

Véralvadási betegségek diagnosztikája

Belgyógyászati Szakvizsga Előkészítő Tanfolyam

2021.05.17-Június 11.

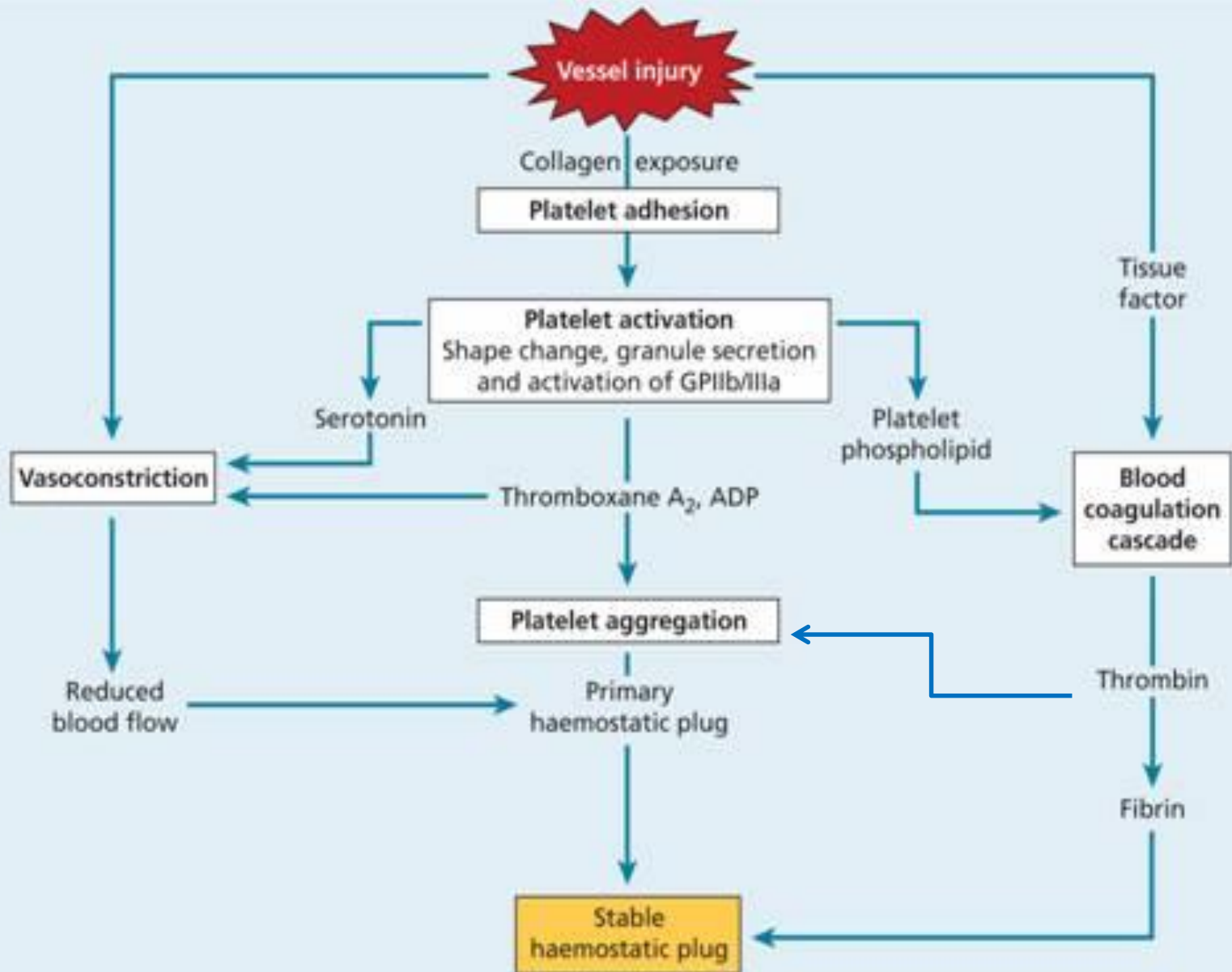
Pécs

On-line tanfolyam

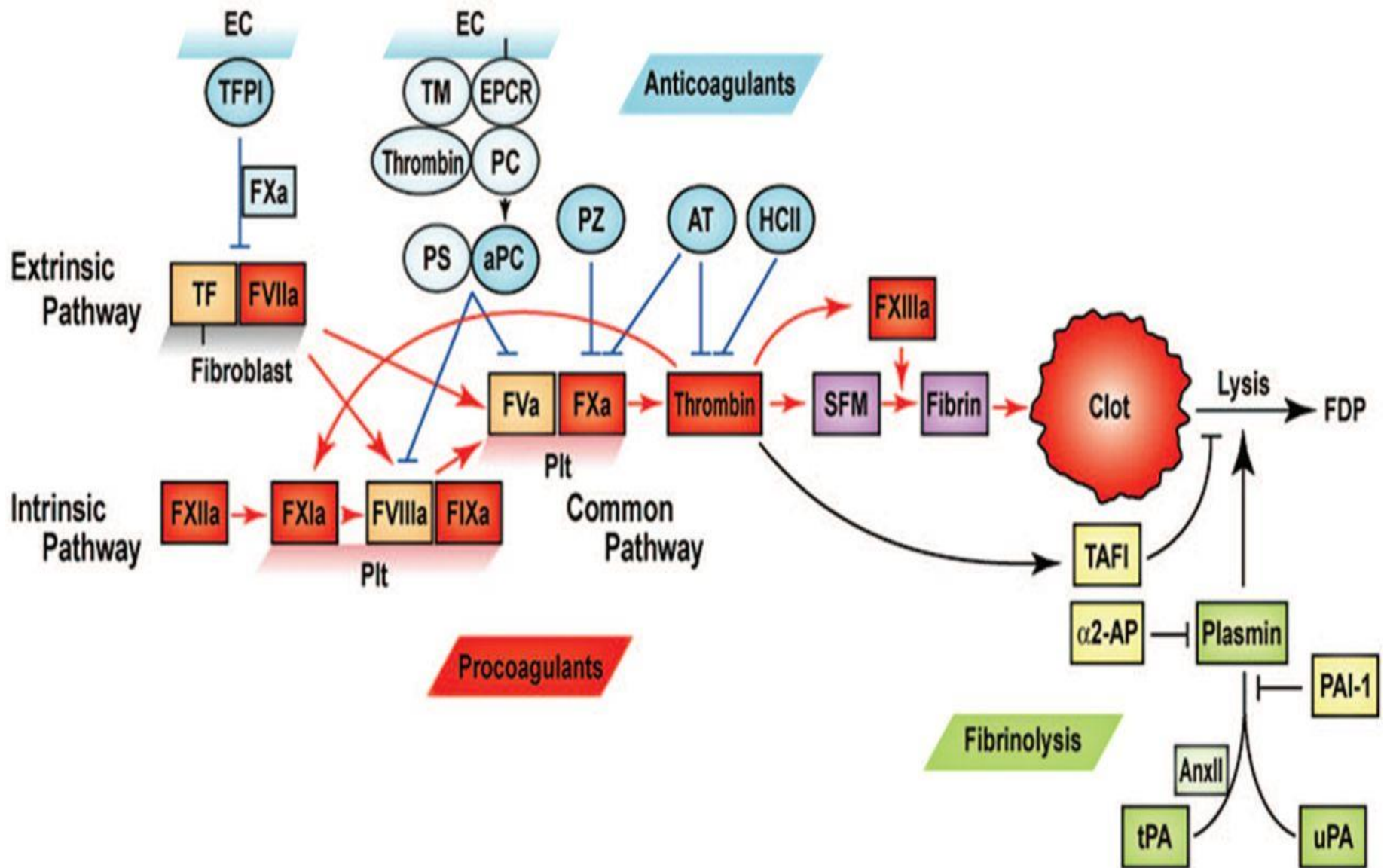
Tartalom

- Bevezetés – Haemostasis
- Kiknél végzünk véralvadási vizsgálatokat?
- Az anamnesisfelvétel főbb szempontjai, klinikum
- Laboratóriumi vizsgálatok
 - Globális tesztek
 - Screening véralvadási vizsgálatok
 - Specifikus véralvadási vizsgálatok
 - Bedside tests
 - Thrombocyta fu. vizsgálata
- Primér haemostasis vizsgálómódszerei
- Sec. haemostasis vizsgálómódszerei

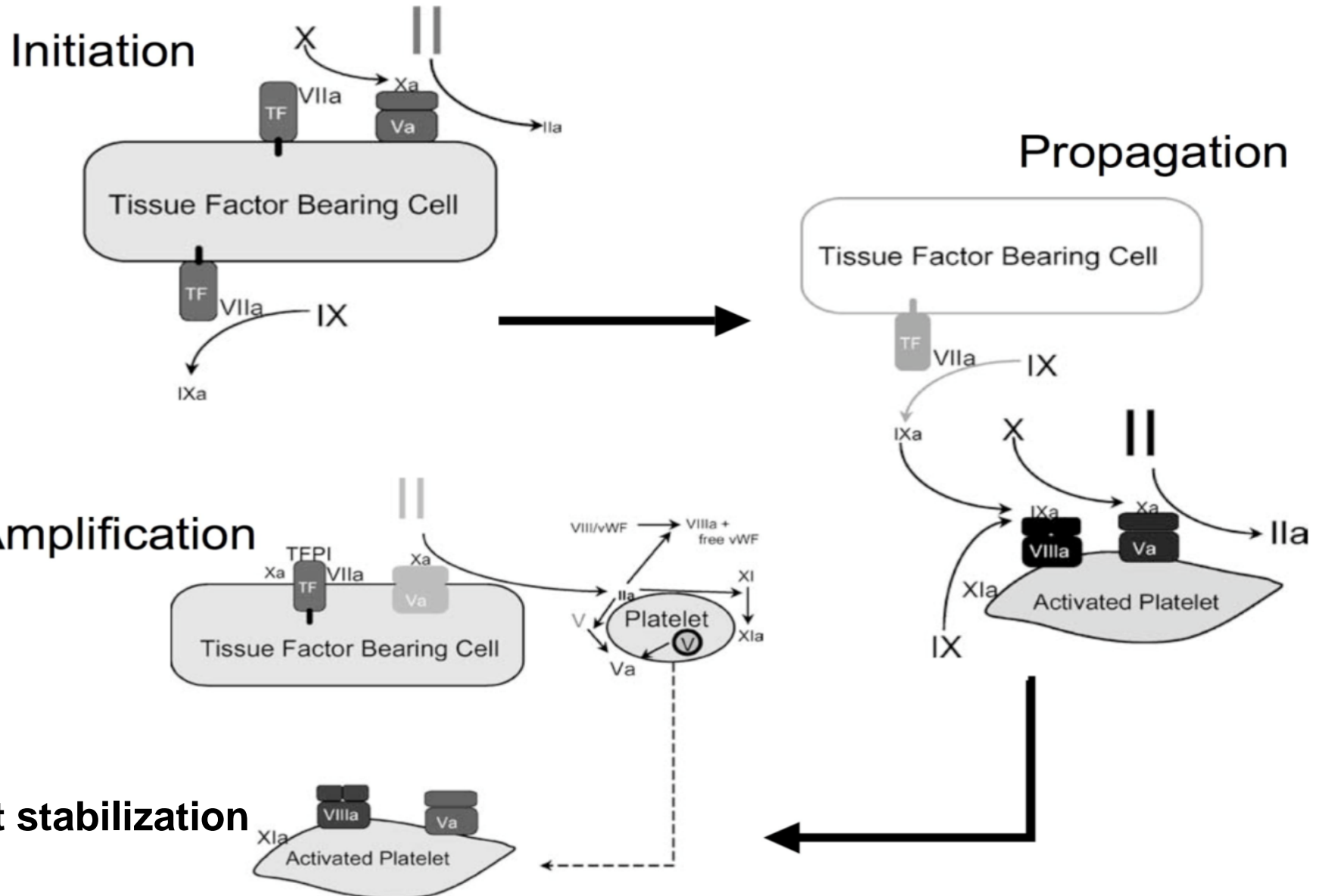
Haemostasis



Haemostasis



Haemostasis – cell based model (plus *in vivo*)

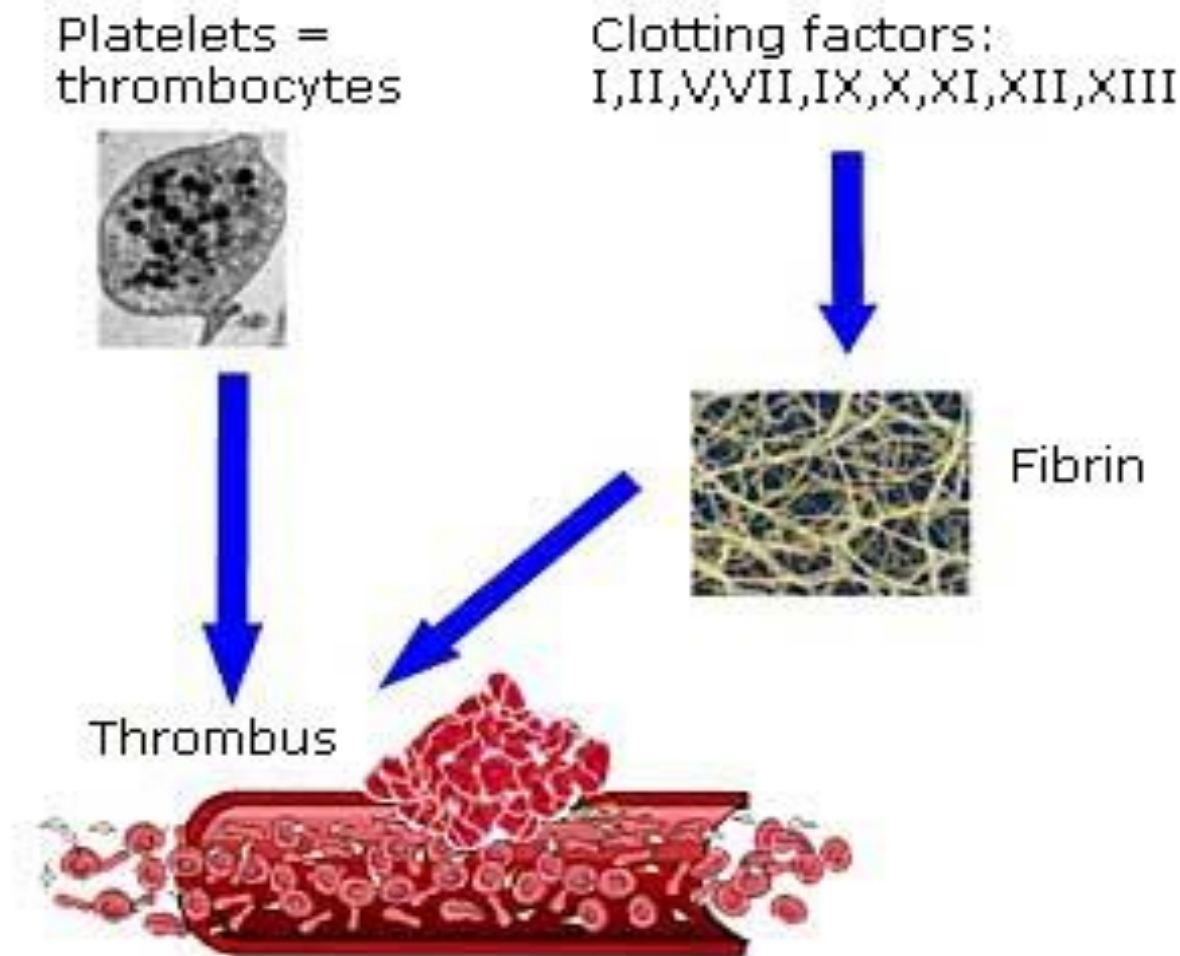


Kit vizsgálunk?

- Vérzékenység klinikai képe
 - Spontán vérzések
 - Invazív beavatkozások, trauma kapcsán fellépő abnormális mennyiségű (nem sebészi) vérzés
- Thrombophilia klinikai képe
- Invazív beavatkozások, műtétek előtt szűrés (vérzékenység irányában)

Vérzékenység

- ▶ A véralvadási rendszer csökkent működéséből adódó betegség, melynek következménye fokozott vérzési hajlam (haemorrhagiás diathesis)



VÉRZÉKENYSÉGEK FELOSZTÁSA

- I. Veleszületett vérzékenység
 - Ritka betegségek!
 - Enyhe – kp. súlyos – súlyos vérzékenység
- II. Szerzett vérzékenység
 - Gyakoribb!
 - Lehetséges okok: Gyógyszerek
(thrombocytaaggregatio gátlók, K-vitamin antagonisták, heparin, új antikoagulánsok, stb.) ,
májbetegség, gátlótestes haemophilia, ITP, leukémia ,
DIC...
 - Változó mértékű

VÉRZÉKENYSÉGEK FELOSZTÁSA

- ▶ I. Vasculopathiák (Marfan sy., steroid th., senilis purpura, Osler kór)
- ▶ II. Thrombocyta rendszer zavarai:
 - mennyiségi
 - minőségi (thrombocytopathiák)
- ▶ III. Coagulopathiák:
 - öröklött (Haemophilia A, B, vWB, egyéb ritka coagulopathiák)
 - szerzett (K vitamin hiány, keringő anticoagulansok, alvadási faktorok felhasználódása, egyéb okok: hematológiai kórképek, vesebetegség, masszív transzfúzió)

Kiket vizsgáljunk vérzékenység irányában?

- ▶ Spontán vérzések
- ▶ Trauma és invazív beavatkozások
következtében az átlagosnál erősebb és
elhúzódóbb vérzés
- ▶ Elhúzódó sebgyógyulás
- ▶ Családi anamnesis alapján/ill.
családvizsgálat (veleszületett vérzékenység
esetén hordozók felkutatása, intrauterin
vizsgálat)

Az anamnesis főbb szempontjai vérzékenység esetén

- Mikor volt az első vérzés?
- Spontán vagy kiváltott?
- Vérzés típusa, lokalizációja, gyakorisága?
- Vérzés súlyossága? Kellett-e transfundálni a beteget?
- Gyógyszerhatás volt-e?
- Menses hossza, erőssége?
- Családi anamnézis, családfa
- Aktuális betegségek, szedett gyógyszerek

Laboratory Tests of Hemostasis

Key Points

- Sample collection and processing has an important impact on the quality of results obtained.
- The sensitivity of any PT or APTT method is highly dependant on the reagents used.
- Clotting factor assays should be performed using several test sample dilutions.
- Specificity of a number of thrombophilia assays may be influenced by interfering substances.
- Oral direct inhibitors have variable effects on coagulation tests and assays, depending on the drug and the laboratory reagents used for testing.

Collection

For normal screening tests, venous blood should be collected gently but rapidly using a syringe or an evacuated collection system, when possible, from veins in the elbow. Application of a tourniquet to facilitate collection does not normally affect the results of most tests for bleeding disorders, although prolonged application must be avoided and the tourniquet should be applied just before sample collection. If there is any delay between collection and mixing with anti coagulant the blood must be discarded because of possible activation of coagulation. Vigorous shaking should be avoided. Any difficulty in venepuncture may affect the results obtained, particularly for activated partial thromboplastin time (APTT) or tests of platelet function. Prior to analysis, the sample should be assessed and discarded if there is evidence of clotting or hemolysis.

Tests of Fibrinolysis

Minimal stasis should be used because venous stasis causes local release of fibrinolytic components into the vein. The needle should not be more than 21 gauge (for infants, a 22 or 23 gauge needle may be necessary).

Venous Catheters

Collection through peripheral venous catheters or nonheparinized central venous catheters can be successful for prothrombin time (PT) and APTT testing, but is best avoided; if used, sufficient blood must be discarded to prevent contamination or dilution by fluids from the line (typically 5–10 mL of blood from adults).

Mixing with Anticoagulant

If there is any delay between collection and mixing with anticoagulant, or delay in filling of the collection system, the blood must be discarded because of possible activation of coagulation. Once blood and anticoagulant are mixed, the container should be sealed and mixed by gentle inversion five times, even for evacuated collection systems. Vigorous shaking should be avoided. Any difficulty in venepuncture can affect the results obtained, particularly for tests of platelet function. Prior to analysis, the sample should be visually inspected and discarded if there is evidence of clotting or hemolysis. Partially clotted blood is typically associated with a dramatic false shortening of the APTT together with the loss of fibrinogen.

Anticoagulant and Sample Filling

The recommended anticoagulant for collection of blood for investigations of blood clotting is normally trisodium citrate.

The volume of anticoagulant required for a 5-mL sample.

Hematocrit (%)	Volume of anticoagulant (mL)	Volume of blood (mL)
25–55	0.5	4.5
20	0.7	4.3
60	0.4	4.6
70	0.25	4.75

added blood varied accordingly to the hematocrit. The volume of blood to be added (to 0.5 mL of 0.109 mol/L citrate) is calculated from the formula:

$$\frac{60}{100 - \text{hematocrit}} \times 4.5$$

Container

The inner surface of the sample container employed for blood sample collection can influence the results obtained (particularly for screening tests such as PT and APTT) and should not induce contact activation (nonsiliconized glass is inappropriate). For factor assays there is evidence that results on samples collected in a number of different blood collection tubes are essentially interchangeable.

Processing and Storage of Samples Prior to Analysis

Centrifugation

For preparation of platelet-rich plasma to investigate platelet function, samples should be centrifuged at room temperature (18–25°C) at 150–200 g for 15 minutes, and analyzed within 2 hours of sample collection. For most other tests related to bleeding disorders, samples should be centrifuged at a speed and time that produces samples with residual platelet counts below $10 \times 10^9/\text{L}$; for example, using 2000 g for at least 10 minutes. Centrifugation at a temperature of 18–25°C is acceptable for most clotting tests. Exceptions include labile parameters, such as many tests of fibrinolytic activity. After centrifugation, prolonged storage at 4–8°C should be avoided, as this can cause cold activation, increasing factor VII and XII activity, and shortening of the PT or APTT.

Stability

Factor VIII and Von Willebrand factor are lost from whole blood stored at 4°C so samples should be stored at room temperature prior to processing. Samples for APTT should be analyzed within 4 hours of collection. This is particularly important for samples containing unfractionated heparin, which is progressively lost from samples as a consequence of neutralization by platelet factor 4 released from platelets. The results of some other clotting tests, such as the d-dimer and the PT of samples from warfarinized subjects, are stable for 24 hours or longer. Unless a laboratory has data on the stability of testing plasmas at room temperature for a specific test, the plasmas should be deep frozen within 4 hours of collection for future analysis.

Some clotting factor test results are stable for samples stored at -24°C or lower for up to 3 months and for samples stored at -74°C for up to 18 months (results within 10% of baseline defined as stable). Storage in domestic grade -20°C freezers is normally unacceptable.

If frozen samples are shipped on dry ice to another laboratory for testing, care must be taken

to avoid exposure of the plasma to carbon dioxide, which may affect the pH and the results of screening tests.

Prior to analysis, frozen samples must be thawed rapidly at 37°C for 3–5 minutes. Thawing at lower temperatures is not acceptable because some cryoprecipitation is possible.

The stability of the sample may be affected by the mechanism of transport and pneumatic tube systems should not be used to transport samples prior to tests of platelet function because the agitation associated with passage through some systems may activate platelets, leading to loss of function.

Use of Coagulation Screening Tests

Laboratories usually offer a set of tests (the coagulation screen) that aims to identify most clinically important hemostatic defects. Invariably this includes the PT, APTT, fibrinogen, and usually thrombin time. It is important to perform a full blood count to quantify the platelet count, but assessment of platelet function is not usually offered or performed in the initial tests. The pattern of abnormalities of the coagulation screen, as shown in Table 2.2, suggests possible diagnoses

Recommendations and summary: sample collection and processing

- Avoid prolonged venous stasis.
- Use a 21-gauge or lower gauge needle for adults.
- Avoid indwelling catheters or lines.
- Mix immediately with 0.105–0.109 mol/L trisodium citrate.
- Discard the sample if there was any delay or difficulty in collection.
- Discard if marked hemolysis or evidence of clotting.
- Underfilling (<80–90% of target volume) prolongs some screening tests.
- If the hematocrit is >55%, adjust anticoagulant: blood ratio.
- The sample collection system can affect results by up to 10%.
- For plasma tests, centrifuge at 2000 g for at least 10 minutes at room temperature.
- Store at room temperature.
- Only centrifuge and store at 4°C if necessary.
- Test within 4 hours (unless there is evidence for longer stability).
- Freezing may affect results depending on the temperature and time of storage.
- Any deep-frozen plasma should be thawed rapidly at 37°C.

Interpretation of abnormalities of coagulation screening tests.

PT	APTT	Thrombin time	Fibrinogen	Possible conditions
Prolonged	Normal	Normal	Normal	Factor VII (FVII) deficiency
Normal	Prolonged	Normal	Normal	Deficiency of FVIII, FIX, FXI, FXII, contact factor, or lupus anticoagulant
Prolonged	Prolonged	Normal	Normal	Deficiency of FII, FV, or FX Oral anticoagulant therapy Vitamin K deficiency Combined deficiency of FV and FVIII Combined deficiency of FII, FVII, FIX, and FX Liver disease
Prolonged	Prolonged	Prolonged	Normal or low	Hypo or dysfibrinogenemia Liver disease Massive transfusion DIC

APTT, activated partial prothrombin time; DIC, disseminated intravascular coagulopathy; PT, prothrombin time .

and allows further tests to be performed to define the abnormality.

Unselected coagulation testing to assess bleeding risk prior to surgery may delay surgery inappropriately, is likely to cause anxiety in patients with “abnormal” test results , and is not cost effective. Coagulation tests are poor predictors of postoperative bleeding so patients with a negative bleeding history do not require routine coagulation screening before surgery. A bleeding history to include details of previous surgery, hemostatic challenges, and family history is much more useful and should be used to identify patients who require further investigation.

Prothrombin Time

Tissue factor (in the form of thromboplastin) and calcium are added to plasma that has been anticoagulated with citrate during collection. Tissue factor reacts with factor VIIa to activate the “extrinsic” pathway and thus form a clot.

Use of the Prothrombin Time Test

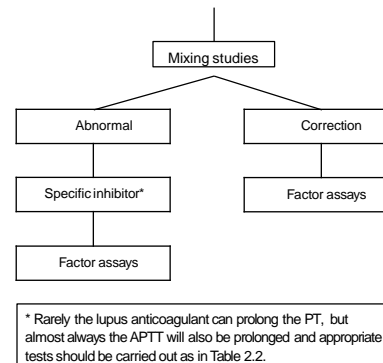
The PT is sensitive to deficiencies of factors VII, X, V, and II, and fibrinogen. The PT is particularly useful in monitoring anticoagulation in patients on vitamin K antagonist therapy such as

warfarin and should be reported as International Normalized Ratio (INR) in such patients.

Figure 2.1 suggests a pathway for investigation of a patient with a prolonged PT.

Activated Partial Thromboplastin Time

Phospholipid (lacking tissue factor, hence the term “partial” thromboplastin) and particulate matter (such as silica or kaolin) or fluid phase activator (such as ellagic acid) are added to plasma to generate a clot. Abnormalities in the “intrinsic” and “common” pathways will result in prolongation of the APTT.



Investigation of a prolonged prothrombin time (PT). APTT, activated partial thromboplastin time.

Use of the Activated Partial Thromboplastin Time Test

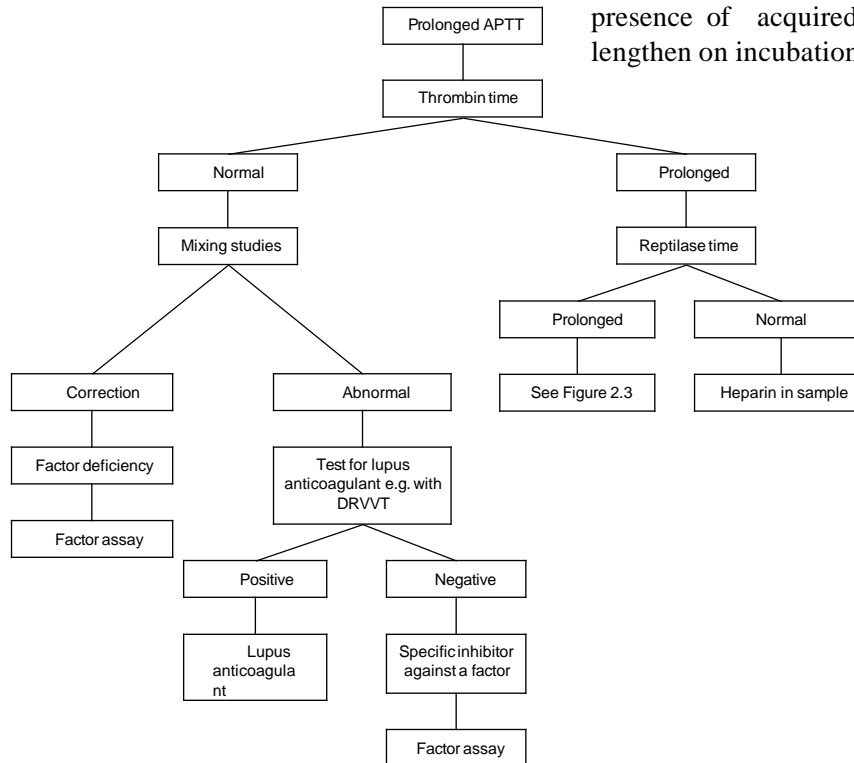
This test is abnormal in patients:

- with deficiencies of prekallikrein (except when ellagic acid is used as activator), high molecular weight kininogen, factors XII, XI, X, IX, VIII, V, II, and fibrinogen;
- on heparin therapy; or
- who have the lupus anticoagulant.

Mixing Studies

These are central in the investigation of a prolonged APTT. The principle is that the test is repeated, with 50% of the test plasma being replaced by normal plasma (which contains normal amounts of all the clotting factors). The result of the mixing study is that the test will have all the clotting factors to a minimum of 50%, and thus should result in:

- a normal APTT if the cause of the abnormality was a deficiency of a clotting factor; or
- a prolonged APTT if an inhibitor (either to a specific factor or a lupus anticoagulant) is present. Occasionally, the APTT on such a mix may initially be normal in the presence of acquired antifactor VIII antibodies, but will lengthen on incubation.



Investigation of a prolonged activated partial thromboplastin time (APTT). DRVVT, dilute Russell viper venom time.

Conditions associated with a prolonged activated partial prothrombin time but without a bleeding diathesis.

Deficiency of:

Factor XII Highmolecularweight
kininogen Prekallikrein

Lupus anticoagulant Excess
citrate anticoagulant

Thrombin Time

The thrombin time measures the rate of conversion of fibrinogen to polymerized fibrin after the addition of thrombin to plasma. It is sensitive to and thus prolonged in:

- hypo and dysfibrinogenemia;
- heparin therapy (or heparin contamination of the sample); and
- the presence of fibrin(ogen) degradation products and factors that influence the fibrin polymerization (e.g., the presence of a paraprotein in myeloma).

Figure 2.3 suggests a pathway for investigation of a prolonged thrombin time. Heparin contamination in a sample can also be confirmed by correction of a prolonged thrombin time after treatment of a sample with heparinase (e.g., heparinase), testing with reptilase, or mixing with protamine sulfate or other agent that neutralizes heparin. Thrombin time reagents vary in their sensitivity to heparin. Generally, thrombin times determined using reagents with a lower thrombin concentration will be prolonged at lower heparin levels. Thrombin time may be prolonged in the presence of low molecular weight heparin (LMWH) depending on the molecular weight of the drug. This is more frequent for LMWHs such as tinzaparin, which contain more of the larger polysaccharide chains that support thrombin neutralization.

Fibrinogen

A number of methods are available for measurement of fibrinogen concentration. Most automated coagulation analyzers now provide a measure of fibrinogen concentration, calculated from the degree of change of light scatter or optical density during measurement of the PT (PT-derived fibrinogen). Although this is simple and cheap, it is inaccurate in some patients, such as those with disseminated intravascular coagulopathy, liver disease, renal disease, dysfibrinogenemia, following thrombolytic therapy, and in those with markedly raised or reduced fibrinogen concentrations. The recommended method for measuring fibrinogen concentration as originally described by Clauss is based on the thrombin time and uses a high concentration of thrombin solution [2].

Screening Tests: Assay Issues

The sensitivity of the PT and APTT to the presence of clotting factor deficiencies is dependent on the test system employed. The degree of prolongation in the presence of a clotting factor deficiency can vary dramatically between reagents [3]. There is no clear consensus on what level of clotting factor deficiency is clinically relevant, and therefore the level that should be detected as an abnormal screening test result has not been defined. In relation to the APTT, one important application is the detection of deficiencies associated with bleeding, in particular factors VIII, IX, and XI.

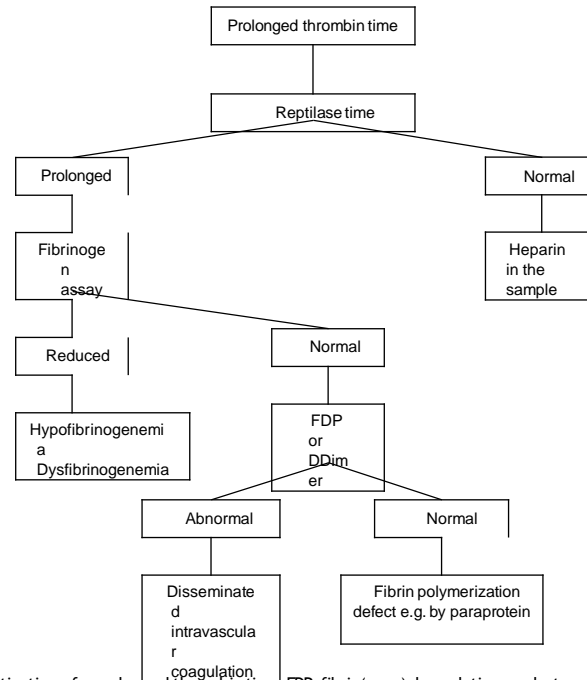


Figure 2.3 Investigation of a prolonged thrombin time: FDP, fibrin(ogen) degradation products.

A number of APTT methods are available for which abnormal results are normally present when the level of clotting factor is below 30 U/dL, and only methods for which this is the case should be used to screen for possible bleeding disorders. In the case of factor VIII, it has been recommended in the past that the APTT technique selected should have a normal reference range that closely corresponds to a factor VIII reference range of 50–200 U/dL. However, it should be noted that, for most methods, normal APTT results will be obtained in at least some patients with factor VIII in the range 30–50 U/dL, and few, if any, reagents will be associated with prolonged results in every patient of this type.

For most techniques, the APTT is less sensitive to the reduction of factor IX levels than for factor VIII, and most, if not all, currently available techniques will be associated with normal APTT results in at least some cases with factor IX in the range 25–50 IU/dL.

Data from published studies and from external quality assessment programs suggest that most widely used current APTT reagents will have:

- prolonged APTT results in samples from patients with factor IX or XI below 20–25 IU/dL; and
- a more mixed pattern of normal and abnormal results when factor IX or XI is in the range of 25–60 IU/dL.

Finally a subgroup of up to 10% of subjects with mild hemophilia A have a molecular defect that results in reduced activity in two stage and chromogenic factor VIII assays whilst retaining normal activity in onestage techniques. These subjects have a normal APTT despite the presence of clinically relevant bleeding tendency consistent with mild hemophilia.

Lower Limit of Normal Range

The lower limit for factor XI activity is probably between 60 and 70 IU/dL. The lower limit of normal for factor VIII or IX is approximately 50 IU/dL. A normal APTT does not always exclude the presence of a mild deficiency. In plasma from subjects with factor IX or XI deficiency, marked elevation of factor VIII, if present, may normalize the APTT.

Recommendations and summary: screening tests	
<ul style="list-style-type: none">• PT and APTT methods vary in sensitivity to factor deficiency.• Mild deficiency may be associated with normal PT or APTT.• For bleeding disorders, select a method for which APTT is normally prolonged when factor VIII, IX, or XI is 30 IU/dL or less.	<ul style="list-style-type: none">• Elevated factor VIII may normalize APTT in mild factor IX or XI deficiency.• Assessments of APTT sensitivity should employ samples from patients.

Factor Assays to Monitor Replacement Therapy in Hemophilia

There are particular issues related to the assay of postinfusion samples if recombinant products are used. Results of chromogenic factor VIII assays may be 20–50% higher than results of onestage assays when plasma standards are used for assay calibration. Results of chromogenic assay are also higher (by approximately 50%) than onestage assay results in the presence of Refacto AF/Xyntha, a B domain deleted factor VIII. This has led to the use of productspecific Refacto AF laboratory standard in combination with one-stage assay reagents in some countries because this delivers agreement with chromogenic assay, and because the chromogenic assay is used to assign potency to this product. An assessment of assay performance in samples containing N8, a B domain deleted factor VIII from a different manufacturer, reported a difference of around 30% and concluded that a product specific standard was not required for assay calibration. A number of modified factor VIII and IX molecules are in clinical trials mainly with the aim of extending the halflife of infused clotting factor. This includes pegylated factor VIII and IX proteins. Early data indicate that several chromogenic assays studied so far may be suitable for monitoring with the usual plasma standard to calibrate assays, but that only a few one-stage assay reagent sets will recover values close to those expected from potency labeling. Other one stage reagent sets grossly underestimate or grossly overestimate the factor VIII or IX activity and are unsuitable for use with conventional plasma standards as assay calibrators. Recent guidance from the ISTH/Science and Standardization Committee states that the optimal approach to postinfusion testing of such concentrates involves use of product specific standards but recognizes that this may be difficult to implement. At the time of writing it remains unclear to what extent productspecific standards will become available.

Recommendations and summary: factor assays	
<ul style="list-style-type: none"> Assays should be calibrated with reference plasmas traceable back to WHO standards where available. Deficient plasmas must have <1 U/dL of the clotting factor being assayed and normal levels of other relevant factors. 	<ul style="list-style-type: none"> No less than three dilutions of test plasmas should be tested. A valid assay requires test and calibration lines to be parallel. Interference by antiphospholipid antibodies can be minimized by use of an APTT reagent with a high phospholipid content.

Factor XIII Testing

Factor XIII circulates as a tetramer of two functional/catalytic A subunits carried by two B subunits. Severe factor XIII A deficiency is associated with severe bleeding events. In severe factor XIII B deficiency the absence of carrier protein leads to a reduced (but not absent) plasma concentration of the A subunit with milder bleeding symptoms [5]. Subunit A is reduced when the B carrier proteins are deficient. Isolated factor XIII deficiency is associated with normal PT, APTT, thrombin time, and platelet function tests. If the clinical symptoms indicate a bleeding tendency then a full evaluation requires inclusion of a test for factor XIII deficiency in the panel of laboratory investigations. Clot solubility screening tests in which clotted citrated plasma is suspended in urea or acid suffer from poor sensitivity. There is published guidance on diagnosis from the factor XIII subcommittee of the International Society on Thrombosis and Haemostasis [5], which recommends that a functional quantitative factor XIII assay should be used as the firstline screening tests. Some factor XIII concentrates used for replacement therapy contain only the A subunit and are not the treatment of choice in the rare cases of B subunit deficiency. The guideline therefore recommends that factor XIII A and XIII B antigen should be measured, and also addresses inhibitor assays in acquired deficiency states.

Thrombophilia Testing

This section addresses some laboratory aspects of testing for heritable thrombophilia: protein C (PC), protein S (PS), antithrombin (AT), activated protein C resistance (APCR), factor V Leiden (FVL), and the prothrombin 20210A allele [6,7].

Thrombophilia Testing

Sample Collection, Processing, and Assay

- A citrate concentration of 0.105–0.109 mol/L should be used for sample collection, because citrate strength may affect results, at least for APCR testing.
- Centrifugation should be as for other coagulation tests described above.
- Residual platelets in plasma following centrifugation can also affect results of APCR tests, and plasmas should be centrifuged as described above, separated, and recentrifuged a second time to ensure maximum removal of platelets. (Such a procedure is not necessary for AT, PC, or PS testing but can be used for convenience without adverse effects if the same plasma is to be used for these investigations in addition to APCR.)
- Such doublecentrifuged plasma can then be stored deep frozen at -70°C prior to analysis for at least 6 months for clotting PS activity and at least 18 months for PC and AT.
- In general, activity assays are preferable to antigen assays because antigen assays will be normal in some patients with type 2 defects where a normal concentration of a defective protein is present.

In the case of PS, this is complicated by the problems associated with interference by FVL in many different activity assays and can lead to important underestimation of the true level, with misdiagnosis a possibility. At present, the standardization of PS activity assays is poor in that results of different assays may differ substantially, even in normal subjects. For these reasons, PS activity assays must be used with caution.

FVL can also cause underestimation of the true PC level in clotting assays. A chromogenic PC assay may be used to avoid this problem although some type 2 defects give substantially different results in clotting and chromogenic PC assays. Alternatively, the PC clotting assay can be modified to include predilution of test sample 1 in 4 in PCdeficient plasma to restore specificity. A similar procedure can be employed to improve performance of clotting PS assays in the presence of FVL.

Clotting assays of PC and PS may also be influenced adversely by elevated factor VIII, causing underestimation. The presence of the lupus anticoagulant may be associated with falsely high results, with the possibility of a false normal result in the presence of deficiency.

When assaying PC, PS, and AT, calibration curves should include a minimum of three dilutions and, in general, the most precise test results will be obtained if a calibration curve is prepared with each group of patient samples. As for other tests of hemostasis, it is important to use a reference plasma traceable back to WHO standards, which are available for AT, PC, and PS.

Testing for APCR is largely based on the APTT in the presence and absence of APC, and therefore many of the variables that affect the APTT will in turn influence APCR test results. These include the presence of heparin or lupus anticoagulant by prolonging clotting times, or elevated factor VIII, which shortens clotting times and manifest as acquired APCR. The original APCR test also requires normal levels of clotting factors, including factor II and X, which are reduced by warfarin therapy. Valid APCR testing as originally used requires a normal PT and APTT.

There is evidence that standardization of results obtained by the original assay can be improved by calculation of the normalized APCR ratio (test APC ratio divided by APC ratio of a pooled normal plasma tested in the same batch of tests). The test can be significantly improved by predilution of test plasma in factor Vdeficient plasma, making the test 100% sensitive to the presence of FVL. This is typically a 1 in 5 dilution in commercial methods but 1 in 10 dilution may improve separation of results between normal subjects and subjects heterozygous or homozygous for FVL. This modification also makes the test specific for FVL, and will be associated with normal results where APC resistance in the classic assay is not a consequence of FVL.

This must be borne in mind when interpreting results. In some versions of the test, there is clear separation between results obtained in heterozygotes and homozygotes but, even for such assays, confirmation by genetic testing may be necessary because it is important to identify homozygotes with certainty. When genetic testing for the FVL or pro thrombin alleles is undertaken, there are fewer relevant preanalytical variables than for phenotypic tests on plasma. Whole blood samples are stable for several weeks, at least for some of the genotyping methods.

Because of the many differences between results of apparently similar assays in thrombophilia testing, it is particularly important to establish locally a reference or normal range.

Recommendations and summary: thrombophilia tests

- | | |
|---|---|
| <ul style="list-style-type: none">• Double centrifugation is required for APC-R testing.• Presence of FVL may cause significant underestimation of clotting PC or PS activity.• Results of PS activity assays are highly dependent on the reagents used.• Elevated factor VIII or lupus anticoagulant can interfere with PC or PS clotting assays. | <ul style="list-style-type: none">• Results of AT assays may depend on the enzyme used in the assay.• APC-R with factor V-deficient plasma dilution is the most sensitive and specific for FVL.• Genetic testing for FVL or prothrombin allele may not be error free. |
|---|---|

Effects of direct oral anticoagulants on tests of hemostasis.

Test	Apixaban		Rivaroxaban		Dabigatran	
	peak	Trough	peak	Trough	peak	Trough
PT/INR	Minimal increase	Unaffected	PT ratio 1.3–1.6	Usually normal	INR/PT ratio <1.5	
APTT	Minor increase	Unaffected	Ratio 1.4–1.6	Normal	Ratio 1.8–2.0	Ratio 1.3–1.4
Thrombin time	Unaffected	Unaffected	Unaffected	Unaffected	>10 fold prolonged	Prolonged
Fibrinogen (Clauss)	Unaffected	Unaffected	Unaffected	Unaffected	False low in some assays	Minimal effects
One stage factor assays	Underestimation (higher levels)	Minimal effects	Underestimation	Minimal effects	Underestimation	Minimal effects
DRVVT	False increase	Minimal effects	False prolongation	Minimal effects	False prolongation	Minimal effects
APCResistance	Elevated ratio in some methods	Minimal effects	Elevated ratio in some methods	Minimal effects	elevated ratios	Minimal effects
AT	Overestimation in Xa based assays	Minimal effects	Overestimation in Xa based assays	Minimal effects	Overestimation in IIa based assays	Minimal effects
Clot based PC and PS assays	Overestimation	Minimal effects	Overestimation	Minimal effects	Overestimation	Minimal effects

APC, activated protein C; APTT, activated partial prothrombin time; DRVVT, dilute Russell viper venom time; INR, International Normalized Ratio; PT, prothrombin time.

Expected plasma concentrations of direct oral anticoagulants (DOACs).

Drug	Dose	Peak levels mean and range	Trough levels mean and range
Apixaban	2.5 mg b.i.d.	0.051 mg/L (CV 27%)	0.014 mg/L (CV 53%)
Apixaban	5 mg b.i.d.	0.082 mg/L (CV 18%)	0.025 mg/L (CV 20%)
Dabigatran	150 mg b.i.d.	0.184 mg/L (95% CI 0.064–0.443)	0.090 mg/L (0.031–0.225)
Rivaroxaban	10 mg o.d.	0.125 mg/L (0.091–0.195)	0.009 mg/L (0.001–0.038)
Rivaroxaban	20 mg o.d.	0.223 mg/L (0.16–0.36)	0.022 mg/L (0.004–0.096)

Recommendations and summary: quality control

- Quality control samples should be analyzed regularly and frequently for screening tests and with each group of factor assays.
- Centers should participate in accredited EQA programs for all tests where available.

Laboratory Testing of Hemostasis

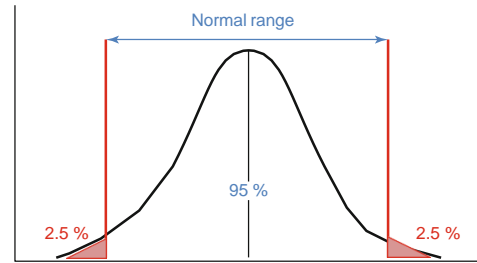
Hemostasis laboratories can carry out large numbers of assays either to obtain accurate and comprehensive diagnoses of hemostatic abnormalities or to monitor antithrombotic treatment. Activated partial thromboplastin time (aPTT) and prothrombin time (PT) are the most prescribed routine tests.

The evaluation of a patient's history, symptoms and clinical signs are essential to assess their bleeding tendency and to determine which laboratory test to use. Moreover, in order to interpret laboratory test results, it is important to understand how the assays are performed and to be aware of their limitations.

A normal range (reference range) is defined as the interval into which 95 % of the values of a reference population fall; thus, 2.5 % of values are inferior to the lower limit, and 2.5 % are superior to the upper limit. Applied to hemostatic testing, this means, for example, that 2.5 % of healthy individuals have a prolonged aPTT (longer than the upper limit).

Pre-analytical variables strongly influence hemostasis test results, and particular attention should be paid to blood collection, sample transportation, and storage. The accuracy of hemostasis tests depends on the sample quality (Lippi et al. [2012](#)).

Normal range definition. When values of the reference population are normally distributed, the reference range is defined as the interval containing 95 % of the values



Blood collection must be as thorough as possible in order to obtain reliable results: samples should be obtained from a peripheral vein using an atraumatic puncture, away from any intravenous perfusion line. Tubes containing 3.2 % (0.109 M) sodium citrate are recommended as other anticoagulants may yield invalid results. These tubes must be carefully filled to predetermined levels in order to respect the blood-to-anticoagulant ratio and then gently inverted five times to mix them together. The first few milliliters of blood collected after the puncture should be discarded.

Coagulation Testing

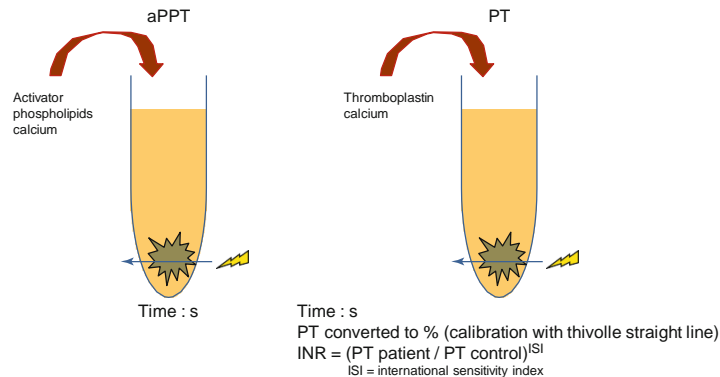
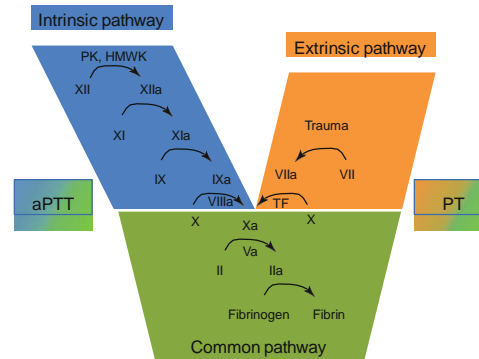
Coagulation Cascade

The cell-based coagulation model focuses on the successive steps of thrombin generation. These are initiation, propagation, and a burst of thrombin generation that occurs in the close vicinity of cell membranes (i.e., platelets) that provide the phospholipids required for the coagulation reaction. Depending on how coagulation is initiated, the classic coagulation cascade describes two somewhat artificial activation pathways that lead to fibrin formation: the intrinsic and extrinsic pathways. The two pathways link up to form a common pathway. This model relates closely to the in vitro coagulation assays usually performed to evaluate the coagulation potency of plasma: aPTT explores the intrinsic pathway, whereas PT explores the extrinsic pathway (Hoffman and Monroe 2005).

Activated Partial Thromboplastin Time (aPTT)

Activated partial thromboplastin time is a clotting assay that measures the intrinsic pathway and is dependent on the concentrations of contact-phase factors (high-molecular-weight-kininogen (HMWK), prekallikrein (PK), factor XII), intrinsic factors VIII, IX, and XI, and on the common pathway factors (II, V, X, and fibrinogen).

Coagulation cascade model



aPTT and PT principles. aPTT is the time it takes to form a clot after adjunction of calcium and a surface-activating agent into plasma. PT is the time it takes to form a clot after adjunction of calcium and thromboplastin into plasma

After blood centrifugation, calcium and a surface-activating agent are added to plasma. The time it takes to form a clot, expressed in seconds, is aPTT. The normal range is specific to each laboratory and depends on the reagents and device used for the coagulation assay. Generally, aPTT is used as a screening test for a deficiency of more than 50 % in factors VIII, IX, and XI, depending on the reagents used – it is less useful for the detection of deficiencies of common factors. The sensitivity of the test depends on the activators used.

<i>Shortened aPTT</i>	
Traumatic/difficult blood puncture	
Pre-analytical problem: handling, storage	
Physical activity	
Obesity	
Pregnancy, postpartum	
Estrogen treatment	
Neoplasia	
Postoperative period	
Venous thromboembolic disease	
Asthma, respiratory failure	
Diabetes	
Hyperthyroidism	
<i>Isolated prolonged aPTT</i>	
Anticoagulant treatment (unfractionated heparin)	Increased hemorrhagic risk
Factor VIII, IX, and XI deficiencies	
Inhibitors of intrinsic pathway factors VIII, IX, and XI	
Factor XII, PK, HMWK deficiencies	No increased hemorrhagic risk
Pre-analytic errors	
Newborns, young infants	
Circulating lupus anticoagulant	Thrombotic risk
<i>Prolonged aPTT and prolonged PT</i>	
Common factor deficiencies (I, II, V, X) or factor inhibitors	Possible
Vitamin K deficiency, malabsorption, VKA treatment	increased
Liver disease	hemorrhagic risk
Some direct thrombin inhibitors	Increased hemorrhagic risk
Disseminated intravascular coagulation	
Dilutional coagulopathy	
Hemorrhage	
Pre-analytic errors	No increased hemorrhagic risk

function of factor VII and of the common factors II, V, and X, and fibrinogen. PT is the time it takes for a clot to form after having added calcium and thromboplastin to the plasma. Expressed in seconds, PT is very short in normal individuals (12–13 s). In some countries, PT is expressed as a percentage of the PT of control plasma. For monitoring the treatment of vitamin K antagonist (VKA), PT is standardized according to the characteristics of the thromboplastin and calibrators used and is expressed as an international normalized ratio (INR).

An isolated prolonged PT may rarely reflect an inherited factor VII deficiency (1 in 500,000 of the general population). More commonly it reflects a moderate deficiency in vitamin K-dependent factors.

Prolonged PT, associated with prolonged aPTT, can be due to (Kamal et al. 2007):

- Deficiencies in factors II, V, and X or fibrinogen
- Presence of factor inhibitors

- VKA treatment
- Direct thrombin inhibitor
- Vitamin K deficiency, malabsorption
- Liver disease
- Disseminated intravascular coagulation
- Dilutional coagulopathy
- Hemorrhage

Fibrinogen

Fibrinogen is the most abundant clotting protein in plasma (2–4 g/l). Fibrinogen abnormalities can be either qualitative (dysfibrinogenemia) or quantitative (total lack, afibrinogenemia, or partial deficiency, hypofibrinogenemia).

Fibrinogen measurement is usually performed using a functional qualitative method (von Clauss chronometric assay); when a high concentration of thrombin is added to diluted plasma, the clotting time is proportional to the level of clottable fibrinogen. Fibrinogen activity levels can also be estimated using a prothrombin time-based kinetic assay, which is a rapid, inexpensive, automated assay, although less specific of fibrinogen activity.

Immunoassays for fibrinogen antigen quantification are also available, but are not used for screening tests. These immunological assays measure the protein concentration rather than the functional activity of fibrinogen.

Fibrinogen measurement is indicated in cases of apparent bleeding symptoms or in cases of suspicion of disseminated intravascular coagulation, hepatic insufficiency, or hyperfibrinolysis (Table 2.2).

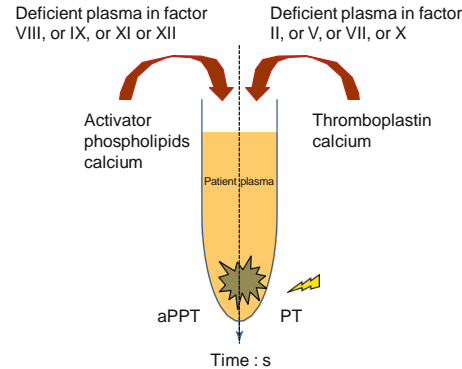
Specialized Testing

A combination of tests for PT and aPTT is usually the first step of a coagulation assessment. Then, according to the results, further assays may be performed (Sié and Steib 2006).

Causes of abnormal fibrinogen levels

Reduced fibrinogen levels	Increased fibrinogen levels
Disseminated intravascular coagulation	Age
Dilutional coagulopathy	Pregnancy, oral contraception, postmenopausal
Liver disease with decreased synthesis	Inflammatory syndrome
Inherited deficiencies	Malignancy
Thrombolytic therapy	
High doses of corticosteroids	

Factor assays principles. Specific factor assays are based on the ability of the patient's diluted plasma to change the clotting times of specific factor-deficient plasmas, measured by aPTT or PT



Mixing Studies

A mixing study mixes patient plasma with control plasma. It is able to distinguish between a prolonged clotting time due to a factor deficiency and one due to an inhibitor. The control plasma contains the coagulation factors that could be deficient in patient plasma. When the mixture corrects the clotting time, it is indicative of one or more factor deficiencies. When the mixture fails to correct the clotting time, it is indicative of an inhibitor (specific factor inhibitor or lupus anticoagulant).

Clotting Factor Assays

Except for the measurement of factor XIII, specific factor assays are based on the ability of patient's diluted plasma to change the clotting time of specific factor-deficient plasmas, measured by PT (for factors II, V, VII, and X) or by aPTT (for factors VIII, IX, XI, and XII). Clotting time is therefore directly proportional to the activity of the factor tested (Fig. 2.4). The result is usually expressed as a percentage of factor activity in control plasma but can also be expressed in IU/dl; one unit of activity being present in 1 ml of normal plasma with 100 % activity, 100 % corresponds to 100 IU/dl. The measurement of factor activity is an essential step in determining the etiology of a prolonged PT or aPTT.

Thrombin Time

Thrombin time is the clotting time of plasma in the presence of exogenous thrombin, expressed in seconds. This test looks solely at the conversion of fibrinogen to fibrin. Thrombin time is prolonged in cases of inherited or acquired dysfibrinogenemias or hypo-/afibrinogenemias or in the presence of thrombin inhibitors (heparin, hirudin, dabigatran, elevated levels of fibrin degradation products, some paraproteins, or thrombin antibodies).

The Reptilase Test

This test is equivalent to the thrombin time test, but is not sensitive to heparin. It measures the conversion of fibrinogen to fibrin by a thrombin-like enzyme (reptilase). Reptilase is not inhibited by heparin, hirudin, antithrombin antibodies, or antifibrinolytics. The test is used to exclude the presence of dysfibrinogenemia in cases with a prolonged aPTT.

D-Dimer Assay

d-dimers are specific breakdown fragments of cross-linked fibrin; they are not produced when non-cross-linked fibrin or fibrinogen are degraded. Increased d-dimer levels reflect the formation of fibrin and subsequent in vivo lysis. There are several methods of measuring d-dimers.

d-dimer levels are elevated in case of thromboembolism (pulmonary embolism, venous thrombosis, arterial thrombosis), malignancy, pregnancy, sepsis, cirrhosis, disseminated intravascular coagulation, and in the postoperative period.

d-dimer levels are widely used to rule out a venous thromboembolic event since a normal result has a high negative predictive value (95–98 %).

Thrombin Generation Test

The thrombin generation test measures the quantity of thrombin produced in response to a calibrated stimulus. It is performed on plasma, with or without platelets. The amount of thrombin reflects the overall functioning of the hemostatic system (activators and inhibitors), without assessing fibrinolysis. The main parameters of the thrombogram are (Dieri et al. 2012; Hemker et al. 2006):

- Lag time (= clotting time)
- Peak (= maximal concentration of thrombin)
- Time to peak
- Area under the curve (endogenous thrombin potential=total amount of thrombin)

The thrombin generation test has numerous drawbacks – including the lack of a standardized procedure – which restrict its use mainly to research programs.

Thrombophilia Testing

Laboratory testing can help understand recurrent thromboembolic venous or arterial diseases, due to inherited or acquired abnormalities of hemostasis. Thrombophilia can be linked to:

- Anticoagulant protein deficiency: antithrombin, protein C, protein S
- Genetic mutation of factor V (including the Leiden mutation), responsible for activated protein C resistance
- Genetic mutation of factor II (mutation G20210A)
- Presence of antiphospholipid antibodies (anticardiolipin, anti- β 2GPI, lupus anticoagulant)

Both functional and antigenic assays are available for antithrombin, protein C, and protein S.

Primary Hemostasis Testing

Primary hemostasis involves the vessel wall, endothelial cells, platelets, and some serine proteins (von Willebrand factor (vWF), thrombin, and fibrinogen).

Many assays are available for platelet function testing; however, no laboratory test can explore vessel walls or endothelial cells. Platelet function assays are time-consuming, difficult, and very sensitive to pre-analytical variables (drugs, food, platelet count, temperature, pH, fibrinogen level, quality of sample, storage, transport).

Platelet Count

Platelet count is measured using an automated counter, on blood drawn in EDTA- anticoagulated samples. In cases of thrombocytopenia (<150 G/l), platelet clumps have to be considered; if clumps are identified, platelet count should be assessed on blood collected in citrate-anticoagulated tubes.

The normal platelet count range for adults is 150–400 G/l. Platelet count, of course, does not evaluate their qualitative performance.

Bleeding Time

Bleeding time is supposed to provide an overall picture of primary hemostasis in vivo. It is determined mainly using the Ivy method (normal Ivy incision bleeding time <10 min). The Duke method (earlobe incision) is no longer used. Bleeding time is prolonged in cases of severe thrombocytopenia (platelets

<50 G/l), thrombopathy, deep hypofibrinogenemia, afibrinogenemia, dysfibrinogenemia, von Willebrand disease (vWD), severe anemia (hematocrit <30 %), or treatment interfering with platelet function. However, a normal bleeding time does not exclude platelet dysfunction or vWD. The bleeding time procedure is delicate, requiring experienced operators, which partly explains why this method has fallen out of use.

Platelet Function Analyzer (PFA-100® and PFA-200®, Siemens)

The platelet function analyzer is a Food and Drug Administration-approved device used to evaluate acquired or congenital platelet dysfunction and, most importantly, to screen for vWD. This device measures the time (closure time) required for platelets to plug an aperture in a membrane after platelet activation by collagen and epinephrine or by collagen and adenosine diphosphate. This closure time is sometimes referred as the “in vitro bleeding time” and is performed using whole blood (Harrison 2005). Closure time is prolonged in cases of anemia or thrombocytopenia or following the intake of drugs that alter platelet function by inducing acquired thrombopathy.

Light Transmission Platelet Aggregometry

Platelet aggregation assays are indicated for the investigation of inherited or acquired qualitative platelet disorders (Dawood et al. 2012).

These tests are useful in patients suspected with disease of primary hemostasis (purpura, cutaneo-mucous bleeding) with a normal platelet count. After centrifugation, the platelet-rich plasma is incubated with various aggregation activators. Activators commonly used for exploring the different activation pathways of platelets are arachidonic acid, ADP, collagen, TRAP (thrombin receptor agonist peptide), epinephrine, and ristocetin. The modification of light transmission due to platelet aggregation induced by the agonist is then studied. Platelet secretion may also be studied after platelet activation with several agonists. Light transmission platelet aggregometry is usually performed in qualified laboratories.

Diagnosis of von Willebrand Disease

vWD is the most frequent inherited bleeding disorder. The diagnosis is based on bleeding symptoms associated with qualitative or quantitative defects of vWF. The patient's bleeding history, in particular, the family history, is of most importance for the diagnosis of vWD. A panel of assays are available (Favaloro 2009):

- vWF antigen measures the amount of vWF; plasma vWF levels vary with blood group; O blood group patients usually have lower vWF levels (up to 40 %) than individuals of other blood groups.
- Factor VIII coagulant activity measures the functional activity of factor VIII.
- vWF ristocetin cofactor activity and vWF collagen-binding activity measure the functional activity of vWF.

Further assays are necessary for the diagnosis of subtypes:

- Factor VIII binding assay measures the affinity of vWF for factor VIII (useful in type 2 N vW disease).
- vWF multimer analysis shows how the vWF monomer is multimerized (joined into chains).
- Ristocetin-induced platelet agglutination measures the sensitivity of vWF to ristocetin (useful in type 2B vW disease).

Monitoring of Antithrombotic Treatment

Several tests are routinely available for assessing the action of anticoagulants such as heparins or vitamin K antagonist. New direct oral anticoagulants (specific inhibitors of thrombin or factor Xa) cannot be monitored using standard coagulation assays and require specific tests. If necessary with regard to antiplatelet therapy, a number of specialized assays are available to assess platelet inhibition, but their utility in clinical and routine practice remains to be determined.

Heparin Monitoring

Since the pharmacodynamic profile of unfractionated heparin (UFH) is poorly predictable, monitoring its anticoagulant effect is essential. The pharmacodynamic profile of low molecular weight heparins (LMWH) is more predictable, and monitoring should be considered only in selected cases (e.g., mild renal insufficiency or extremely high or low body weight).

Unfractionated Heparin (UFH)

aPTT is widely used for monitoring UFH. The target time range is two or three times longer than the basal aPTT value. It must be stressed that the aPTT clotting assay depends on several coagulation factors. Thus, in some instances, aPTT can under- or overestimate the degree of anticoagulation conferred by UFH. This may occur in the case of an important inflammatory syndrome with a very short basal aPTT or in cases of factor deficiency or lupus anticoagulant with longer basal aPTT, for example. In such situations, the use of a specific chromogenic assay that measures the activity of factor Xa provides a more reliable assessment of the anti-coagulant effect. Anti-Xa activity measurement requires calibration: a standard curve is constructed using different plasma samples with different concentrations of heparin. After adding known quantities of factor Xa, any residual Xa activity is inversely proportional to the concentration of heparin in the sample. The result can be reported either in international units of heparin per ml (IU/ml) or in anti-Xa units per ml (anti-Xa U/ml).

Low Molecular Weight Heparin (LMWH)

LMWHs have a poor antithrombin effect and do not usually affect aPTT. The anti-Xa assay is thus required for monitoring with specific calibrators. Anti-Xa levels are usually measured 3–5 h after a dose of LMWH, when its concentration in the blood is expected to be at its highest level (peak level). Residual anti-Xa tests may also be carried out when accumulation is suspected (e.g., in renal failure).

Fondaparinux and Danaparoid Monitoring

Fondaparinux is a synthetic direct Xa-inhibitor that does not usually require monitoring. In some cases, anti-Xa measurement may be prescribed when there are concerns that fondaparinux may be accumulating. A fondaparinux standard curve is required for reporting fondaparinux levels when using an anti-Xa assay.

Danaparoid is a heparinoid containing heparan sulfate, dermatan sulfate, and chondroitin sulfate. Its activity should be monitored using an anti-Xa assay with danaparoid calibrators.

Vitamin K Antagonist Treatment

See Sect. [2.3.3](#).

Direct Oral Anticoagulants(DOACs)

Direct oral inhibitors of thrombin (dabigatran) or of factor Xa (rivaroxaban, apixaban) cannot be monitored using simple standardized laboratory assays. At pharmacological doses, these drugs may interfere on different ways with aPTT and PT (depending of the reagents), but aPTT and PT are poorly correlated with NOAC blood concentrations. It should be noted that the standard INR refers to VKA treatment and cannot evaluate the effectiveness of NOACs: the therapeutic ranges of INR used to monitor VKA do not apply to NOACs.

Specific assays for the measurement of NOAC plasma concentrations are available in specialized laboratories and should be ordered in case of hemorrhagic events or emergent surgeries (Sié et al. 2011; Pernod et al. 2013).

Standard Tests and Predicting Perioperative Bleeding Risk

The goal of preoperative screening for congenital or acquired hemostatic disorders is to prevent perioperative hemorrhagic complications by using appropriate medical and surgical management. When prescribing hemostatic tests prior to invasive procedures, the objective should be to identify those patients with an increased risk of perioperative bleeding. Unfortunately, the standard tests (PT, aPTT, platelet count) have very low positive predictive values for bleeding risk in the general population (Chee et al. 2008; Segal et al. 2005).

The percentage of abnormal test results depends on the indication (systematic testing or clinically indicated), on the reference values, and on the types of patients (Kitchens 2005):

- In selected patients (hemostasis assessment clinically indicated), the percentage of abnormalities may be up to 40 %.
- In patients not selected on the basis of history and clinical examination, standard hemostatic testing revealed 0.5–16.0 % abnormalities.

The performance of systematically prescribed standard hemostatic tests in predicting the bleeding risk during surgery or other invasive procedure is very poor since normal results do not preclude the possibility of hemostatic disease or perioperative hemorrhage.

References

- Chee YL, Crawford JC, Watson HG et al (2008) Guidelines on the assessment of bleeding risk prior to surgery or invasive procedures. British Committee for Standards in Haematology. *Br J Haematol* 140:496–504
- Dawood BB, Lowe GC, Lordkipanidze M et al (2012) Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel. *Blood*. doi:[10.1182/blood-2012-07-444281](https://doi.org/10.1182/blood-2012-07-444281)

References

- Dieri AI R, de Laat B, Hemker HC (2012) Thrombin generation: what have we learned? *Blood Rev* 26:197–203
- Favaloro EJ (2009) Toward a new paradigm for the identification and functional characterization of von Willebrand disease. *Semin Thromb Hemost* 35:60–75
- Harrison P (2005) The role of PFA-100 testing in the investigation and management of haemostatic defects in children and adults. *Br J Haematol* 130:3–10
- Hemker HC, Dieri AI R, De Smedt E, Béguin S (2006) Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost* 96:553–561
- Hoffman MM, Monroe DM (2005) Rethinking the coagulation cascade. *Curr Hematol Rep* 4:391–396
- Kamal AH, Tefferi A, Pruthi RK (2007) How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. *Mayo Clin Proc* 82:864–873
- Kitchens CS (2005) To bleed or not to bleed? Is that the question for the PTT? *J Thromb Haemost* 3:2607–2611
- Lippi G, Salvagno GL, Ippolito L et al (2010) Shortened activated partial thromboplastin time: causes and management. *Blood Coagul Fibrinolysis* 21:459–463
- Lippi G, Salvagno GL, Montagnana M et al (2012) Quality standards for sample collection in coagulation testing. *Semin Thromb Hemost* 38:565–575
- Olson JD (1999) Addressing clinical etiologies of a prolonged aPTT. *CAP Today* 13:28, 30, 32 passim
- Pernod G, Albaladejo P, Godier A et al (2013) Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: proposals of the working group on perioperative haemostasis (GIHP) – March 2013. *Arch Cardiovasc Dis* 106:382–393
- Segal JB, Dzik WH, Transfusion Medicine/Hemostasis Clinical Trials Network (2005) Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. *Transfusion* 45:1413–1425
- Sié P, Steib A (2006) Central laboratory and point of care assessment of perioperative hemostasis. *Can J Anaesth* 53:S12–S20
- Sié P, Samama CM, Godier A et al (2011) Surgery and invasive procedures in patients on long-term treatment with direct oral anticoagulants: thrombin or factor-Xa inhibitors. Recommendations of the Working Group on perioperative haemostasis and the French Study Group on thrombosis and haemostasis. *Arch Cardiovasc Dis* 104:669–676
- Wayne PA. *Collection, Transport, and Processing of Blood Specimens for Testing of Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline*, 5th edn. CLSI H21A5. Clinical and Laboratory Standards Institute, 2008:28.
- Mackie I, Kitchen S, Machin S, Lowe GDO. Guidelines on fibrinogen assays. *Br J Haematol* 2003; 121: 396–400.
- Marlar RA, Cook JC, Johnston M, et al. *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline*, 2nd edn. CLSI H47A2. Clinical and Laboratory Standards Institute, 2008.
- Hubbard AR, Dodt J, Lee T, et al. Factor VIII and Factor IX Subcommittee of the Science and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haem* 2013; 11: 988–989.
- Kohler HP, Ichinose A, Seitz R, et al. on behalf of the FXIII and Fibrinogen SSC Subcommittee of the ISTH. Diagnosis and classification of factor XIII deficiencies. *J Thromb Haem* 2011; 9: 1404–1406.
- Jennings I, Cooper P. Screening for thrombophilia: a laboratory perspective. *Br J Biomed Sci* 2003; 60: 39–51.
- Walker ID, Greaves M, Preston FE. Investigation and management of heritable thrombophilia. *Br J Haematol* 2001; 114: 512–518.
- Kitchen S, Gray E, Mackie I, et al. Measurement of noncoumarin anticoagulants and their effects on tests of haemostasis: guidance from the British Committee for Standards in Haematology. *Br J Haem* 2014; 166: 830–841.

Vasculopathiák, thrombocyta rendszer zavarai - tünetek

- ▶ Petecchiák
- ▶ Purpurák
- ▶ orr-, fogíny-,
nyálkahártya-
vérzések
- ▶ sc.
haematomák



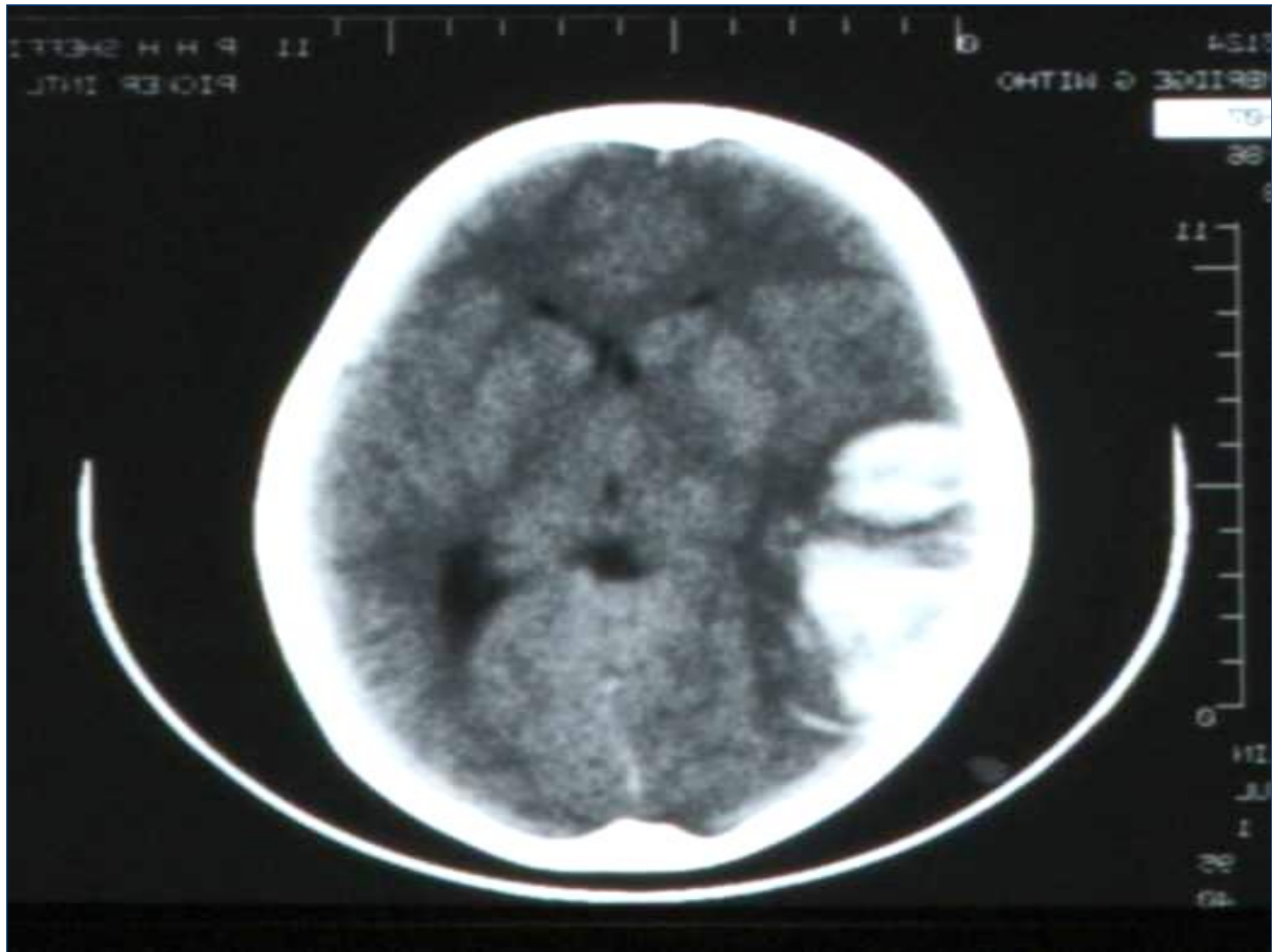
Petechiák & purpurák



Nyálkahártya vérzések



Agyvérzés



Coagulopathiák: ízületi bevérzések, intracranialis vérzés



Coagulopathiák: haematomák, intramuscularis vérzések, GI vérzés, haematuria



Jellemző vérzéstípusok

	Koagulopathiák	Vasopathiák és thrombocytopathiák
Purpura:	Ritka	Jellemző
Haematoma:	Jellemző	Ritka
Haemarthros:	Jellemző	Ritka
Utóvérzés:	Gyakori	Ritka
Vérzés felszínes vágásokból:	Minimális	Perzisztáló
Beteg neme:	80-90 % ffi	80-90% nő
Pozitív családi anamnesis:	Gyakori	Ritka

Javasolt vizsgálatok vérzékenység esetén

- Anamnézis, fizikális vizsgálat
- Vértkép, vércsoport
- Klinikai kémia (májfu., vesefu.)
- Rumpel-Leede teszt
- Vérzési idő/PFA-100
- Alap alvadási paraméterek (szűrés): APTI, TI, PT, fibrinogén; keveréses tesztek
- Thrombocyta-aggregometria (Born vagy impedancia)

Javasolt vizsgálatok vérzékenység esetén

- VWD diagnosztika (APTI nem mindig megnyúlt! Vérzési idő sem!)
- Specifikus alvadási tesztek
 - Izoláltan megnyúlt APTI esetén: FVIII, FIX, FXI, FXII (+APA, LA!)
 - Izoláltan megnyúlt PI esetén: FVII
 - Mindkettő megnyúlt: FV, FX, FII, ill. K-vitamin dep. faktorok
 - TI is megnyúlt: fibrinogén, D-dimer, heparinok
 - Inhibitor kimutatás
 - Anti Xa teszt, NOAC kimutatás
- ROTEM: globális teszt
- FXIII meghatározás: anamnesis alapján (különböző fokú vérzékenység – spec: KIR vérzés, infertilitás, köldökcsonk vérzés, sebgyógyulási zavar) A szűrőtesztek nem mutatják ki!

Primer haemostasis vizsgálómódszerek

- Rumpel-Leede teszt
- Vasculopathiák kimutatására alkalmas módszer
- Vérnyomásmérővel pangást hozunk létre a karban – a megjelenő petecchiák számát vizsgáljuk



Vérzési idő – Ivy szerint



Vérzési idő

- 1912 Duke – fülcimpa
- 1941 Ivy – alkar volaris felszíne, 40 Hgmm
- 1969 Meckel – Template standardizáció
- Ref. Tart: 4-9 min
- Hátrányok:
 - Nehéz standardizálni, nem konzisztensek az eredmények
 - Nincs összefüggés a sebészi vérzéssel
 - Hegképződés

Table 1 A summary of the types of platelet function tests currently available and used in diagnostics*

Procedure	What it measures/detects	Strengths/benefits/advantages	Limitations/weaknesses/disadvantages
Light transmission aggregometry (LTA)	Low shear platelet-to-platelet aggregation in response to a range of agonists and concentrations	Gold standard. Widely used in specialized laboratories. High publication and evidence base around usage	Time-consuming, complex, sample preparation, poorly standardized, and requires specialized equipment. Limited IQC and EQA
Whole blood aggregometry (WBA)	Monitors changes in impedance in response to a range of agonists, sometimes including a range of agonist concentrations	Simplified whole blood test, multichannel version available. Widely used in specialized laboratories although less than LTA	Dependent on platelet count, older instruments require electrodes to be cleaned and recycled. Simplified system has limited utility in diagnostics, and perhaps has some utility in monitoring anti-platelet therapy. Requires specialized equipment. Not standardised. Limited IQC and no EQA available
Lumi-aggregometry	Combines LTA or WBA with measurement of nucleotide release	Monitors release reaction with secondary aggregation. Widely used in specialized labs, although less than LTA or WBA	Semiquantitative. Requires specialized equipment. Not standardised. Limited IQC and no EQA available
PFA-100/-200	High-shear platelet adhesion and aggregation during formation of a platelet plug	Whole blood test, high shear, small blood volumes, simple, rapid, POC feasibility. Very sensitive to VWD. Widely used. EQA available	Inflexible; VWF, hematocrit and platelet count dependent, meaning not specific for platelet function. Requires specialized equipment. May miss some forms of mild VWD (e.g., mild type 1, alternatively called 'low VWF as a cause of bleeding')
Flow cytometry	Measurement of platelet glycoproteins and activation markers by fluorescence	Whole blood test, small blood volumes, wide variety of tests. Increasingly used in specialized labs	Specialized operator, expensive, samples prone to artifact unless carefully prepared. Mainly in realm of research at the moment. Not standardised. Requires specialized equipment. Limited IQC and no EQA available

*Table is not meant to be an exhaustive list of options, but rather expresses the main procedures used in diagnostics for platelet function assessment. Many other procedures may also be used to monitor anti-platelet therapies and/or in research settings. For a detailed listing, please refer to other excellent reviews (3,6,7,14). IQC, internal quality control; EQA, external quality assessment; POC, point of care; PFA, platelet function analyser; VWD, von Willebrand disease; VWF, von Willebrand factor.

Table 3 Expected PFA-100/200 test patterns for different clinical scenarios^a

C/Epi	C/ADP		
	Normal	Mildly Prolonged	Grossly Prolonged (or non-closure)
Normal	Normal (mild defect ^b)	Rare event	Shouldn't happen (repeat tests)
Mildly prolonged	Aspirin, mild defect ^b , mildly reduced hematocrit +/- platelet count	Mild defect ^b , mildly reduced hematocrit +/- platelet count	Shouldn't happen (repeat tests)? Severe defect ^c
Grossly prolonged (or non-closure)	Aspirin	Moderate to severe defect ^c , reduced hematocrit +/- platelet count (aspirin)	Severe defect ^c , severely reduced hematocrit +/- platelet count, gross sample hemolysis

a, table summarizes expected PFA-100/-200 test patterns for various clinical scenarios as may be encountered by laboratories undertaking PFA-100/200 testing. b, for example, mild type 1 von Willebrand disease, mild platelet dysfunction. c, for example, type 2A, 2B, 2M, or 3 von Willebrand disease, severe platelet dysfunction.

Bleeding Time Abnormalities:

Collagen disorders	e.g. Ehlers Danlos syndrome
Thrombocytopaenia	It is important to check the platelet count before performing a bleeding time. A platelet count of $<50 \times 10^9/L$ is generally considered to prolong the BT.
Qualitative platelet disorders	Inherited and acquired platelet disorder including the use of anti-platelet drugs such as aspirin and clopidogrel will prolong the BT. However, the BT cannot reliably predict the risk of peri-operative bleeding in patients taking these drugs. Paraproteinaemias can also lead to defective platelet function and may, therefore, prolong the BT. Other acquired disorders of platelet function such as seen in uraemia and the myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD) will also prolong the BT.
Von Willebrand Disease (VWD)	A deficiency of Von Willebrand Factor (VWF) may prolong the BT but not in all cases. The BT is no longer recommended as a test for the diagnosis of Von Willebrand Disease (VWD)- see UKHCDO guidelines on Diagnosis of VWD.
Severe anaemia	In patients with anaemia, there is a change in the distribution of platelets and a decreased interaction of the platelets with the vascular endothelium resulting in a prolonged BT. Correction of the anaemia will improve the BT.
Hypofibrinogenaemia	Fibrinogen is required for platelet-platelet interaction and the BT will, therefore, be prolonged in cases of hypofibrinogenaemia.

PFA100/200

- Platelet function analyser
- Point-of-care
- Minta: citráttal antikoagulált teljes vér
- A high-shear-dependens TCT funkciót méri
- $TCT > 80$, $HTC > 30\%$
- Cartridge membrán
 - Coll-epi (screening)
 - Coll-ADP (diff)



PFA100

Advantages of the PFA-100 include:

Only **small volumes of citrated venous blood** [800 μ L) are needed and so the test is useful for investigating platelet function in children.

Can be used by non-skilled personnel and is both **rapid and automated**

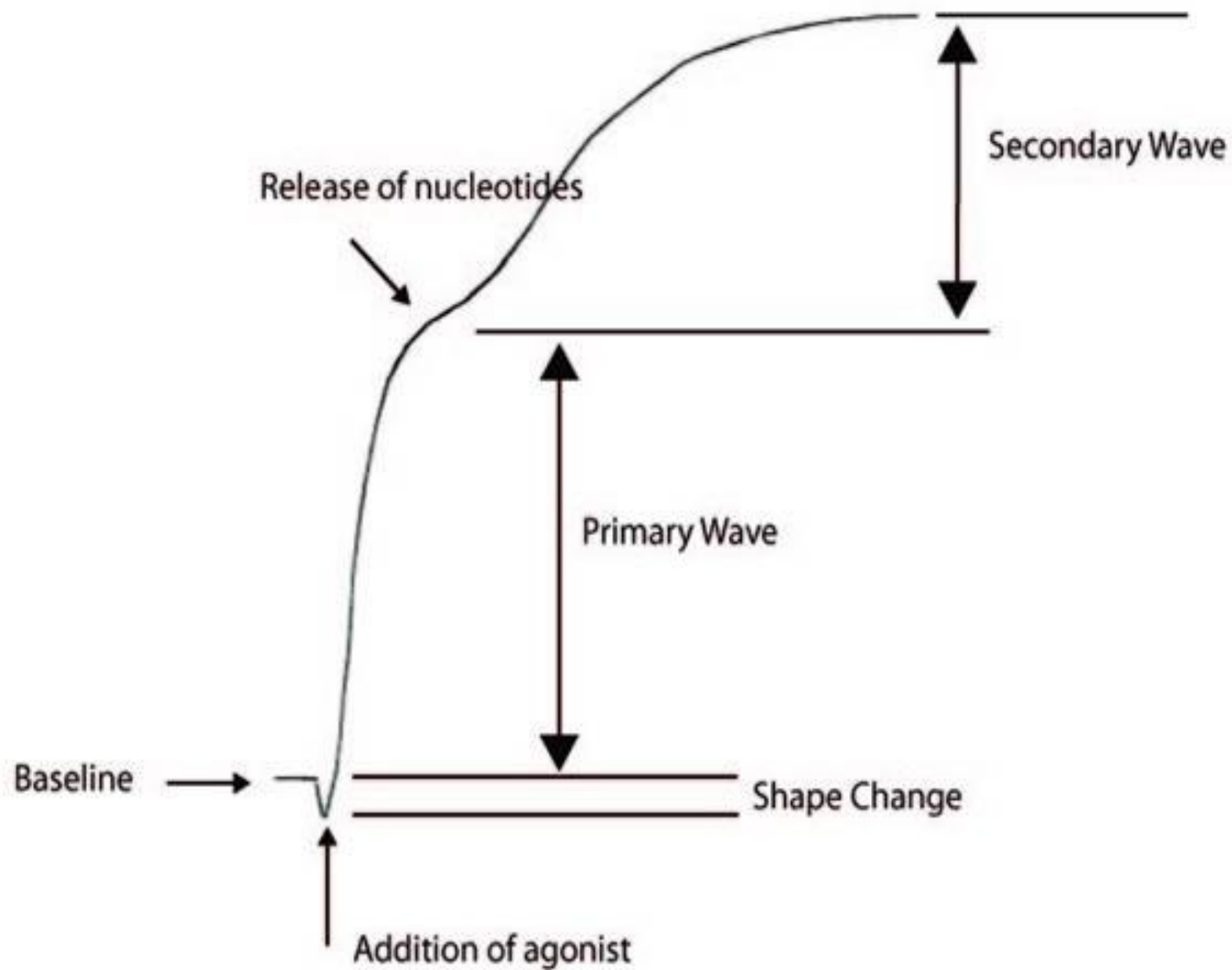
The PFA-100 was designed as a screen to detect problems with primary haemostasis and in part to **replace the bleeding time and in this respect it is better standardised.**

Measurement of platelet function at **high shear [physiological] rates** whereas LTA measures platelet function at low shear rates i.e. less physiological.

Relatively insensitive to clotting factor deficiencies

High negative predictive value – i.e. if the PFA-100 gives a normal result then with some exceptions primary haemostasis is intact [Exceptions: SPD, Primary Secretion Defects, mild Type 1 VWD]

Disorder	CT Collagen-ADP	CT Collagen-EPI
Normal	N	N
Aspirin and NSAIDs	N	↑
ADP receptor disorders including the use of Clopidogrel	N or ↑	N or ↑
BSS	↑	↑
GTT	↑	↑
VWD	↑	↑
Platelet-Type VWD	↑	↑
Dense Granule Deficiency	N or ↑	N or ↑
Primary Secretion Defects	N or ↑	N or ↑
Gray Platelet Syndrome	↑	↑
MYH9-related Disorders	N	↑
Scott Syndrome	N	N
MDS	N or ↑	N or ↑
Liver Disease	↑ [possibly as a result of ↓Hb]	↑ [possibly as a result of ↓Hb]
Uraemia	↑ [possibly as a result of ↓Hb]	↑ [possibly as a result of ↓Hb]



TCT agonisták

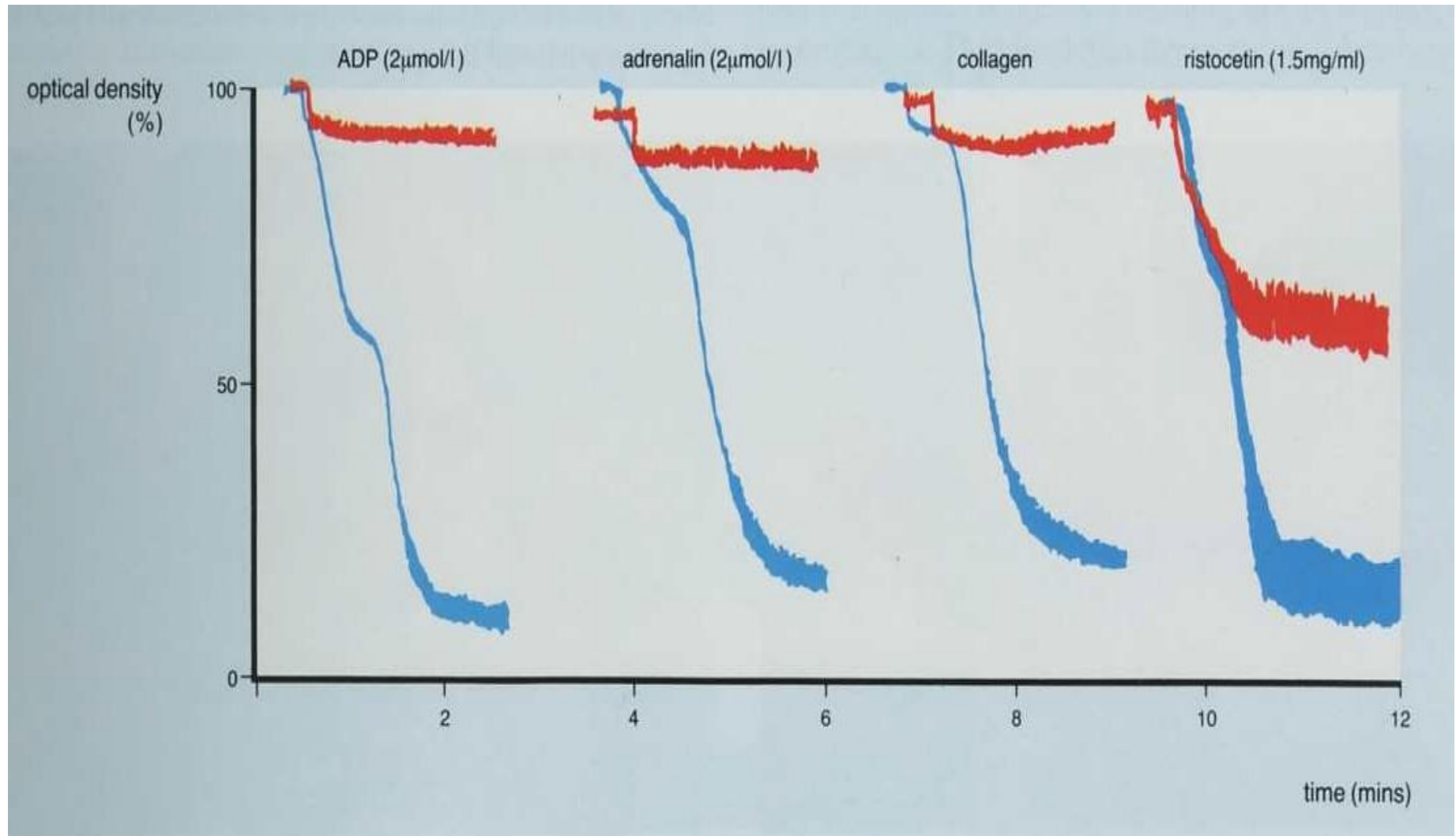
Agonist	Working Concentration
ADP	Low dose: 1, 2.5, 5 μ M High dose: 10 μ M
Collagen	1, 4 μ g/mL
Ristocetin	Low dose: 0.5 mg/mL High dose: 1.5, 5 mg/mL
Adrenaline	5, 10 μ M
Arachadonic Acid	500 μ g/mL
Thrombin	Low dose: 50 nmol/L High dose: 100 nmol/L

Light transmission aggregation (**LTA**) is the gold standard for the diagnosis of platelet function disorders (PFDs), but it is time-consuming and limited to specialized laboratories. Whole-blood impedance aggregometry (Multiplate) and platelet function analyzer (PFA) may be used as rapid screening tools to exclude PFDs.

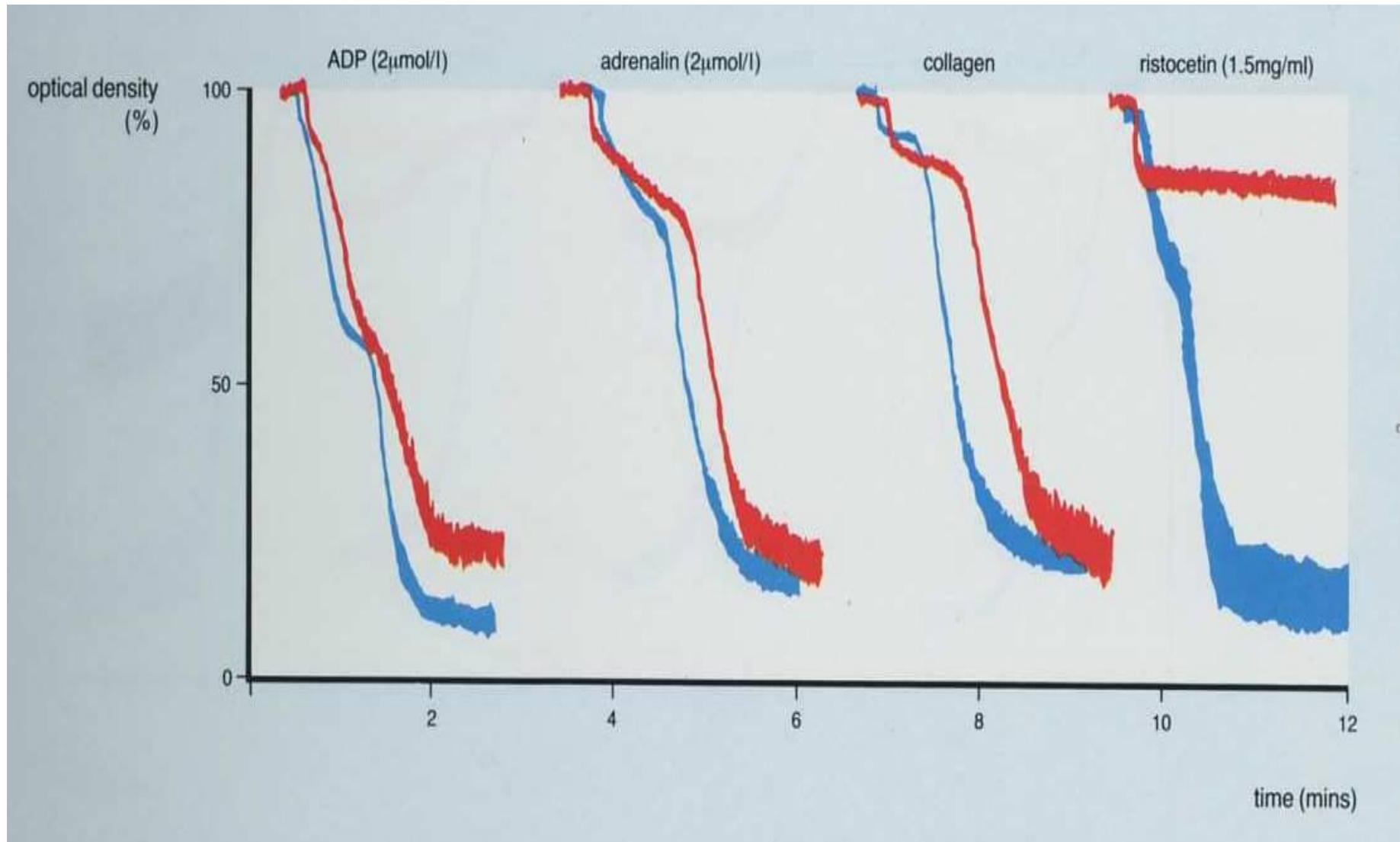
LTA

Disorder	Characteristic Findings on LTA
Glanzmann's Thrombasthenia OR afibrinogenaemia	Absent or markedly impaired aggregation to all agonists except ristocetin. Ristocetin-induced agglutination shows only primary wave - aggregation cannot occur because fibrinogen cannot bind. Afibrinogenaemia gives similar results.
Bernard Soulier Syndrome OR Von Willebrand Disease	Absent or markedly reduced platelet agglutination with ristocetin.
Storage Pool Disorder OR Platelet Release Defect	Primary aggregation only with ADP, adrenaline and collagen and only partial agglutination with ristocetin suggesting a failure of granule release or a deficiency of platelet granules.
Aspirin [or defects in the COX pathway]	Absent aggregation to arachadonic acid. Primary wave aggregation only with ADP. Decreased or absent aggregation with collagen.
Clopidogrel	Absent aggregation with ADP
2B VWD/Platelet-type [pseudo]VWD	Aggregation with low dose ristocetin e.g. 0.5 mg/mL.

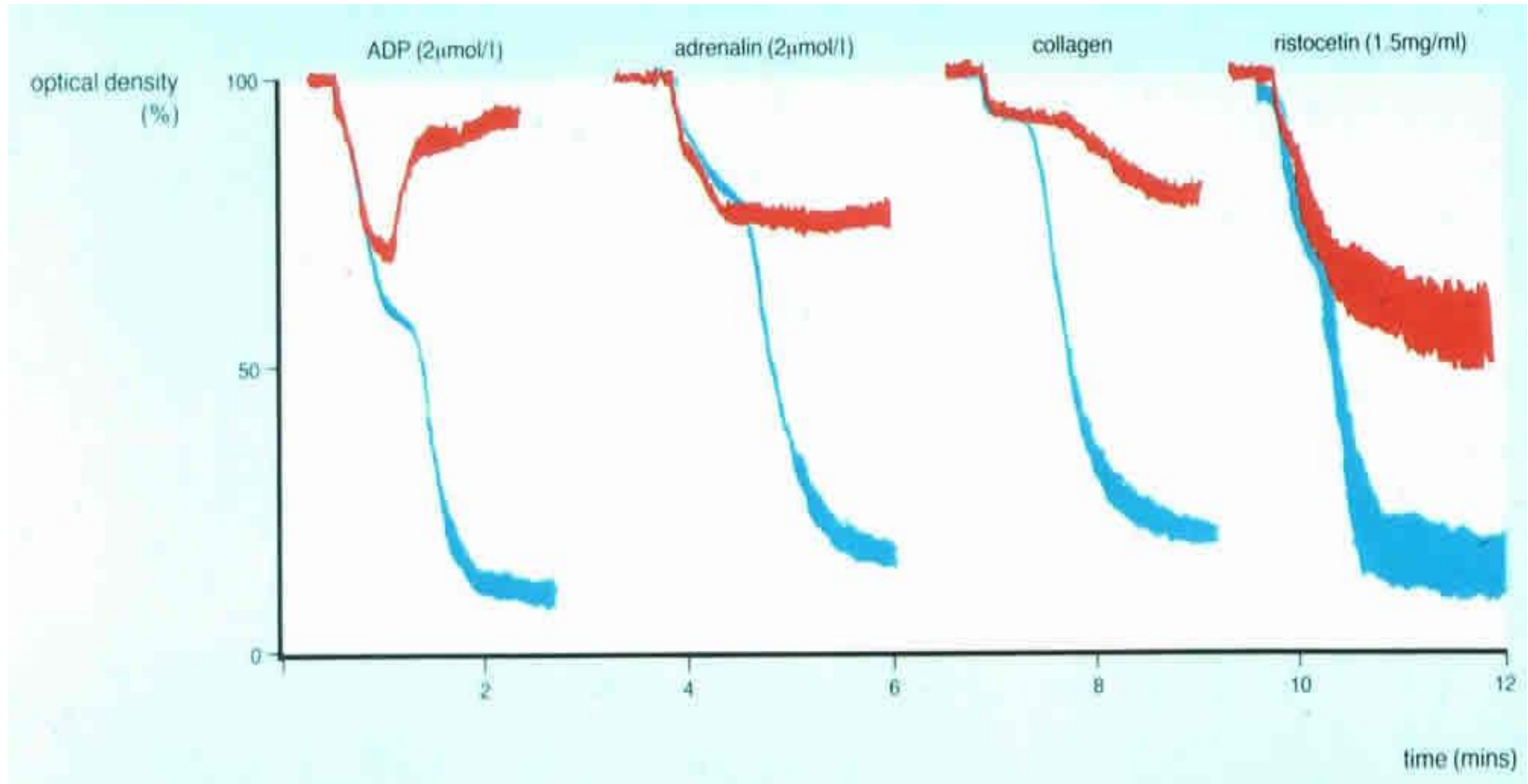
Glanzman-thrombasthenia



Bernard-Soulier Sy. Vs. VWD



Storage pool disease



Teljes vér aggregometria

○Előnyei:

- kisebb vérigény
- nincs szükség centrifugálásra (thrombocyta aktiváció, deszenzitizáció!),
- a vér összes alakos eleme, ezek interakciója vizsgálható

○Single platelet counting: idő- és munkaigényes, egy mintából több meghatározás szükséges, microaggregációt nem különbözteti meg a macroaggregációtól

(Fox et al, Thromb Haemost 1982; 48:327-9)

○Impedancia aggregometria: gyors, érzékeny, DE: drága, mérések között tisztítandó platina elektródákat használ

(Cardinal és Flower, J Pharmacol Methods 1980; 3:135-58)

○Multiple electrode aggregometry

- POC alkalmazásra lehetőség

Multiplate – „Multiple electrode aggregometry”

- Impedancia aggregometrián alapul
- Minta:
 - (Na₃-citráttal vagy)
 - **hirudinnal antikoagulált teljes vér**
- Az anticoagulált vért 1:1 arányban 0,9%-os NaCl-dal hígítjuk az eldobható küvettában
- 3 perc inkubálás 37°C-on keverés közben (PTFE-vel bevont mágneses keverő, 800 U/min)
- 6 perc mérés - az elektródák között detektálja a felrakódott vérlemezkék miatt kialakult ellenállásfokozódást

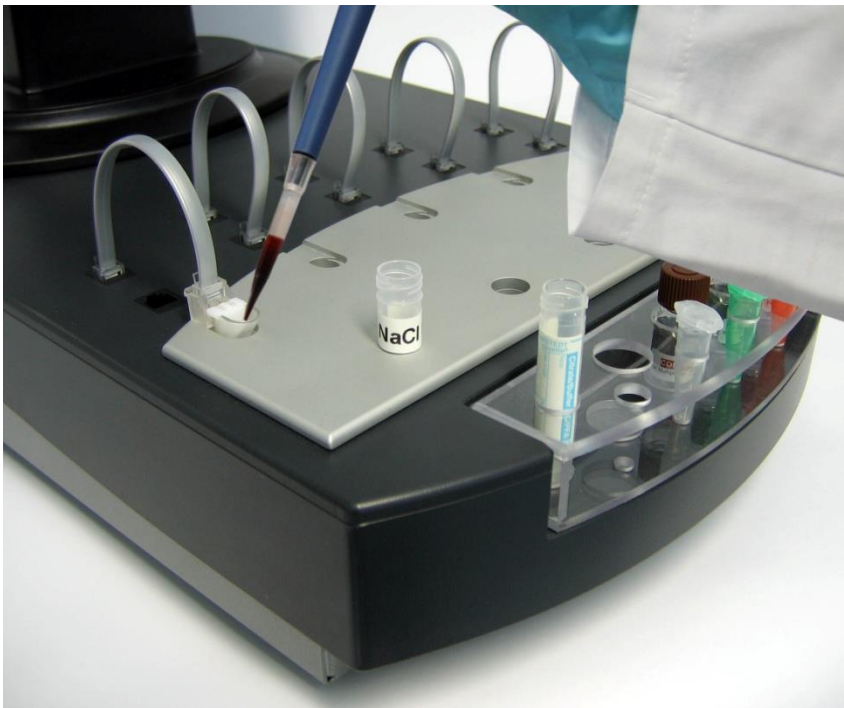
> Multiplate[®]- készülék

multiple
platelet
function
analyzer

- Kompact (10" x 15" x 4")
- 5 különálló csatorna
- Integrált Windows XP alapú computer
- Beépített automata pipetta



Tesztek kivitelezése



Előkészítés

Reagens feloldás

fiziológiás só előkészítése

> Multiplate® - Teszt kivitelezése

a küvétát a megfelelő helyre helyezzük



csatlakoztatjuk az érzékelő kábelt



300 µl fiz. só + 300 µl vért pipetázunk a küvétába



hozzáadjuk az aktivátort

3 perc inkubáció

6 perc után:

- eredményeket kinyomtatjuk
- a küvétát eldobjuk

Multiplate

- Előny: teljes vér, kis mennyiségű minta, POCT
- Hátrány: küvetta ára, bizonyos thrombocytopathiákra nem elég érzékeny
- Alkalmazási terület:
 - Thienopyridin kezelés monitorizálása (randomizált tanulmánnyal igazolt klinikai hatás) (4. Aradi, D. et. al. (2015), Throm Haemost, 113:221-230
5. Sibbing, D. et. al. (2017), Lancet, Aug 27 [e-pub])
 - Pre- ill. perioperatív vérzés predikciója és vérzés esetén haemostatikus kezelések monitorizálására

Tesztek

Teszt	Agonista	Fő alkalmazási terület
ASPItest	Arachidonsav (0.5 mM)	ASA és reverzibilis COX-1 gátlók
COLtest	Kollagén (3.2 ug/ml)	ASA és reverzibilis COX-1 gátlók
TRAPtest	TRAP-6 (32 uM)	GpIIb/IIIa gátlók (pl. tirofiban, eptifibatid)
ADPtest	ADP (6.5 uM)	ADP receptor antagonisták (direkt vagy thienopyridinek)
ADPtest HS	ADP+PGE1 (9.4 nM)	+ clopidogrel

Flow cytometria

- Monitoring of GpIIb/IIIa antagonist therapy
- Diagnosis of inherited deficiencies of platelet surface glycoproteins
- Diagnosis of storage pool disease
- Diagnosis of heparin-induced thrombocytopenia
- Diagnosis of Scott syndrome

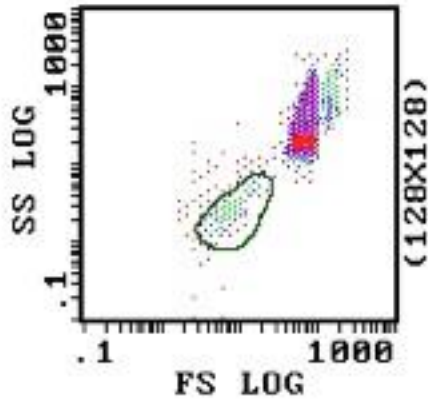
FCM

Antibody	Specificity
CD41	CD41 recognises the platelet membrane glycoprotein GpIIb (the integrin alpha IIb chain) which is non-covalently associated with GpIIIa (the integrin beta 3 chain) to form the GpIIb/IIIa complex.
CD61	CD61 recognises the platelet membrane glycoprotein GpIIIa (the integrin beta 3 chain).
CD42b	CD42b reacts with GpIb on megakaryocytes and platelets. CD42b also inhibits ristocetin-dependent binding of Von Willebrand Factor to platelets and ristocetin-induced platelet agglutination.

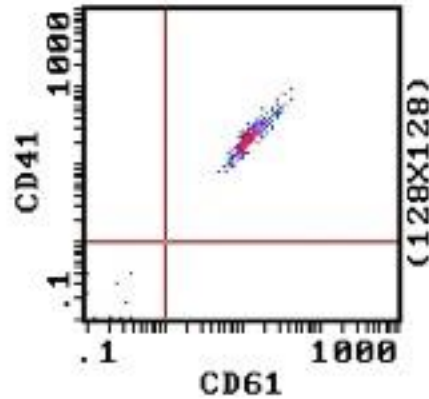
FCM – Glanzman thrombasthenia

Normal

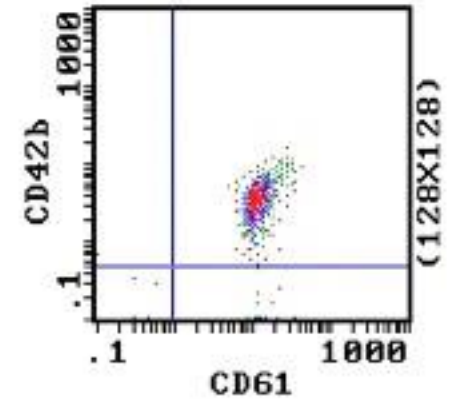
1:



2: Gated

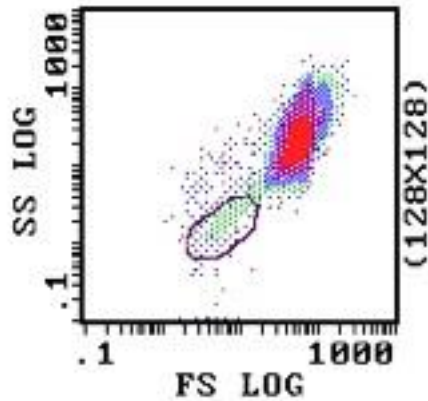


3: Gated

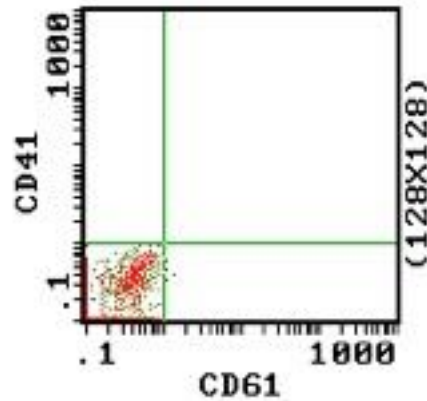


GT

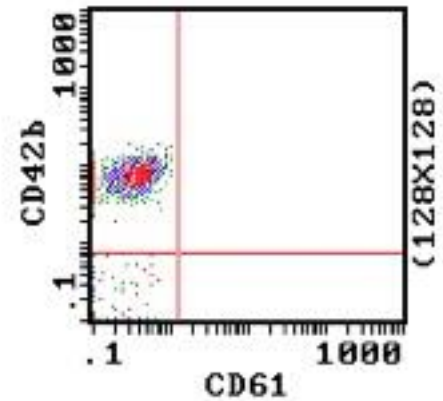
4:

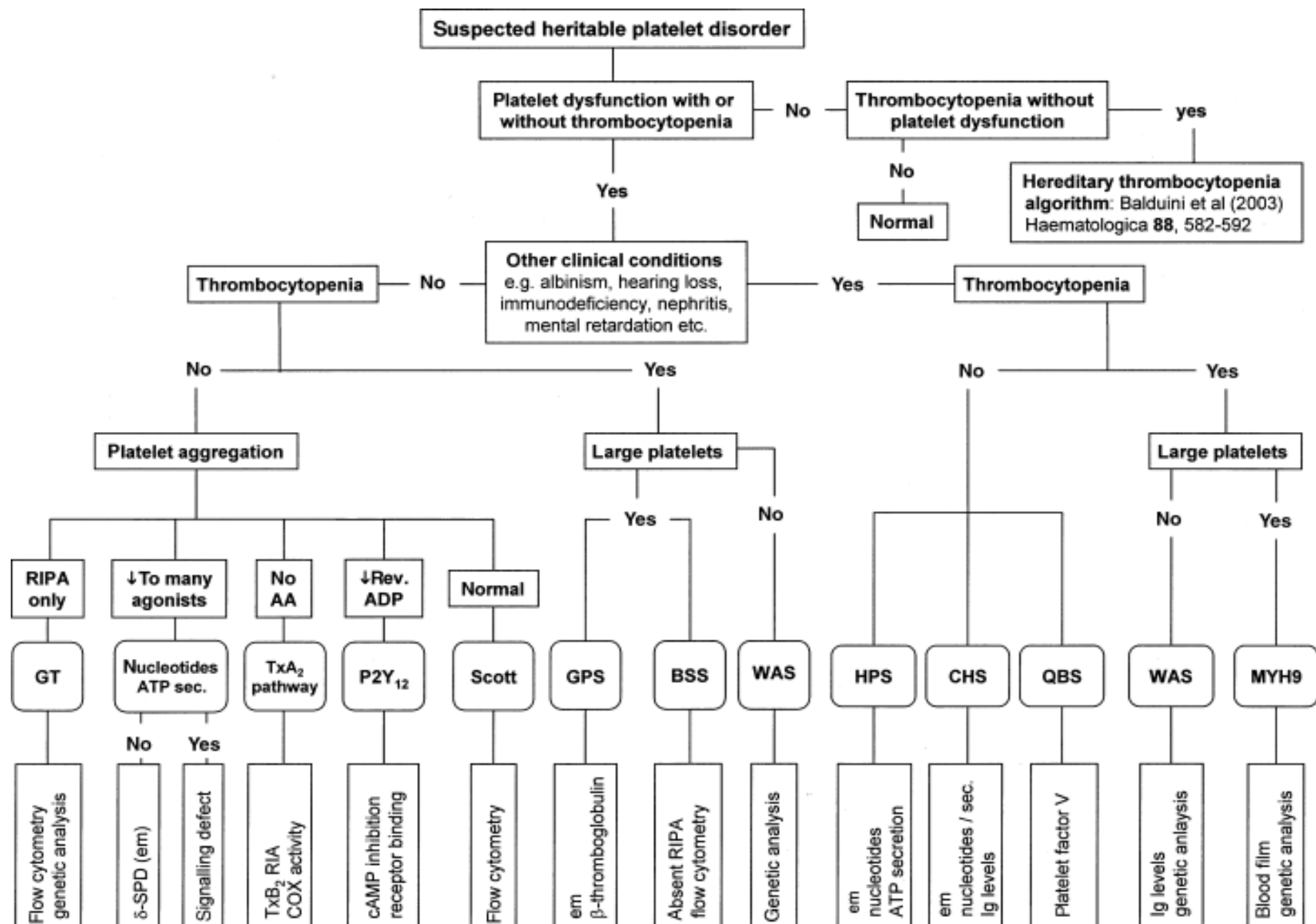


5: Gated



6: Gated





A suggested classification of the heritable platelet disorders

<i>Disorders of platelet number</i>				
MYH9 disorders				
May–Hegglin anomaly	155100	<i>MYH9</i>	<100	<1000
Sebastian syndrome	605249			
Fechtner syndrome	153640			
Epstein syndrome	153650			
Congenital amegakaryocytic thrombocytopenia	604498	<i>MPL</i>	<10	<100
Amegakaryocytic thrombocytopenia with radioulnar synostosis	605432	<i>HOXA11</i>	<10	<100
Thrombocytopenia absent radius syndrome	274000	Unknown	<10	<100
X-linked thrombocytopenia with dyserythropoiesis	300367	<i>GATA1</i>	<10	<100
<i>Severe disorders of platelet function</i>				
Wiskott–Aldrich syndrome	302000	<i>WAS</i>	<100	<1000
Glanzmann thrombasthenia	273800	<i>ITGA2B, ITGB3</i>	<100	<1000
Bernard–Soulier syndrome	231200	<i>GP1BA, GPIBB, GP9</i>	<100	<1000
<i>Disorders of receptors and signal transduction</i>				
Platelet cyclo-oxygenase deficiency	605735	Unknown	<10	<100
Thromboxane synthase deficiency	274180	Unknown	<10	<100
Thromboxane A2 receptor defect	188070	<i>TBXA2R</i>	<10	<100
ADP receptor defect (P2Y ¹²)	600515	<i>P2RY12</i>	<10	<100
<i>Disorders of the platelet granules</i>				
Idiopathic dense-granule disorder (δ -storage pool disease)	185050	Unknown	<100	<1000
Hermansky–Pudlak syndrome	203300	<i>HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, HPS8</i>	<100	>1000
Chediak–Higashi syndrome	214500	<i>LYST</i>	<100	<1000
Grey platelet syndrome	139090	Unknown	<10	<100
Paris–Trousseau/Jacobsen syndrome	188025 and 147791	<i>11q23 deletion (FLI1)</i>	<10	<100
Idiopathic α - and dense-granule storage pool disease	185050	Unknown	<100	<1000
<i>Disorders of phospholipid exposure</i>				
Scott syndrome	262890	<i>ABCA1</i>	<10	<10

Sec. haemostasis vizsgálómódszerek

- Alap alvadási paraméterek/screening
 - PT
 - aPTT
 - TT
 - Fibrinogén

Véralvadási mérések

- Mintavétel:
 - Éhgyomorra
 - Trinatrium-citráttal kell alvadásgátlolni (3,8 % 1:9 arányban, azonnal keverni)
- Mintaelőkészítés:
 - Centrifugálás szobahőn 2000 g 10 perc → PPP
 - 4 órán belül fel kell dolgozni a mintát, vagy azonnal lefagyasztani
 - -20 °C 1 hónap
 - -80 °C 1 év

Alapvető véralvadási vizsgálatok

Vizsgálat	mechanizmus	kóros érték
prothrombin idő (PTR)	extrinsic út közös út	K vitamin dependens fakt. ↓ májbetegség DIC , Syncumar th.
APTI	intrinsic út közös út	hemofília A,B ,Von Willebrand sy. keringő antikoaguláns heparin th. DIC, XI-es XII-es , X –es fakt,↓ fibrinogén ↓
thrombin idő (TI)	fibrinogén- fibrin átalakulás	FDP ↑ DIC, heparin hatás hypofibrinogenaemia
Vérzési idő	primer hemosztázis tct. funkciók	tct. funkciós zavar tct-penia, von Willebrand sy.

Alapalvadási paraméterek

- PI (prothrombin idő)
 - Plasma (PPP) + thromboplastin (TF) + PL+ Ca
 - Extrinsic és közös út (FVII, FX, FV, FII)
 - Különböző reagenseknek különböző az érzékenysége az egyes faktorokra
- $INR = (\text{beteg PI} / \text{normál PI})^{ISI}$
 - Cumarin kezelés monitorizálására

Abnormality	Interpretation
Isolated Prolonged PT	Factor VII deficiency
Prolonged PT in association with other coagulation abnormalities	<p>Vitamin K deficiency</p> <p>Vitamin K antagonists e.g. warfarin, phenindione, rodenticides</p> <p>Liver disease</p> <p>Malabsorption (leading to vitamin K deficiency)</p> <p>High concentrations of unfractionated heparin</p> <p>Direct thrombin inhibitors e.g. Lepirudin, argatroban</p> <p>Afibrinogenaemia and dysfibrinogenemia</p> <p>Dilutional coagulopathy e.g. massive blood transfusion</p> <p>Multiple clotting factor deficiencies e.g. FV and FVIII deficiency</p> <p>Abnormalities of the vitamin K cycle e.g. mutations within the <i>VKORC1</i> gene</p> <p>Chromosomal aberrations - the <i>F7</i> and <i>F10</i> genes are located on the long arm of chromosome 13 - deletions of which are associated with reduced FVII and FX levels.</p>
Shortened PT	Following treatment with rVIIa

Alapalvadási paraméterek

- APTI
- PPP + kontakt aktivátor (kaolin, Micronized silica, Celite, Ellagic acid) + phospholipid + Ca
- Intrinsic és közös út
- Izolált megnyúlás: FVIII, IX, XI, XII hiány; LA, heparin, inhibitorok
- PI + APTI megnyúlás: ld. Fenn
- Különböző reagenseknek különböző az érzékenysége az egyes faktorokra (20-40%)
- Megnyúlás esetén teendő: keveréses teszt

Alapalvadási paraméterek

- TI (thrombin idő)
 - Plasma + thrombin + Ca
 - Fibrinogén, heparin, FDP
- Reptilase idő: módosított TI, a thrombint reptilázzal helyettesítik (kígyóméregből izolált enzim – Bothrops atrox – fibrinopeptin A lehasítása)

Lándzsakígyó



	Thrombin Time	Reptilase Time
Presence of unfractionated heparin	↑	Normal
Presence of LMWH	May show some prolongation	Normal
Presence of direct thrombin inhibitors	↑	Normal
Warfarin	Normal	Normal
Decreased/absent fibrinogen	↑	↑
Dysfibrinogenaemia	↑	↑
DIC	↑	↑
Liver disease	↑	↑
Heparin-like anticoagulants	↑	Normal
Paraproteinaemias	↑	↑
Thrombolytic therapy	↑	↑
Neonate	↑	↑
Amyloid	↑	↑
Hyperfibrinogenaemia	↑	↑
Hypoalbuminaemia	↑	↑

Fibrinogén szint meghatározás

- Clauss módszer: funkcionális assay, hígított plazmában thrombin aktiváció után mérik a fibrinképződésig eltelt időt (kalibrátor)
- Vagy: a prothrombin idő mérésből származtatott érték (nephelometria)
- Vagy: immunológiai módszerrel a fibrinogen antigén szint mérésére (dysfibrinogenaemia esetén diszkrepancia!)
- Csökkent fibrinogen szint okai:
 - Veleszületett: hypofibrinogenaemia, afibrinogenaemia
 - Szerzett: májbetegség, DIC, dilutio, thrombolysis után,
 - Qualitativ zavar: dysfibrinogenaemia (ritka), májbetegség: túlzott glycolyzáció, emelkedett FDG szint
- Emelkedett szint: gyulladás, tumor, terhesség, női nem, OC, idős kor.

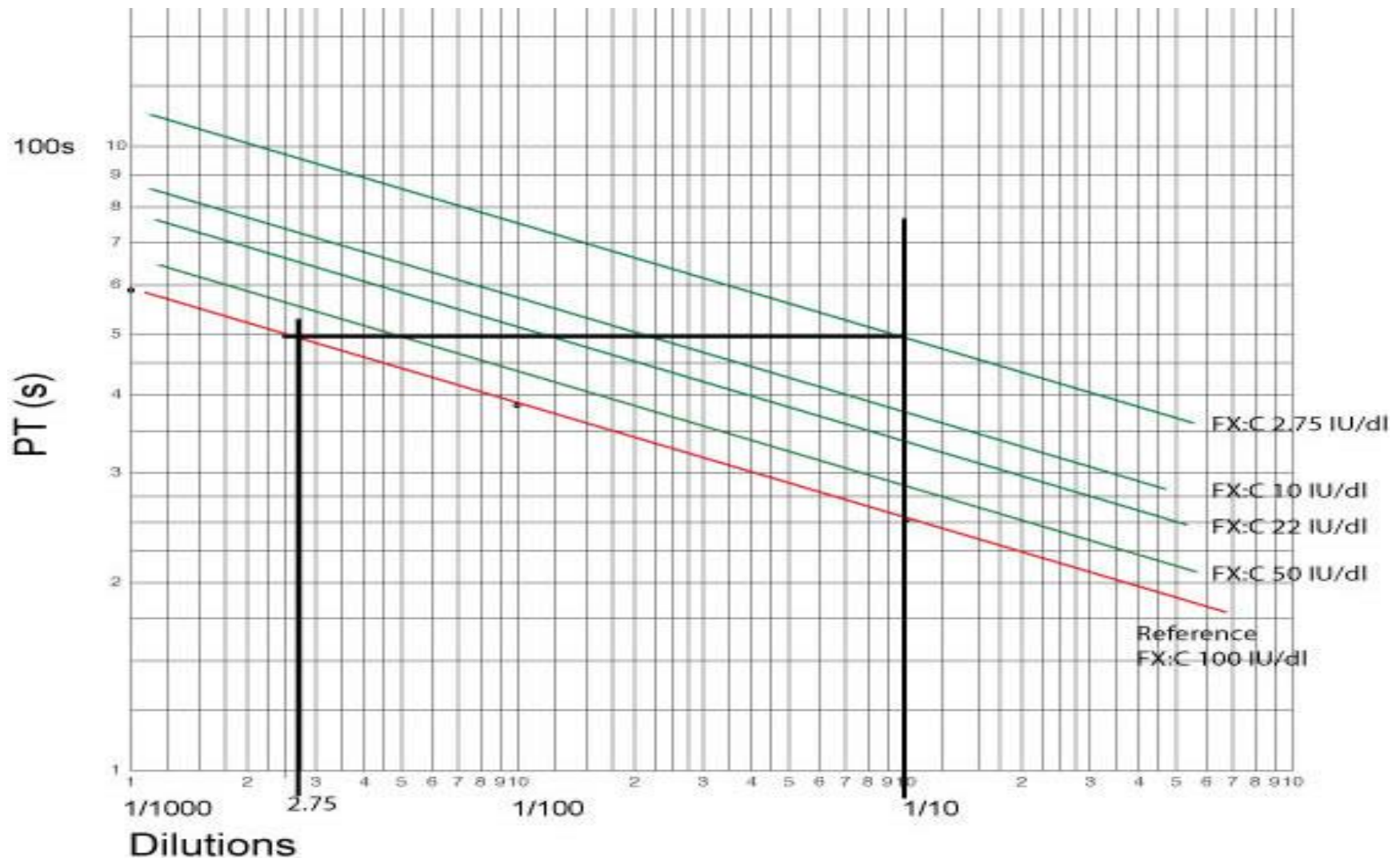
Vizsgálómódszerek

- Vérzési idő – Ivy szerint (standardizált módszer)
- Alapalvadási paraméterek
 - PTR
 - aPTI
 - TI
 - Fibrinogén
- Keveréses teszt
- Faktormeghatározás
- Inhibitor kimutatás

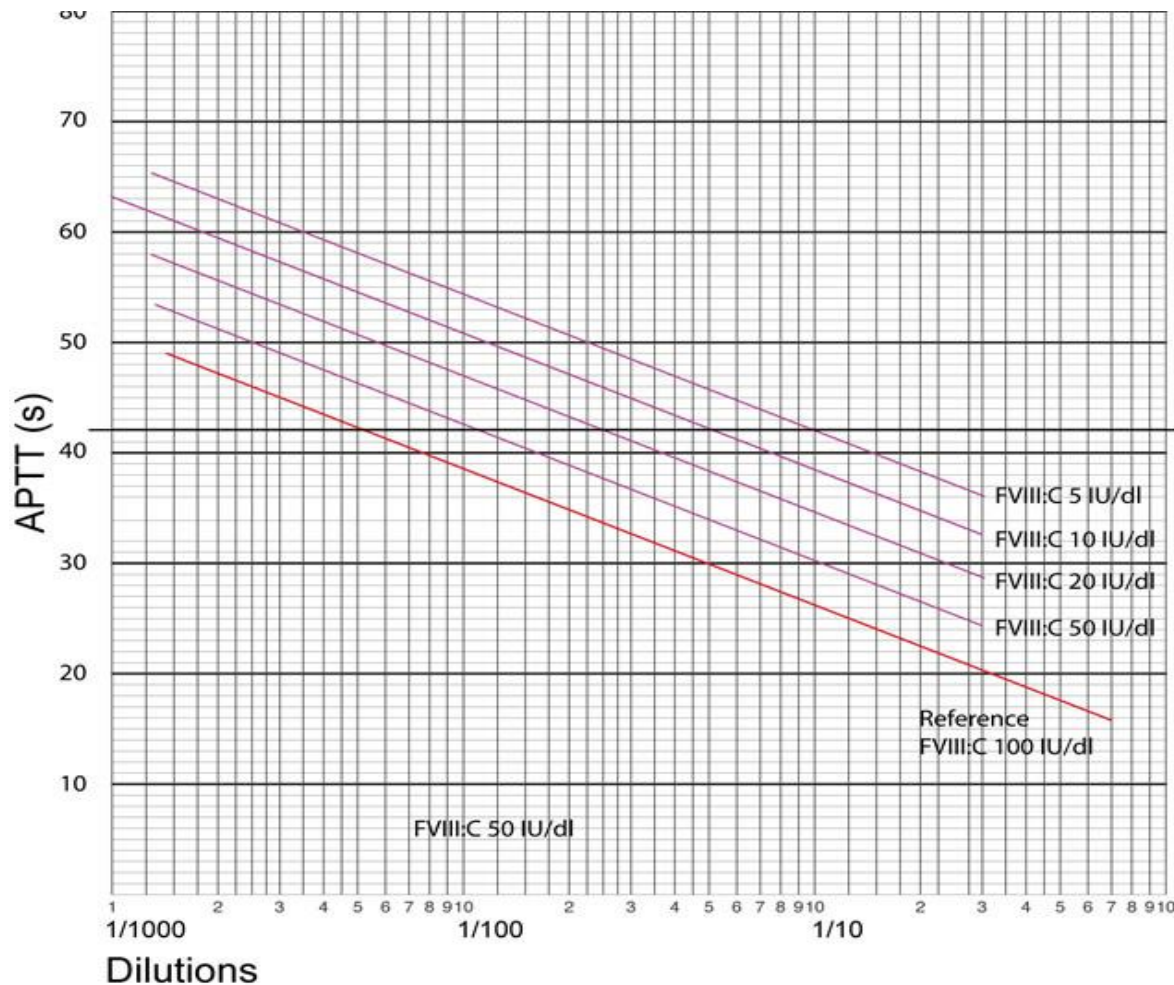
Faktormeghatározás – 1-stage

- Hiány plazma (a mérendő alvadási faktort nem tartalmazó plazma) segítségével a beteg és a normál plazmából hígítási sor készítése (100-50-25% vagy 6,25-3,125-1,56%)
- Mérés:
 - Intrinsic (aPTI alapú) one-stage assay
 - Extrinsic (PI alapú) one-stage assay
 - Chromogen tesztek
- Calibrations görbe
- A beteg plasma mérése → a cal. görbe alapján meghatározható az aktuális faktorszint

FX meghatározás – PT-based 1-stage assay



FVIII aktivitás mérés – APTT-based 1-stage assay



A tesztet zavaró főbb tényezők:

- Lipidek
- Heparin
- FVIII preaktivatioja
- LA
- Lepirudin
- egyes mutációk

Inhibitorok esetén a vonalak nem lesznek paralellek!

FVIII - 2-stage assay

- 1. Adsorbed **patient's plasma** is mixed with activated serum, factor V, calcium and phospholipid to initiate coagulation and **generate factor Xa**.
- 2. After a fixed period of incubation an aliquot of the mixture is added to an aliquot of normal plasma and the time to clot formation measured. Clotting times are plotted on double log paper and from which the factor VIII level in the patient sample can be derived.

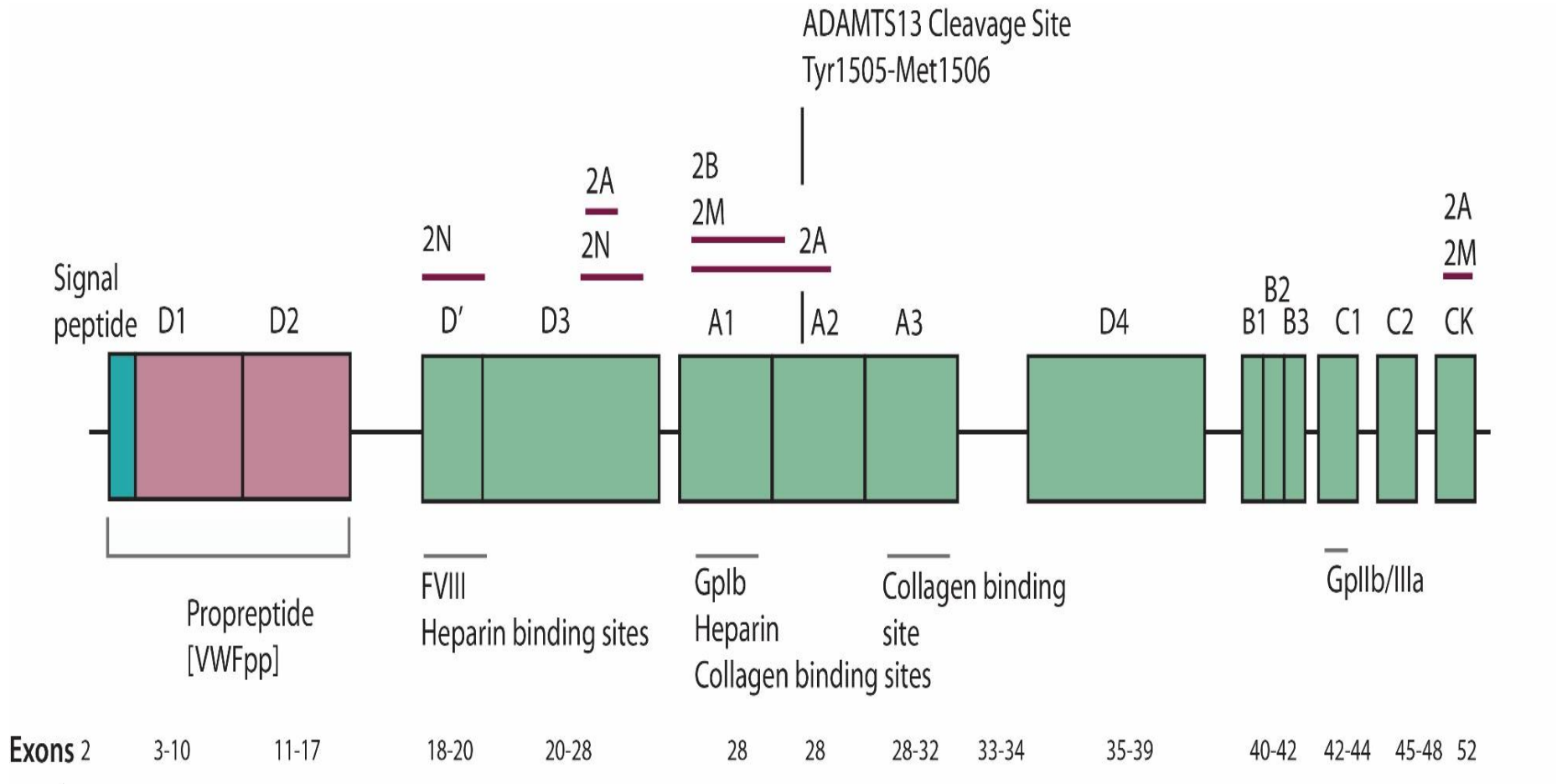
FVIII – chromogen assay

- 1. The patient's plasma is incubated with the reagent cocktail at 37°C. The thrombin in the cocktail activates the FVIII to FVIIIa and, in the presence of Ca^{2+} and phospholipid, this acts as a co-factor for FIXa for the conversion of FX to FXa. FVIII is the rate limiting step.
- 2. The chromogenic substrate is added. After a second incubation period the absorbance at a specific wavelength is measured and compared to a reference curve to give the FVIII level.
- The chromogenic assay is now the reference method for potency determination of FVIII concentrates
- Előny: alacsony tartományokban pontosabb meghatározást tesz lehetővé (jobb a felbontás), nagy hígítású plazmát használ, ezért kevésbé befolyásolják egyéb faktorok, mint a 1-stage assay-t; általában jobban korrelál a klinikummal, ha diszkrepancia van az eredmények között.

FVIII hiány - mit vizsgáljunk még?

- 1. If factor VIII deficiency is a new and unexpected finding then **Von Willebrand Factor levels and Factor V levels** should be checked. This is unnecessary if a finding of factor VIII deficiency is expected (e.g. known person with a haemophilia A).
- 2. There are rare cases in which patients with factor VIII deficiency have discrepant factor VIII levels when measured by a 1-stage assay compared to a 2-stage or **chromogenic FVIII assay**. The levels are generally higher with a 1-stage assay but the bleeding phenotype correlates better with a 2-stage or chromogenic FVIII assay
- 3. **Acquired Factor VIII deficiency**. An otherwise healthy individual may develop an autoantibody against factor VIII leading to acquired haemophilia A. Such antibodies are also seen in patients with immunological disorders e.g. rheumatoid arthritis.
- 4. You can also find a low FVIII in **acquired Von Willebrand Syndrome [AVS]**.
- 5. Factor VIII is an **acute phase protein** and the levels may be high in individuals who are stressed for any reason and this includes pregnancy.
- 6. The concentration of FVIII is a major determinant of the APTT and low FVIII levels will prolong the APTT and conversely **high FVIII levels will shorten the APTT**.
- 7. In a female with a low FVIII or FIX no relevant family history, the karyotype should be established e.g. **Turners syndrome** should be considered.

vWF diagnosztika



vWF diagnosztika

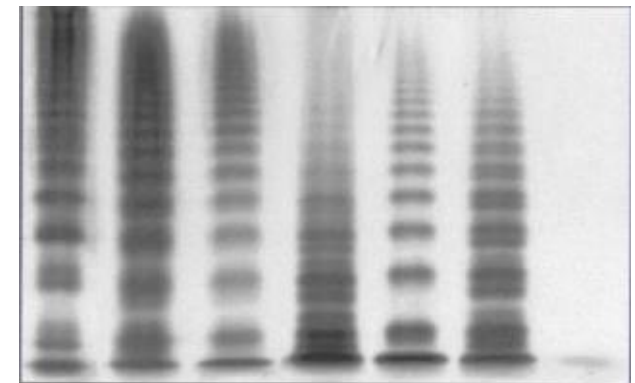
- Anamnesis, családi anamnesis, családfa
- Vérkép, PI, APTI, fibrinogen, (PFA100)
- vWF aktivitás mérés: vWF:Rco (Ristocetin cofactor activity) vagy vWF:CB (collagén kötő aktivitás –ELISA)
- Ha ez alacsony: vWF:Ag (antigén szint meghatározás);
- Ha a vWF:Akt/ vWF:Ag arány $<0,7$ -nél: 2-es típusú hiány

Type 2A VWD: the ratio is usually low.

Type 2B VWD: the VWF:RCO/VWF:Ag ratio is usually low but may be normal.

Type 2M VWD: the VWF:Ag concentration may be reduced or normal, but the VWF:RCO/VWF:Ag ratio will be <0.7 .

- FVIII binding assay: 2N subtípus kimutatása
- Multimer analysis
- Low dose RIPA: 2B subtípus kimutatása
- VWFpp/vWF:Ag arány: 1-es típus vWD elkülönítése 1C vWD-től (Vicenza)



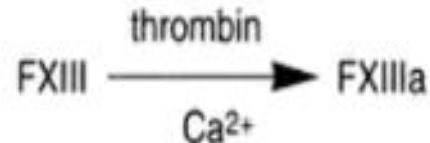
Plt NP 1 2A 2A 2B 3

Image by Marlies Ledford. In Hoffman's Hematology, 5th Edition

FXIII activity measurement

Table 1. The general principle of kinetic spectrophotometric **FXIII** activity assays based on the measurement of released ammonia

1. Activation of **FXIII**:

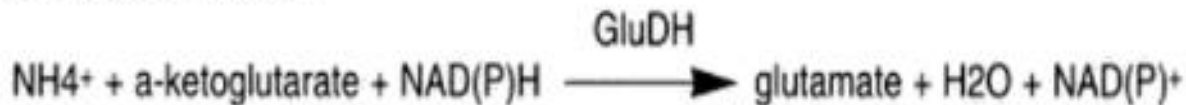


Polymerization of fibrin produced by thrombin is prevented by an inhibitory peptide.

2. Transglutaminase reaction:



3. Indicator reaction:



Continuous spectrophotometric measurement of the decrease of NAD(P)H concentration at 340 nm.

Inhibitor kimutatás

- Előfordulás: veleszületett hemofíliásokban a kezelés kapcsán alloimmunizáció, szerzett gátlótestes hemofíliában autoimmun inhibitor
- Inhibitor szűrés: keverékes teszt (a kontrollhoz képest az aPTI megnyúlása)

APTT –based screening test

	Tube 1	Tube 2	Tube 3
	Normal plasma	Test plasma	Equal volumes of normal + test plasma
Step 1	Incubate at 37°C for 120 minutes	Incubate at 37°C for 120 minutes	Incubate at 37°C for 120 minutes
Step 2	Place samples on ice	Place samples on ice	Place samples on ice
Step 3	Prepare equal volumes of normal + test plasma i.e. Tube 1 and Tube 2 [=Tube 4]		
Step 4	Perform APTT on Tube 1, Tube 2, Tube 3 and Tube 4.		

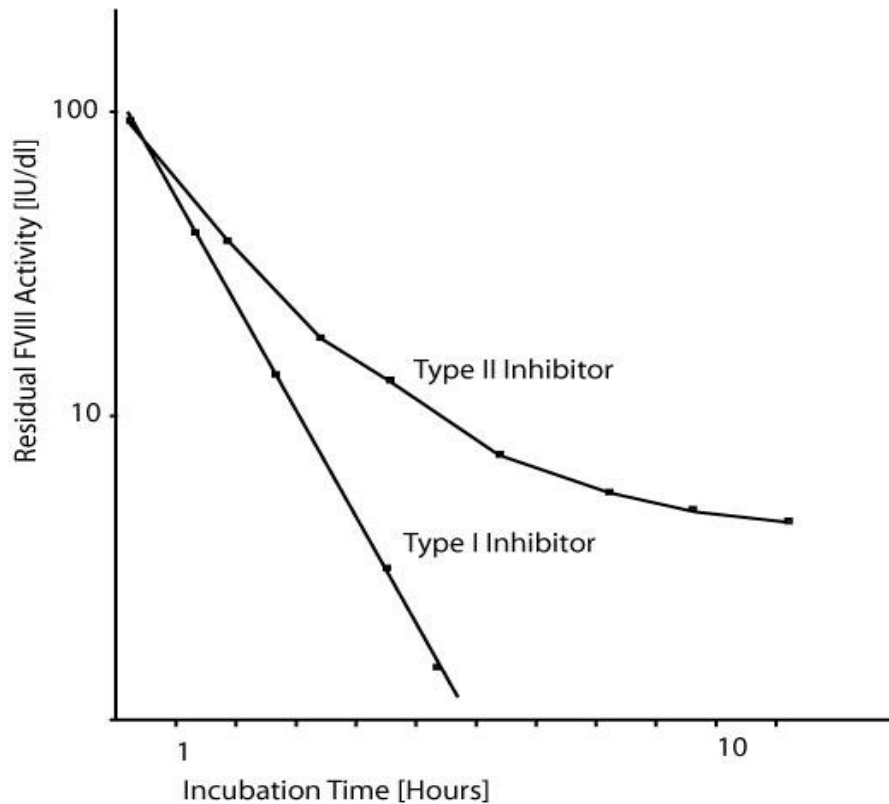
APTT –based screening test

Tube	APTT			
Tube 1 [Normal plasma]	Normal	Normal	Normal	Normal
Tube 2 [Test plasma]	Normal	Prolonged	Prolonged	Prolonged
Tube 3 [Incubated test + normal plasma]	Normal	Normal	Prolonged	Prolonged
Tube 4 [Non-incubated test + normal plasma]	Normal	Normal	Prolonged	Normal
Interpretation	Normal	Clotting factor deficiency	Immediate acting inhibitor	Time-dependent inhibitor

Inhibitor kimutatás

- Inhibitor mennyiség mérése: dilutios módszerrel- a hígított és normál plazmával összekevert beteg plasma reziduális FVIII aktivitását kell mérni
 - 1 Bethesda egység: az az inhibitor mennyiség, amely 37°C-on, 2 óra alatt a rendszerben lévő normál plasma FVIII aktivitásának 50%-át inaktiválja.
- Nijmegen módosítás:
 - Normál plazma pufferolása 0,1 M imidazole pufferrel pH 7,4
 - Immunodepletált FVIII def. Plazma használata a kontroll keverék során.
 - Álpozitív eredmények kizárása

Inhibitorok típusai



Type	Kinetics	Inhibition of FVIII	Seen with:
Type I	Simple - First Order	Complete	Alloantibodies arising in a person with haemophilia treated with FVIII concentrates and who makes an antibody to the foreign protein.
Type II	Complex - Second order	Incomplete	Autoantibodies – seen in acquired haemophilia A

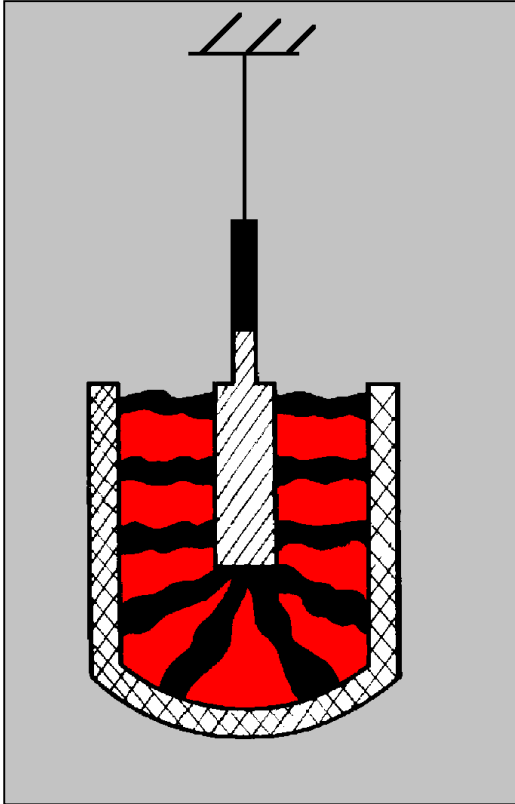
Anti-Xa assay-k

- Antikoaguláns hatás monitorozálása – UFH, LMWH, (direkt Xa inhibitorok)
- Ismert mennyiségű antikoagulánssal standard görbe készítés
- A plazmához fix mennyiségű FXa-t adunk
- Inaktív AT-Xa képződés
- A maradék FXa aktivitást mérjük pl. chromogen assay-vel
- Általában csúcshatásban mérjük – azaz LMWH beadása után 4 órával (th. tart: 0,5-1 U/ml; prophylaxis: 0,1-0,5 U/ml)
- Csak bizonyos helyzetekben kell mérni! (túl kicsi, túl nagy testsúly, terhesség, vesebetegség)

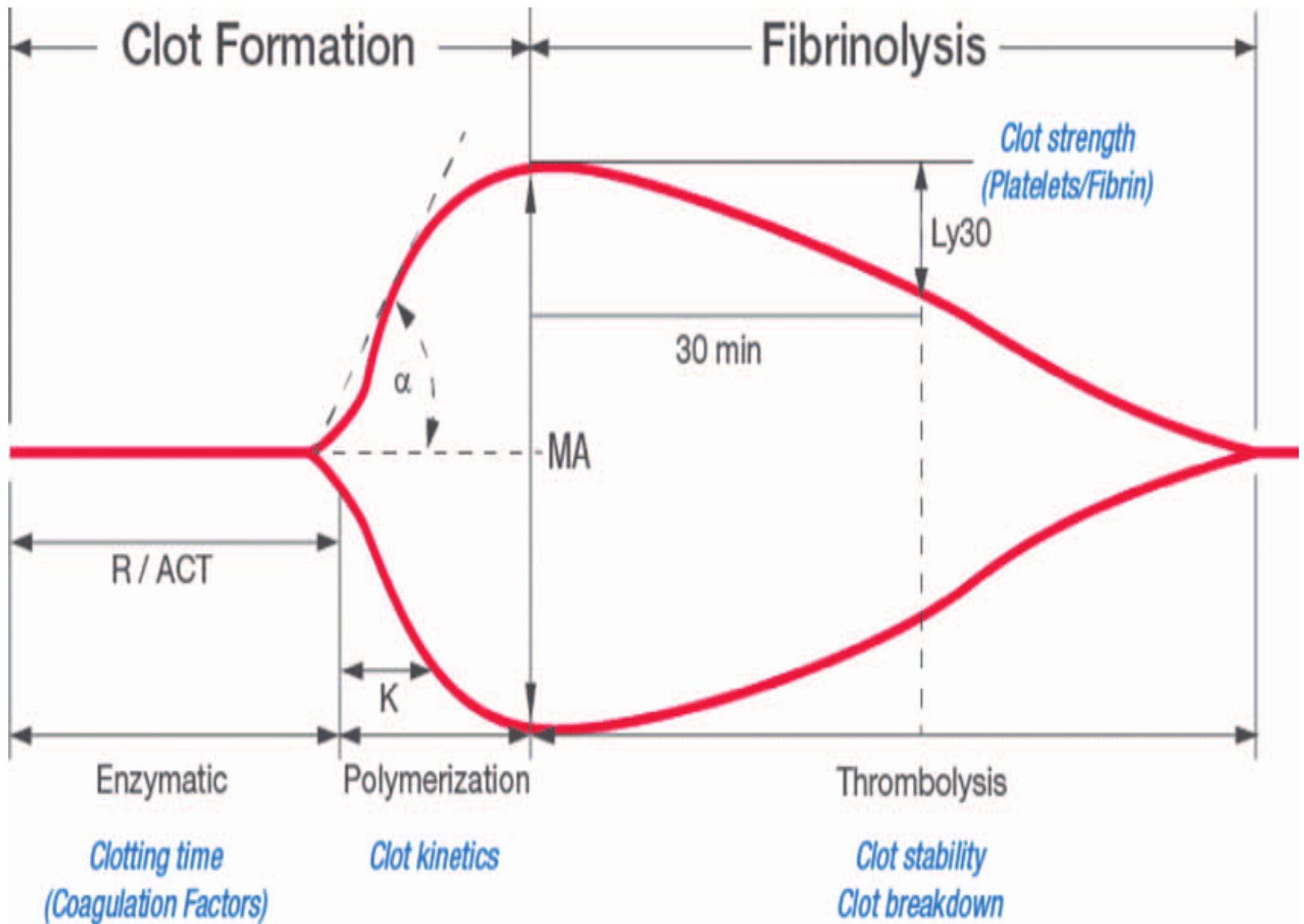
Thrombelastographia

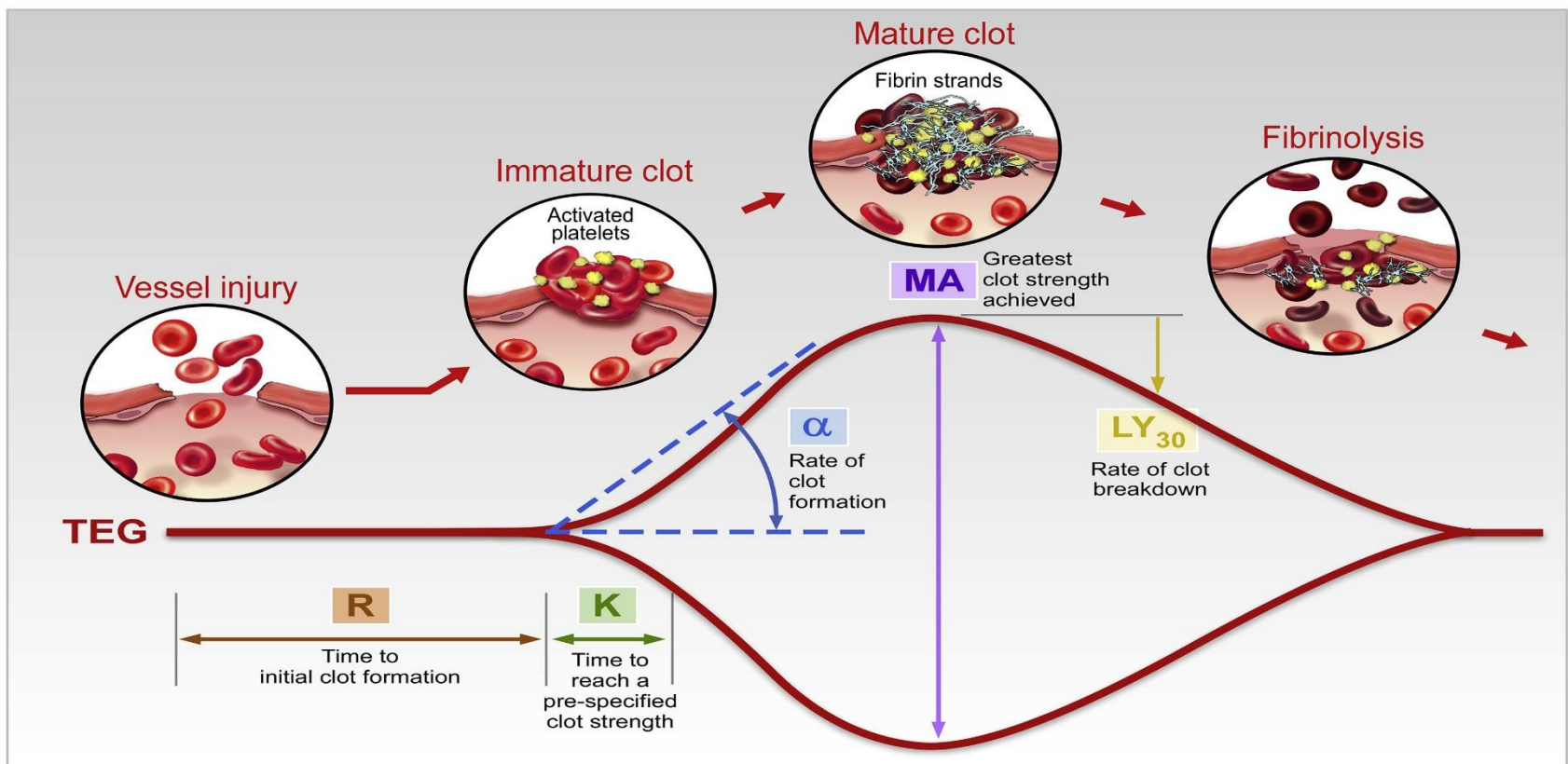
- 1948-ban Hartert fejlesztette ki Heidelbergben
- Eredetileg nem antikoagulált vérre alk.
- Technikai nehézségek miatt sokáig háttérbe szorult
- Az utóbbi időben az intraoperatív véralvadási vizsgálatok arany standardja lett

TEG technikája



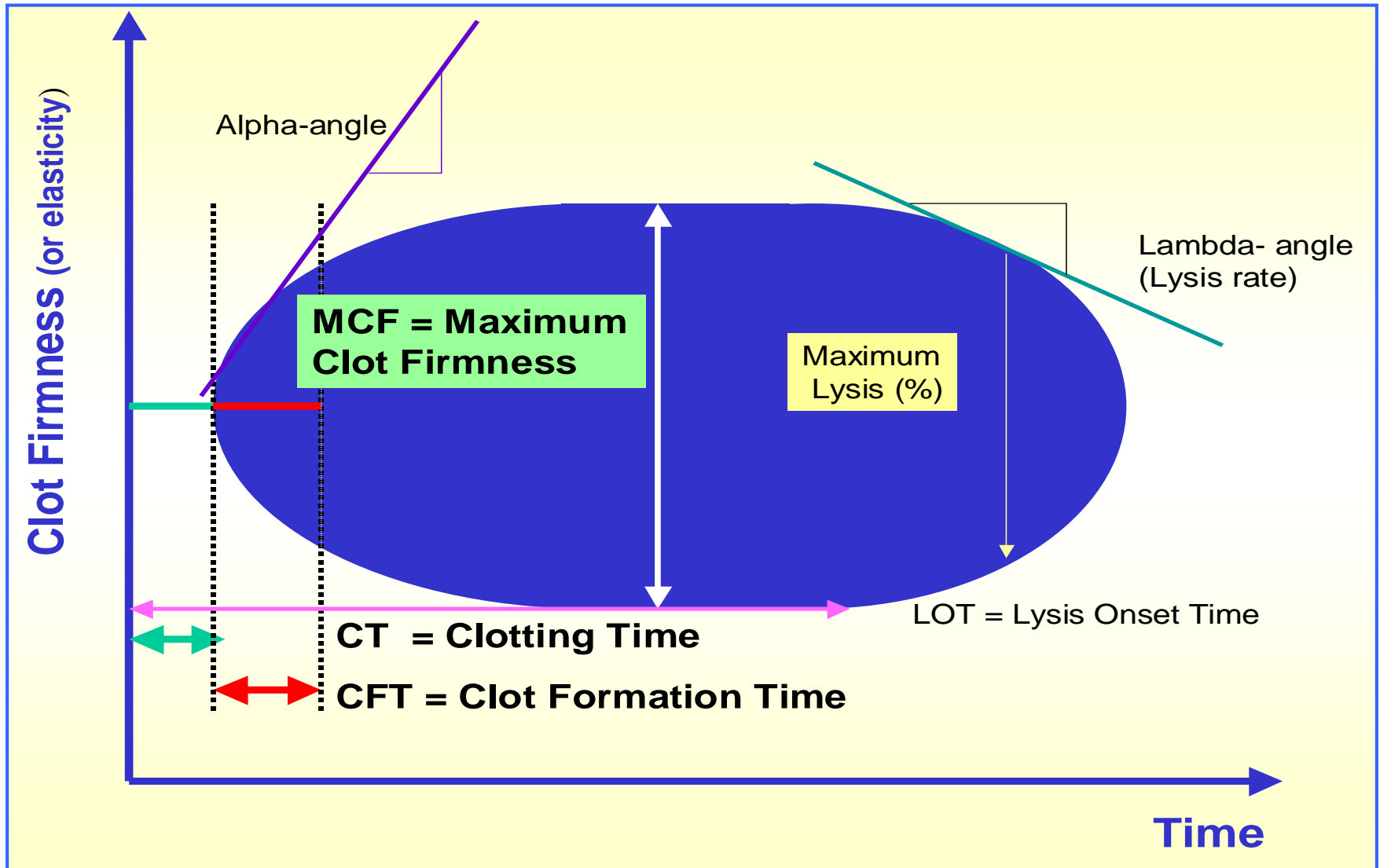
- A vér egy nem fiziológiás felszínű küvetta kerül, ezáltal a véralvadás aktiválódik
- A küvette és a szenzor egymáshoz képest $6\text{x/min} \pm 5^\circ$ elfordul
- A szenzor egy torziós rugóhoz van rögzítve
- A véralvadás megkezdődése az elasticitás megváltozásához vezet, amely befolyásolja a szenzor mozgását, amit a fényvisszaverés változásával detektálnak.





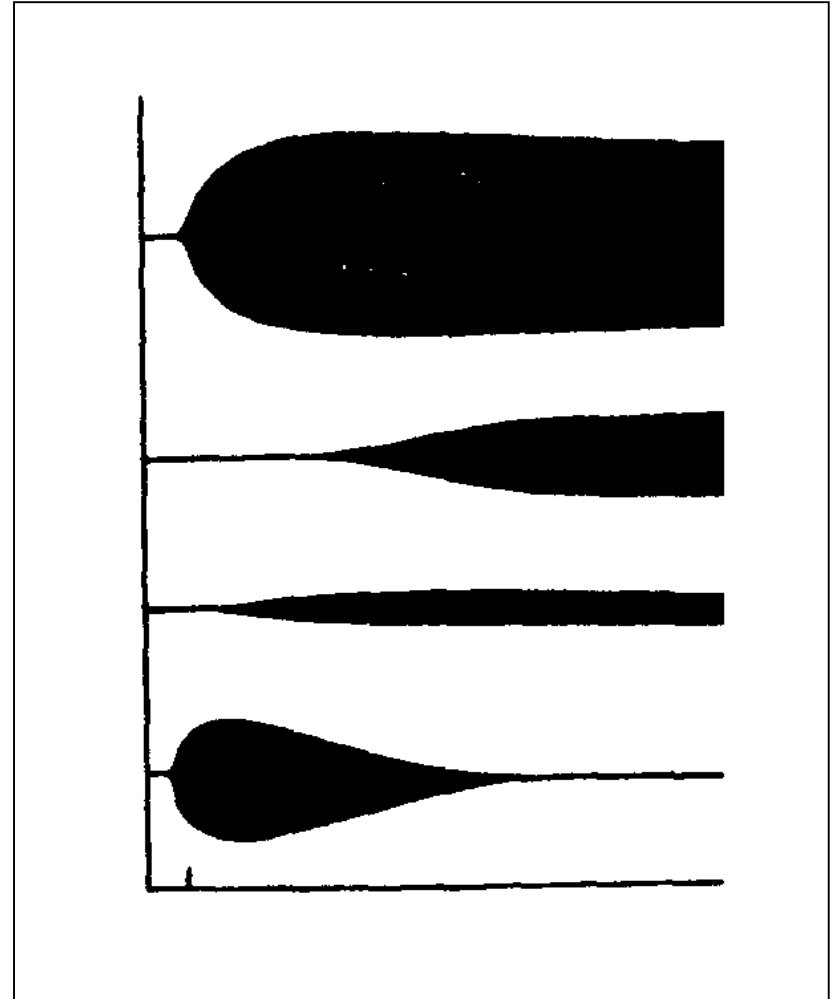
	R-time (R)	K-time (K)	Alpha angle (α)	Maximum Amplitude (MA)	Lysis % at 30 mins. (LY30)
Definition	Time to first deviation from baseline	Time for tracing to reach 20 mm amplitude	Angle between baseline and tangent line that intersects initial deviation	Maximum deviation of tracing from baseline	Decrease in curve amplitude (relative to MA) at 30 minutes
Controlling Pathways	Coagulation cascade	Fibrinogen cleavage Fibrin polymerization	Fibrinogen cleavage Fibrin polymerization	Fibrinogen activity Platelet count / quality	Fibrinolysis
Interpretation	↑ = hypocoagulable ↓ = hypercoagulable		↓ = hypocoagulable ↑ = hypercoagulable		
Therapeutic Implications	↑ = administer fresh frozen plasma	If K time is ↑ or alpha angle is ↓, then: administer cryoprecipitate or fibrinogen concentrate		↓ = administer platelets	↓ = administer tranexamic acid

TEG eredmények



TEG eredmények

- Normál TEG
- Hemofília
- Thrombocytopenia
- Fokozott fibrinolysis



THROMBOELASTOGRAPHY (TEG)

by Nick Mark MD



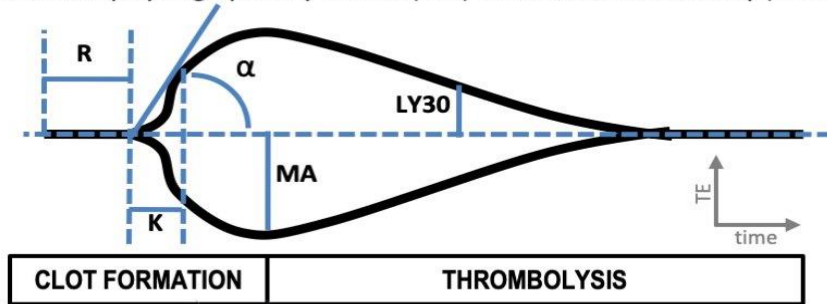
onepagericu.com
@nickmmark

Link to the
most current
version →



General Principle

- A small cuvette is rotated to simulate sluggish venous flow and stimulate clot formation.
- The resistance to rotation due to the mass of the clot is measured, which allows the kinetics of clot formation to be assessed.
- This provides information about clot formation and breakdown, and also reflects problems with coagulation cascade and platelet function.
- This is displayed graphically as time (min) versus thromboelasticity (in mm).



Explaining the numbers

- **R - reaction time** - latency until clot formation begins as defined by an amplitude of 2 mm (normal 3-9 min)
- **K - K value** - time from the end of R until the clot reaches 20mm – reflects speed of initial clot formation (normal 0.5 to 3 min)
- **α - alpha angle** - the angle tangent to the curve at K (normal 54-80 degrees)
- **MA - maximum amplitude** - reflects total clot strength (normal 51-78 mm)
- **Lysis time (LY30)** - % lysis after 30 min - reflects the fibrinolysis stage of clot development (normal 0-9%)

TEG guided resuscitation protocols

Advantage: Results available promptly: K, R within 5 minutes, MA and α-angle within 15 minutes, and LY30 within 45 minutes.

What do do?

- Increased R time => FFP
- Decreased α angle => cryoprecipitate
- Decreased MA => platelets (consider DDAVP)
- Fibrinolysis => tranexamic acid (or aminocaproic acid)

Specific Pathologies

NORMAL



ANTICOAGULANTS FACTOR DEFICIENCY

Delayed clot formation

- R prolonged
- α angle decreased
- K prolonged
- MA decreased

PLATELET BLOCKERS

Decreased clot strength

- R normal
- α angle normal
- K prolonged
- MA decreased

FIBRINOLYSIS

Accelerated clot breakdown

- R normal
- MA decreased
- LY30 markedly increased

HYPERCOAGULABLE STATE

Accelerated clot formation

- R decreased
- α angle increased
- K shortened
- MA increased

DIC (early)

Accelerated clot formation & breakdown

- R decreased
- α angle increased
- K shortened
- MA increased
- LY30 increased

(late)

- R prolonged
- α angle decreased
- K prolonged
- MA decreased

Vérzékenységek diff. dg-ja

Alapvető véralvadási vizsgálatok

Vizsgálat	mechanizmus	kóros érték
prothrombin idő (PTR)	extrinsic út közös út	K vitamin dependens fakt. ↓ májbetegség DIC , Syncumar th.
APTI	intrinsic út közös út	hemofília A,B ,Von Willebrand sy. keringő antikoaguláns heparin th. DIC, XI-es XII-es , X –es fakt,↓ fibrinogén ↓
thrombin idő (TI)	fibrinogén- fibrin átalakulás	FDP ↑ DIC, heparin hatás hypofibrinogenaemia
Vérzési idő (vi)	primer hemosztázis tct. funkciók	tct. funkciós zavar tct-penia, von Willebrand sy.



Köszönöm a megtisztelő figyelmet!