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Research Paper

Correlation of fibrinogen level and absorbance change in both PT and APTT clotting curves on BCS*XP*

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Abstract

Objective: To investigate the correlation of fibrinogen level and absorbance change in both PT and APTT clotting curves on BCS*XP* Analyzer. Methods:A serial of standard fibrinogen and 250 patient plasma samples with different qualities(normal, hemolysis, icterus, and lipemia) were run on BCS*XP* for assays PT, APTT and Fibrinogen. The absorbance change(DeltaA) from baseline to plateau in clotting curve was retrieved and analyzed on its correlation with the Fibrinogen result. Influence of plasma quality and PT/APTT result on this correlation was also studied respectively. Results:Both PT-DeltaA and APTT-DeltaA showed good linear regression with fibrinogen level in the sample, with R^2 close to 0.90 in both standard and patient samples. Hemolysis(H), itcterus(I) and lipemia(L) of the sample with valid clotting curves were found to have no significant difference in this correlation from normal(N) sample(R^2: 0.83_H, 0.92_I, 0.81_L and 0.79_N in PT; 0.89_H, 0.95_I, 0.91_L and 0.89_N in APTT) in either PT or APTT curve. PT or APTT result also has little impact on this correlation(0.71 in range 7 ~ 10 sec, 0.56 in10 ~ 20 sec, and 0.70 in 20 sec~; R^2 in APTT: 0.88 in 20~30 sec, 0.92 in 30~40 sec, and 0.95 in 40 sec~). Conclusion:The absorbance change in either PT or APTT clotting curve correlates well with the fibrinogen level in plasma, which is independent of plasma quality PT or APTT results. The absorbance change can be used as an alternative way to roughly estimate fibrinogen level in either PT or APTT clotting curve when the result of clauss-based fibrinogen measurement is not available.

Key words: PT; APTT; fibrinogen; clotting curve; absorbance change; BCS*XP*

INTRODUCTION

Using absorbance changes in the kinetic clotting curve produced on photo-optical coagulation analyzers to derive fibrinogen has already been published in some papers[1-11], in which only PT(prothrombin time) clotting curve was studied. BCS*XP*, a new modern coagulometer which is increasingly utilized in clinical coagulation labs[13-16], provides more comprehensive raw data recording real-time absorbance change during clot formation not only in PT assay, but also in APTT(activated partial thromboplastin time). We believe that APTT clotting curves can also reflect fibrinogen level in the sample plasma.

A typical kinetic clotting curve in PT/APTT on BCS*XP* is shown in Fig 1. In this kinetic clotting curve, the absorbance recording(the real-time turbidity change in the sample plasma) starts from the very beginning (called initial absorbance or baseline). It then progressively increases with the ongoing clot formation and reaches a plateau, where all the fibrinogens in the sample plasma are converted into cross-linked fibrins. In this study, the absorbance change is the height from the baseline to plateau, and can also be called "DeltaA" (delta Absorbance) hereafter.

Some researchers have made tremendous progress on so-called "PT-derived fibrinogen", in which the fibrinogen level is calculated by DeltaA. However this

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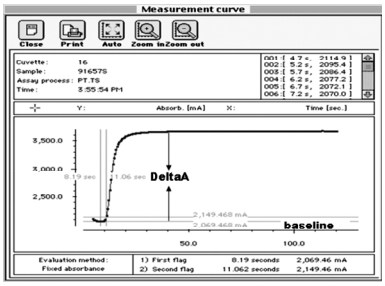


Fig 1 A typical clotting curve on BCSXP

calculated fibrinogen method was not recommended to replace the classical clauss-based fibrinogen measurement which has been world widely utilized in clinical laboratories as the assay "Fibrinogen" for about 50 years[1-11,12].

Despite this, fibrinogen is not a routine requirement in coagulation screening assays and sometimes is missed by clinical doctors. When patients are diagnosed to be DIC or any other fibrinogen-related diseases[17], to look back into the records of lab test results for the change of fibrinogen level in history is essential. In cases that Fibrinogen result is not available, absorbance change in clotting curve is useful to estimate fibrinogen level. In addition, high concentration of thrombin inhibitors in the sample plasma, such as hirudin, argatraban, bivalirudin and ximelagatran, may interfere in clauss thrombin assay[18-19], but can be overcome in PT derived assay.

In this study, we investigated the correlation of DeltaA to fibrinogen in PT and APTT kinetic clotting curves respectively on the new generation of coagulation analyzer BCS*XP* as well as the influences of sample quality (hemolysis, icterus and lipemia) and the PT/APTT result range on this correlation.

MATERIALS AND METHODS

Sample collection

100 patient samples which were apparently clear without any abnormal color or turbidity, 50 hemolytic, 50 icteric, and 50 lipemic plasma samples were collected from the clinical coagulation lab within 4 hours after the drawing time. The hemolysis, icterus, and lipemia of the sample plasma were graded according to a criteria chart provided by the company Beckman Coulter.

Standard curve

0.5ml normal pooled plasma(A1019, PrecisionBiologic, Canada) was mixed with 10 μl appropriately diluted (no dilution,1/2, 1/4, 1/8, 1/16, 1/32) thrombin reagent (537579) and incubated in a refrigerator at 4℃ for 1 hour and then centrifuged to remove the clot. The supernatant plasma was tested for PT(Innovin, 536932), APTT(Actin FSL, 527338A; calcium 506850D) and Fibrinogen. In order to make sure that the clotting factors in the plasma were least affected, those with nearly normal PT and APTT results were kept, among which the one containing the lowest fibrinogen level was finally chosen as the defibrinated plasma. All the reagents employed hereby were from Dade Behring/ Siemens of Germany.

Defibrinated plasma was mixed with plasma from a patient with high concentration of fibrinogen(800 mg/dl) at different ratio in volume: 0%, 2%, 4%, 6%, 8%, 10%, 20%, 40%, 60%, 80%, and 100%.

All the standard plasma was tested on BCSXP for PT, APTT and Fibrinogen to prepare the standard curve for DeltaA-fibrinogen correlation study.

PT, APTT, Fibrinogen assay of the 250 patient plasma samples on BCS*XP*

After the BCSXP machine(Dade Behring/Siemens, Germany) was prepared and the quality control tests were passed, all the patient samples were loaded and run for the three assays PT, APTT and Fibrinogen. All the reagent preparation and machine operating procedures were exactly following the instructions and documentations by the manufacture and lab SOPs.

Data and clotting curve transfer and analysis

All the raw data were transferred to computer by the software "Dade Importing Tool" for further analysis. All the samples with normal valid kinetic clot curve were selected. Those tagged with question marks in their results were ruled out. The data of deltaA from the kinetic clotting curve of both PT and APTT were plotted with Fibrinogen result respectively, and analyzed on basis of the different range of PT/APTT results, and as the plasma property: normal, hemolysis, icterus and lipemia as well. Considering that the clotting curve of a plasma sample with critically low fibrinogen should have no change in the absorbance(DeltaA =0), the linear regression curve in all the DeltaA-fibrinogen plots were set with "*intercept* = 0", although this may have impaired the coefficient correlation to some extent.

RESULTS

The correlation between DeltaA and fibrinogen level in a standard pattern

In both PT(Fig 2A) and APTT(Fig 3A) kinetic clotting curves, each plateau increases with elevated concentration of fibrinogen in the plasma sample. PT clotting curve starts sooner, progresses faster, and reaches plateau earlier. Although the baseline varies slightly due to the initial absorbance of samples mixed at different ratio, the absorbance change(DeltaA) from the baseline to the plateau in each kinetic curve enlarges with elevated fibrinogen level and in linear correlation to the fibrinogen level with $R^2 = 0.98$ in both standard plots (Fig 2A, Fig 3B). Unexpectedly, for the same level of

fibrinogen in the sample, the DeltaA in the APTT clotting curve is considerably larger than that in PT curve, displaying a much higher slope(7.27 in Fig 3B), but only 1.86 in standard curve of PT DeltaA-fibrinogen(Fig2 B).

Correlation of DeltaA and fibrinogen level in patient samples

After the clotting curves of all the samples were checked and evaluated by the machine, those without question marks in PT or APTT results were plotted(Fig 4). All the valid results have a linear correlation between the DeltaA and its fibrinogen level, although the R^2 was slightly reduced to 0.83 and 0.91 respectively from 0.98, compared to the standard DeltaA-fibrinogen(*Fig 2B, Fig 3B*). More interestingly, all the DeltaAs in APTT clotting curves of each sample are higher than it in PT curve in terms of the slopes: 6.71 in APTT and 1.54 in PT.

Influence of plasma quality on the correlation of DeltaA and fibrinogen

The sample qualites, hemolysis, icterus, and lipemia, were not seen with any influence on the linear correlation of DeltaA and fibrinogen level in both PT and APTT

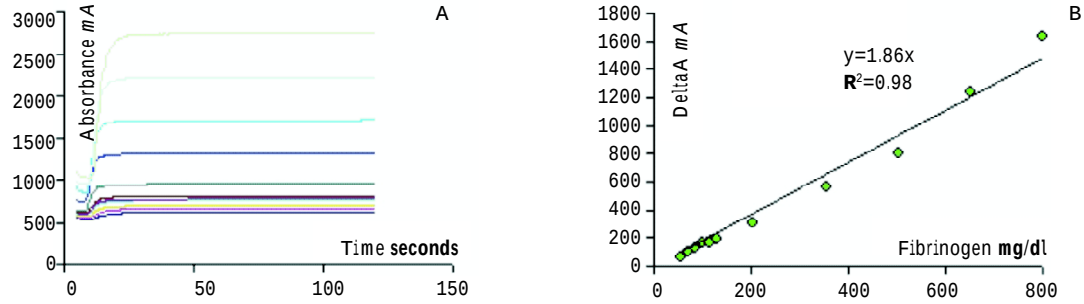


Fig 2 PT kinetic clotting curves of fibrinogen standard plasma(A) and correlation of DeltaA to fibrinogen level(B)

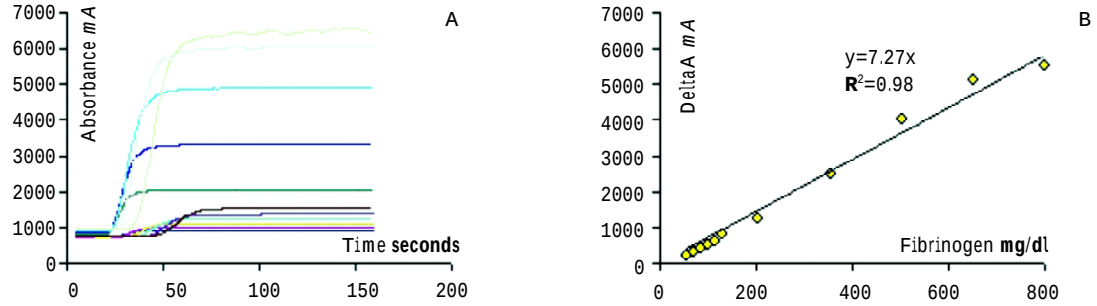


Fig 3 APTT kinetic clotting curves of fibrinogen standard plasma(A) and correlation of DeltaA to fibrinogen level(B)

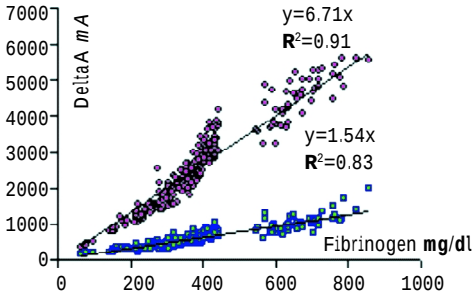


Fig 4 PT-and APTT-DeltaAs of patient samples with different fibrinogen levels

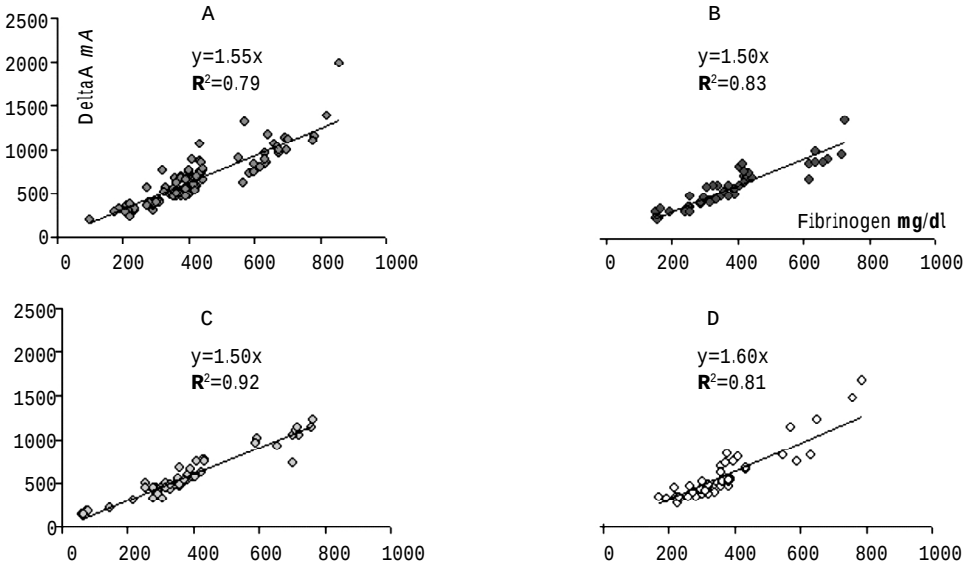
clotting curves(Fig 5, Fig 6) since they have very close R^2s and slopes. And there is no significant statistical difference in their regressions.

Influence of clotting time results on the correlation of DeltaA and fibrinogen

When the plots of deltaA-fibrinogen were divided by different ranges of clotting time results, little discrepancy was seen among them, and this was then confirmed by statistic analysis. In the result ranges of 7.1~10.0 s, 10.1~20.0 s and longer than 20.1 s, the PT deltaA-fibrinogen linear regression has 0.71, 0.56 and 0.70 in R^2, and 1.47, 1.50, 1.67 in slopes(Fig 7) ;in APTT delta-fibrinogen plots, the R^2 were 0.88, 0.92, 0.95 and slopes were 6.28, 6.55, 7.24 respectively in the APTT result ranges of 20.0~30.0 s, 30.1~40.0 s and 40.1 sec~.(Fig 8)

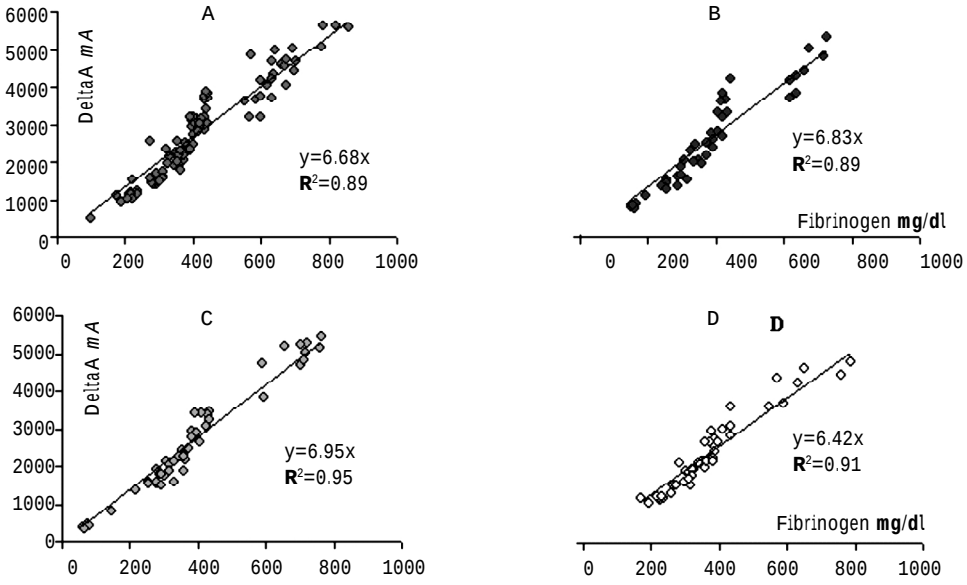
DISCUSSION

Using light absorbance changes in kinetic clotting curves on optical clotting measuring machines is not an innovative approach to determine the concentration of



A: normal plasma; B: hemolytic plasma; C: icteric plasma; D: lipemic plasma

Fig 5 PT deltaA-fibrinogen of patient samples with different quality



A: normal plasma; B: hemolytic plasma; C: icteric plasma; D: lipemic plasma

Fig 6 APTT deltaA-fibrinogen of patient samples with different quality.

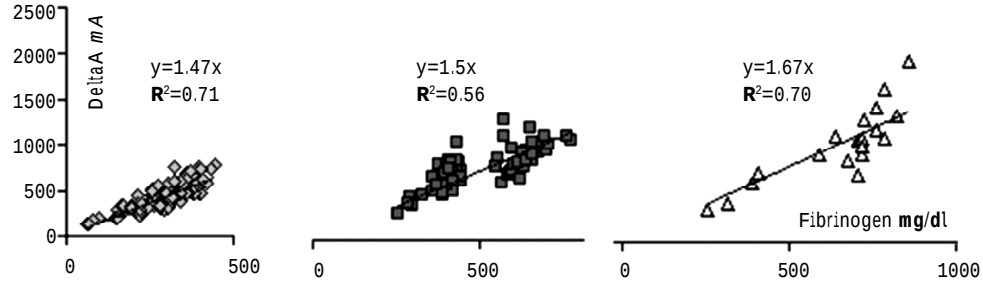


Fig 7 PT deltaA-fibrinogen in result ranges: 7.1~10.0 s, 10.1~20.0 s, 20.1 s~(from left to right)

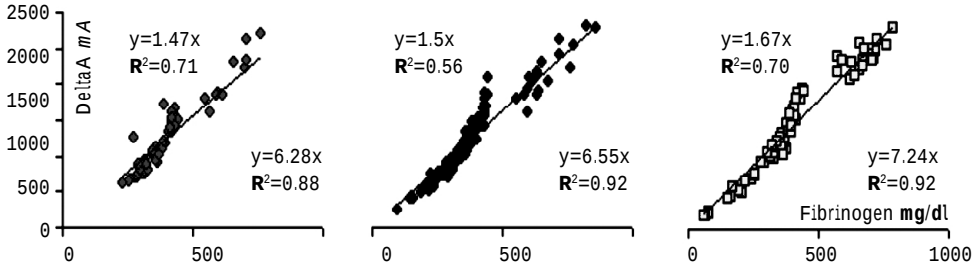


Fig 8 APTT deltaA-fibrinogen in result ranges: 20.0~30.0 s, 30.1~40.0 s, 40.1 s~(from left to right)

fibrinogen(which is called "PT derived fibrinogen"). But BCS*XP*, the latest generation of coagulation analyzer is equipped with a sophisticated light emitting and sensing system which makes it sensitive to light absorbance changes over the clot formation during testing. And its advanced software provides comprehensive checking and evaluating methods. Any unusual pattern in the kinetic clotting curve can be found out and then marked with question marks followed by possible explanations. These significant features may confer BCS*XP* higher accuracy in PT derived fibrinogen.

Although intensive work has been done on PT (absorbance change) derived fibrinogen, APTT derived fibrinogen(which has the same mechanism as PT derivation for fibrinogen) is rarely reported[20]. In some cases, like monitoring heparin administration, PT does not have to be always available. Thus, APTT can also be employed as an alternative to estimate the fibrinogen concentration to track the variation of fibrinogen level during diseases courses like DIC, or hyper/hypo-fibrinogen.

In this study, both PT and APTT were found to have good correlation in absorbance change with fibrinogen concentration, which means fibrinogen can be roughly determined by the height of the clotting curve. The slope of PT derived fibrinogen is close to that reported by Dr. Lawrie[1] in which both employed the same PT reagent-Innovin. One of the limitations in our work is other thromboplastins were not tested to see the discrepancy in PT reagents to derive fibrinogen. But in a clinical coagulation laboratory, since one single PT/APTT reagent with the same lot number is usually used for a period, it's practicable to compare the DeltaAs in clotting curves and find fluctuations of fibrinogen level in history.

The most interesting thing in this study is that the absorbance change in APTT clotting curve is more sensitive to that in the PT curve, which is not found in any other publications. That the same fibrinogen in plasma failed to yield similar amounts of absorbance change is confusing. When we tried to figure out this interesting question, we compared all the PT and APTT clotting curves of each sample and found the initial absorbance of APTT curve is always greater than that of PT curve. This can be explained as being due to the yellow dye in APTT reagent Actin FSL. But this should not have any role in expanding DeltaA during clotting. Therefore the reaction of clotting process initiated by PT and APTT reagent in the sample must be different. This is not because of the different phospholipids in both reagents, since modification either in quantity or quality of the phospholipids did not dramatically alter the absorbance change. Besides, the height of absorbance change does not reflect of the clot firmness, which was demonstrated from the experiment performed with the same reagents and protocol on a thromboelastography machine, yielding a totally opposite result: clot in PT assay is much stronger than that in APTT assay. However, when we ran the same plasma with diluted PT reagent Innovin, the clotting was delayed and slower, but found reaching a higher level. This may shed a little light on the mechanism of larger APTT DeltaA than PT. However, this question remains unclear and to unmask the real truth needs more research.

Optical machines are vulnerable to interference like hemolysis, icterus and lipemia that can cause unusual pattern in clotting curves[13-16,19]. When all the questionable clotting curves were ruled out in this study, these interferences had little effect on PT/APTT derived fibrinogen, since their coefficient correlations lacked significant statistical difference from normal plasma samples. This suggests that, as long as the machine yields a normal clotting curve, it should be used to predict fibrinogen level regardless of the sample quality.

It is also showed that PT and APTT results were not associated with the correlation between absorbance change and fibrinogen level. No matter how long the beginning of the clotting is delayed, fibrinogen will be reflected in the height of the clotting curve from baseline to plateau. But prolonged lag time of clotting may result in late endpoint of clot formation, in which plateau is not yet reached, when the clotting reading is stopped at the time defined in assay protocol.

In this study, the good correlation was demonstrated between DeltaA and fibrinogen level which is not interfered by either sample quality(hemolytic, icteric, and lipemic), or the results of clotting time. Although we also do not suggest "PT or APTT derived fibrinogen" as a substitute for the classical clauss-based assay "ibrinogen", they can be used to roughly estimate the fibrinogen level when "Fibrinogen" assay result is not available, especially for the purpose to see the change of fibrinogen level during the course of a related disease.

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