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Laboratory diagnosis and clinical manifestations of patients with dysfibrinogenemia[1)]

Laboratoriumsdiagnostik und klinische Manifestation der Dysfribrinogenämie

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Abstract

Hereditary dysfibrinogenemia is a rare clotting disorder due to a structural defect in the fibrinogen molecule that results in a tendency for bleeding and thrombosis, as well as obstetric complications. We describe the laboratory results and clinical manifestations for 50 patients with a diagnosis of dysfibrinogenemia. Various different laboratory measurements of fibrinogen were performed on samples from these patients, including fibrinogen (Clauss), heat fibrinogen precipitation according to Schulz and immunological fibrinogen. Fifty patients were found with dysfibrinogenemia (52% female; median age 52, range 9–89 years). The fibrinogen level according to Clauss was low, with a median of 51 mg/dL (range 15–86 mg/dL; normal range 150–450 mg/dL). Determination of other fibrinogen levels revealed normal results: heat fibrinogen precipitation according to Schulz, 240 mg/dL; and immunological fibrinogen, 244 mg/dL. The median reptilase time was longer than normal, at 55 s (normal 20 s). Some 50% of the patients reported a distinct bleeding tendency, mostly a tendency for hematoma (60%) and secondary bleeding (44%). Thirteen patients had thrombotic events, of which 54% were located arterially. Some 12% of the patients reported a tendency for bleeding and for thrombosis, whereas 19% had miscarriages, sometimes recurrent. We found that functional fibrinogen levels (Clauss) were generally lower in patients with bleeding manifestations (43 vs. 57 mg/dL in other patients).

[1)]Zusammenarbeit von DGKL-Fachredaktion und GTH-Arbeitsgruppe MAISTHRO (Main-Isar-Thromboseregister).

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Zusammenfassung

Die angeborene Dysfibrinogenämie ist eine sehr seltene Gerinnungsstörung, basierend auf einem strukturellem Defekt im Fibrinogenmolekül, worunter eine Neigung zu Blutungen, Thrombosen und Aborten entstehen kann. Wir beschreiben die Ergebnisse der Labordiagnostik und die klinischen Manifestationen bei 50 Patienten mit Dysfibrinogenämie. Es wurden folgende Untersuchungen vorgenommen: Fibrinogen (Clauss), immunologisches Fibrinogen und Hitze-Fibrinogen nach Schulz. Unter den 50 Patienten mit Dysfibrinogenämie (52% weiblich; Altersmedian 52 Jahre, 9–89 Jahre) war die Fibrinogenkonzentration nach Clauss erniedrigt im Median mit 51 mg/dL (15–86 mg/dL; Norm 150–450 mg/dL). Die Bestimmungen der Fibrinogen-Antigen-Konzentrationen ergaben Normalbefunde: Hitze-Fibrinogen nach Schulz, 240 mg/dL; und immunologisches Fibrinogen, 244 mg/dL. Die Reptilasezeit war mit 55 s verlängert (Median; Norm $<$20 s). Fünfzig Prozent der Patienten hatten ein oder mehrere Blutungsereignisse, meistens eine deutliche Neigung zu Hämatomen (60%) oder Nachblutung (44%). Bei 13 Patienten ist ein thromboembolisches Ereignis aufgetreten. Bei 12% der Patienten war eine Neigung gleichzeitig zu Blutung und Thrombosen vorhanden. Bei 19% der weiblichen Patienten hatten einen oder mehrere Aborte. Bei Patienten mit stattgehabten Blutungssymptomen war die Konzentration von Fibrinogen nach Clauss mit 43 mg/dL im Median niedriger als bei Patienten ohne Blutungsproblemen (57 mg/dL).

Schlüsselwörter: Abort; Blutung; Dysfibrinogenämia; Thrombose.

Overview

Fibrinogen is a 340,000-Da protein that is synthesized in hepatocytes. Fibrinogen is the precursor of fibrin and is thus a key component in clot formation. The development of the three-dimensional network in fibrin can be

divided into three steps [1]. First the fibrinogen molecule is activated by thrombin, which removes two pairs of small peptides from the Aα and Bβ chains, creating two polymerization sites known as A′ and B′ [2, 3]. The second step is the formation of intermediate polymers. The fibrin monomers associate in a half-staggered manner through E and D regions and end-to-end through the D regions of different monomer units, giving rise to longitudinal growth of a polymer with a width of two fibrin monomers, yielding the fibrin monomer unit called protofibril. The third step is lateral association of these intermediate polymers, forming the three-dimensional fibrin network [1].

The fibrinogen molecule is a homodimer; each half consists of three non-identical polypeptide chains that are termed Aα, Bβ and γ chains [4]. The genes for all three chains have been localized to the long arm of chromosome 4. The amino termini of the three pairs of polypeptide chains are symmetrically arranged in a disulfide knot to form a central E-domain that is flanked by two large D-domains to form a tri-nodular structure [5]. The D region is divided into several domains, including the β and γ nodules, which constitute the C-terminal parts of these chains that fold independently [6]. The C-terminal α-chain, spanning the sequence from Aα-208 to Aα-610, contains a nodule and a connecting strand [7]. The αC domains have several functions in the hemostatic process and act as substrates for activated factor XIII [8–12]. The αC domains contribute to the polymerization process, accelerating it and influencing the final clot structure [13, 14]. Amino acids Aα 572–575 are involved in the adhesion of other proteins and structures such as endothelial cells.

Congenital dysfibrinogenemia is caused by genetic defects in fibrinogen, resulting in a decrease in functional clotting activity despite normal circulating levels of fibrinogen antigen. Congenital dysfibrinogenemia may be transmitted in a homo- or heterozygous manner, and inheritance is mostly autosomal dominant or co-dominant. Most homozygous carriers are symptomatic, whereas some heterozygous carriers are asymptomatic [15]. The most common structural defects involve the fibrinopeptides and their cleaving sites; the second most common involves the γ-chain polymerization region. These defects are primarily the result of DNA point mutations with substitutions of single amino acids. Other dysfibrinogenemias are caused by formation of new stop codons, and small base additions or deletions. Currently, a database of human fibrinogen variants includes more than 430 molecular abnormalities (http://www.geht.org/databaseang/fibrinogen). Dysfibrinogenemia may also be acquired secondary to liver disease, the use of certain drugs (e.g., acetaminophen) [16, 17], autoimmune disease, and malignancies, and may even occur spontaneously without a known underlying cause.

There are case reports of patients presenting with newly acquired abnormal laboratory coagulation results, including prolonged thrombin and reptilase time and low functional fibrinogen (Clauss) levels [18, 19]. Studies have shown that IgG antibodies directed against the fibrinogen molecule interfere with the binding of thrombin [18] and that monoclonal immunoglobulin γ light chains bind to fibrinogen and interfere with all stages of polymerization [19]. Acquired dysfibrinogenemia may have a poorer prognosis than congenital dysfibrinogenemia owing to possible underlying co-morbidities. A wide variety of symptoms from bleeding to thrombosis are observed in affected patients [16]. Besides some case studies, there are only a few studies on the clinical manifestations in a large number of patients with dysfibrinogenemia.

The obstetric complications of dysfibrinogenemia include miscarriage, mostly during the first trimester, thrombosis, bleeding and placental abruption during pregnancy. There are very few studies on the impact of dysfibrinogenemia on pregnancy or on treatment approaches. For patients with congenital dysfibrinogenemia, effective treatment has proven extremely difficult, since there is a broad spectrum of symptoms that are sometimes contradictory. Our study was designed to establish a correlation between variations in clinical manifestations and laboratory test results.

Patients and methods

We investigated 50 patients from 19 families with dysfibrinogenemia, of whom 26 (52%) were female. The median patient age was 52 years (range 9–89 years). Each patient was interviewed regarding history of clinical manifestations.

Laboratory diagnosis of dysfibrinogenemia was based on criteria established by the International Society of Thrombosis and Hemostasis (Subcommittee on Fibrinogen; Scientific and Standardization Committee) [20]. Testing started with sensitive but non-specific screening tests and then included more specific confirmatory tests. Initially, all patients had to undergo standard screening tests: thrombin time, prothrombin time, and activated partial thromboplastin time. Plasma concentrations of fibrinogen were analyzed for all patients using several methods. In most cases, laboratory diagnosis of dysfibrinogenemia can be established from a discrepancy between fibrinogen activity and fibrinogen antigen levels [23].

Fibrinogen activity was measured in terms of fibrin polymerization function by the Clauss method, which measures the rate of clot formation after adding a high concentration of thrombin to citrated plasma. Fibrinogen antigen concentrations were determined by immunologic (radial immunodiffusion) [22, 23] and precipitation (heat according to Schulz) methods [23].

Results

All 50 patients showed abnormal laboratory results in terms of low functional fibrinogen levels (Clauss) in con-

trast to normal values for heat precipitation fibrinogen (Schulz) and immunologic fibrinogen (Table 1).

The median fibrinogen concentration (Clauss) was 51 mg/dL (range 15–86 mg/dL; normal range 150–450 mg/dL). The median immunological fibrinogen concentration was 244 mg/dL (normal range 200–450 mg/dL) and the median concentration of heat precipitation fibrinogen (Schulz) was 240 mg/dL (normal range 200–450 mg/dL). The median reptilase time was 55 s (normal <20 s).

We found that functional fibrinogen (Clauss) was generally lower in patients with bleeding manifestations (43 mg/dL) compared to other patients (57 mg/dL). The clinical parameters for the patients are summarized in Table 2.

Despite having abnormal laboratory results, 18/50 study participants showed no symptoms of dysfibrinogenemia. Various degrees of bleeding were described in 25/50 patients (50%). Six of 50 patients (12%) manifested with both hemorrhage and thrombosis. The most common hemorrhagic manifestation was a tendency for bruising/hematoma, found in 15/25 (60%) of patients with hemorrhagic symptoms. This was followed by excessive post-traumatic bleeding (including post-surgical and post-partal) in 11/25 (44%) of the patients, oral (dental, gums) bleeding in 6/25 (24%), epistaxis in 5/25 (20%), excessive menstrual bleeding in 4/25 (16%), and gastro-intestinal bleeding in 2/25 (8%).

In 13/50 (26%) of the patients, thrombotic events occurred. The overall number of patients with deep venous thrombosis (DVT) was six, with pulmonary embolism in 50% of these. Arterial thrombosis occurred in seven patients. In patients with arterial thrombosis events, 28% suffered from myocardial infarction (MI), 42% from ischemic stroke and 28% of the patients had peripheral artery disease. In two patients, both venous and arterial thrombosis was found.

Table 1 Laboratory criteria for diagnosis of patients with dysfibrinogenemia.

Parameter	Dysfibrinogenemia		Normal
	Median	Range	
Fibrinogen (Clauss), mg/dL	51	10–86	150–450
Heat fibrinogen, mg/dL	240		200–450
Immunological fibrinogen, mg/dL	244		200–450
Reptilase time, s	55		<20

Table 2 Clinical manifestations in patients with dysfibrinogenemia.

Clinical manifestation	Patients (%)
Asymptomatic	18/50 (36)
Bleeding	25/50 (50)
Thrombosis	13/50 (26)
Arterial thrombosis	9/13 (69)
Bleeding and thrombosis	6/50 (12)
Abortion	5/26 (19)

Miscarriages occurred in five of 26 female patients (19%). Three of these patients suffered from recurrent pregnancy loss.

There were several additional laboratory findings in patients with dysfibrinogenemia. Von Willebrand syndrome was present in one family, with a combination of bleeding and thrombotic features (two patients with bleeding symptoms, one patient with bleeding and DVT). Heterozygous factor V-Leiden mutation was found in two patients from one family (one patient with MI and the other patient was asymptomatic). Heterozygous factor II mutation (prothrombin 2021 GA) was found in one family (one patient with stroke and gastrointestinal bleeding).

Discussion

The clinical manifestations in patients with dysfibrinogenemia include bleeding symptoms, venous or arterial thrombosis and abortions. It is not clear, however, which factors precipitate the development of one of these symptoms in patients. All of our patients had low levels of functional fibrinogen but normal fibrinogen antigen levels, which is significant for the laboratory diagnosis of dysfibrinogenemia [21].

We compared the results of our study to a meta-analysis of over 260 reported cases of dysfibrinogenemia, which revealed that approximately 55% of patients had no clinical manifestation; approximately 25% of patients exhibited a tendency for hemorrhage and 20% had a tendency for thrombosis [20]. Interestingly, 27% of patients with a history of thrombosis also experienced bleeding. Some 50% of our study patients suffered from bleeding, mostly recurrent, spontaneous hematomas not caused by injury. These findings confirm the results of a previous study that bleeding is the most frequent symptom in dysfibrinogenemia [20]. We found that functional fibrinogen level (Clauss) was generally lower in patients with bleeding manifestations.

The rate of clinical manifestations, particularly bleeding, was strikingly higher than in previous studies. This might be explained by selection bias. Our study population was recruited from outpatients attending our clinic and their family members.

In general, patients with a known history of previous bleeding should be treated with a fibrinogen substitute prior to surgery, after trauma or post partum. Fibrinogen substitution needs to be sufficient to raise the plasma level of fibrinogen to hemostatic levels. In patients with thrombosis the prevalence of dysfibrinogenemia is rather low. In a study of 2376 patients with DVT the prevalence of dysfibrinogenemia was 0.08% [24]. In many cases, the patients had family members with a history of thrombosis.

In patients with dysfibrinogenemia, thrombosis is usually mild and arterial thrombosis is very rare. Surprisingly, the majority of patients with thrombotic events in our study suffered from arterial thrombosis (54%). Several mechanisms may be responsible for the occurrence of

thrombosis in patients with dysfibrinogenemia [24, 25]. One is defective binding of abnormal fibrinogen to thrombin, which results in elevated levels of thrombin. Abnormal fibrinogen may also form a fibrin clot that is resistant to plasmin degradation. Moreover, decreased tissue plasminogen activator binding and/or plasminogen binding in some dysfibrinogens may result in lower plasminogen activation [26]. It has been suggested that altered clot architecture resulting in proteolytic resistance is responsible for thrombosis [27].

We had a lower number of asymptomatic patients in our population, most likely owing to the selection bias already noted for patients with bleeding symptoms. Moreover, investigation of fibrinogen gene mutations was not within the scope of our study. Therefore, we cannot relate the variability of manifestations to underlying gene defects.

Our results demonstrate the variability of clinical manifestations in families with congenital dysfibrinogenemia. The distribution of manifestations within families is as variable as the disease itself. This variability of clinical manifestations of dysfibrinogenemia may reflect the genetic heterogeneity of the syndrome.

Conclusions

Patients with dysfibrinogenemia demonstrated a high rate of clinical manifestations compared to previous studies, mostly with a tendency for easy bruising and bleeding. Although the overall percentage of thrombosis in our population was at the expected level, arterial thrombosis was unexpectedly high, as the majority of thrombotic events were of arterial origin. The high tendency for miscarriage is striking. Management of patients may be complicated by the variablility in clinical manifestations and combinations thereof. Therapeutic recommendations should be evaluated on the individual basis of the patient. Very careful treatment recommendations have to be provided for patients with simultaneous presentation of both bleeding and thrombosis.

In conclusion, consistent correlation between variations in clinical manifestations and laboratory results could not be established in our study. Further studies of dysfibrinogenemia may lead to a better understanding of hemostasis and thrombosis dependent on the underlying fibrinogen gene mutation and of optimal treatment options for patients.

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