Investigation Of A Prolonged APTT – Different Approaches Adopted By Participants In The WFH External Quality Assessment Programme

Authors I Jennings, S Kitchen, DP Kitchen, TAL Woods, FE Preston, ID Walker

UK NEQAS for Blood Coagulation, 3rd Floor Pegasus House, 463A Glossop Road, Sheffield S10 2QD

UK NEQAS

Background:

The APTT is a useful screening test in the investigation of haemostatic abnormalities. A prolonged APTT may be indicative of specific or multiple factor deficiencies, the presence of a factor-specific inhibitor, or a non-specific inhibitor or lupus anticoagulant, and a logical algorithm for investigation of a prolonged APTT is useful.

Proficiency testing programmes commonly assess one specific analyte in an EQA challenge. However, we have previously reported a study in which UK NEQAS participants were asked to determine their own course of investigation of a prolonged APTT. This challenge faces all laboratories involved in the diagnosis of haemophilia, and we describe here the same exercise carried out with participants in the World Federation of Hemophilia EQA programme in 2013.

Study:

Vials of a lyophilised plasma sample, WS2013, were sent to 80 participants in the WFH programme; results were received from 36 (45%). WS2013 was from a donor with severe haemophilia A, and no known inhibitor. Participants were provided with a clinical scenario of a 1 year old child with a history of bleeding into joints, and asked to investigate the cause of a prolonged APTT.

Results from each centre, together with the laboratory diagnosis, were collated, and data were compared to the UK NEQAS participant data, in which the same material (with a different code) was employed.

Results:

Table 1 shows the overall interpretations made by the WFH centres for this sample from a donor with severe haemophilia A. 25/36 centres provided a diagnosis for this patient. All 25 identified haemophilia A, though only 15 reported severe haemophilia. The number of tests employed by different centres ranged from 1 to 9, and a total of 17 different investigations were employed (table 2). FVIII:C assay, FIX:C assay and APTT were most widely performed.

Table 3 shows the median results obtained by participants in the WFH programme, and corresponding results reported by UK NEQAS participants for this sample. Data were comparable for the two groups.

Incomplete investigations revealed a number of potential errors in the diagnosis of a patient with a prolonged APTT. These are detailed in table 4..

Table 1. Diagnosis by WFH participants

Sample WS2013	**n**
Total Returning Results	36
Total Returning Overall Interpretations	25
Severe Haemophilia A (3 noted no presence of an inhibitor)	15
FVIII deficiency/Haemophilia A	6
Haemophilia or VWD (no VWF assay available)	1
Moderate Haemophilia A	2
Moderate Haemophilia A and latent Haemophilia B	1

Table 2. Tests employed in investigation of the prolonged APTT * Inc – incubated Abs – adsorbed plasma

Test	**n**	**Test**	**n**
APTT	22	APTT 1:1 immediate mix	15
PT / INR	12	APTT inc*. mix/inhibitor screen	3
Thrombin Time	2	APTT 1:1 Abs* plasma mix	1
Fibrinogen	4	APTT 1:1 serum mix	1
FVIII:C assay	33	APTT 1:1 FVIII def plasma mix	3
FIX:C assay	24	APTT 1:1 FIX def plasma mix	3
FXI:C assay	8	FVIII inhibitor assay	2
FXII:C assay	2	Lupus Anticoagulant screen	1
VWF:Ag	7	VWF:RCo	4

Table 3.

Test	**UK NEQAS**		**WFH**	
	n	**Median**	**N**	**Median**
APTT (s)	96	3.56	22	3.38
PT / INR (ratio)	35	1.19	12	1.10
Thrombin Time (ratio)	47	1.06	2	1.00
Fibrinogen (g/)l	24	2.27	4	2.34
FVIII:C assay (u/dl)	67	0.9	33	0.8
FIX:C assay (u/dl)	90	75.5	24	72.8
FXI:C assay (u/dl)	80	75.0	8	71.4
FXII:C assay (u/dl)	75	61.0	2	73.2
VWF:Ag (u/dl)	57	80.8	7	82.7
VWF:Rco (u/dl)	41	79.1	4	77.9

Table 4.

Investigation	**Possible diagnostic pitfall**
APTT (21/34 did not repeat the APTT)	For samples referred from another centre, repeat testing can confirm the abnormality
Thrombin time (only 2 centres performed TT)	Sample contamination with heparin, and some anticoagulant therapy, can prolong the APTT
Only FVIII:C performed, no other assays	Multiple factor deficiency is unlikely, and further investigation with limited resources is not warranted, but a non-specific inhibitor (eg Lupus anticoagulant) could be picked up with other assays
VWF assays (12 centres reporting haemophilia A did not perform VWF assays)	Type 3 VWD (and 2N VWD) should be excluded – clinical details did not indicate the gender of this child
Mixing studies	Useful to exclude inhibitors – some centres did not include incubated studies, and therefore could not exclude a time-dependent inhibitor

Conclusions:

- All centres returning an interpretation for this sample identified haemophilia A or FVIII deficiency, with the majority reporting severe haemophilia A.
- There are important differences in the management of patients with moderate and severe deficiencies. Centres reporting moderate haemophilia correctly interpreted their FVIII assay result, but should review their assay sensitivity and precision at low levels to ensure severe deficiency can be successfully diagnosed.
- One centre additionally reported latent haemophilia B in this sample.
- There were a number of different approaches and testing patterns employed by participants in this exercise, similar variability was seen when UK NEQAS participants carried out investigations on the same sample.
- In some cases, the relatively small volume of plasma may have restricted the testing that would otherwise have been undertaken.
- However, there are potential problems in some of the investigative approaches taken, which may lead to diagnostic errors. Laboratories should ensure an effective approach to investigation of a prolonged APTT.

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Laboratory Issues

Ian Jennings

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