

Clinical Cases of Haemostasis

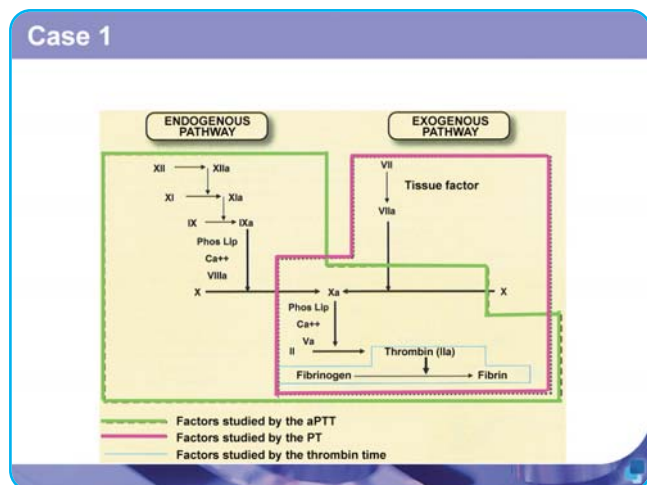
Case number 1

A patient, aged 28, suffering from Down's syndrome (trisomy 21) and congenital heart disease undergoes a pre-op screen before having cardiac surgery. The prothrombin time, which was found to be low in two laboratories, is sent to Biomnis for confirmation testing. The prothrombin ratio result: 42%, confirmed (Reference values: 80-130%).

What further testing could be performed?

1. PTT?
2. Fibrinogen?
3. Factor II, V, VII, X?

Answer: 1. Firstly, the PTT needs to be measured. Indeed, the prothrombin time should never be interpreted (or PTT) on its own, except in special cases (treatment, deficiency follow-up). The combination of the two tests directs us to a type of anomaly, endogenous, exogenous or a common cause.



The PTT (Cephascreen® Stago on the STA-R) is measured as 50 secs (ratio: 1.78). The prothrombin time and the PTT are extended; it is necessary to measure the fibrinogen level. The result is: fibrinogen activity: 2 g/L.

What is your interpretation? What other tests would you recommend?

A decreased prothrombin time on its own suggests a factor II, V, VII, X anomaly or a fibrinogen anomaly. An increased PTT on its own is suggestive of the presence of a circulating anti-coagulant or a factor VIII, IX, XI or XII anomaly. When faced with an abnormal PTT or prothrombin time result, fibrinogen must be measured: If the result is normal, measure factor II, V and X and perform

a lupus anticoagulant screen (LA).

Quantitative measurements of factors II, V and X (common pathway) revealed the following:

Factor II: 44%, Factor V: 50%, Factor X: 47%.

(Reference values: 50 - 150%)

For information, the results of factor VIII, IX, XI and XII quantification were as follows:

Factor VIII: 45%, Factor IX: 39%, Factor XI: 57%, Factor XII: 42%.

All the factor results are abnormal. Firstly, an analytical error must be ruled out, however the prothrombin time has already been confirmed as low in two other laboratories. Could these results correspond to a hepatic condition? This is possible, but our patient's liver enzyme profile result is normal.

Please note: If the patient is suffering from a liver condition, the factor VIII level (and the Willebrand factor level) is normal or increased (not decreased). A complete blood count (also known as a full blood count) test is therefore requested. The CBC result was as follows:

WBC: 10 G/L, RBC: 7.8 T/L, Hb: 20 g/dl, Ht: 0.82, Platelets: 200 G/L.

What is the link between these results and the haemostasis results? What possible interpretation can be made from the prothrombin time and PTT results obtained?

One must remember that the volume of anticoagulant (citrate) in the tube for samples for haemostasis testing is adapted to a specific haematocrit (Ht) value, which lies between 0.45 and 0.50. This anticoagulant/sample blood ratio is valid if the Ht > 0.30 and < 0.55. However the ratio is no longer valid if we exceed these values. Our patient had a very high Ht level (this corresponded to a poorly filled tube), the coagulation time was falsely increased; contrarily, in subjects with a low Ht (a similar situation is found in a hypo citrated tube) the time is decreased.

According to the recommendations of the specific Societies, results must be released under reserve if the haematocrit result is > 55% or < 30% (GEHT Study Group on Haemostasis and Thrombosis); an adjustment is required if the Ht > 0.65 (Clinical and Laboratory Standards Institute CLSI).

To adjust the citrate volume according to the haematocrit level, there are several possibilities:

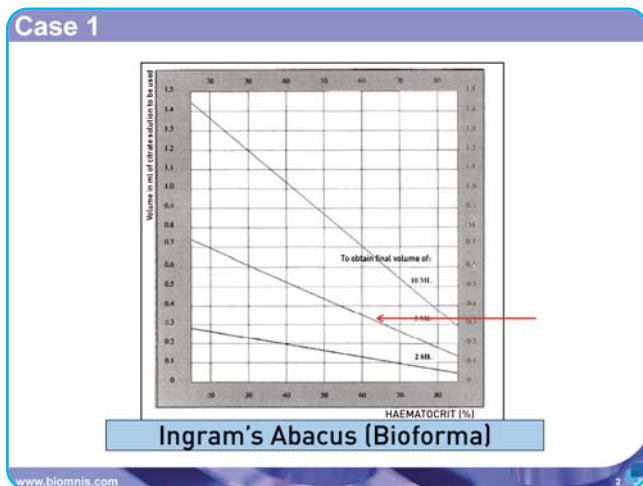
1. Mac Gann formula: $C = (0.00185 \times \text{volume of blood in mL}) / (100 - \text{Ht})$ (with an Ht %)

2. Ingram formula: $C = \text{volume of blood in (mL)} \times (100 - \text{Ht}) / (595 - \text{Ht})$ (with an Ht %)

3. Simplified method: remove 0.1 mL of the citrate from the outset

4. Use of graphs: volumes calculated (Ingram graphs)

Case 1



For our patient, the Ht is 0.8 for a tube of 5 mL, the optimal citrate volume is 0.2 mL. A 'classic' citrate tube is manually adapted to 0.2 mL and the sample collected by a nurse (the tube is no-longer under vacuum).

The readjusted results are:

PT: 80% **PTT: 32 sec (ratio 1.14)**

The haemostasis profile is normal.

Case number 2

A patient, aged 60, with non-alcoholic cirrhosis underwent pre-op PT/PTT testing. This profile was sent to Biomnis for confirmation testing of an increased PTT time found in two samples (PT is normal).

Which tests should be requested when faced with an isolated increased PTT result?

1. Lupus anticoagulant screening?
2. Factors VIII, IX, XI and/or XII?
3. Willebrand factor antigen (VWF)?
4. Willebrand factor activity?
5. Factors II, V, VII, X?

Answers: 1, 2. The VWF quantifications will be performed only if the factor VIII level is decreased. Factor II, V, VII, and X quantification is not necessary because the PT result is normal.

The confirmation haemostasis profile gave the following results:

PTT (Synthasil® IL) : 56 sec (ratio: 1.75)
 aPTT (APTT-SP® IL) : 101 sec (ratio: 3.25)
 PT: 83%

Lupus anticoagulant screening:

PTT (PTT-LA® Stago): 97 sec (mixed 78 sec) Rosner: 48
 dRWV Lac screen® IL: 1.72 dRWV Lac confirm® IL: 1.11
 Standardised ratio LS/LC: 1.55

Conclusion: Lupus anticoagulant (LA) positive.

Should quantification testing for factors VIII, IX, XI (XII) be performed? What analytical interference can be caused by LA? What should be done in the case of interference?

According to the ISTH recommendations, 4 steps must be followed to screen for and identify a circulating anticoagulant (S Miyakis et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome, *J.Thromb Haemost*,2006;4:295-306).

1. Screening: increase in phospholipid dependant (PL) coagulation tests (using reagents with low levels of PL).
2. Evidence of an inhibitory activity (mini-pool testing): LA testing

should be performed if the time has not been corrected on adding normal plasma. LA or anti-factor?

3. Confirmation of the inhibitors dependence on phospholipids: if when using a reagent rich in phospholipids, the time is reduced, it is LA.
4. Exclusion of another coagulation anomaly (specific inhibitors). Yes, factor VIII, IX, X, XI and XII should be measured (at different dilutions) in order to eliminate all other anomalies that could cause an increased coagulation time in the screening test: a real deficit in the intrinsic pathway factor (or prothrombinic complex) or the presence of an isolated anti-factor: e.g. anti-VIII. **NB:** no test detects the isolation of all of the lupus anticoagulants. The consensus is to use at least 2 screening tests, preferably those based on different methods.

	"Pure" plasma: 1/10	Diluted plasma 1/20	Diluted plasma 1/40	Diluted plasma 1/80	Diluted plasma 1/160
Factor VIII : C (%)	25	40	53	72	80
Factor IX : C (%)	5	10	19	40	54
Factor XI : C (%)	3	4	4	9	35*
Factor XII : C (%)	2	14	35	60	75

* The dilutions extended to 1/320 and 1/640 for factor XI with a plateau at 35%.

The results of the quantification tests performed on 'pure' plasma show an LA interference (which increases the coagulation time) in the factor quantification tests (measure of activity by the chromometric test): the ratios are false and under-estimated. The dilutions allow us to decrease the quantity of LA in the sample and as such, to decrease the analytical interference.

By increasing the dilutions, we note a normalisation of the factors with an isolated decrease in factor XI. What is your interpretation? Is there a risk of haemorrhage?

Indeed, our patient had a deficit in factor XI previously discovered when a LA screen was performed. A new sample must be requested to confirm the deficit and is needed, to measure factor XI antigen.

Constitutional deficit in factor XI: reminder

This belongs to the group of rare haemorrhage deficits: 1 case/1 million, but its frequency is greater in Ashkenazi Jews and the Basque population. A risk of haemorrhage does exist, but is unlikely: it is not dependant on the ratio, it is variable with time and within the same family, it is rarely spontaneous, usually provoked (especially in ENT surgery, genital or urinary surgery or labour).

Two types exist: severe ('total' deficit: 0 - 20%) or moderate (partial deficit: 21-60%).

Pre-op risk and factor XI:

There is no specific recommendation, however the factor XI threshold usually kept in mind before surgery is 30 - 45%. The available treatments include: tranexamic acid (Exacyl®; efficient in moderate forms), freshly frozen plasma or factor XI concentrates (Hemoleven® in severe deficits; caution: thrombotic risk).

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