Causes of isolated prolonged activated partial thromboplastin time in an acute care general hospital

W J Chng, C Sum, P Kuperan

ABSTRACT

Introduction: To determine the causes of isolated prolonged activated partial thromboplastin time (APTT) in an acute care general hospital setting so as to rationalise fresh frozen plasma usage.

Methods: A prospective study of consecutive patients with isolated prolonged APTT presenting to our hospital between February 2002 and January 2004 was performed. All patients had normal prothrombin time and thrombin time. For all patients, an initial 50:50 correction with plasma was done and a standard panel of tests was performed. These included detection of lupus anticoagulant using two different sensitive tests; measurement of coagulant factors VIII (FVIII), IX, XI and XII, which are involved in the intrinsic arm of haemostasis; von Willebrand factor antigen (vWF:Ag) levels and for those with FVIII levels less than 10 percent, an inhibitor assay using the Nijmegan modification of the Betheseda method.

Results: 177 patients were included in the study. The cohort was typical of an acute care general hospital patient population in Singapore in terms of age, sex and racial distribution. The most common cause of an isolated prolonged APTT in our study was the presence of lupus anticoagulant (53.1 percent of cases). In 31.6 percent of cases, obvious cause could be detected after our panel of tests. These patients mostly had mildly prolonged APTT that could be both correctable and non-correctable by normal plasma. Prolonged APTT due to factor deficiency was relatively rare with those that may potentially cause haemorrhagic problems only accounting for 4.5 percent of cases.

Conclusion: Our study suggests that most of the causes of isolated prolonged APTT do not lead to haemorrhagic complications. In fact, in a majority, it may signify an underlying thrombophilic condition. As a result, prolongation of APTT should be fully investigated and correction with fresh frozen plasma should be used only when appropriate.

Keywords: coagulation factors, fresh frozen plasma, lupus anticoagulant, prolonged activated partial thromboplastin time

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Department of Pathology and Laboratory Medicine
Tan Tock Seng Hospital
11 Jalan Tan Tock Seng
Singapore 380433

W J Chng, MBChB, MRCP, MRCPath
Associate Consultant

C Sum, MSc
Scientific Officer

P Kuperan, FRCP, FRCPA, FRCPath
Senior Consultant and Head

Correspondence to:
Dr Chng Wee Joo
Department of Haematology-Oncology
National University Hospital
5 Lower Kent Ridge Road
Singapore 119074
Tel: (65) 6772 4971
Fax: (65) 6872 3137
Email: chngwj@nuh.com.sg

INTRODUCTION

Activated partial thromboplastin time (APTT) is a commonly requested coagulation test to assess the intrinsic pathway of coagulation, with 4,000 requests a month on average, at our institution. Although originally designed to confirm suspected haemophilias[1], its use has been expanded over the years to include monitoring of unfractionated heparin therapy[2], investigation of disseminated intravascular coagulation[3], and as a pre-operative haemostatic screen[4]. Fresh frozen plasma (FFP) is a blood product rich in various coagulation factors that have often been used to correct the coagulopathy with prolonged APTT. In our previous study, we found that probably about two-thirds of FFP requests were for inappropriate indications and a significant number of these requests were for correction of prolonged coagulation tests, often without establishing the causes of the underlying coagulation abnormalities[5]. We decided to investigate the causes of an isolated prolonged APTT in an acute care general hospital in an attempt to facilitate guidelines for proper investigations and to further rationalise FFP usage.

METHODS

Consecutive patients with prolonged APTT (>43s) and normal prothrombin time (PT<15s), based on our laboratory normal reference ranges and confirmed by a second blood draw performed by a dedicated phlebotomist, were enrolled into the study after informed consent, between February 2002 and January 2004. This was to ensure that pre-laboratory variables which can alter APTT, such as inadequacy of

venepuncture[6], deterioration of factors due to delay in analysis[7], heparin contamination[8], wrong ratio of plasma to anticoagulant[9], were minimised. Patients with prolonged thrombin time (TT) were further excluded from the study to exclude cases resulting from heparin contamination. All included patients underwent a battery of investigations specifically to identify a cause for the isolated prolonged APTT. These included a 50:50 plasma correction of the APTT, detection of lupus anticoagulant (LA), coagulant factor VIII (FVIII), FIX, FXI, FXII, plasma von Willebrand factor antigen (vWF:Ag) assays and FVIII inhibitor assay when FVIII is less than 10%. APTT is deemed correctable if it shortened to within 5s of control APTT or it was shortened by more than 50% from the original APTT compared to control[10]. The schema for investigation is shown in Fig. 1.

Samples were drawn by fresh venepuncture with a clean rapid draw into commercial vaccum tubes (Vacutainer Plus, Becton Dickinson, CA, USA). Nine parts of whole blood were mixed with one part of 3.2% sodium citrate within 20 seconds from the beginning of venepuncture. PT, APTT and TT were done immediately on unfrozen platelet-poor plasma prepared by centrifugation. Other tests were performed on plasma that had been frozen immediately to -70ºC and stored without thawing until indicated. APTT, PT and TT were performed using Actin FSL (Dade Behring, Marburg, Germany), STA $CaCL_2$ 0.025M, STA Neoplastine CI Plus, and STA Thrombin 2 (Diagnostica Stago, France) reagents, respectively.

LA detection was based on prolongation of lupus anticoagulant sensitive APTT (Diagnostica Stago, France), and correction by addition of phospholipids. Two different assays were used. A dilute Russell's Viper Venom time-based assay with calculated ratio using reagent, without and with phospholipids, where a normalised ratio of more than 1.2 was considered positive (Dade Behring, Germany). Using this assay, strength of LA can be further classified into weak (normalised ratio of 1.2 to 1.5), moderate (normalised ratio of 1.6 to 2) or strong (normalised ratio greater than 2). Presence of LA was confirmed by a second test using hexagonal phase phosphatidyle-ethanolamine (HPE) as the source of phospholipid. A difference in APTT of more than 8s with and without HPE was considered positive (Staclot LA Kit, Diagnostica Stago, France).

FVIII, IX, XI, and XII assays were APTT-based assays using factor deficient plasma (Diagnostica Stago, France). In cases where FVIII level was less than 10%, FVIII inhibitor was quantified by mixing the test plasma with control plasma containing a known amount of FVIII. The level of inhibitor present was then calculated by comparing the residual FVIII activity of a patient-control mixture and a buffer-control mixture and expressed in Bethesda Units. vWF:Ag was quantitfied by immuno-turbidimetric method (STA Liatest vWF, Diagnostica Stago, France). All the above tests were performed using automated machine STA Compact, except for Staclot LA, which was performed using ST Art (both by Diagnostica Stago, France).

The causes of prolonged APTT were assigned according to the following criteria. If LA was positive, the cause was LA regardless of presence of low factor levels. This was because of the well-documented effect of LA on one stage clotting-based factor assays[11,12]. However, if the test for the specific FVIII inhibitor was positive, then the cause was FVIII inhibitor. Factor deficiencies were diagnosed when factor VIII, IX, XI and XII levels were less than 41%, 42%, 56% and 44%, respectively, based on the sensitivity of our APTT reagent. Probable von Willebrand disease (vWD) was assigned if vWF:Ag level was less than 50% based on previously established laboratory reference range. The cause was classified as unknown, if the above criteria were not met. Stratification based on perceived haemostatic severity of the underlying haemostatic defect is the same as that previously proposed and published[13] and presented in Table I. This stratification is based on expert consensus and their reading of evidence from literature.

RESULTS

204 patients had confirmed isolated prolonged APTT and were included in the study. Of these, 27 cases were excluded due to prolonged TT, leaving 177 for the final analysis. The median age of the cohort was 52 years old (range 13 to 104 years old). The female to male ratio was 1:1.27. Racial distribution was as follows: 133 Chinese, 20 Malays, 16 Indians and eight belonging to other races, hence our cohort was representative of a general hospital population in Singapore.

The most common cause of an isolated prolonged APTT in our cohort was LA (53.1%) and in 31.6% of cases, no obvious cause was found. The factor deficiencies were rare (Fig. 1). Not all cases positive for LA had non-correctable APTT, and there was no correlation between the strength of LA, APTT and correctability of APTT. Among the 56 with unknown causes, 40 had correctable APTT while 16 had non-correctable APTT after 50:50 plasma

Fig. 1. Study schema and causes of isolated prolonged APTT detected.

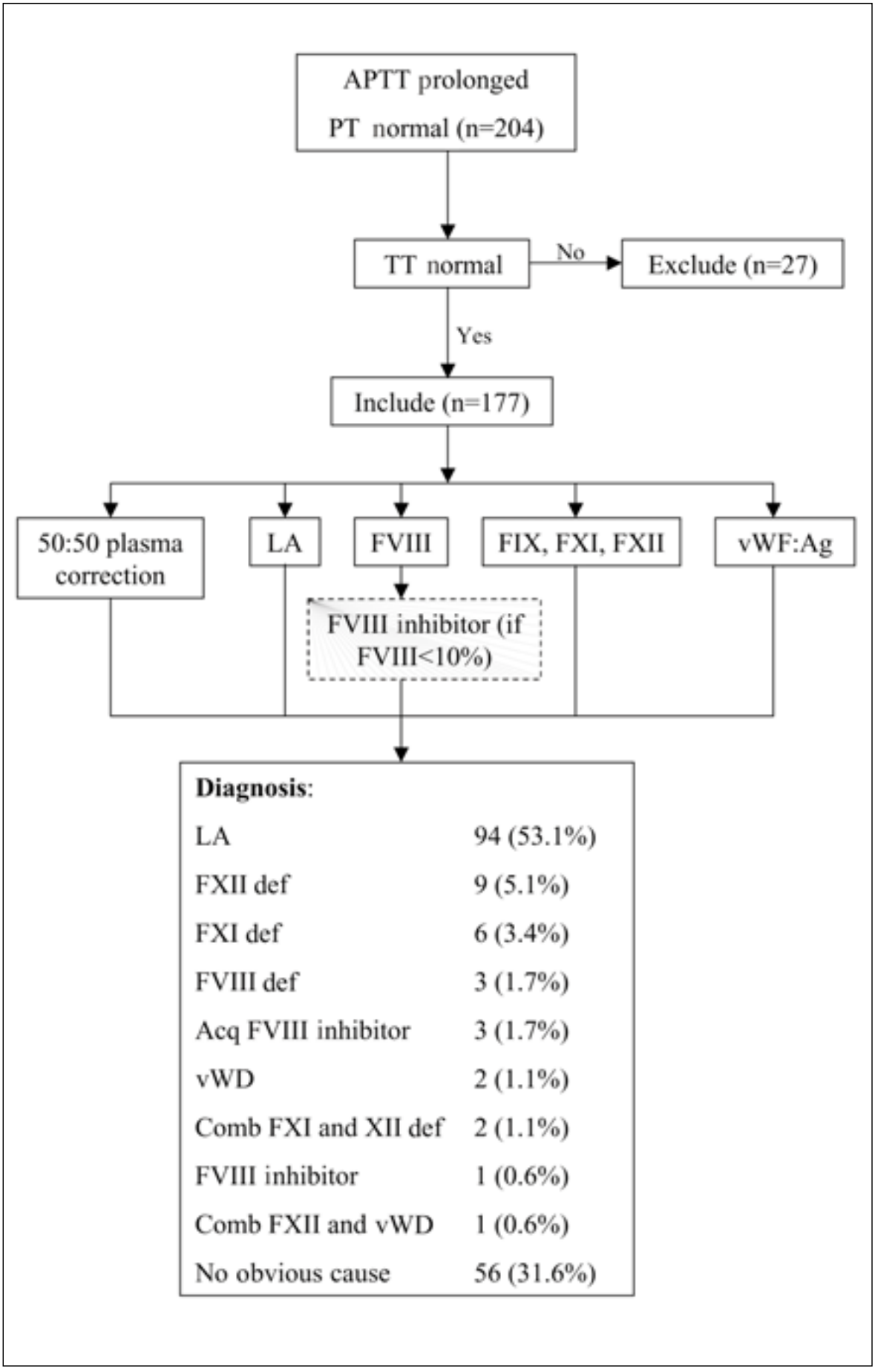


Table I. Patients (excluding those with unknown causes) in different haemorrhagic risk stratification.

Haemorrhagic risk groups	Number (%)
Group 1: No risk of haemorrhage with surgery	103 (58.2%)
Lupus anticoagulant	94 (53.1%)
Factor XII deficiency	9 (5.1%)
Group 2: Mild risk of bleeding with surgery	8 (4.5%)
Factor VIII deficiency 30-50%	3 (1.7%)
Factor IX deficiency 30-50%	0 (0%)
Factor XI deficiency 30-50%	5 (2.8%)
Group 3: Moderate risk of bleeding with surgery	3 (1.7%)
Factor VIII deficiency 5-30%	0 (0%)
Factor IX deficiency 5-30%	0 (0%)
Factor XI deficiency 5-30%	2 (1.1%)
von Willebrand disease, type I	1 (0.6%)
Group 4: Severe risk of bleeding with surgery	6 (3.5%)
Factor VIII deficiency <5%	1 (0.6%)
Factor IX deficiency <5%	0 (0%)
Factor XI deficiency <5%	1 (0.6%)
Factor VIII inhibitor	3 (1.7%)
von Willebrand disease, type III	1 (0.6%)

correction. There was no difference in the mean APTT between those with correctable and non-correctable APTT (47.4s versus 47.5s). In this group, the APTT prolongation was usually mild and less than 1.5 times of mean APTT (51s for our laboratory).

All of those deemed to have probable vWD, factor deficiency or FVIII inhibitor had prolonged APTT which were correctable with plasma (Table II). Haemostatic factor levels stratified to moderate to high risk of bleeding were found in those with APTT greater than 1.5 times of mean APTT (Table I).

DISCUSSION

To our knowledge, this is the first study looking specifically at the causes of isolated prolonged APTT in a clinical setting. A previous study had also looked at the causes of prolonged APTT but included in their study were patients with prolonged PT and TT. Another important difference is that the previous study included a significant proportion of patients with "spuriously' prolonged

Table II. Investigation results of cohort deemed to have factor deficiency, probable vWD or FVIII inhibitor.

Age	Sex	Race	APTT (s)	FVIII (%)	FVIII inhibitor (Betheseda unit)	FIX (%)	FXI (%)	FXII (%)	vWF:Ag (IU/dL)	LA result	Diagnosis	APPT-C
36	M	C	49.80	37		99	82	62	188	Negative	FVIII def	Y
65	M	C	48.90	47		87	42	62	54	Negative	FVIII def	Y
64	M	C	45.20	37		73	96	94	74	Negative	FVIII def	Y
37	F	C	47.00	30		78	56	52	37	Negative	vWD	Y
42	M	C	66.80	3		82	51	79	8	Negative	vWD	Y
77	M	C	80.10	3	12	91	17	18	212	Positive	FVIII inh	Y
73	F	C	77.60	1	22	191	83	46	293	Positive	FVIII inh	Y
77	F	O	59.90	9	2	151	80	62	260	Negative	FVIII inh	Y
28	F	C	59.60	3	0.4	61	64	94	163	Negative	FVIII def + inh	Y
70	F	C	45.50	109		153	37	47	153	Negative	FXI def	Y
64	M	C	51.60	210		114	35	43	185	Negative	FXI def	Y
47	M	O	44.00	86		106	31	87	71	Negative	FXI def	Y
49	M	C	44.70	169		136	39	42	138	Negative	FXI def	Y
22	M	C	57.40	51		72	18	41	53	Negative	FXI def	Y
23	M	I	100.90	142		126	<1	73	106	Negative	FXI def	Y
85	M	C	99.90	250		125	12	14	351	Negative	FXI, FXII def	Y
91	M	C	49.30	134		73	31	32	235	Negative	FXI, FXII def	Y
61	M	C	46.40	160		115	61	32	350	Negative	FXII def	Y
29	F	My	45.90	81		79	59	36	67	Negative	FXII def	Y
66	M	C	49.30	300		174	82	39	465	Negative	FXII def	Y
21	F	My	45.00	88		101	62	38	75	Negative	FXII def	Y
62	M	O	45.80	204		148	86	17	142	Negative	FXII def	Y
67	F	C	47.20	124		113	58	30	197	Negative	FXII def	Y
91	F	C	50.30	145		204	78	22	393	Negative	FXII def	Y
79	M	C	49.70	235		119	86	27	162	Negative	FXII def	Y
48	F	C	48.80	149		140	97	28	246	Negative	FXII def	Y
16	M	C	50.5	73		81	50	18	7	Negative	FXII def, vWD	Y

inh = inhibitor; def = deficiency; APTT-C = APTT correctable by plasma; Y = yes; My = Malay; C = Chinese; I = Indian; O = Others

Table III. APTT analysis of cohort with LA.

	Weak LA	Moderate LA	Strong LA
Total number	52	25	17
No. with correctable APTT	24	5	3
No. with incorrectable APTT	28	20	14
Median APTT (s)	48.0	52.7	67.2
APTT range (s)	43.4 to 63.2	46.1 to 75.6	44.4 to 97.1

APTT due to pre-analytical factors. This cause of prolonged APTT was minimised in our study by including only patients with confirmed isolated prolonged APTT after repeat venepuncture by a dedicated phlebotomist. Our study therefore included patients mainly with "true" prolonged APTT.

We found that the most common cause of isolated prolonged APTT was LA which may predispose patients to thrombosis rather than haemorrhage[14]. There was a wide overlap between the APTT of patients with weak, moderate or strong LA (Table III). The second most common group was those without detectable causes. The APTT was usually only mildly prolonged in this group and possible causes may include deficiency of contact factors that are clinically insignificant or very weak LA. None of these conditions presented a haemorrhagic risk and therefore FFP was not indicated. There remains the possibility that pre-analytical factors may have caused these prolongations of APTT despite our best efforts to minimise them. Most importantly, none of these patients developed any clinical bleeding.

Significant factor deficiency was in fact a relatively rare cause of an isolated prolonged APTT, accounting for just 11.3% of all the patients. FXII deficiency, alone or in combination with probable vWD or FXI deficiency, accounted for 54.5% of these patients. Factor XII deficiency is more often associated with thrombosis[15] and is relatively prevalent in the normal population[16]. Among the rest, only patients with significant factor VIII, IX or XI deficiency may benefit from FFP treatment and these constitute a very small number (6.2%). Even then, haemophilia A and B should preferably be treated with specific factor concentrates rather than FFP. This is also true for vWD. Usually only factor XI level below its haemostatic level of 30% is associated with bleeding[17].

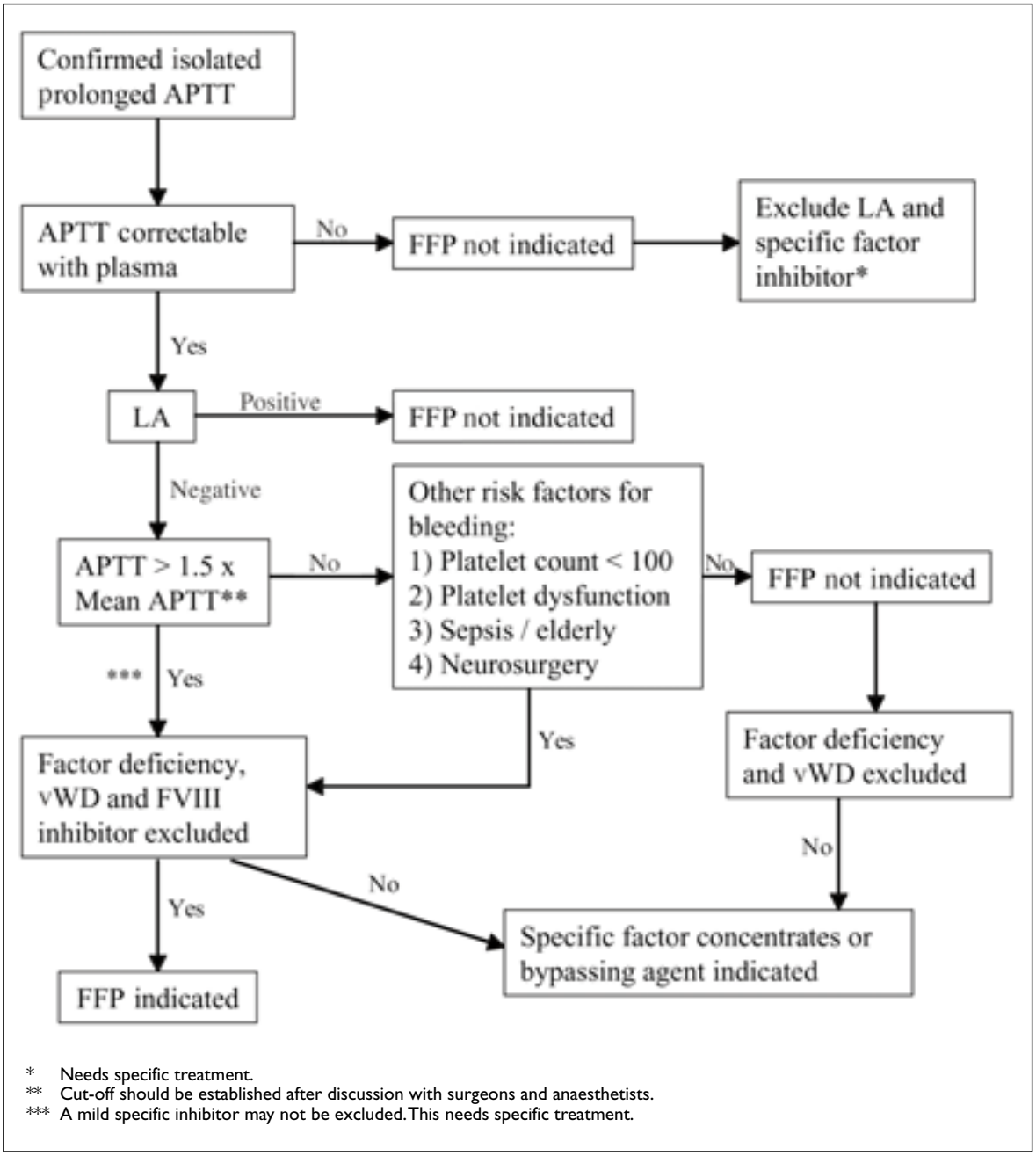
In our cohort, all three patients with FXI levels less than 30% had APTT more than 1.5 times our laboratory's mean APTT (51s), whereas those with FXI levels greater than 30% had APTT less than 51s. Most guidelines state that FFP transfusion is indicated in bleeding patients or pre-operatively, only if APTT is more than 1.5 times of mean APTT[18,19]. Although previous studies have shown that the degree of prolongation of the APTT does not have any predictive value regarding haemostasis[13], significantly low levels of coagulation factors may be associated with an APTT level above this level. After categorising the patients into the different risk groups for bleeding complications, most patients had no or mild risk (Table I). Our study therefore suggests that FFP is rarely indicated for isolated prolonged APTT.

We found that the degree of prolongation of APTT is neither predictive of bleeding risk nor the underlying diagnosis with significant overlap in APTT between the different risk groups and diagnoses. This is consistent with findings from previous studies[13]. We also studied the utility of plasma correction of APTT as a surrogate for the presence of inhibitor or factor deficiency. We found that among 94 patients with positive LA, 32 had correctable APTT. Most of these LA positive patients with correctable APTT had weak LA (Table III). All the patients in our cohort with FVIII inhibitor had correctable APTT although all of them also had APTT greater than 51s. Therefore, immediate correctability of APTT by plasma does not help in differentiating between patients with inhibitors or factor deficiency. In contrast, all patients with factor deficiency had correctable plasma, and factor deficiency may be excluded as a cause if the prolonged APTT was not correctable by normal plasma.

Based on our findings, we have proposed an algorithm for investigating isolated prolonged APTT and the use of FFP to correct the coagulation defect (Fig. 2). Using this schema, patients with FVIII inhibitor may sometimes be inappropriately given FFP, but FVIII inhibitors usually arise in known haemophiliacs who will be monitored for inhibitor development, or if they are acquired, then these patients usually present with evidence of bleeding. In this situation, factor assays will usually be done in the setting of a prolonged APTT (usually non-correctable with plasma). Patients with FVIII inhibitor should be recognised and managed accordingly to prevent uncontrollable bleeding.

One shortcoming of our study is a lack of clinical correlation to the reason for APTT request and clinical information on the patients. However, as the objective of this study was not to determine the sensitivity and specificity of APTT in diagnosing specific conditions or predicting bleeding risk, we felt that this information was not necessary. Previous studies had already shown that APTT had low sensitivity in detecting haemophilic disorders[20] or

Fig. 2 Investigation algorithm for isolated prolonged APTT and decision for FFP usage.



* Needs specific treatment.
** Cut-off should be established after discussion with surgeons and anaesthetists.
*** A mild specific inhibitor may not be excluded. This needs specific treatment.

yield as a pre-operative screening test in the absence of clinical evidence of coagulation disorder[21].

In conclusion, our study suggests that isolated prolonged APTT in an acute care hospital setting is usually due to causes that do not lead to increased bleeding risk. An investigational algorithm may help in determining the cause of the coagulation defect as well as rationalising FFP use.

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