Methods for Differential Diagnosis of
Consumption Coagulopathy*

Masahiro Maki, Katsuji Sasaki and Susumu Sato

Department of Obstetrics and Gynecology (Prof. S. Shinagawa).
Hirosaki University School of Medicine, Hirosaki

In order to find out the sensitive tests to differentiate intravascular coagulation from intravascular proteolysis, the changes in factors of coagulation-fibrinolysis were studied on experimentally produced fibrinolysis, fibrinogenolysis and intravascular coagulation.

Fibrinogen, and factors V and VIII were decreased; the prothrombin time, thrombin time and partial thromboplastin time were prolonged in both intravascular proteolysis and coagulation. The reliable factors to be tested for the differentiation were the prothrombin determined by the two stage method or by employing prothrombin-free plasma, plasminogen, platelets and the split products derived from either fibrinogen or fibrin. Namely, the platelets and prothrombin were decreased in intravascular coagulation, whereas these factors were normal in proteolytic stage. Plasminogen was low in both fibrinolytic and fibrinogenolytic states, but normal in intravascular coagulation.

Basing on the facts that the split products from fibrinogenolysis were heat-labile at 56°C for 15 minutes, while those from fibrinolysis were heat-stable, the differentiation between the split products from fibrinogen and fibrin was possible by measuring the thrombin clotting on a mixture of control plasma and heated or non-heated test sample, and by employing the simple radial immune diffusion technique.

Consumption coagulopathy due to intravascular proteolysis or intravascular coagulation is often associated with obstetrical hemorrhage, leukemia and other various diseases, and is one of the most serious hemorrhagic diatheses. A choice of therapeutic agent, either antiplasmin or anticoagulant, depends on the etiology of the consumption coagulopathy. It is, therefore, very important to make a differential diagnosis between intravascular proteolysis and intravascular coagulation.

In order to obtain what tests are most sensitive to establish the differential diagnosis between them, changes in the coagulation-fibrinolysis system are studied on experimentally produced intravascular coagulation and plasma proteolysis. In addition, a possibility of making the differential diagnosis of consumption coagulopathy will be discussed from the difference in heat-stability between fibrinogenolysis products and fibrinolysis ones.

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To avoid confusion in terminology, the definitions of the terms described in this paper are presented as in the following.

1. Primary intravascular (plasma) proteolysis: Digestion of clotting factors such as fibrinogen (fibrinogenolysis), factors V and VIII.


3. Fibrinogenolysis: Digestion of fibrinogen.

4. Fibrinolysis: Dissolution (digestion) of fibrin clot.

5. Intravascular coagulation: Disseminated intravascular coagulation (defibrination); unless otherwise noticed this refers to intravascular coagulation which is not associated with (secondary) fibrinolysis.


7. Fibrinolysis products: Split products from fibrinolysis.

**MATERIALS AND METHODS**

1. **Preparation of test plasma**
   
   Nine volumes of blood obtained by venipuncture were mixed with 1 volume of 3.8% sodium citrate. The plasma was prepared by centrifugation at 2,000 g for 15 minutes at 4°C. The plasma should be subjected to the tests immediately after separation. If not, antiplasmins such as Trasylol (250 KIU) or trans-aminomethylycloclohexane carboxylie acid (AMCHA, 5 mg) should be added to 1.0 ml of whole blood to avoid the advance of plasma proteolysis after collecting blood sample.

2. **Thrombin time**
   
   **Reagents:** (1) Bovine thrombin (Mochida) was diluted with normal saline to contain 10 units per milliliter; (2) Normal saline.
   
   **Procedure:** The thrombin clotting time was determined by transferring 0.1 ml of citrate plasma to a small Pyrex tube containing 0.1 ml of the normal saline. After 3 minutes keeping the tube in a water bath at 37°C, 0.1 ml of thrombin was blown forcibly into the tube and stopwatch was started simultaneously. The tube was put in a water bath and shaken gently. A few seconds before the expected clotting time, the tube was tilted very gently to permit detecting the incipient web of fibrin, which was the endpoint.

3. **Antithrombin activity**
   
   This was evaluated by measuring the thrombin clotting time on a mixture of 0.1 ml each of control and test plasma.

4. **Prothrombin time by Quick's one stage method**
   
   **Reagent:** Lyoplastin (Mochida) was used as a preparation of thromboplastin.
   
   **Procedure:** The prothrombin time was measured on 0.1 ml of citrate plasma by adding 0.2 ml of thromboplastin-calcium.

5. **Recalcification time**
   
   **Reagent:** 0.025 M calcium chloride
   
   **Procedure:** The recalcification time was determined by adding 0.2 ml of 0.025 M calcium chloride into a tube containing 0.1 ml of citrate plasma.

6. **Partial thromboplastin time**
   
   **Reagents:** (1) Activated Cephaloplastin from Dade; (2) 0.02 M calcium chloride.
   
   **Procedure:** Into a test tube containing 0.1 ml of activated Cephaloplasin incubated for a minimum of 1 minute, 0.1 ml of test plasma was added and mixed. The mixture was allowed to stand for 2 minutes. The stopwatch was started with forcible addition of
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0.1 ml of calcium chloride into the tube. The tube was incubated for 30 seconds at 37°C, and the clotting time was recorded.

7. **Thrombotest by Ouren**

8. **Platelets count by Fonio-Sahli or Rees-Ecker**

9. **Prothrombin**

   This was measured by using prothrombin-free rabbit plasma.

   **Reagents:** (1) Activated thromboplastin (Dade), (2) 0.04 M calcium chloride, and (3) prothrombin-free rabbit plasma (Dade).

   **Procedure:** A reaction mixture consisting of 0.1 ml each of activated thromboplastin, calcium chloride and prothrombin-free rabbit plasma was prepared as rapidly as possible and kept at 37°C. Test plasma (0.1 ml) was forcibly blown and simultaneously stopwatch was started. The activity was expressed by per cent activity.

10. **Factor V**

    **Reagents:** (1) Factor V-deficient plasma; The deficient plasma was obtained by incubating EDTA-plasma (a supernatant obtained by centrifuge of a mixture of 1 volume of 0.5% disodium ethylenediamine tetraacetic acid and 9 volumes of normal whole blood) at 37°C for 24 hours. (2) Adsorbed plasma with barium sulfate (BaSO₄-plasma); Citrate test plasma (1 ml) was adsorbed by stirring with 200 mg barium sulfate for 10 minutes, followed by centrifugation. (3) Lyoplastin (Mochida)

    **Procedure:** Factor V-deficient plasma from normal plasma (0.1 ml) was mixed with 0.1 ml of BaSO₄-plasma, and the clotting time was measured by adding 0.2 ml of Lyoplastin-calcium. The activity was expressed by per cent from the standard curve obtained preliminarily.

11. **Factor VII complex**

    **Reagents:** (1) Factor VII-deficient plasma (Bentonite-plasma); The deficient plasma was obtained by stirring normal citrate plasma (1 ml) with 50 mg of Bentonite (Kanto Chemical) for 10 minutes, followed by centrifugation. (2) BaSO₄-plasma; This was prepared by the same way as above.

    **Procedure:** Diluted (1:10) citrate plasma (0.1 ml) was mixed with 0.1 ml each of Bentonite- and BaSO₄-plasma, and the clotting time was measured by adding 0.2 ml of Lyoplastin-calcium. The activity was expressed as per cent from the standard curve obtained preliminarily.

12. **Factor VIII by thromboplastin generation test**

13. **Fibrinogen estimation by phenol reagent method, turbidimetry and heating procedure**

    **(A) Phenol reagent method of Ratnoff & Menzie and Maki**

    **Reagents:** (1) Thrombin (Mochida, 100 units/ml of normal saline), (2) N sodium hydroxide and hydrochloric acid, (3) 0.55 M sodium carbonate, and (4) Folin-Ciocalteu’s phenol reagent which was diluted 1:3 with distilled water before use.

    **Procedure:** Citrate plasma (0.25 ml) was diluted to 5 ml with normal saline, and 0.1 ml of thrombin was added. When fibrin began to form, glass powder (20-50 mg) was added. The fibrin clot formed was completely precipitated with glass powder, and was washed several times with normal saline. Any drop of water was removed by turning the tube upside down. The fibrin was hydrolysed with 1 ml of N sodium hydroxide in a boiling water bath for 20 minutes. The hydrolysate was neutralized with 1 ml of N hydrochloric acid, and the optical density was measured at 640mμ after developing color with 5 ml of 0.55 M sodium carbonate and 1 ml of the phenol reagent. Fibrinogen concentration was calculated from the standard curve.

    **(B) Turbidimetry by Parfentjev et al.**

    **Reagents:** (1) Ammonium sulfate reagent; Ammonium sulfate (133.33 g), sodium
chloride (10 g) and merthiolate (0.025 g) were dissolved in distilled water. The solution was made to pH 7.0 and a final volume of 1,000 ml; (2) Normal saline.

**Procedure:** Citrate plasma (0.5 ml) was mixed with 4.5 ml ammonium sulfate. The turbidity was measured at 490 μm 3 minutes after adding the ammonium sulfate against the blank which was prepared by adding saline instead of the reagent. The fibrinogen concentration was calculated from the standard curve.

(c) **Fibrinogen determination by heating procedure**

This was done by heating plasma at 56°C for 15 minutes, and the precipitate was measured by using a specific tube of Schulz or by colorimetry by phenol reagent.

14. **Plasminogen estimation by a modified method of Hedner & Nilsson**

**Reagents:** (1) 4% Casein; Four g of casein (Hammersten, Merck) were suspended in 1/7.5 M disodium phosphate and heated in a boiling water bath for 20 minutes. The solution was diluted to 100 ml with distilled water. (2) 0.1 N sodium hydroxide and hydrochloric acid, (3) Streptokinase (Varidase-Lederle) or Urokinase (Midori-Juji), (4) 0.44 M TCA (Trichloroacetic acid), and (5) phosphate buffer (Soerensen, pH 7.4) containing 0.9% sodium chloride.

**Procedure:** To a test tube containing 0.5 ml of citrate plasma, 1 ml of 0.1 N hydrochloric acid was added to destroy antiplasmin and allowed to stand for 30 minutes at room temperature, followed by neutralization with 1 ml of 0.1 N sodium hydroxide. To the resultant reaction mixture 1.5 ml of phosphate buffer and 0.5 ml of streptokinase (500 units) or urokinase (500 Ploug units) were added and incubated for 5 minutes. The reaction was continued for 1 hour after the addition of 2.5 ml of casein, and was stopped by adding 5 ml of TCA. The mixture was filtered through the Toyo filter paper No. 5. The optical density was measured at 275 μm against the blank which was prepared in the same way except for adding TCA before incubating with casein substrate. The activity was expressed by Kunitz unit.

15. **Antiplasmin assay by a fibrinolytic method**

**Reagents:** (1) 0.2% Bovine fibrinogen (Armour) in 1/15 M phosphate buffer pH 7.4, (Soerensen) containing 0.9% sodium chloride, (2) Plasmin activated spontaneously in 50% glycerol-phosphate buffer, (3) Thrombin (Mochida, 100 units/ml normal saline), and (4) Trasylol (Bayer).

**Procedure:** Into a test tube containing 0.5 ml of plasmin, 0.5 ml of inhibitor (1:10 diluted citrated plasma or standard Trasylol) were added and the mixture was incubated for 10 minutes at 37°C. After incubation time of 8–9 minutes, 0.1 ml of thrombin was added. The lysis time was measured after adding 1 ml of fibrinogen. The antiplasmin level was calculated from the standard curve which was prepared simultaneously with standard Trasylol.

16. **Preparation of rabbit antiserum against human fibrinogen**

**Reagents:** (1) Human fibrinogen (Kabi) was prepared so as to contain 5 mg in 5 ml of normal saline, (2) Freund’s adjuvant, complete (Difco), and (3) sodium azide or merthiolate.

**Procedure:** Using a plastic disposable syringe and No.18 needles, 5 ml of complete Freund’s adjuvant were taken and added to 5 ml of fibrinogen. Emulsion was prepared by filling and emptying it back into a small beaker repeatedly. It was convenient to use a homogenizer for the preparation of a large amount of emulsion. The emulsion was ready to use when a drop of the emulsion held completely together on the surface of normal saline in a Petri dish.

Each rabbit was injected with 1 ml of emulsion in 6 sites sterilized previously with 70% alcohol; each foot pad, the scruff of the neck and the intraperitoneal cavity. The use of antibiotics was recommended to avoid bacterial infection. After the first injection, a minimum of a 2 week period was necessary before administration of challenging dose of the
same material. Two weeks thereafter, blood was taken by venipuncture, followed by centrifugation after clotting. Sodium azide (1 mg) or merthiolate (0.1 mg) was added to 1 ml of serum. To remove the nonspecific antibodies, 1 volume of normal human serum was added to 10 volumes of antiserum. The mixture was kept at 37°C for 90 minutes and then in a refrigerator for 24 hours. The precipitate formed was removed by centrifugation and decantation. The similar procedure, if necessary, was repeated. The non-specific antibodies should be checked by Ouchterlony plate.

17. Simple radial immunodiffusion by Mancini et al.11

The principle of the method is based on the fact that the diameter of immune circle formed around well is directly proportional to a concentration of antigen.

Reagents: (1) Diethylbarbiturate buffer (pH 8.6); This was prepared by adding 14.8 ml of 0.1 N hydrochloric acid to 100 ml of 0.1 M sodium diethylbarbiturate. (2) 2% agar solution; Purified agar from Behringwerke or Difco was dissolved in the buffer in a concentration of 2%, and 100 mg of sodium azide or 10 mg of merthiolate was added to 100 ml solution. (3) Rabbit antiserum against human fibrinogen, (4) normal saline, (5) dye solution; One % solution of aniline blue black or azocarmine-G in methanol-acetic acid-water (50–10–50 v/v), and (6) washing solution; Methanol-acetic acid-water (50–10–50 v/v).

Procedure: The antiserum was usually diluted 2-fold with the diethylbarbiturate buffer; however the dilution was made depending on the titre of antiserum. Ten ml of agar solution which was preliminarily dissolved in a boiling water bath, and 5 ml of antiserum dilution were kept at 48°C for 5–10 minutes, and were combined gently so as not to foam. The mixture was poured onto warmed (60°C) Petri dish and allowed to stand for 30 minutes at room temperature. Wells of equal size (2 or 5 mm) were cut into the agar gel. Using a capillary tube attached to water pump, agar in each well was removed by aspiration. Test solution of 0.002 ml for 2 mm diameter or 0.05 ml for 5 mm diameter well was placed with micropipet. The plate was allowed to stand for 24 hours on a level surface, and the diameter of immune circle around the well was measured.

The immune circle can be copied directly onto photographic paper on one-to-one scale. It is however recommended to be copied after washing the plate repeatedly with normal saline, so that one can obtain a sharper photograph.

Preservation of the record is possible after staining. The washed plate was stained by aniline blue black for 30–60 seconds or by azocarmine-G for 2–3 minutes. The excess dye was removed by changing washing solution until getting clear back ground. The plate was dried at 37°C and preserved.

18. Tanned red cell hemagglutination inhibition immune assay (TRCHII)

The TRCHII was performed by a simplified method of Merskey et al.12 The detailed technique was presented elsewhere.13

RESULTS

I. Differentiation of intravascular proteolysis from intravascular coagulation by measuring factors of coagulation-fibrinolysis system


Citrate plasma (20 ml) was incubated with 4,000 units of streptokinase in 0.4 ml of normal saline for various incubation time. At the given time, 4 ml of the reaction mixture were taken into a test tube containing 20 mg AMCHA to stop the reaction. The presence of AMCHA of this amount did not produce any significant effect on the tests described above, except for the anti-plasmin assay. Therefore, AMCHA was not added for the antiplasmin assay. The changes in
various factors for blood coagulation and fibrinolysis were studied on the resultant reaction mixture.

The results are shown in Figs. 1–3. The thrombin clotting time, prothrombin time, partial thromboplastin time, recalcification time and thrombotest were prolonged, of which the thrombin clotting time and partial thromboplastin time were most sensitively prolonged. Fibrinogen and plasminogen were decreased rapidly, while antiplasmin was not changed. Factors V and VIII were inactivated with prolongation of incubation time, while prothrombin and factor VII remained unchanged.

Fig. 1. Changes in thrombin time, partial thromboplastin time (P.T.T.), recalcification time, thrombotest and prothrombin time during experimental plasma proteolysis in vitro.

Fig. 2. Changes in plasminogen, antiplasmin and fibrinogen during experimental plasma proteolysis in vitro.

Fig. 3. Changes inprothrombin, factors V, VII and VIII during experimental plasma proteolysis in vitro.
2. *Experimental intravascular proteolysis in dogs.*

Human fibrinolysin (Lyovac, streptokinase-activated fibrinolysin, 50,000 units, Merck, Sharp and Dohme) was injected into a dog, and the factors of coagulation-fibrinolysis system were determined at the given time. The average values obtained in 4 dogs are shown in Fig. 4. The thrombin time, prothrombin time and thrombotest were prolonged. These changes might be mainly due to decrease in fibrinogen and increase in fibrinogen split products which had an activity to inhibit fibrin polymerization by thrombin. Prothrombin, factor VII and antiplasmin were not changed, while factor V, fibrinogen and plasminogen were markedly decreased. These changes were consistent with those obtained *in vitro* tests of the above experiment. Platelets were slightly decreased to an average value of 85%, ranging 65–125%.

![Diagram of Fig. 4](image)

*Fig. 4.* Changes in coagulation-fibrinolysis system during experimental plasma proteolysis in dogs. The values before infusion were expressed as 100%.


Intravascular coagulation was produced in dogs by intravenous infusion of saline extract of the placenta according to a method of Mori. The results in Fig. 5 show the changes of coagulation-fibrinolysis system in average values of 5 dogs.

![Diagram of Fig. 5](image)

*Fig. 5.* Changes in coagulation-fibrinolysis system during experimental intravascular coagulation in dogs.

The values before infusion were expressed as 100%.
Fibrinogen, prothrombin and factor V were decreased, while factor VII was not changed. No significant change in fibrinolytic system was observed.

II. Differentiation of fibrinogenolysis products from fibrinolysis products

Theoretically, fibrinogenolysis products in fibrinogenolytic state, fibrinolysis products in intravascular coagulation with secondary fibrinolysis and none of the split products in intravascular coagulation which is not associated with secondary fibrinolysis could be detected in patient’s plasma. If it is possible to differentiate fibrinogenolysis products from fibrinolysis ones, this may give a decisive clue to differentiate intravascular proteolysis from intravascular coagulation with or without secondary fibrinolysis.

Beller and Maki\textsuperscript{15} demonstrated that in vitro fibrinogenolysis products were heat-labile, while fibrinolysis ones heat-stable at 56°C for 15 minutes.

To re-investigate this problem, fibrinogenolysis was induced by incubating 20 ml of citrate plasma with 2 ml of 20,000 units streptokinase for various incubation time, and fibrinolysis by incubating 20 ml of citrate plasma with 2 ml of a mixture of 20,000 units streptokinase and 10 units of thrombin. The reaction mixture (2 ml) at given incubation time was taken into a tube containing 0.05 ml of 500 units Trasylol.

As native material, blood sample from the vasa efferentia of the uterus, which was very high in fibrinolytic activity,\textsuperscript{16,17} was obtained during gynecological surgery, and incubated for 3–20 hours. The incubated whole blood with sodium citrate (a model of fibrinogenolysis) and one without sodium citrate (a model of fibrinolysis) were subjected to the various tests. Nearly complete or partial fibrinolysis was noted during this incubation time. To make the same condition

\begin{table}
\centering
\caption{Fibrinogen concentration, thrombin clotting time whole blood in the presence of sodium citrate (a)}
\begin{tabular}{|l|c|c|c|c|}
\hline
No.* & Incubation time & Fibrinogen concentration (mg/100 ml) determined by & Ratnoff & Menzie
\hline
 & & Before incub. & After incub. & Heating after incub.
\hline
1 & 20 hrs. & 315 & 0 & 110
\hline
2 & 20 hrs. & 213 & 35 & 120
\hline
3 & 20 hrs. & 155 & 41 & 100
\hline
4 & 20 hrs. & 213 & 132 & 200
\hline
5 & 20 hrs. & 155 & 125 & 150
\hline
6 & 3 min. & 320 & 0 & 50
\hline
7 & 10 min. & 320 & 0 & 0
\hline
\hline
\end{tabular}
\end{table}

* Blood samples of Nos. 1–5 were from vasa.
Fibrinogenolysis was induced by addition
† The test was carried out after removing
‡ As the diameter of well was 5 mm, the or very trace of the split products.
as in the model of fibrinogenolysis, 1 volume of 3.8% sodium citrate was added to 9 volumes of the incubated whole blood after incubation finished. The reaction was stopped by adding Trasylol.

The antithrombin activity was determined on a mixture of 0.1 ml of control citrate plasma and 0.1 ml of heated (56°C for 15 minutes) or unheated sample. The results are shown in Tables 1 and 2.

The fibrinogen concentration was decreased by incubation of citrate blood depending on difference of samples. Fibrinogen concentration determined by the method of Ratnoff and Menzie was lower than that obtained by the heat precipitation procedure or turbidimetry. The thrombin clotting time was markedly prolonged after incubation. By adding the incubated plasma to control plasma, the thrombin clotting time was increased depending on the fibrinogenolytic activity. This prolongation effect on the thrombin clotting time was corrected by heating the test plasma.

In contrast to the fibrinogenolysis products the influence of the fibrinolysis products on the thrombin clotting time was not marked, and was often shortened. This shortening of the thrombin clotting time might be due to a clot promoting effect of serum or defibrinated plasma. However, the thrombin clotting time of control plasma in the presence of unheated materials (fibrinolysis products).

The diameter of immune circle observed by the simple radial immune diffusion technique was markedly decreased after heating in fibrinogenolysis products, while it was slightly increased or not changed in the fibrinolysis products (Fig. 6). However, the heat-stability of fibrinogenolysis products was increased with prolongation of incubation time and higher plasmin activity, and after reacting with thrombin.15

<table>
<thead>
<tr>
<th>Thrombin time (sec)</th>
<th>Influence of incubated plasma on thrombin time (sec)</th>
<th>Diameter of immune circle of incubated plasma (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before incub.</td>
<td>After incub.</td>
<td>Before heating</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>19</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>16</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>16</td>
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<tr>
<td>12</td>
<td>30</td>
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<tr>
<td>12</td>
<td>60</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>25</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>30</td>
</tr>
</tbody>
</table>

and diameter of immune circle of plasma obtained from incubated model of fibrinogenolysis) before and after incubation.

efferentia of the uterus.
of streptokinase in Nos. 6 and 7.
clottable protein with thrombin.
value of 5 mm indicated that there is no
### Table 2. Influence of serum obtained from incubated whole blood in the absence of sodium citrate (a model of fibrinolysis) on thrombin clotting time and diameter of immune circle before and after heating

<table>
<thead>
<tr>
<th>No.</th>
<th>Incubation time</th>
<th>Influence of serum on thrombin clotting time (sec)</th>
<th>Diameter of immune circle (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before heating</td>
<td>After heating</td>
</tr>
<tr>
<td>1</td>
<td>3 hrs.</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>3 hrs.</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3 hrs.</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>3 hrs.</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>3 hrs.</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>18 hrs.</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>6 hrs.</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>3 hrs.</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>3 min.</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>10 min.</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Blood samples of No. 1–8 were from vasa efferentia of the uterus. Fibrinolysis was induced by adding thrombin-streptokinase into citrate plasma in No. 9 and 10.

![Fig. 6. Immune circle of fibrinogenolysis products and fibrinolysis products before and after heating at 56°C for 15 minutes.](image)

**Upper wells** (From left to right): Unheated fibrinogenolysis products (Tab. 1, No. 7), Heated fibrinogenolysis products (Tab. 1, No. 7), Unheated fibrinolysis products (Tab. 2 No. 10)

**Middle wells**: Unheated fibrinolysis products (Tab. 2, No. 6), Unheated fibrinolysis products (Tab. 2, No. 7), Heated fibrinolysis products (Tab. 2, No. 8), Heated fibrinolysis products (Tab. 2, No. 10)

**Lower wells**: Heated fibrinolysis products (first 3 of the middle wells)
The split products from both fibrinogen and fibrin were sensitively detected by the TRCHII. However, it was difficult to differentiate fibrinolysis products from fibrinogenolysis ones by heating procedure.

**DISCUSSION**

Regarding the etiology of acute acquired hypofibrinogenemia, there have been controversial discussions about whether this situation results from primary proteolysis (digestion of fibrinogen and other clotting factors but not of fibrin) or from disseminated intravascular coagulation (defibrination) with or without secondary fibrinolysis. Also a possibility of fibrinogen consumption due to hemorrhage into extravascular space, e.g., the retroplacental space, has been presented. The treatment of this situation is, therefore, quite different depending on the etiology. An anticoagulant such as heparin should be used for intravascular coagulation, and antiplasmin preparations for intravascular proteolysis. For this reason, it is a very important problem to establish the differential diagnosis between them.

The purpose of this paper is to find out what tests are most sensitive to make a differential diagnosis, and to obtain a correct criterion for the interpretation of laboratory tests in consumption coagulopathy.

Consumption of the clotting factors due to intravascular coagulation results in decrease in fibrinogen, prothrombin, factors V and VIII and platelets, because these factors are consumed during coagulation, while the clotting factors called as the serum factor (factors VII, IX and X) are normal.

In consumption coagulopathy due to plasma proteolysis by plasmin, fibrinogen, factors V and VIII are lowered, because these substances are digested by plasmin. Plasminogen is converted to plasmin followed by inactivation with naturally occurring antiplasmin. As the result, plasminogen is lowered in proteolytic state. As to prothrombin and antiplasmin, there have been controversial discussions. Our result showed that both prothrombin and antiplasmin were not changed by plasmin. In spite of the insignificant decrease in antiplasmin in the experimental proteolysis, we found frequently a lowered antiplasmin level in clinical cases of pathologic proteolysis. It was therefore suspected that the lowered antiplasmin was not due to secondary change of pathologic proteolysis but a primary factor in a development of proteolysis.

The changes in factors of coagulation-fibrinolysis system in intravascular coagulation and intravascular proteolysis can be summarized as illustrated in Table 3. The reliable differentiation can be made by measuring platelets and prothrombin determined by the two stage method or by employing prothrombin-free plasma.

It has been known that fibrinolysis is very often noted as a secondary response to intravascular coagulation. In this case, split products from fibrin by digestion with plasmin can be detected from patient's plasma. On the other hand, the presence of split products from fibrinogen is indicative of primary proteolysis (fibrinogenolysis).
TABLE 3. Tests for differential diagnosis between intravascular coagulation and intravascular proteolysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Intravascular coagulation</th>
<th>Intravascular coagulation with secondary fibrinolysis</th>
<th>Intravascular proteolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin time</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Prothrombin*</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Normal</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Lowered</td>
</tr>
<tr>
<td>Factors V and VIII</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Normal</td>
</tr>
<tr>
<td>Factor VII</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>Normal</td>
<td>Lowered</td>
<td>Normal</td>
</tr>
<tr>
<td>Antiplasmin</td>
<td>Normal</td>
<td>Normal†</td>
<td>Normal†</td>
</tr>
<tr>
<td>Platelets</td>
<td>Lowered</td>
<td>Lowered</td>
<td>Normal</td>
</tr>
<tr>
<td>Split products</td>
<td>Absent</td>
<td>(Fibrinolysis products)</td>
<td>(Fibrinogenolysis products)</td>
</tr>
</tbody>
</table>

* Prothrombin determined by two stage method or by employing prothrombin-free plasma
† Antiplasmin level was normal in in vitro test, but in the clinical case usually lowered.

To differentiate the split products from fibrinogen and those from fibrin, the measurement of the thrombin clotting time and the immune assay seem to be effective. It was noted that fraction D from fibrinolysis, which locates immunoelectrophoretically in the same position of mother fibrinogen, was heat-stable. On the contrary, it was found that fraction D derived from fibrinogenolysis was heat-labile. The stabilization of fraction D seemed to be due to the presence of thrombin.15

Lewis and Wilson23 provided evidence that heating inactivated the antithrombin effect of fibrinogenolysis products, whereas fibrinolysis products even without heating had not antithrombin effect. Fibrinolysis products, however, contain thrombin as a contamination, and the antithrombin effect may be masked. The appearance of antithrombin effect after heating fibrinolysis products seemed to be due to inactivation of contaminated thrombin and heat-stability of fibrinolysis products.

It was also noted that diameter of immune circle was decreased in fibrinogenolysis products but was not changed in fibrinolysis ones.15 From these facts, fibrinolysis products can be differentiated from fibrinogenolysis products as illustrated in Table 4.

There are, however, some questions to be discussed.

(1) Since the nature of immune anticoagulant, which is occasionally released in obstetrical condition, has not been clarified completely, it is unknown how this anticoagulant influences the thrombin clotting time method.

(2) Pathologically elevated secondary fibrinolysis may induce simultaneously intravascular proteolysis. In such a combined situation, the differentiation may be difficult.
TABLE 4. Differentiation of fibrinogenolysis products from fibrinolysis products

<table>
<thead>
<tr>
<th></th>
<th>Fibrinogenolysis products</th>
<th>Fibrinolysis products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin clotting time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Control plasma</td>
<td>Prolonged</td>
<td>Shortened or increased</td>
</tr>
<tr>
<td>+ patient's plasma</td>
<td>Normalized</td>
<td>Prolonged</td>
</tr>
<tr>
<td>2 Control plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ heated patient's plasma</td>
<td>Disappeared or decreased</td>
<td>Did not disappear or slightly increased</td>
</tr>
<tr>
<td>Immune circle after</td>
<td></td>
<td></td>
</tr>
<tr>
<td>heating at 56°C for 15 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The presence of anticoagulant such as heparin or immune anticoagulant should be neglected before performing the tests.

(3) The split products derived from both fibrinogen and fibrin, which are formed by digestion with highly active plasmin for a long incubation time, are close in heat-stability.

In other words, fibrinogenolysis products acquire heat-stability after incubation with highly active plasmin for a long time. This, however, may not occur in vivo, because (1) excess amount of naturally occurring antiplasmins is present in plasma, and (2) such a strong proteolysis found as in vitro may not develop in vivo.

For these reasons, the reliability of the tests in making the differential diagnosis needs further investigation on clinical materials.

References


