Fibrinogen disorders

Congenital fibrinogen disorders comprise two classes of plasma fibrinogen defects: type I, afibrinogenemia or hypofibrinogenemia, in which there are absent or low plasma fibrinogen antigen levels (quantitative fibrinogen deficiencies); and type II, dysfibrinogenemia or hypodysfibrinogenemia, in which there are normal or reduced antigen levels associated with disproportionately low functional activity (qualitative fibrinogen deficiencies).

The diagnosis of congenital dysfibrinogenemia is generally based on the assessment of functional and antigenic fibrinogen.

Plasma fibrinogen could be examined by several laboratory test as described above table. Claus assay have been widely used as a primary screening test. However, Claus assay can examine only fibrinogen activity, which is shown in fibrin polymerization induced by thrombin. Although type I fibrinogen defects shows low fibrinogen activity with normal fibrinogen antigen, we cannot distinguish “low fibrinogen activity due to low fibrinogen antigen” and “low fibrinogen activity due to abnormal fibrinogen” in only Claus assay. We have to perform both Claus assay and immunological assay to diagnose fibrinogen deficiency.

**Objective**

**Can we diagnose fibrinogen deficiency only using Claus assay?**

It is difficult to assess both fibrinogen activity and antigen in a routine testing, especially in a small laboratory. We need the other laboratory testing to diagnose fibrinogen abnormalities.

To diagnose fibrinogen abnormalities without antigen determination, we focused on Claus fibrinogen assay and clot waveform analysis (CWA), and tried to establish a new laboratory test for screening of fibrinogen deficiencies.

**Methods**

1. **Claus assay and clot waveform analysis (CWA)**
   - Reagent: Thrombocheck Fib(L) (Sysmex, Japan)
   - Instrument: CS-5100 (Sysmex, Japan)

   Claus assay was performed using automated coagulation analyzer CS-5100. The light transmission was analyzed, and fibrin formation was detected by reduced absorbance at 450 nm. A clot waveform was made from the light transmittance, and several parameter was calculated by differential analysis. The 1st derivative curve was obtained from the raw clot waveform, the 2nd derivative curve was from 1st derivative curve. We investigated additional parameters described follows.

2. **Latex Immunoagglutination Assay (LIA)**
   - Reagent: FactorAuto Fibrinogen (Q-may, Japan)
   - Instrument: CS-5100 (Sysmex, Japan)

   We determined fibrinogen antigen by latex immunoagglutination assay (LIA) using anti-fibrinogen antibody.

**Conclusion**

The “[min1]” value, which was provided from CWA in Claus assay, could be an alternative value of fibrinogen antigen. The [min1] values were conserved in dysfibrinogenemia despite their fibrinogen activity (Ac) were low. The estimated antigen (eAg) and Ac/eAg ratio, which were calculated by [min1], could be an indicator for screening of fibrinogen abnormalities. Taken together, our study suggested a new laboratory test, which enables to detect abnormal fibrinogen with no additional costs in Claus assay.

**Results**

**Fibrinogen activity (Ac) strongly correlated fibrinogen antigen (Ag) in normal plasma**

We examined fibrinogen activity (Ac) and antigen (Ag) in normal plasma (N=91). As shown in left figure, fibrinogen Ac and Ag were strongly correlated (r=0.9472). The ratio of Ac and Ag (Ac/Ag, indicating specific activity) was calculated, and the average was 0.987 (0.800-1.262). These results showed that Ac and Ag values were almost equal in normal plasma.

**The “[min1]” values of CWA associated with fibrinogen Ag**

We also performed CWA in normal plasma, and investigated the correlation between CWA parameters and fibrinogen Ag.

**Estimated antigen using [min1] value from CWA**

We tried to estimate plasma fibrinogen Ag using [min1] values from normal CWA. We prepared the calibration curve as described left, and calculated estimated antigen (eAg) and estimated specific antigen shown in ratio of activity/eAg (Ac/eAg) as follows.

**Estimated specific antigen = activity (Ac) / estimated antigen (eAg)**

First, we investigated the correlation between Ag and Ac in normal plasma. As shown in left figure below, eAg, which is calculated by [min1] values, were highly correlated with Ag (r=0.9654). The mean Ac/eAg ratio was 1.103 (0.971-1.285), while the mean Ac/Ag ratio was 0.987 (0.800-1.262).

**Low levels of the Ac/eAg ratio in dysfibrinogenemia**

The Ac/eAg ratio in dysfibrinogenemia patients was markedly reduced compared with normal (P<0.0001). This results showed abnormal fibrinogen represented high [min1] values in spite of low Ac, and suggested that Ac/eAg ratio by CWA had a potential to detect abnormal fibrinogen.

*Corresponding to A. Suzuki, Ph.D./ E-mail: asuzuki@med.nagoya-u.ac.jp

The authors have no conflicts of interest to declare.