Original article

COMPARATIVE ANALYSIS OF THE QUANTITATIVE AND QUALITATIVE METHOD FOR DETERMINATION OF D - DIMER

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SUMMARY

Introduction: D - dimer is a product released during the process of blood clotting and degradation, which can be measured by blood sample analysis. There is usually the minimal activity of the pro/anticoagulant system in the human body, which generates low levels of D-dimer in healthy individuals. Normal values for plasma D-dimer are ≤ 0.50 mg / l.

Aim: The aim of the present study is to determine to what extent the quantitative and qualitative method for determination of D - dimer can be interchangeable and what is their diagnostic reliability in the normal and pathological area of measurement.

Materials and methods: We studied the levels of D-dimer by two methods - quantitative and qualitative, in 91 patients aged 25 to 86 years, of which 59 men and 32 women. To determine the D-dimer, we used venous blood taken in a vacuette containing sodium citrate. We used a Roche test for quantitative determination and a Latex agglutination test for qualitative determination.

Results: It was found that in positive samples above 0.5 mg/l, there is a very high percentage of coincidence. There is a discrepancy in the values obtained by the two methods at the negative values below 0.5 mg/l. We determined the sensitivity, specificity and accuracy of both methods.

Conclusion: The correlation in the results of the two methods is very good, but the quantification of D-dimer is more specific and accurate. We recommend that the value of 0.5 mg/l should be used as a cut off value for D-dimer.

Keywords: D-dimer, qualitative, quantitative, cut off

INTRODUCTION

The D - dimer is a product released during the process of blood clotting and degradation, which can be measured by blood sample analysis. It is usually released when the blood clot begins to break down.

Thrombin converts fibrinogen to soluble fibrin by splitting of fibrin peptides A and B. Fibrin monomers polymerize spontaneously. Active factor XIII binds two D-domains and generates a solid fibrin clot. A new plasmin-resistant antigen determinant ("D-dimer") is obtained. During the degradation of the fibrin clot, fragments contain-ing D-dimer are formed from plasmin, respectively. Most fibrin degradation products contain high molecular weight X-oligomers [1].

D-dimer testing is clinically relevant when deep vein thrombosis (DVT), pulmonary embolism (PE) or disseminated intravascular coagulation (DIC) are suspected [2, 3].

False negative and false positive results may occur in some cases. Due to the frequency of false-negative results, some authors recommend that D-dimer be used only at low probability of pulmonary embolism or deep vein thrombosis.

It should be noted that there are some physiological and medical conditions that can lead to elevated D-dimer in patients without pulmonary embolism, deep vein thrombosis, or DIC. These include, but are not limited to, pregnancy, malignancy, smoking, trauma, infection, or sepsis. In addition, elderly patients, immobilized patients, patients with autoimmune disorders, or those who have recently undergone surgery may have an elevated D-dimer [4].

In new studies, it is proposed to use corrected age limits for D-dimer, as D-dimer values †may increase with age, even in the absence of pathology [5].

There is usually the minimal activity of the pro/anticoagulant system in the human body, which generates low levels of D-dimer in healthy individuals. The normal plasma D-dimer values are ≤ 0.50 mg/l. A D-dimer above 0.50 mg/l is considered positive [6].

The sensitivity and specificity of the D-dimer vary depending on the type of assay method. However, D-dimer tests generally have high sensitivity but low specificity. There are currently various methods for determining plasma D-dimer levels, both quantitative and qualitative [3, 7].

PURPOSE

Our goal is to determine to what extent the quantitative and qualitative method for the determination of D - dimer can be interchangeable and what is their diagnostic reliability in the normal and pathological field of measurement.

PATIENTS AND METHODS

In our study, we measured the levels of D-dimer in the plasma of patients at the University Hospital - Pleven. All of them have signed informed consent at the hospital

admission. The studied patients were 91, aged 25 to 86 years, of which 59 men and 32 women. The mean age for men was 59 years (SD = 15.4) and for women 64 years (SD = 13.3).

The patients we selected for the control group included 30 healthy individuals, 15 men and 15 women, aged 31 to 77 years.

To determine D-dimer, we used venous blood taken in a vacuette containing sodium citrate the (blood-anticoagulant ratio is 9: 1). The blood is mixed well with the anticoagulant, then centrifuged, and the separated plasma is used for analysis. D-dimer was measured in all plasmas by two methods - quantitative and qualitative. We quantified the D-dimer with a Roche test on a biochemical analyzer Cobas 6000, and with a qualitative Latex agglutination test.

The Roche assay is an immunoturbidimetric assay in which latex particles of the same size are encapsulated in monoclonal antibodies (F (ab) 2 fragments) and attached to the D-dimer epitope. The antigen/antibody complexes obtained by adding samples containing D-dimer cause an increase in the turbidity of the test reagents. The change in absorption over time depends on the concentration of D-dimer epitopes in the sample. The precipitate is determined turbidimetrically. Reference value ≤ 0.50 mg/l.

The qualitative D-dimer test is a latex agglutination test using latex particles combined with a highly specific D-dimer monoclonal antibody. XL-FDP present in the plasma binds to the coated latex particles, resulting in visible agglutination occurring when the D-dimer concentration is above the detection threshold of the assay. A result below 0.2 mg/l is considered negative and a result above 0.2 mg/l - positive.

In order to be as accurate as possible to measure semi-quantitatively higher concentrations, as far as the test allows, we worked with the dilutions offered by the manufacturer of the test, namely - 1:2; 1:4; 1:8. The interpretation of the results is as follows: no agglutination when mixing undiluted plasma with reagent, the result is negative - less than 0.2 mg/l; in the presence of agglutination with undiluted plasma, but no agglutination with a dilution of 1: 2, the result is 0.2 - 0.4 mg/l; in the presence of agglutination with undiluted plasma and with a dilution of 1:2, but no agglutination with a dilution of 1:4, the result is 0.4 - 0.8 mg / l; in the presence of agglutination with undiluted plasma and with 1:2 and 1:4 dilutions, but no agglutination with 1:8 dilution, the result is 0.8 - 1.6 mg/l; in the presence of agglutination with undiluted plasma and with all dilutions, the result is 1.6 - 3.2 mg/l;

RESULTS

In the control group, the levels of D-Dimer were measured by a quantitative method, and it was found that the mean value was 0.287 (0.12 - 0.5), and the median was 0.295. All measured values were below the cut-off value of 0.5 mg/l (from reagent manufacturer Roche). This gave us reason to choose the cut off for our study 0.5 mg/l.

During the study of D-dimer in our laboratory was conducted daily intra-laboratory quality control in two levels for both methods.

From the 91 samples for D-dimer studied by both methods, it was found that in the case of positive samples above 0.5 mg/l, there is a very high percentage of coincidence of the results. We have a 100% match in the results for D-dimer above 1.6 mg/l.

There is a discrepancy in the values obtained by the two methods for negative D-dimers below 0.5 mg/l. At these values, it was found that in 28 cases, the qualitative test gave a positive result (> 0.2 mg/l) and the quantitative test a negative result (<0.5 mg/l). This means that in 25.48% of cases, the quality test gives a positive result in samples in which the value of the D-dimer is in the range 0.2 - 0.5 mg/l (Fig. 1). For this reason, in the discussion, we will focus on more detail on the results obtained in this area. It was also found that the quantitative method for measuring D - dimer has practically 100% sensitivity, specificity and accuracy. In terms of quality test, the results are different; specificity - 49.68% (95% CI - 32.04% to 65.75%); accuracy - 69.23% (95% CI - 58.68% to 78.49%) and sensitivity - 100%, (Table 1), (Fig. 2).

**Fig. 1.** Percentage of true and false positive D-dimers in the range up to 0.5 mg/l

2,73%

25,48%

54,60%

■ True positive D- Dimer

■ False positive D- Dimer

■ True negaitive D- Dimer
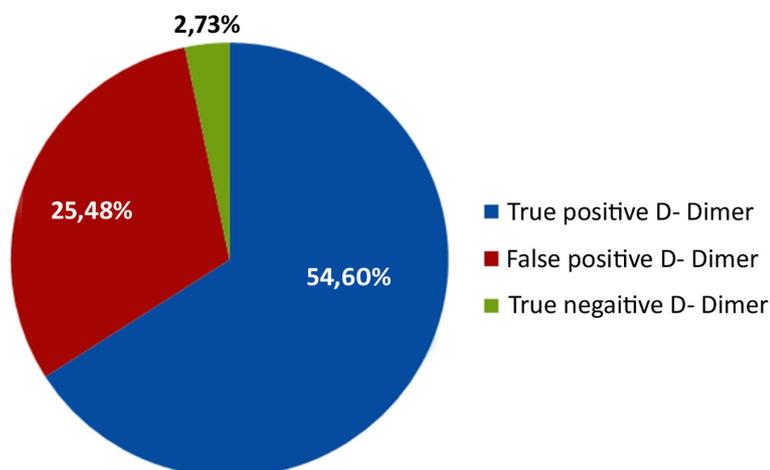
Table 1. Comparative analysis of sensitivity, specificity and accuracy between the two methods for D-dimer

Statistic	D – dimer Quantity		D – dimer Qualitative	
	Value	95% CI	Value	95% CI
Sensitivity	100%	94.04% to100.00%	100 %	94.04% to 100.00%
Specificity	100%	88.78% to 100.00%	49.68%	32.04% to 65.75%
PPV	100%	-	68.18%	65.63% to 70.63%
NPV	100%	-	100 %	-
Accuracy	100%	96.03% to 100.00%	69.23%	58.68% to 78.49%

Fig. 2. Sensitivity, specificity and accuracy of both methods for the determination of D-dimer
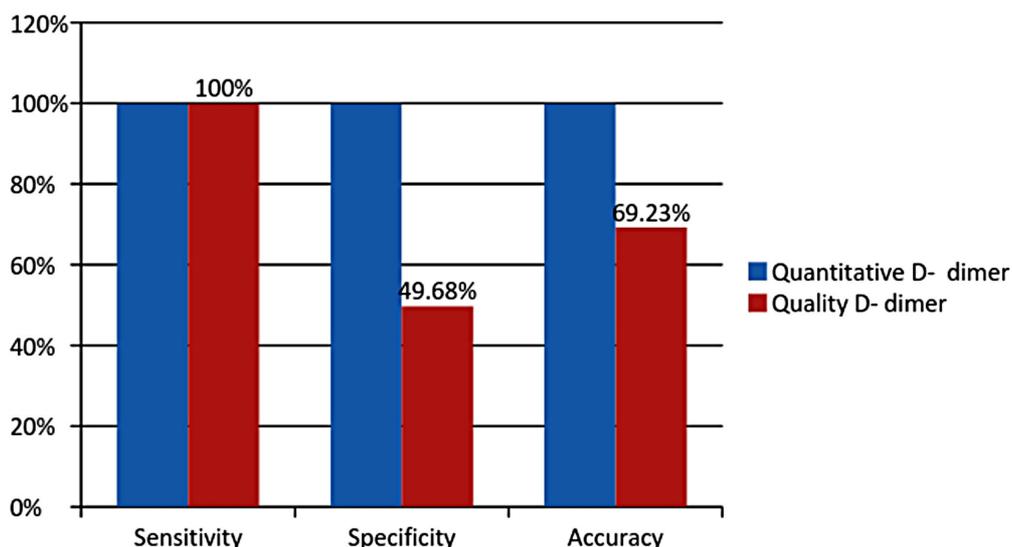
Fig. 3. PPV and NPV of the quantitative and qualitative method for the determination of D-dimer
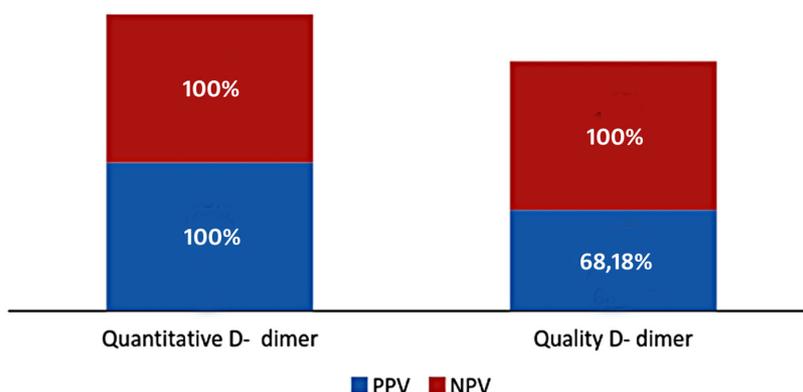
DISCUSSION

From the obtained results, we can conclude that the results for D-dimer from the two methods are very well correlated, especially in the range above 0.5 mg/l. In the area above 1.6 mg/l, there is practically 100% agreement of the results. The only problem is that above 3.2 mg/l, the quality test cannot give a specific value, which is a serious problem in patients with high levels of D-dimer in the blood that must be followed, including the duration of treatment. There is a very good correlation in the results with strongly negative D-dimers below 0.2 mg/l.

Regarding the results in the range of 0.2 - 0.5 mg/l, there are serious discrepancies. We found that with a quality test, there is a percentage of probability of giving false positive results. In practice, we found that in 25.48% of cases, the qualitative test gave a positive result in samples in which

the value of the D-dimer is in the range 0.2 - 0.5 mg/l (Fig. 1). First of all, the problem arises from the difference in the reference values of the two tests - the quantitative up to 0.5 mg/l, and the qualitative up to 0.2 mg/l. The cut off value imposed not only by us but also by most authors for D-dimer is 0.5 mg/l. The problem would be serious if both methods work in the same laboratory, and the reference values are not unified [8, 9, 10, 11, 12].

In terms of sensitivity, both methods are 100% sensitive (95% CI- 94.04% to100.00%). As a note, it should be noted that with regard to the performance of the qualitative test, the purity of the plates where the reaction between the latex reagent and the patient sample takes place must be observed very strictly [8, 13]. The accuracy of the quantitative method is 100% (95% CI - 96.03% to 100.00%), while the qualitative one is 69.23% (95%CI- 58.68% to 78.49%)

In addition, we measured the positive and negative predictive value of the methods. As can be seen in Fig. 3, the negative predictive value of both methods is 100%, while the positive predictive value of the quantitative method is 100%, and the qualitative - 68.18% [14].

CONCLUSION

In conclusion, we can say that the correlation in the results of the two methods is very good. The quantification of D-dimer is more sensitive, and specificity is preferred. Especially in cases where it is necessary to monitor treatment. We recommend that the cut off value for D-dimer be the value - 0.5 mg/l. In order to avoid discrepancies in the results, if one laboratory uses both methods for determination of D-dimer, we recommend mandatory unification of the reference values.

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