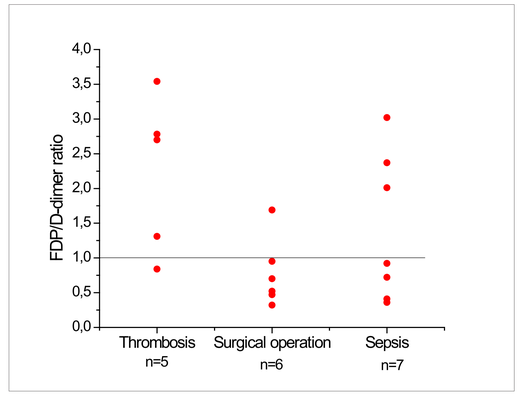

FIGURE 4. Ratios of the FDP to DD levels in the blood of patients suffering from different diseases measured by the DD189-DD255 assay.

Conclusions

1. The DD189-DD255 assay equally detects DD and HMW FDP
2. HMW FDP to DD ratio is different in patients suffering from different diseases
3. Taking into consideration the fact that patients can have different spectrums of fibrin degradation products in their blood, it is our belief that only those assays with equal specificities to DD and HMW FDP can adequately detect the quantities of all forms of fibrin degradation products.

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