News Release

Title

Development and validation of a novel qualitative test for plasma fibrinogen utilizing clot waveform analysis

Key Points

- Plasma fibrinogen is commonly examined by Clauss fibrinogen assay, which cannot distinguish between quantitative and qualitative fibrinogen anomalies.
- Our previously reported Clauss fibrinogen assay utilizing clot waveform analysis (Clauss-CWA) provides additional information that contributes to the classification of fibrinogen anomalies. In this study, we adopted the Clauss-CWA method for an autoanalyzer to automatically measure the antigenic estimate (eAg) of fibrinogen in addition to the functional amount (Ac), and to thus provide the Ac/eAg ratio as a qualitative indicator.
- We obtained an optimal cutoff of 0.65 for Ac/eAg by ROC curve analysis of the training cohort, offering superior sensitivity (>0.9661) and specificity (1.000). This cutoff was validated in the validation cohort, providing positive predictive value >0.933 and negative predictive value >0.998.
- The Clauss-CWA method may represent a useful approach for detecting qualitative fibrinogen abnormalities in routine laboratory testing.

Summary

Plasma fibrinogen is commonly examined by Clauss fibrinogen assay, which cannot distinguish between quantitative and qualitative fibrinogen anomalies. However, our previously reported Clauss fibrinogen assay utilizing clot waveform analysis (Clauss-CWA) provides additional information that contributes to the classification of fibrinogen anomalies. In this study, we adopted the Clauss-CWA method for an autoanalyzer to automatically measure the antigenic estimate (eAg) of fibrinogen in addition to the functional amount (Ac), and to thus provide the Ac/eAg ratio as a qualitative indicator. Performance was validated by receiver operating characteristics (ROC) and precision recall (PR) curve analyses using a patient cohort, consisting of a training cohort (n=519) and a validation cohort (n=523), both of which contained cases of congenital (hypo)dysfibrinogenemia as qualitative defects. We obtained an optimal cutoff of 0.65 for Ac/eAg by ROC curve analysis of the training cohort, offering superior sensitivity (>0.9661) and specificity (1.000). This cutoff was validated in the validation cohort, providing positive predictive value >0.933 and negative predictive value >0.998. PR curve analysis also showed that Clauss-CWA provided excellent performance for detecting qualitative fibrinogen anomalies. The Clauss-CWA method may represent a useful approach for detecting qualitative fibrinogen abnormalities in routine laboratory testing.

Research Background

Plasma fibrinogen can be examined by several assays. In routine laboratory testing, thrombin time and the Clauss fibrinogen assay have each been recommended as an initial screening test. The Clauss fibrinogen assay is well optimized for a large population of automated coagulation analyzers and is in wide use, and

allows determination of functional fibrinogen levels.

We sometimes encounter low levels of plasma fibrinogen and suspect fibrinogen abnormalities from routine screening tests. The majority of cases of congenital dysfibrinogenemia and hypodysfibrinogenemia are diagnosed incidentally, and most patients diagnosed with congenital dysfibrinogenemia actual appear asymptomatic. Such individuals show significantly low levels of functional fibrinogen, but discriminating between low levels of functional fibrinogen due to qualitative defects and low levels of fibrinogen antigen due to quantitative defects by Clauss fibrinogen assay alone is quite difficult. Hence, for the laboratory diagnosis of fibrinogen abnormalities, antigenic measurement of fibrinogen is required. However, measurement of fibrinogen antigen incurs extra costs over functional fibrinogen testing and is unsuitable for routine testing due to its low throughput. Furthermore, the precise prevalence of qualitative fibrinogen deficiencies has not been established because of the large number of asymptomatic cases, representing another reason antigenic fibrinogen determination is not carried out in every laboratory.

We recently reported that clot waveform analysis (CWA) in the Clauss fibrinogen assay could be useful to detect functional fibrinogen abnormalities with no additional measurement of fibrinogen antigen. This method of Clauss fibrinogen assay utilizing CWA (Clauss-CWA) employs a value of maximum velocity (commonly represented as “Min1”) and calculates the estimated fibrinogen antigen (eAg) levels introduced from the Min1 value. This eAg can be used as an alternative for actually measured fibrinogen antigen determined by immunological assays, and the ratio of functional fibrinogen level (Ac) to eAg (Ac/eAg ratio) can be used to identify qualitative fibrinogen defects. The clinical utility of this novel analytical method requires validation.

The original method requires the export of data from an autoanalyzer and external calculation of the Ac/eAg ratio using spreadsheets. We therefore developed and installed novel autoanalysis software in a CN-6000 automated blood coagulation analyzer (Sysmex, Kobe, Japan). This software, which is still under development, immediately provides the Ac/eAg ratio from internal auto-calculations performed with each measurement. The present study aimed to validate this novel application and assess its usefulness in clinical settings using a larger cohort.

Research Results

During the study period, plasma samples from 1,044 patients were used, distributed across training and validation cohorts without any sample overlap between the two cohorts. From June 2020 to July 2020, a total of 530 samples were randomly collected and investigated for fibrinogen Ac and Ag. Two participants (samples) were excluded due to low Ac/Ag ratios in the absence of a previously confirmed diagnosis of congenital (hypo)dysfibrinogenemia (CD). After excluding overlapping participants, 504 samples were enrolled as a normal group representing normal Ac/Ag ratios, and a final total of 519 participants were enrolled after including 15 samples from 15 patients with CD in the training cohort. In addition, from August 2020 to October 2020, a total of 558 plasma samples were randomly collected. After excluding extra samples from the same individuals, 509 samples were enrolled as a control group. Plasma samples from 14 patients with CD diagnoses were also included and randomly assorted among the samples. In total, 523 participants were included in this validation cohort.

Ac/eAg ratio was evaluated as an alternative index to distinguish qualitative from quantitative

abnormalities of plasma fibrinogen. The results of receiver operating characteristics (ROC) analyses are shown in Table 2. When TCFibL was used, the area under the ROC curve (AUROC) of the Ac/eAg ratio for distinguishing CD from controls was 0.9661 (95% confidence interval [CI], 0.9018–1.000), and the optimal cutoff value was predicted as 0.62–0.66. Sensitivity and specificity were 0.9286 (95%CI, 0.6853–0.9963) and 1.000 (95%CI, 0.9925–1.000), respectively. The positive likelihood ratio (+LR) was infinity and the negative likelihood ratio (-LR) was 0.071 (95%CI, 0.01–0.5). When Dade was used, AUROC was 0.9962 (95%CI, 0.9890–1.000) and the predicted cutoff of Ac/eAg ratio was 0.60–0.68. Sensitivity, specificity, +LR, and -LR were 0.9333 (95%CI, 0.7018–0.9960), 1.000 (95%CI, 0.9925–1.000), infinity, and 0.067 (95%CI, 0.01–0.4), respectively.

Optimal cutoffs revealed from the training cohort were used to evaluate the predictive performance of Ac/eAg ratio. We used the suggested cutoff of 0.65 for both reagents and found that CD could be clearly distinguished with high sensitivity and specificity (Figure 2A). When TCFibL was used, sensitivity and specificity were 1.000 (95%CI, 0.7847–1.000) and 1.000 (95%CI, 0.9925–1.000), respectively, and positive predictive value (PPV) and negative predictive value (NPV) were both 1.00 (Table 3). When using Dade, sensitivity, specificity, PPV, and NPV were 1.000 (95%CI, 0.7847–1.000), 0.9961 (95%CI, 0.9858–0.9993), 0.933, and 0.988, respectively.

Research Summary and Future Perspective

We found that Clauss-CWA offers a novel approach to detecting qualitative fibrinogen abnormalities, and is now ready for use in routine laboratory testing. This method, made available simply by upgrading the software, is expected to see use in many laboratories and may help detect cryptic fibrinogen abnormalities. We hope that the method we have developed will contribute to all laboratories, including those that do not specialize in thrombosis and hemostasis.

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