ECAT Newsletter

Diagnosis of Hypofibrinogenemia (Case study on Bleeding Disorder 2024-CBD)

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Introduction

Each year the ECAT Foundation organises a special survey based on a case study for bleeding disorders. This case study focuses on both analytical aspects and the clinical interpretation of laboratory results. In 2024 seventy-five participants received a case description together with a corresponding plasma sample. They were asked to perform relevant diagnostic laboratory tests and to interpret the clinical outcome of the case. Sixty-four participants responded and submitted the results of laboratory tests performed. The aim of this case study is to enhance the capability of laboratories to establish a robust strategy of laboratory testing which will benefit the patient.

Case description

The participants received the following case description:

"A 20-year-old woman, reports to the haematologist that she has been referred by her dentist for coagulation analysis because wisdom teeth need to be extracted. The patient indicates that she suffers from gum bleeding while brushing her teeth. She also has a history of severe menstrual blood loss, for which she takes tranexamic acid but no history of spontaneous bleeding. The patient does not take other medication".

The sample used in this case study was from a patient with hypofibrinogenemia.

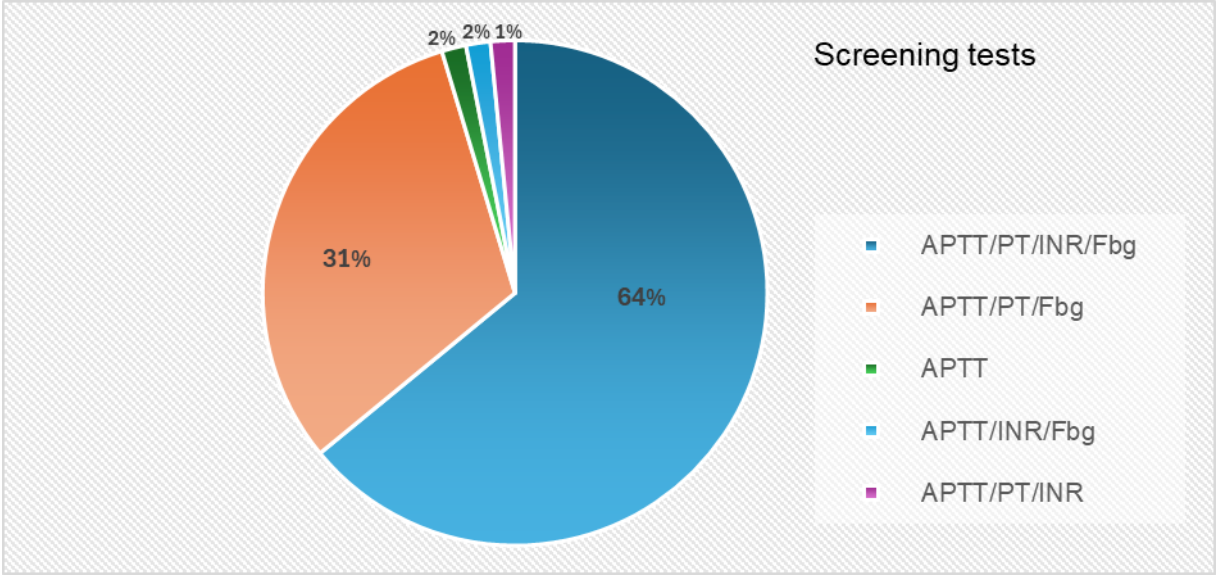
Strategy on laboratory testing

On the basis of this case description, participants were asked to perform the appropriate laboratory tests and to clarify their follow-up strategy for laboratory testing. After they had perfomed the screening tests, the participants selected additional laboratory tests in a systematic way resulting in follow-up strategies. The outcome of the screening tests and follow-up strategies are given in this case report.

General routine screening testing

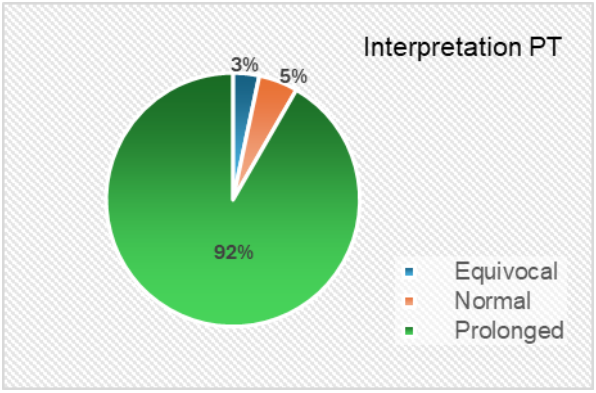
The vast majority of participants performed the following screening tests: Prothrombin Time (PT), International Normalized Ratio (INR), Activated Partial Thromboplastin Time (APTT) and Fibrinogen (Fbg). Figure 1 shows the percentage of participants using a certain combination of screening tests.

Figure 1: General routine screening tests performed by the participants



Most participants found a **prolonged PT, normal APTT and detected an abnormal (low) fibrinogen level**. The interpretations for PT are shown in figure 2.

Figure 2: Interpretations given by the participants for PT



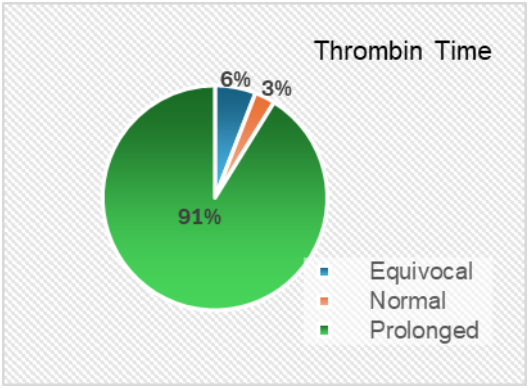
From the participants who performed the PT (n=60), ninety-two percent found a prolonged PT, which was 1.5 to 2 times higher than the reported mean of the reference range for PT (mRR-PT). The range of the mRR-PT* runs from 8.2 – 14.8 sec. Fourteen participants found PT results above the measuring range and reported no coagulation results.

*The mRR-PT depends on the combination of reagent and analyser used.

All participants reported for this sample an abnormal fibrinogen level within a range from 0.06 – 1.28 g/L. An abnormal fibrinogen level is to be found in the plasma of a patient diagnosed with hypofibrinogenemia.

From the participants who performed the Thrombin Time (n=34) ninety-one percent found a prolongation of the Thrombin Time within a range of 19.8 – 68.0 sec. There were thirteen participants who reported Thrombin Time results above the measuring range [see figure 3].

Figure 3: Interpretations given by the participants (n=34) for Thrombin Time.

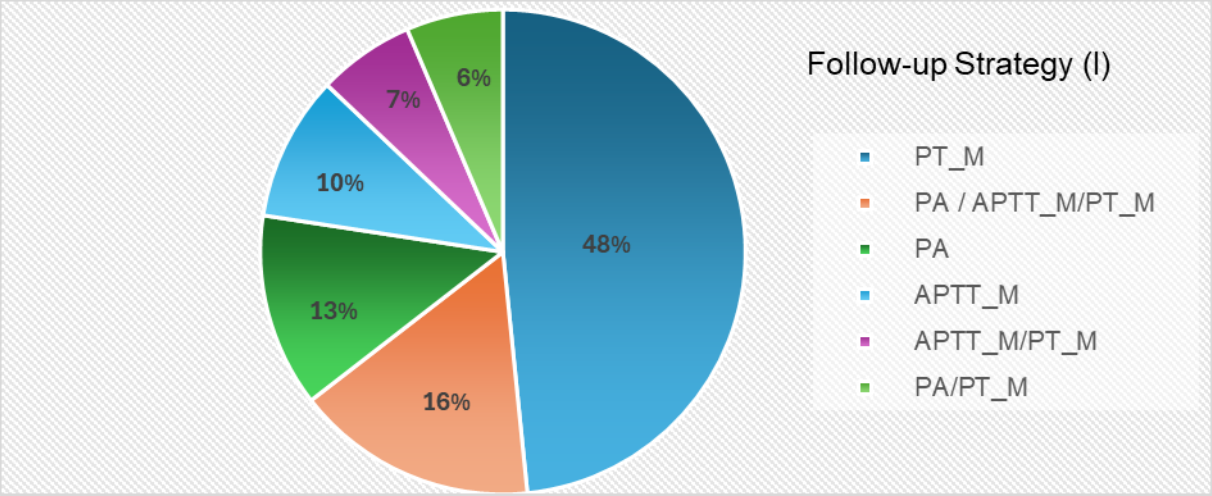


A prolonged PT and Thrombin Time and a decreased fibrinogen level are indicative of a patient diagnosed with hypofibrinogenemia.

Follow-up Strategy (I) for laboratory testing

On the basis of the outcome of the screening tests presented above, additional laboratory tests were carried out, the so-called Follow-up Strategy (I). In figure 4 the follow-up strategy indicated by the participants is shown.

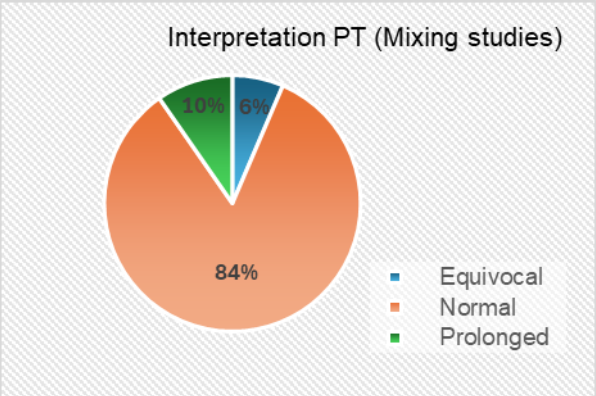
Figure 4: Follow-up Strategy (I): Mixing studies and Presence of an Anticoagulant.



The Follow-up Strategy (I) includes the Laboratory tests: PT Mixing (PT_M), APTT mixing (APTT_M) and performance of Unfractionated and/or Low Molecular Heparin assays to check the Presence of an Anticoagulant (PA).

On the basis of the outcome of the routine screening tests (**prolonged PT, normal APTT, low fibrinogen and prolonged Thrombin Time**) performance of PT mixing studies is a logical choice. The PT mixing studies were carried out by thirty-one participants. Figure 5 shows the interpretation given by the participants.

Figure 5: Interpretations given by the participants (n=31) for PT mixing studies.



In the PT mixing studies eighty-four percent of the participants reported a normalisation of the PT result. A normalisation in the PT mixing studies, corresponds with a sample with a low fibrinogen level presented in this case study.

A small number of participants (n=15) performed the APTT mixing studies which is not a logical choice. Mixing studies are only relevant in the case of a prolongation of the PT and/or APTT. In this case study for a sample with a low fibrinogen and prolonged PT, performing only a PT Mixing test is the most obvious choice.

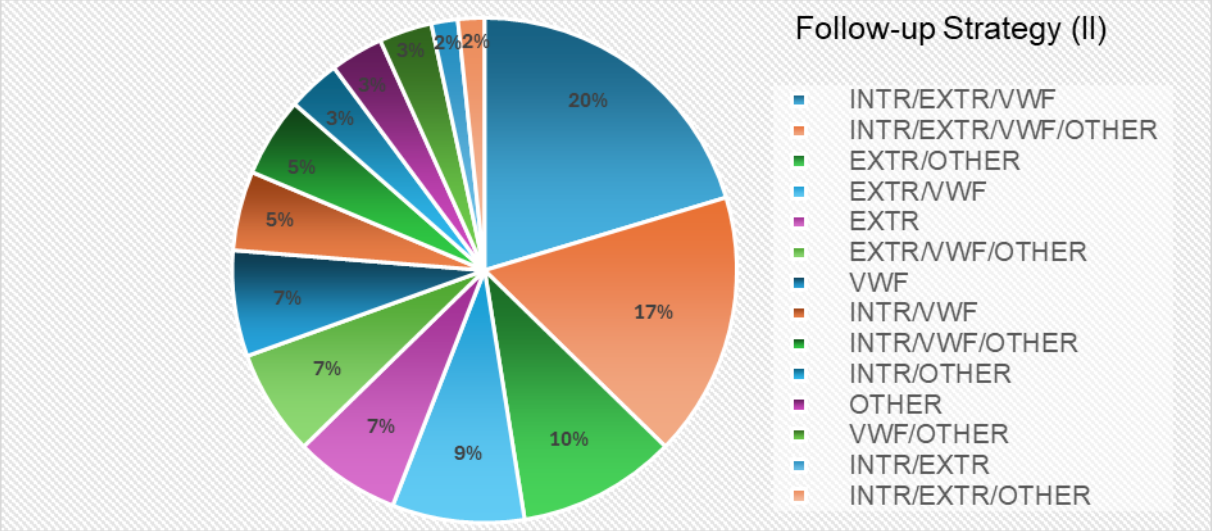
Twenty-eight participants looked for heparin contamination and performed an Unfractionated (UFH) and/or Low Molecular Weight heparin (LMWH) assay. All concluded there was no anticoagulant present.

On the basis of the normal APTT and APTT mixing test heparin contamination is not to be expected. Therefore testing for heparin contamination was irrelevant and not necessary in this sample.

Follow-up Strategy (II) for laboratory testing

After the screening tests and mixing studies the participants were asked to indicate the relevant follow-up testing which they used. This is presented in the Follow-up Strategy (II). In figure 6 the follow-up strategy indicated by the participants is shown.

Figure 6: Follow-up Strategy (II): Additional laboratory tests.



A combination of various additional tests were selected by the participants. Intrinsic (INTR) and Extrinsic (EXTR) factors, VWF (FVIII:c, VWF:Ag and VWF:act) and other additional tests.

Although most APTT results were classified as normal, the majority of the participants performed the intrinsic factor tests [see figure 7]. Given the outcome of a normal APTT, it is not logical or necessary to perform intrinsic factor assays.

The majority of the participants looked into the levels of clotting factors II, V, VII and X and found normal results [see figure 8]. As a result of the prolonged PT and the correction of the PT in the mixing studies to measure extrinsic factors this was a logical choice.

Figure 7: Intrinsic factors.

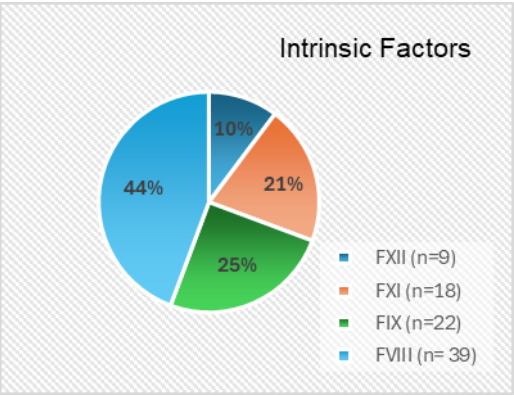
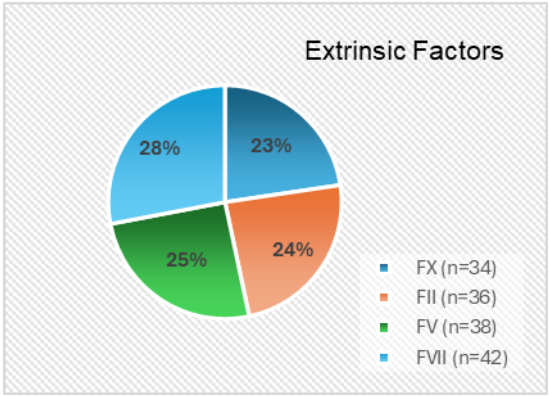


Figure 8: Extrinsic factors.



The vast majority of the participants also investigated whether there was an abnormality in the Von Willebrand Factor (VWF) and found normal results. It is plausible that participants performed Von Willebrand Factor assays to explain the cause of the clinical symptoms, present in the case description. However, with a decreased VWF level a prolonged APTT is expected because of the associated decreased Factor VIII level.

On the basis of the outcome of their Follow-up Strategies, participants performed additional specific coagulation assays (the so called “Other” tests) for this case. The additional specific coagulation assays are presented in table 1.

Table 1: Schematic scheme with additional laboratory tests.

Additional specific coagulation assays	Number of participants
Fibrinogen antigen	18
FDP	1
Fibrine monomeer	3
Fibrinogen Schultz (heat precipitation)	1
Proteïn Electrophoresis	1
Fibtem	1
Thrombin Time polybrene	1
Thrombin Time diluted	1
D-Dimer	9
Reptilase	6
APTT Synthasil	1
Chromogenic Factor VIII activity	3
Factor V activity diluted	1
Factor XIII activity	6
Factor XIII antigen	1
Alpha 2-antiplasmin	1

Eighteen participants added the fibrinogen antigen test which is related to the low level of fibrinogen activity. In this specific case of Hypofibrinogenemia it is relevant to add the fibrinogen antigen assay.
It is not clear why nine participants performed the D-Dimer test, as this is not a logical choice. The D-Dimer test is mostly used in the case of Deep Vein Thrombosis (DVT or venous thrombosis), Pulmonary Embolism (PE), Disseminated Intravascular Coagulation (DIC) or stroke. For this case study the patient suffers from gum bleeding and has a history of severe menstrual blood loss, though no history of spontaneous bleeding. Given the clinical information described for this case study there is no reason for D-Dimer testing.
Six participants performed the Reptilase Time (RT) which is not surprising. RT is used to detect deficiency or abnormalities in fibrinogen in cases of heparin contamination. RT is not prolonged in samples containing heparin whereas the Thrombin Time will be prolonged if there is heparin contamination in the sample.
There were also six participants who performed the FXIII assay, which is not the most logical choice. In the case of an FXIII deficiency there would have been normal screening results. Clinical symptoms of an FXIII deficiency manifest in most of the cases in umbilical cord bleeding or spontaneous intracranial haemorrhage which are not relevant to the clinical symptoms described in this case description.

Diagnosis

This is the case study of a patient who was diagnosed with Hypofibrinogenemia, which commonly occurs as part of a wider coagulation abnormality. Dysfibrinogenemia cannot be excluded. However, this diagnosis is rare and additional immunological testing would be required.

The vast majority of the participants indicated the diagnosis of Hypofibrinogenemia, which is the **correct diagnosis**. From the participants who selected “Unexplained bleeding Disorder” or “Other factor deficiencies” shown in figure 8, both categories were further subdivided into the provided diagnosis by the participant, which is shown in figure 9.

Figure 8: Selected diagnose given by the participants.

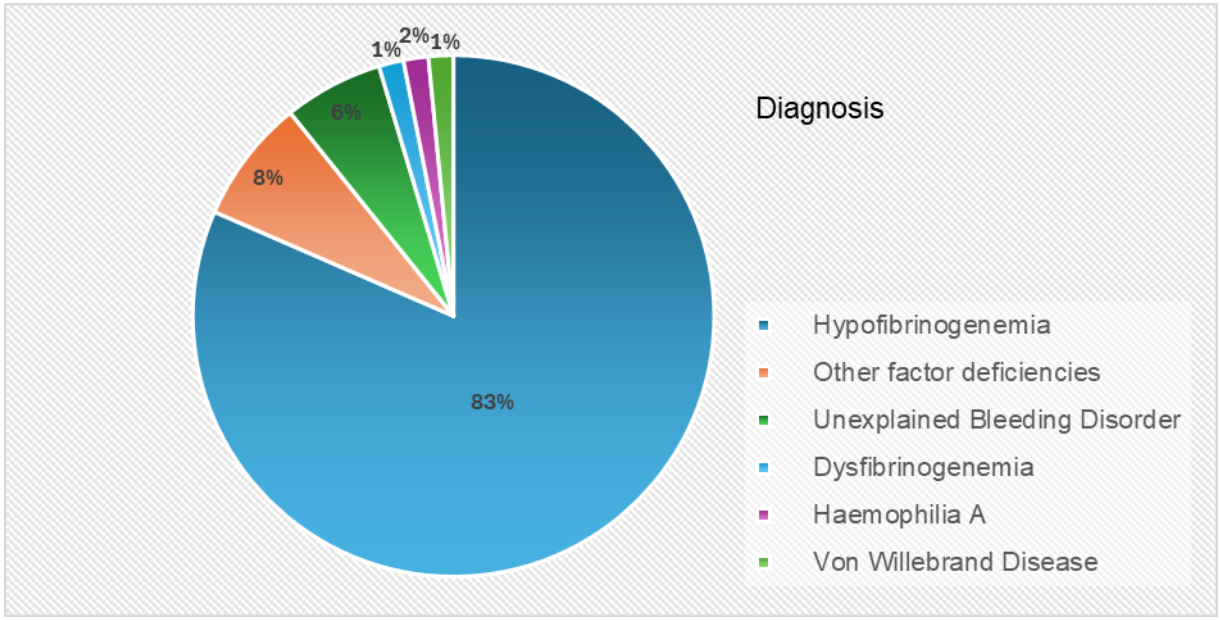
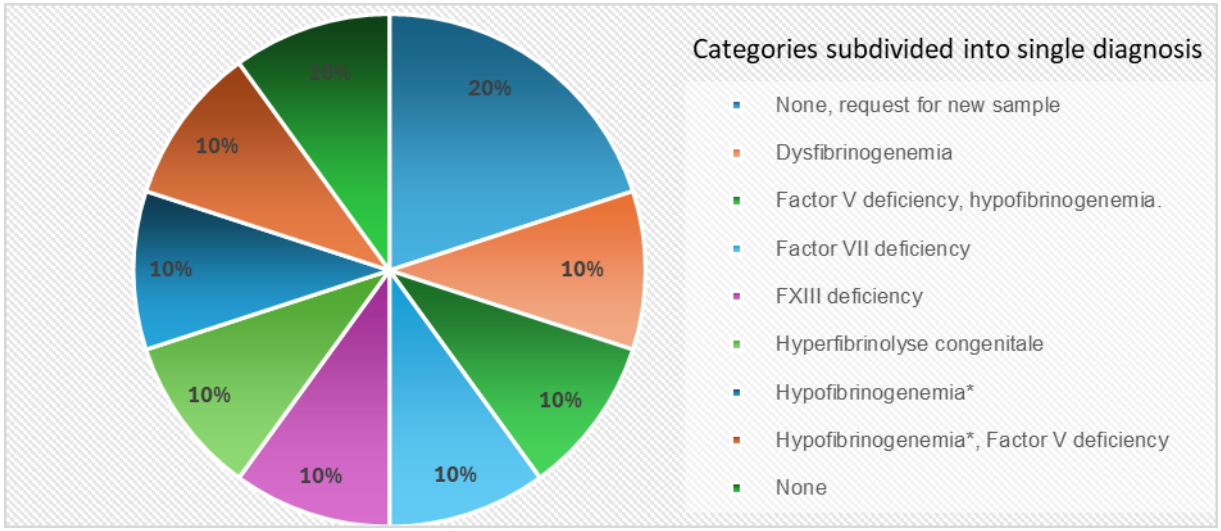


Figure 9: The provided diagnosis within the categories "Other factor deficiencies" and "Unexplained bleeding Disorders".



* There were some participants who indicated Hypofibrinogenemia as a second diagnosis

Discussion

On the basis of the outcome of the screening tests and Follow-up Strategies, (**prolonged PT, normal PT in the mixing study, normal APTT, low Fibrinogen and prolonged Thrombin Time**) it is not logical to perform the APTT mixing study and intrinsic factor assays.

Mixing studies are only relevant in the case of a prolongation of the PT and/or APTT. In this case study, for a sample with a low fibrinogen and prolonged PT, the PT Mixing test is the most obvious choice. There were participants who performed the Extrinsic factors test. This was a logic choice

Investigation of Von Willebrand Factor resulted in normal VWF results. It is plausible that participants performed Von Willebrand Factor assays because of the clinical symptoms, described in the case description. However, with a decreased VWF level a prolonged APTT is expected because of an associated decreased Factor VIII level.

Almost all participants (n=62) included fibrinogen in the screening panel and found a low fibrinogen level. On the basis of the low fibrinogen level, eighteen participants performed the fibrinogen antigen test also resulting in an abnormal low result.

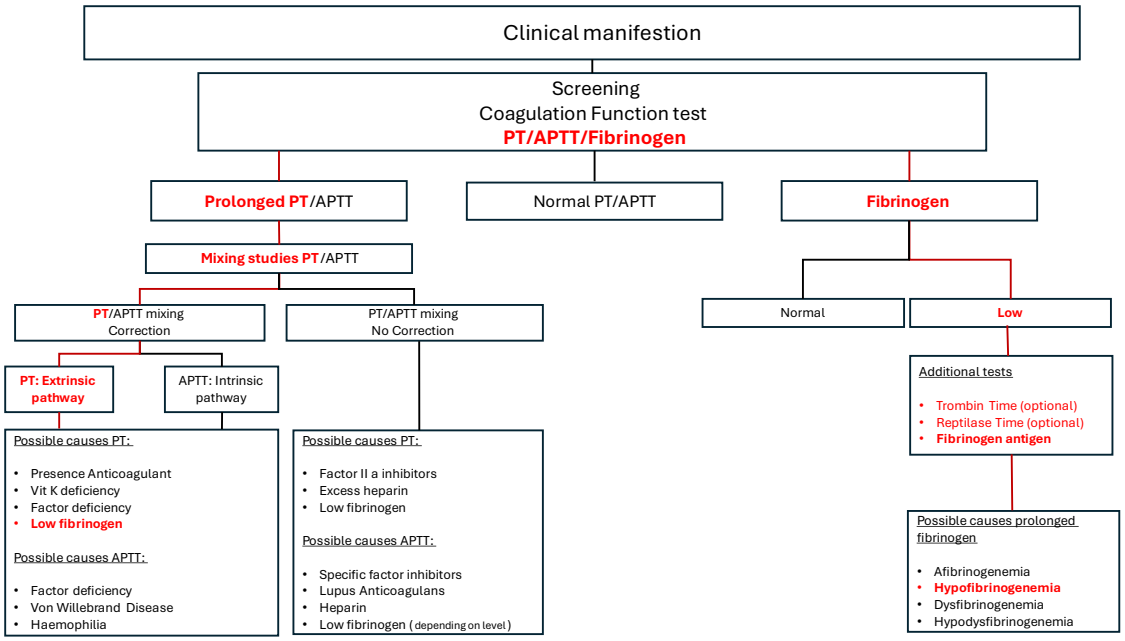
It is not clear why some participants performed the D-Dimer and FXIII assay. Neither of the two assays are relevant in this case study. The D-Dimer is mostly used in case of blood clotting conditions such as Deep Vein Thrombosis (DVT or venous thrombosis), Pulmonary Embolism (PE), Disseminated Intravascular Coagulation (DIC) or stroke. In the case of a FXIII deficiency there would have been normal screening results. Clinical symptoms of a FXIII deficiency in most cases present in umbilical cord bleeding or spontaneous intracranial haemorrhage. For neither of the assays are the clinical symptoms related to the clinical symptoms described in this case description.

Performance of the Reptilase Time (RT) assay is not surprising. RT is used to detect deficiency or abnormalities in fibrinogen in cases of heparin contamination. RT is not prolonged in samples containing heparin whereas the Thrombin Time will be prolonged if there is heparin contamination in the sample.

The vast majority of the participants indicated the diagnosis of Hypofibrinogenemia, which is the **correct diagnosis**.

To arrive at the correct patient diagnosis and prevent unnecessary diagnostic testing, it is recommended that a robust strategic testing scheme be used. A robust strategic scheme should start with the screening tests (PT, APTT, Fibrinogen). On the basis of the outcome of the screening tests and the clinical information about the patient, the follow-up strategy should be carried out. If a robust strategic testing scheme is used, it is likely to produce savings in time and cost, which will benefit the patient [see Recommended strategy for a specific case of hypofibrinogenemia].

Recommended strategy for a specific case of hypofibrinogenemia



Reference Source Scheme [1,2,3,4]

References:

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