CLINICAL UTILITY OF ISTH BAT (INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS, BLEEDING ASSESSMENT TOOL) AND THROMBOELASTOGRAPHY FOR ASSESSMENT OF PATIENTS REFERRED FOR EVALUATION OF BLEEDING DISORDERS.

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REQUIREMENTS FOR THE M.D. DEGREE BRANCH XXI(TRANSFUSIONMEDICINEANDIMMUNOHEMATOLOGY)EXAMINATION OF THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY CHENNAI TO BE HELD IN APRIL 2017.

CERTIFICATE

This is to certify that this dissertation titled "CLINICAL UTILITY OF ISTH BAT (INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS, BLEEDING ASSESSMENT TOOL) AND THROMBOELASTOGRAPHY FOR ASSESSMENT OF PATIENTS REFERRED FOR EVALUATION OF BLEEDING DISORDERS." is a bonafide work done by Dr.Pragya Kafley, in part fulfilment of rules and regulation from the M.D. BRANCH XXI (Transfusion Medicine and Immunohaematology) Degree examination of the Tamil Nadu Dr. M.G.R Medical university, to be held in April 2017.

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The candidate has independently reviewed the literature, the data collection methodology and carried out the evaluation toward completion of the thesis.

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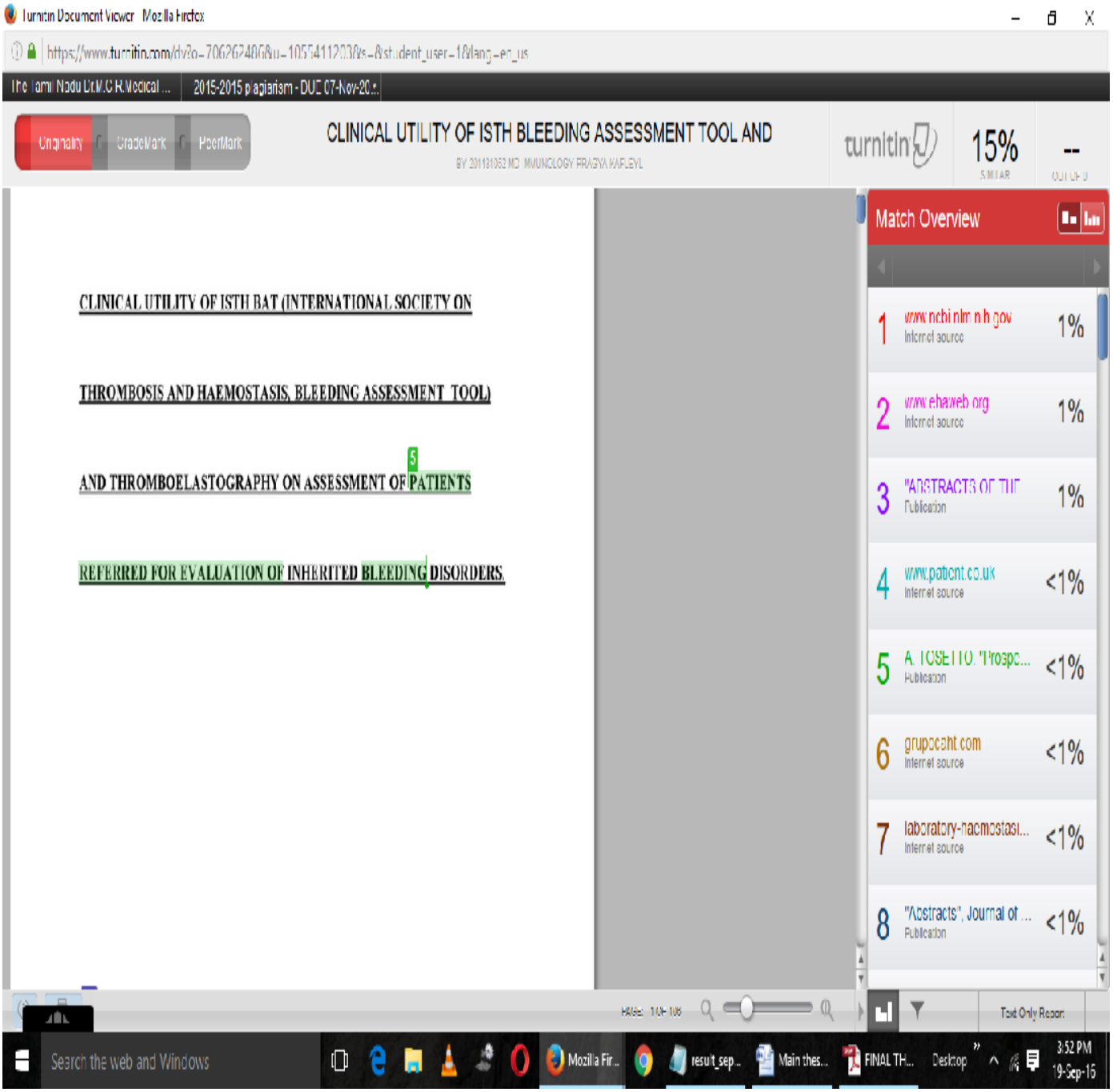
CONTENTS

PLAGIARISM CHECK: ORIGANILITY CERTIFICATE



ACRONYMS

ISTH	**International Society on Thrombosis and Hemostasis**
BAT	**Bleeding Assessment Tool**
BS	**Bleeding Score**
TEG	**Thromboelastography**
ROTEM	**Rotational Thromboelastometry**
R Time	**Reaction Time**
K Time	**Kinetics Time**
MA	**Maximum Amplitude**
Ly 30	**Lysis at 30 minutes**
VWD	**Von Willebrand Disease**
VWAg	**Von Willebrand antigen**
Vwf	**Von Willebrand Factor**
F	**Clotting factor**
PT	**Prothrombin Time**
BT	**Bleeding Time**
APTT	**Activated partial thromboplastin time**
HMWK	**High molecular Weight Kininogen**
RBDs	**Rare Bleeding Disorders**
NPV	**Negative Predictive value**
PPV	**Positive predictive value**
LR	**Likelihood Ratio**

Introduction

Introduction

Bleeding disorders are said to affect 1 out of 1,000 men and women all over the world (1). Hemophilia A and Von Willebrand Disease amongst them rank as the most common(2,3). Indian data are maintained by the HFI (Hemophilia Federation of India). As per data that was released in 2011 , 14,718 patients who presented with bleeding disorders 11,586 patients were patients of Haemophilia A. (4)India reports third largest number of patients with bleeding disorders , and also the second highest number of patients having Haemophilia A .(4) Other bleeding disorders include the rare factor deficiencies and Platelet function disorders. RBDs are reported in almost all populations, incidence varies from 1 out of 500 000 for deficiency of Factor VII to 1 in every 2 to 3 million for deficiency of Factor II and Factor XIII .(5,6) The incidence of inherited platelet function disorders are much lower as compared to other bleeding disorders.(7)

Given the prevalence and the morbidity associated with bleeding disorder, proper diagnosis and early management will give the patients a better quality of life. While diagnosing severe bleeding disorder may not be problematic, diagnosing mild bleeding disorder remains a challenge.(8) Adding to the challenge is the fact that severity of bleeding disorder is highly subjective and studies have shown that even healthy volunteers when asked for the presence of bleeding symptoms answer in the affirmative, that was as high as 65 % in females and 35 % in males(9). Hence it is important to ask precise guided questions that will help us arrive at a better conclusion. With the

importance of a well taken history in the diagnosis of bleeding disorder in mind and in an attempt to standardize bleeding history many bleeding assessment tools(BAT) have been introduced. Standardized questions in combination with an interpretation grid, which is well defined and used for finally computing the bleeding score is referred to as BAT.(10) In 1995 Sramek et al published their findings and in 2005 International Society on Thrombosis and Hemostasis(ISTH) set out a provisional criteria for diagnosis of Von Willebrand Disease.(10,11)

Building on this criteria investigators from Vicenza Italy developed and validated a questionnaire in 2005, to improve the sensitivity of the bleeding score further

two different bleeding assessment tool by the name European Molecular and

Clinical Markers for the Diagnosis and Management of type 1 VWD (MCMDM-1

VWD) was developed but owing to its long administration time a shortened version of the same was developed. In order to develop a consensus bleeding score by combining the results obtained from all the assessment tools mentioned above ISTH published the ISTH- BAT in 2010.(12) The aim of our study is to see the utility of ISTH-BAT in evaluation of hemostasis among patients who get referred to our laboratory for the same .

Rare bleeding disorders eludes detection by routine coagulation tests and abnormality in these tests do not always translate to clinical bleeding.(13) So it was decided that as an adjunct to BAT and other lab tests a global test of hemostasis namely Thromboelastography (TEG) would be included in these patients. The limitations of test being routinely done for work-up of a bleeding patient is that there are a number of tests each evaluating a single aspect of coagulation. On the other hand global assays of coagulation like TEG measures the effect of various deficiencies in coagulation while retaining the interactions of other components of blood like erythrocytes, platelets and leucocytes as the test is done in whole blood. In contrast to other routine tests TEG offers information about all phases of coagulation.(13)

AIMS AND OBJECTIVES

AIM:

Determintion of the clinical utility of Thromboelastography and ISTH BAT for evaluating patients referred for complete coagulation work-up.

OBJECTIVES:

1) To calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive (NPV) of ISTH BAT in evaluation of patients referred for complete coagulation work-up.

2) To calculate the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of Thromboelastography in patients referred for complete coagulation work-up.

3) To calculate the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of Thromboelastography and ISTH BAT in patients referred for complete coagulation work-up.

REVIEW OF LITERATURE

HEMOSTASIS:

The classical also sometimes referred to as the cascade based model of hemostasis describes series of reactions involving sequential activation of various clotting factors by one of the two pathways, either intrinsic or extrinsic pathway, the endpoint of these activation resulting in copious amount of thrombin generation and the eventual conversion of fibrinogen to fibrin clot.(14)(Fig.1)

Although this explanation supports laboratory investigations of hemostasis it is not very useful in understanding the events of hemostasis in-vivo. The model also is incapable of explaining why some patients with factor deficiencies bleed while others with low levels of Factor XII and High molecular weight kininogen(HMWK) do not inspite of an APTT that is prolonged which would normally mean the defect in the intrinsic pathway.(15)This model also failed to explain that in haemophiliacs with normal PT , how is it that the normal extrinsic pathway fails to compensate for prolonged bleeding.

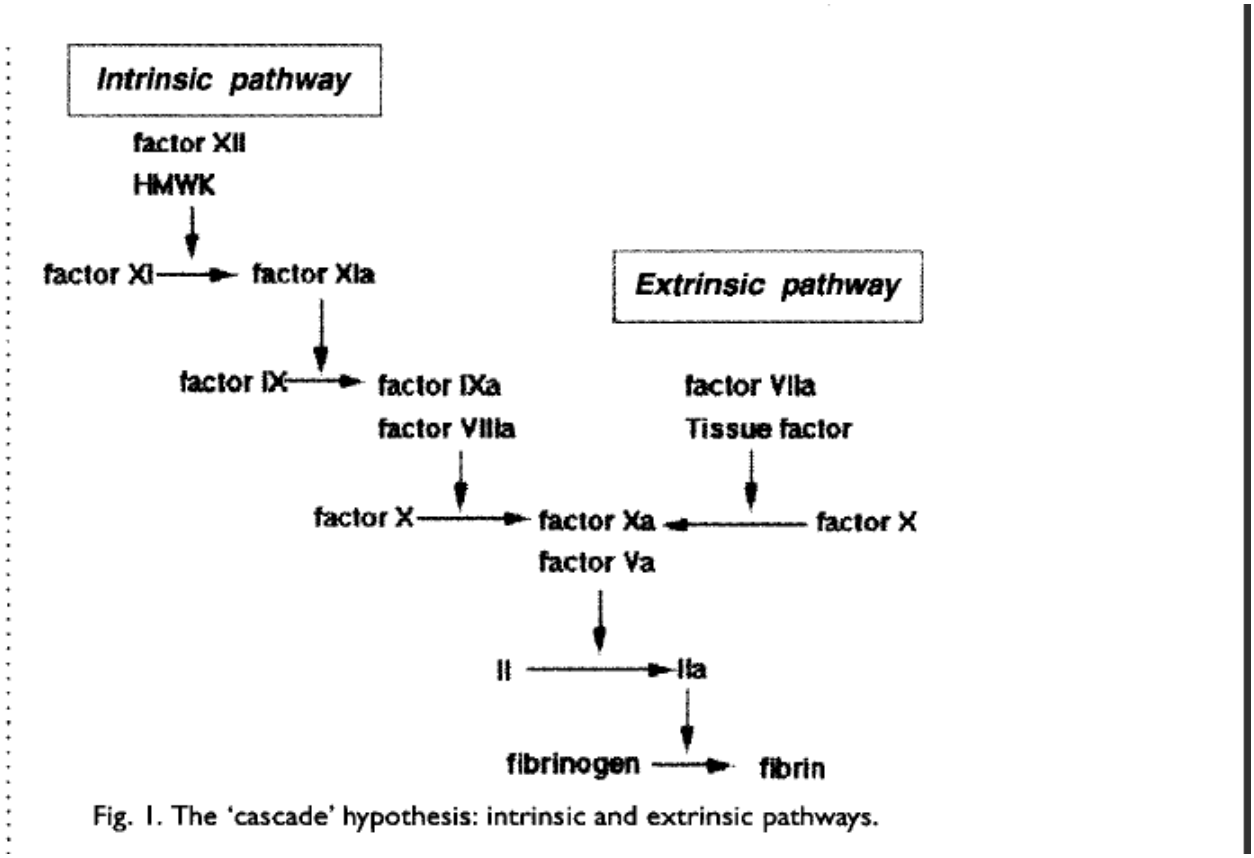


Fig. 1. The 'cascade' hypothesis: intrinsic and extrinsic pathways.

Figure 1: Adapted from Maureane Hoffman. A cell-based model of coagulation ,Blood reviews 2003:17;51-55

These questions necessitated the introduction of a cell based model of hemostasis. This model emphasizes that coagulation in vivo occurs in three steps that occur on a surface of a cell, and these steps usually are overlapping.(15)

The three phases are **Initiation, Amplification and Propagation**.(15)

Initiation: This event takes place on the surface of cells that bear tissue factors. This may refer to the extrinsic pathway of the earlier model as normally tissue factor bearing cell remains outside of the vascular compartment, therefore extrinsic or outside to blood.

Although many cells may produce tissue factor it is only in the setting of inflammation, that they do come in contact with blood. However some evidences point to the fact that initiation of coagulation in the extrinsic system occurs even in healthy individuals all the time but coagulation is prevented as the intact vessel wall creates a barrier of sorts between tissue factor and other components of coagulation.(15)

Amplification: Vascular damage results in components of the vascular system previously unable to leave the vascular compartment owing to their large size to come out. Notable among these are platelets and Factor VIII and vWF. As they exit the vessel compartment they come in contact with tissue factor present on the surface of cells that bear them. Platelets form a plug after attaching to these. The thrombin present activates the platelets and also activation of the clotting factors namely FVIII that ultimately helps in the cleavage of VWF. Factor XI also gets activated. This may explain why Factor XII is not essential in hemostasis . Though insufficient to form a clot by itself, thrombin that is present on the tissue factor bearing cells surface help in activating platelets that are now layered with co-factors which are procoagulant. The product is now ready for propagation.

PROPAGATION:

During propagation, on the surface of activated platelets, Factor IXa combines with its cofactor Factor VIIIa on the surface of activated platelets. This complex then goes on to a to form Factor Xa, as a result of activation of FX. The FXa then combines with its co-factor.

The Factor Xa /Va complex then converts large amount of fibrinogen to fibrin that helps in stabilization of the clot. The cell based model of coagulation thereby helps in better understanding of the mechanism than the earlier postulated hypothesis,otherwise known as the cascade hypothesis.(16)

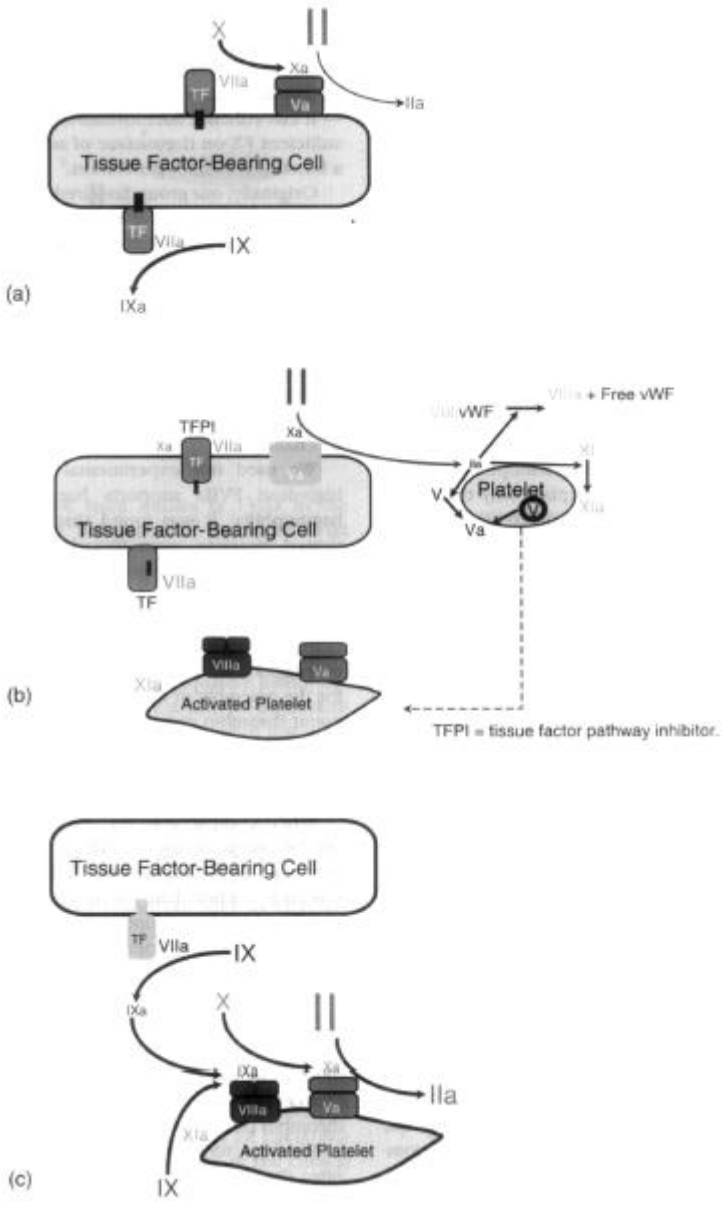


Fig. 3. The cell-based model of haemostasis: (a) initiation, (b) amplification, (c) propagation.

Figure 2:Adapted from Maureane Hoffman. A cell-based model of hemostasis . Blood reviews 2003:17;51-55

OVERVIEW OF COMMON AND RARE BLEEDING DISORDERS:

1) Disorders of soluble clotting factor: The most common among these are classical Hemophilia or Hemophilia B due to deficiency of FVIII and FIX respectively.

2) Other disorders include:

Fibrinogen Abnormalities: Normally pattern of inheritance being autosomal recessive . Two forms known are Afibrinogenemia and Dysfibrinogenemia.(17) Fibrinogen is composed three polypeptide chains, each with two sets: Aα, Bβ and γ, and when any of the chains are defective, it results in a rare bleeding disorder called Afibrinogenemia. The dysfibrinogens may be the result of one of the following mutations, missense, nonsense, or splice junction mutations.(18)

Prothrombin (Factor II) Deficiency: It is one of the rare bleeding disorder and the pattern of inheritance is Autosomal Recessive .

Factor V Deficiency: It is an autosomal recessive condition caused by an abnormality in Factor V gene. More than 40 mutations have been described in Factor V gene, even then they are more common than the mutations seen in other clotting factors.(19)

Factor VII Deficiency: An autosomal recessive disorder seen to occur in mild, moderate and severe forms. Factor VII is the main initiator of clotting mechanism along with other co-factors. There are more than 100 mutations in the gene identified so far.(19)

Hemophilia A and Hemophilia B : These conditions have a unique pattern of inheritance, X linked recessive. Conversion of pro-thrombin to thrombin is done by these two factors. They are of mild moderate and severe forms.(18) Several hundred mutations have been identified.(19)

Factor X Deficiency: Factor X deficiency also shows an autosomal recessive mode of inheritance. It is also classified as mild, moderate severe and the symptoms bear resemblance to Hemophilia A.(18)

Factor XI Deficiency: Bleeding in this disorder may be of varying severity, this is probably due to normal levels of Factor VIII and IX that forms tenase complex and Factor V and X that forms pro-thrombinase complex.(18) This exhibits AR mode of inheritance.

Factor XII deficiency, Prekallikrein and HMWK deficiency:

Autosomal recessiveinheritence . Most patients come to notice because of incidental prolonged APTT.

Factor XIII Deficiency: Deficiency of plasma transglutaminase results in this particular disorder, FXIII is required for cross-linking of fibrin chains to form a clot of Fibrin that is impermeable. 2 A chains and 2 B chains together constitute FXIII, A being the active subunit, B acts as a carrier for the A subunit.

Multiple Clotting Factor Deficiencies : Factor VIII and Factor V deficiency occur together. This is followed by combined deficiency of Factors II, IX,VII and X. Mode of inheritance being AR.(18)

von Willebrand Disease : Most common amongst hereditary clotting factor deficiency. von willebrand factor facilitates platelet adhesion is a carrier of clotting factor VIII.

Table 1: Classification of vWD

Classification of vWD

Type	Description
1	Partial quantitative deficiency of vWF
2	Qualitative vWF defects
2A	Decreased vWF-dependent platelet adhesion and a selective deficiency of high molecular weight vWF multimers
2B	Increased affinity for platelet GPIb
2M	Decreased vWF-dependent platelet adhesion without a selective deficiency of high molecular weight vWF multimers
2N	Markedly decreased binding affinity for FVIII
3	Virtually complete deficiency of vWF

vWF, von Willebrand factor.
From Sadler JE, Budde U, Eikenboom JC, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand factor. *J Thromb Haemost* 2006;4(10):2103–2114.

INHERITED PLATELET DISORDERS:

Bernard Soulier syndrome: It is one of the syndrome that comprises the Giant platelet syndrome. This is an autosomal recessive condition and affects one of the genes encoding the Gp Ib/V/IX complex. Fechtner, May Hegglin, Sebastian,Epstein are other macrothrombocytopenia syndromes that arise secondary to MYH9 gene.

Glanzmann Thrombasthenia: This is caused by the defect in platelet membrane complex Gp IIb/IIIa. The defect may sometimes be seen in other gene, it exhibits molecular heterogeneity.

Storage Pool Deficiency: ADP, ATP, calcium and serotonin are the contents of dense granules whereas alpha granules have fibrinogen, FV, thrombospondin , platelet derived growth factor,fibronectin. Failure to release the contents of platelets on activation results in Storage pool deficiency.(18)

Given the varied presentation of bleeding disorder and the inherent challenges in its diagnosis, the importance of well taken relevant history cannot be overemphasized.

Table 2: Features of Inherited deficiencies of coagulation factors .(Adapted from

N Engl J Med, Vol. 344, No. 23 · June 7, 2001)

Deficient Coagulation Factor	Incidence in General Population	Chromosome Involved	Mode of Inheritance
Fibrinogen	1:1 million	4	Autosomal recessive
Prothrombin	1:2 million	11	Autosomal recessive
Factor V	1:1 million	1	Autosomal recessive
Factor VII	1:500,000	13	Autosomal recessive
Factor VIII	1:10,000	X	X-linked recessive
Factor IX	1:60,000	X	X-linked recessive
Factor X	1:1 million	13	Autosomal recessive
Factor XI	1:1 million	4	Autosomal recessive
Factor XIII	1:1 million	6 (subunit A) 1 (subunit B)	Autosomal recessive

A clear, correct and precise bleeding history is the most useful tool for diagnosing a bleeding disorder, based on the history further investigations should be decided. Diagnosing bleeding disorder especially in its milder forms pose a big challenge to the physicians as bleeding is a symptom found in healthy as well as diseased population. It has been seen that when people are merely asked for the presence or absence of bleeding disorder a high percentage of healthy people say yes, this shows the importance of a well taken history with guided questions in distinguishing pathological bleeding from normal bleeding. (9) Attempts to standardize history taking in bleeding disorders have been going on and in the process many different types of bleeding assessment tools that are a set of questionnaire pertaining to bleeding episodes, severity and treatment received for the same have been developed.(12). In 1995 the first bleeding assessment tool was developed and subsequently validated.(11) Thereafter BATs have gone many evolutions and in 2010 ISTH published a BAT(10).

Vicenza based bleeding score:

When ISTH first laid down the criteria for BAT a group of investigators that was lead by Rodeghiero developed a bleeding assessment tool for VWD Type 1 , in this the bleeding symptoms present were given scores ranging from 0 to 3 and the final score were to be summated ,when this bleeding score was subsequently validated with a cut off score of 3 for males and 5 for females the specificity for the disease was found to be 98% and sensitivity of 65%.(20).

To increase the sensitivity of BAT further revision of scoring system was done and a negative score for absent bleeding despite significant hemostatic challenge was introduced and the score now ranged from -1 to 4. This model was used for European Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD (MCMDM-1 VWD) Study.(21) Owing to its long administration time the score was further shortened version of the same was introduced. This particular BAT was prospectively analyzed one in the setting of primary care and another in two groups of referred population. In the setting of primary care patients the questionnaire gave a sensitivity of 100% and specificity of 87%, However in the other set the sensitivity and specificity obtained was widely variable.(22,23). With a view of consolidating the knowledge that was acquired from all these published studies and the need for a consensus bleeding assessment tool , ISTH established a scientific sub –committee(SSC) which with inputs received from women's health issue SSC published a questionnaire known as the ISTH BAT in 2010(10). There is an ongoing attempt to validate this tool has been and a web based version encouraging the researchers to share their data is available.(12)

ISTH BAT

ISTH BAT was developed and published by the ISTH SSC in 2010. The issues that led to the development of this tool was to establish a single bleeding assessment tool that would be useful for the standardization of bleeding symptoms reporting and would be useful in

adults and the pediatric population alike. The problems with the already existing BATs were that they were validated using the normal population as a reference so the chance of missing mild disorders were high and also because the primary focus was in the diagnosis of mild bleeding disorder(MBD) there was every chance of the score getting saturated in severe bleeding disorder. ISTH BAT was created to overcome these problems.(10). ISTH BAT has a score of 0 to 4 for each symptom and has a maximum score of 56 in females and 48 in males. A score of 3 in pediatric population, 4 and more in adult males and 6 and more in adult females are considered significant.(24)(ISTH BAT in annexure)

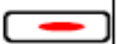
Other bleeding assessment tools:

Apart from the BATs developed by the Italian group various other BATs have been developed and published.(12) These include:

Menorrhagia specific tools:

Menorrhagia is an important hemostatic challenge and it is seen that of all the women who experience excessive bleeding symptoms 15% have an underlying bleeding disorder.(25) Menorrhagia also remains one of the most frequently reported bleeding symptom among women. Menorrhagia specific BAT involves a Pictorial Bleeding assessment chart (PBAC) which enables women to document the number of tampons ans sanitary napkins used and the extent of soiling on each, based on which a score is obtained, score of 185 and more is considered significant.(12)

Month: _____

	Pads			Tampons			Clots		Flooding	Score
Date	Light  (1 pt each)	Medium  (5 pts each)	Heavy  (20 pts each)	Light  (1 pt each)	Medium  (5 pts each)	Heavy  (10 pts each)	5 cent size (1 pt each)	50 cent size (5 pts each)	1 pt each episode	
1										
2										
3										
4										
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29										
30										
31										
									Total	

Figure 3a: PBAC , Menorrhagia specific tool.

PBAC Scoring System

Pads		
1 point	For each lightly stained pad	
5 points	For each moderately stained pad	
20 points	For each completely saturated pad	
Tampons		
1 point	For each lightly stained tampon	
5 points	For each moderately stained tampon	
10 points	For each completely saturated tampon	
Clots/Flooding		
1 point	For each small clot (Australian 5 cent coin)	
5 points	For each large clot (Australian 50 cent coin)	
5 points	For each episode of flooding	

Figure 3b: PBAC , Menorrhagia specific tool.(Scoring system)

Pediatric bleeding assessment tools:

While the diagnosis of mild and rare bleeding disorder is in itself very challenging, bleeding disorders in pediatric population pose a whole set of fresh challenges. The classical bleeding symptoms usually seen in adult population are usually lacking in children this combined with the obvious absence of hemostatic challenge makes it further more difficult to know the bleeding symptoms. Some early symptoms like umbilical cord bleeding could have been overlooked or forgotten. Hence a specific BAT meant to overcome these challenges in pediatric population was warranted.(12)

Since ISTH endorsed Vicenza based BATs Bowman et al created a pediatric specific BAT by adding symptoms specific to pediatric population into MCMDM-1 VWD questionnaire. The pediatric bleeding questionnaire thus created and subsequently tested showed a sensitivity and specificity of 83% and 70 % respectively.(26)

Composite clinical score for clinical severity of Hemophilia.:

Plasma levels of Factor levels poorly correlates with phenotypic expression of Hemophilia (27) Assessment of probable factors that influence phenotypic expression of Hemophilia has garnered considerable interest. Several Factors namely half life of Factor VIII , Thrombin generation, vWF and also the components of fibrinolytic systems have been evaluated.(28–31)

Since there are several aspects of severity it was decided to construct a composite score for Hemophilia severity which may be useful. The items in the Hemophilia Severity Score were:

1) Joint bleeds-annual incidence

2) Orthopedic joint score given by WFH

3) Factor consumption per annum.

They concluded that Hemophilia Severity Score was a tool that was able to provide information about the clinical severity of Hemophilia in adults.(32)

This scoring system was further validated by Tagliaferri et al ,where they found it to be a useful tool in assessing the phenotypic severity of Hemophilia.

Evaluating clinical utility:

While evaluating the BATs for its clinical utility the objective and the setting where it is used must be borne in mind. It may be used for screening patients during first visit in primary and tertiary centers or it can be used in assessing disease severity. Although the focus of Vicenza based BAT s have been VWD but many prospective studies have taken place to evaluate the clinical utility of BATs in evaluation of other bleeding disorders. (12) Also while evaluating the clinical efficacy it is important to recognize how study of a particular population affects the results, for example if a known bleeder were to be enrolled into the study it would falsely increase the sensitivity. Rodegheiro in 2005 eliminated this problem by enrolling subjects who werev obligate carriers of VWD rather than studying the cases of the same.(33). Tossetto et al in 2006 considered only the symptoms present prior to the diagnosis of Type 1 VWD for calculating the score whereas Bowman et al in 2006 with MCMDM 1 and PBQ used only the first time cases. Also the controls used in all were healthy population.(21,22,26).

While the clinical utility of Vicenza based BATs have mainly be done with respect to VWD several prospective studies analyzing its utility in other inherited bleeding disorders have been carried out. The Condensed MCMDM VWD Type 1 has been used for prospective evaluation of platelet function disorder with specificity, sensitivity, PPV and NPV of 86%, 65%, 0.50 and 0.92 respectively(12). Tosetto et al in 2011 used the BAT for various bleeding disorder and found that there was heightened sensitivity when the bleeding score was used in conjunction with Activated partial thromboplastin time.(34)

ISTH BAT has also been evaluated for diseases other than VWD. Lowe et al prospectively analyzed bleeding assessment tool in patients with inherited platelet disorder and concluded that though for documentation of bleeding symptoms, ISTH BAT was an effective however the score thus obtained was not indicative of a platelet function disorder and there was no correlation with the defect observed on platelet aggregometry. (35).

More recently in 2016 Rashid et al administered ISTH BAT in patient with Platelet function disorder in a setting with resource constraint and concluded that apart from being a very useful tool for documentation of bleeding symptoms over time the BAT was also predictive of platelet function defect as eventually identified by aggregometry with odds ratio 3.25 [95% confidence interval 2.13-4.37, $p = < 0.001$](36)

Kaur et al also evaluated the utility of ISTH BAT in platelet function disorder especially Glanzmann Thrombasthenia and Bernard Soulier Syndrome, they even compared the web version and the normal version and the result they obtained showed a sensitivity, specificity, PPV and NPV of ISTH BAT in platelet function disorder of 100%, 76.2%, 0.9 and 1 respectively.(37)

TABLE 3: DEPICTS THE EVOLUTION OF VICENZA TYPE BLEEDING

ASSESSMENT WITH ITS YEAR OF PUBLICATION AND THE RANGES:

BAT and YEAR OF INTRODUCTION	SCORING SYSTEM	POSSIBLE RANGE OF SCORE	ABNORMAL BLEEDING SCORE	ADMINISTRATION TIME
Vicenza bleeding questionnaire (VBQ) 2005	0 to + 3		BS of >3 in males and>5 in females	40 Minutes
MCMDM1-VWD 2006	−1 to +4	–3 to +45	>4	40 Minutes
Condensed MCMDM-1VWD BQ 2008	-1 TO +3	–3 to+ 45	>4	5 to 10 mins
Pediatric Bleeding Questionnaire 2009	−1.5 to +2.5	-3 TO 48	(BS) ≥2	20 MINS
ISTH BAT 2010	0 to 4	0 to 48 in Men and children. 0 to 56 in women	BS of ≥4 for a Males, ≥6 for females and ≥3for children	20 MINS

VISCO ELASTIC TESTS:

Two major systems in place are using this technology are Thrombelastograph (TEG) (Haemoscope Corporation, Niles,Illinois, USA) and the ROTEM (Pentapharm GmbH, Munich,Germany).(38) While the parameters measured by these machines are identical they differ in their primary mechanism. In TEG there is an oscillating cup that moves in a limited arc with sample inside and there is a pin/wire transduction system whereas in ROTEM the cup is immobile and pin transduction system oscillates. The analysis of TEG is called Thromboelastography whereas that of ROTEM is called Thromboelastometry. Both ROTEM and TEG come under a category of test called the test of global hemostasis. Global tests of coagulation includes tests namely the TEG, ROTEM and Thrombin generation test.(39) Traditionally hemostasis evaluation were being carried out by clotting times of plasma , APTT and prothrombin time (PT), these tests determine the conversion of Fibrinogen to Fibrin that is dependent on thrombin and assesses only the clot formation initiation and not the entire length of the process. This combined with the fact that these tests are performed at un-physiological conditions and these tests segregate coagulation into different compartments thereby precluding the study of other factors that impact coagulation process as a whole the conventional tests are riddled with limitations(40–42).

At present, there is lack of easily available and standardized tests that can measure the hemostatis. Tests measuring global hemostasis can with greater sensitivity and accuracy capture generation of thrombin and formation of Fibrin clot. These include thrombin generation assay(TGT), TEG and ROTEM.(43–45)

THROMBOELASTOGRAPHY:

Thromboelastography/TEG is the method described by Dr Helmutt Hartert . The term refers to the obtained trace as a result of viscoelastic changes produced during fibrin polymerization. TEG allows the evaluation of all the steps of hemostasis from initiation to formation and stability of clot.(39) As has been earlier mentioned that the TEG has a cup with a sample that rotates in a limited arc and there is a pin transduction system and the values are traced . The tracing thus obtained has the following parameters. .While the initial utility of TEG was meant to reduce transfusion requirement in complicated surgeries but now its utility has expanded to bleeding and thrombotic disorder.(39) TEG and its utility in bleeding disorder has also been greatly studied.

Table 4: Parameters measured by TEG.

R	R time is the period of time of latency from the time that the blood was placed in the TEG® analyzer until the initial fibrin formation. This represents the enzymatic portion of coagulation.
K	K time is a measure of the speed to reach a certain level of clot strength. This represents clot kinetics.
α	α measures the rapidity of fibrin build-up and cross-linking (clot strengthening). This represents fibrinogen level.
MA	MA, or Maximum Amplitude, is a direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot. This represents platelet function/aggregation.
LY30	LY30 measures the rate of amplitude reduction 30 minutes after MA. This represents clot lysis.

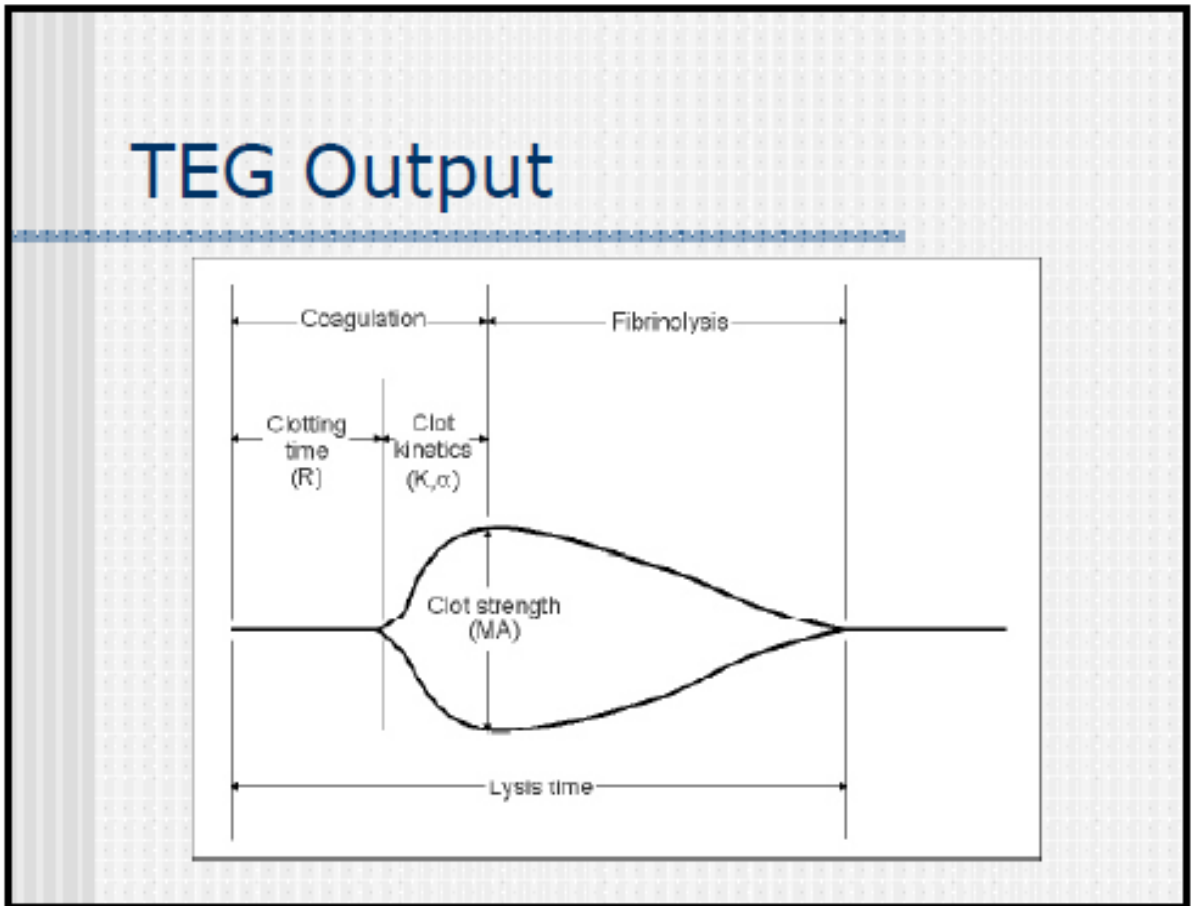

Figure 4: Normal tracing of TEG.

TEG IN HEMOPHILIA:

Classical Hemophilia A and Christmas disease or Hemophilia B are inherited deficiency of Factor VIII(FVIII) and Factor IX(FIX) respectively. Hemophilias are classified based on Factor levels into mild, moderate and severe. (Mild FVIII or FIX >5%, Moderate 1-5%,Severe <1%). Even though considered rare,they are the most common when it comes to bleeding disorders.

Traditionally diagnosis of Hemophilia and its monitoring has been based on measurable levels of clotting factors in plasma. The advent of whole blood coagulation assay has meant that the whole dynamic process of coagulation is not compartmentalized and it can

be studied in an environment the interaction between enzymatic factors and platelet interaction is conserved.(46)

In a study done by Chitlur_et al , where they studied 58 children with severe Hemophilia and their TEG profiling was done found that the R time in children with Severe Hemophilia without inhibitors was significantly prolonged than the normal controls ($p<0.001$) and when they compared the time taken for clot formation in Severe Hemophilia A with inhibitors as against those without inhibitors, the R time was significantly prolonged in the former catergory.($p<0.001$)The discrepancy often observed between phenotype and genotype in severe Hemophilia A have been studied and it was seen that patients who had less bleeding had better thrombin generation capacity(46). The utility of TEG in titration of recombinant FVII has also been evaluated.(44)

TEG IN RARE BLEEDING DISORDERS:

RBDs comprise of coagulation factor deficiencies and platelet function defects. The inheritance of these disorders are usually AR.(47,48) Patients with RDB often have variable phenotypes that cannot be predicted by levels of clotting factors alone.(48,49).

As is the inherent problem with all the coagulation tests commonly used that they compartmentalize the different facets of coagulation ,as a result RBD may evade detection by these conventional tests. Global coagulation assays measure the effects of factor deficiency while the interactions of other factors like Leucocytes, erythrocytes and Platelets intact.(13)

Zia et al studied TEG in the diagnosis of RBDs. This was a retrospective study done on 26 patients with rare bleeding disorder. They had 4 patients of Fibrinogen deficiency, 1 patient of pro-thrombin deficiency, 1 Factor V deficiency,3 patients of combined Factor VIII and Factor V deficiencies, 1 of Factor XI and 4 of Factor XIII deficiency. Other disorders included in the study were 1patient of PAI 1 deficiency, 1 of Vitamin K dependent clotting factor deficiency, 14 cases of Glanzmann Thrombasthenia and 1 patient of Bernard Soulier Syndrome. Apart from this there were 2 patients, 1 each of High Molecular Kininogen deficiency and 1 of FXII deficiency. (13)

Fibrinogen Deficiency:

TEG in these patients showed K time that was prolonged, and a decreased MA.

Prothrombin deficiency

Showed a prolonged R and K time.

FV deficiency:

Showed extremely prolonged R time and K time and reduced α angle.

FV + FVIII deficiency:

This study evaluated 3 patients with combined factor V and VIII deficiency and along with prolonged R time and low normal MA. Another interesting observation in the study was that, that out of 3 patients two were siblings and had identical factor levels ,but one of the sibling was severely symptomatic while other was not.

In the asymptomatic sibling it was found that she had less prolongation of

R time was less severe and normal K time., this pointed to the fact that TEG could predict bleeding phenotypes.

FVII deficiency:

There were three patients of Factor VII deficiency identified in this study. Two out of three patients were severe bleeders and had markedly prolonged R and K time whereas the moderate bleeder among the three had lesser degree of derangement.

FXI deficiency:

The Factor XI deficiency patient had R time and K time both prolonged.

FXII deficiency:

TEG abnormality associated with FXII deficiency R time that was prolonged .

FXIII deficiency:

When four patients of FXIII deficiency were evaluated TEG showed low normal MA and fibrinolysis.

Glanzmann thrombasthaenia:

Four patients with thrombasthenia revealed markedly decreased MA .

Bernard Soulier Syndrome:

Showed increased K and decreased angle.

PAI-1 deficiency:

Showed increased fibrinolysis.

HMWK deficiency:

The abnormality seen was R time that was prolonged.

While genetic testing may be confirmatory in rare bleeding disorders there is a poor correlation between genotype and phenotype. There is also a lack of universal screening test for rare bleeding disorders. In a study done by Zia et al where they retrospectively analyzed 26 patients with rare bleeding disorders they found that TEG was abnormal in all 26 patients, the major limitation of this study was that it was retrospective in nature and owing to the rare nature of RBDs in general the small sample size and poor representation of some of the conditions a conclusive inference cannot be drawn. Nevertheless TEG may be an effective screening tool for these patients. (13)

The heterogeneity of bleeding disorder makes it very cumbersome for practicing clinicians to diagnose these disorders accurately, added to that fact is the limitations of all the screening tests. Zia et al in their retrospective study found TEG to be of limited utility as a screening test for Inherited coagulation disorder. In their retrospective study they found that on 195 patients with 29 cases of diagnosed bleeding disorders that comprised TEG to have very poor sensitivity and specificity.(50)

A study has shown that when a BAT score is taken in conjunction with APTT, a normal BAT and a normal APTT can exclude the presence of a minor bleeding disorder(34) we decided to check the sensitivity and specificity of BAT score in conjunction with parameters of thromboelastography.

MATERIALS AND METHODS

MATERIALS AND METHODS

This is a prospective study done in the Department of Transfusion Medicine and Immunohematology, Special Tests Laboratory Christian Medical College and Hospital,Vellore,India. The study was approved by Institutional Review Board.

A total of 223 patients who were referred for coagulation work-up in between November 2015 and May 2016 were enrolled into this study. An informed consent was taken.All the patients who are referred to our lab for the said work-up undergo a set of tests based on the protocol of our lab. Before the sample for the test is taken registrars posted there take a detailed clinical history. For this study before the procedure began an informed consent and in case of children an informed parental consent and a children assent was also obtained by the registrars.

Patients referred to our lab mainly fall into one of the three categories.

1. Patients referred for increased bleeding tendency.
2. Patients referred for further evaluation of abnormal lab parameters.
3. Patients referred because of history of bleeding disorders

All the patients who gave their consent were enrolled into the study. There was no exclusion criteria for enrollment into the study. Post enrollment a detailed history was taken and the questionnaire administered by the registrars. A bleeding score was calculated based on the answers obtained after administration of questionnaire. Thereafter as our routine protocol the complete coagulation work-up was carried out. The baseline tests that we do in our laboratory include:

1. Complete Blood count(CBC)
2. Bleeding Time(BT) in patients 3 years and older
3. Platelet Function assay(PFA 200)
4. Prothrombin Time(PT)
5. Activated partial thromboplastin time(APTT)
6. Thrombin Time(TT)
7. Fibrinogen activity(Claus assay)
8. Ristocetin cofactor assay(RicoF)
9. Factor VIII levels

For the purpose of doing the tests mentioned above the following samples were collected from the patients. The samples were collected using a vacutainer .

1. 5 citrated tubes(5 ml) containing 3.2% Sodium citrate as anticoagulant.
2. 4 citrated tubes(2.7ml)
3. 1 Serum separator tube
4. 1 EDTA tube containing 7.2 ml K2 EDTA as anticoagulant.

After the samples were collected all of the above mentioned tests were done. The tests of our baseline tests helped guide further line of investigations. The reference methods used were Factor assays if after baseline investigations we suspected deficiency of coagulation factors. Light transmittance aggregometry was done if baseline investigations pointed towards Von willebrand disease or Platelet Function disorder which was further confirmed by Von Willebrand Antigen and Collagen or Factor VIII binding assay incase

of the former and Flow cytometry incase of the former. On every patient sample a global test of hemostasis namely TEG is also done.

Thromboelastography or TEG is done on Haemoscope Thromboelastography analyzer using citrated whole blood,320 microlitre, 20 microitre recombiplastin and 0.2 M calcium chloride . The trace thus obtained was analyzed.

All the patients whose baseline tests including TEG were normal were given the diagnosis of No intrinsic hemostatic defect and they served as the control of our study. Baseline parameters if abnormal were further investigated as mentioned above and the final diagnosis arrived at thereafter was noted.

A bleeding score of 3 or more for pediatric patients, 6 or more for adult females and 4 or more for adult males was taken as significant or positive bleeding score.

Any abnormality in any one of the parameters obtained from the trace of TEG was taken as positive TEG.

PROCEDURE FOR TEG:

Collection of specimen:

1)Sample is collected within from the ante-cubital vein within 1 minute of application of tourniquet to avoid venous stasis.

2) Proper needle must be used 19 to 21 G for adults, 22 to 23 G for children

3) . Clear venipuncture.

4). Sample must be drawn in a plastic syringe. If glass syringes cannot be avoided, they must be siliconized using silica spray or they must be smeared with Vaseline to prevent contact activation.

5). The Coagulation tube should be filled without formation of foam. Fast and sufficient mixing with anticoagulant (Citrate) solutions should be done by 5 to 6 inversions.

6). Correct labeling of the tube with hospital number.

7). Add only the second syringe blood for Haemostatic workup.

8). Transport whole blood to laboratory as quickly as possible, as some coagulation factors are labile, and undue delay will affect coagulation testing. For practical purposes, blood should be tested within four hours of collection. In general, samples should be transported at room temperature (~22^{o}C). Transport of whole blood at extreme temperatures (eg 4^{o}C or >30^{o}C) should be avoided as this may have an effect on test results.

9) . Add 9 parts of blood to 1 part of buffered 3.2% trisodium citrate and mix well. A suitable commercially supplied citrate anticoagulant tube will suffice (eg Becton Dickinson VACUTAINER systems [these are also often referred to as “blue top” tube], Greiner Vacuette, etc). Typically, 4.5ml of blood is added to 0.5ml-buffered trisodium citrate for normal coagulation studies. For paediatric purposes use 1ml mini collect Greiner Vacuette. Note that underfilled tubes may not be acceptable for testing.

TEG:

Procedure:

Citrated Whole blood- 320 microlitre + 20 microlitre of Recombiplastin is added.

(Recombiplastin-1/2000 is diluted in 20 microlitre of Imidazole buffer, so that the final concentration reaches 1/36000 in 340 microlitre of blood sample.)

With this, 20 microlitre of 0.2 M calcium chloride is added to warmed cup.

The sample is loaded on to the machine and the tracing obtained.

STATISTICAL METHOD:

The sample size was calculated on the basis of retrospective values of TEG in 15 patients with Hemophilia A which is the bleeding disorder we commonly encounter in our laboratory, so the sample based on 90% sensitivity and 90% specificity is 36 cases and 36 controls with 95% confidence interval and 10% precision. Another objective of the study is to look at the difference between some parameters among cases and controls generated +by ROTEM. The parameters with the sample size for corresponding difference is tabulated below.

	control		Case		
Parameters	mean	sd	mean	Sd	sample size per arm
R min	6.9	2	27.2	36.7	28
K min	2	2	8.98	11	22
Alpha	66.3	3	36.6	17.3	7
MA	66.3	3	55.4	18.9	27
Ly30	0.5	0.1	0.009	0.03	53

All the calculations were made for 80% power and 5% error. A sample size of 53 in each arm(total=106) is the highest for the difference in ly30.

The data have been summarized using mean(Standard Deviation) for continuous variables,frequency (percentage) for categorical data. The Thromboelastgraphy parameters were compared among cases and controls using independent t-test. Based on validated cut-off values Thromboelastography parameters were classified and diagnostic accuracies were presented with 95% Confidence interval . All statistical analysis were done using STATA/IC 13.1 software.

RESULTS

RESULTS

A total of 223 patients were enrolled into the study of which 97 were females and 126 were males. Females comprised 43.5% of the patients and males comprised 56.5 % of the patients enrolled . Of the 223 patients 201 patients that is 90.1 % of the patients were referred for increased bleeding tendency and a total of 18 patients comprising 8.07 % were referred for coagulation work-up as a result of incidentally detected abnormal coagulation parameters. Only 4 patients were referred for evaluation of hemostasis because of abnormal family history.

The mean age of the patient was 18.31 years ranging from 0-67 years. There were 133 patients in the pediatric age group(<18 years), and 90 patients who were older than 18 years, .they comprised 59.6 and 40.3% of the enrolled patients respectively. The mean bleeding score was 6.91 ranging from 0 to 32. Ninety patients amongst 223 patients had normal baseline investigations who were then labeled as patients with no intrinsic hemostatic defect, and served as the control in our study, this constituted 40% of our study population. 72 patients or 32.2% of the total patients enrolled were those of Clotting Factor deficiencies and among them 54% were those patients with Severe Hemophilia A. Other patients in the group were 8 and 3 patients each of Mild and Moderate Hemophilia A respectively. There were 3 patients of Severe Hemophilia B and 2 of Mild Hemophilia B. There were 1 patient each of Severe Factor II and Factor XI deficiency. There were 2 patients each of Factor VII and Factor X deficiencies and 3 patients of Factor V deficiency. A total of 7 patients had Factor XIII deficiency which

accounted for a total of 10% of patients with clotting Factor deficiency. 2 patients had combined Factor deficiency. After coagulation factor deficiency the most common disorder encountered was Platelet Function Defects, 23 patients had Platelet Function disorder of which 18 were patients of Glanzmann Thrombasthenia, 2 patients with Bernard Soulier Syndrome and 3 patients with Platelet Function Disorder.

11 patients with Von Willebrand Disease were enrolled into the study. Of the 11 patients 6 patients had Von Willebrand Disease Type 3, 2 Patients had Von Willebrand Disease Type 2A/M and there were 1 patient each of Von Willebrand Disease Type 2b and Von willebrand Disease Type 1. There were 7 patients with Fibrinogen disorders. 3 patients each of Afibrinogenemia and Hypodysfibrinogenemia and 1 patient of Hypofibrinogenemia.(Table 4)

Table5: Frequency of Diagnosis

Diagnosis	Frequency
1.No intrinsic hemostatic defect	90
2. Clotting Factor Deficiency	72
A. Severe Hemophilia A	39
B. Moderate Hemophilia A	03
C. Mild Hemophilia A	08
D. Severe Hemophilia B	03
E. Moderate Hemophilia B	02
F. Severe Factor V deficiency	03
G. Mild Factor X deficiency	02
H. Factor XI deficiency	01
I. Factor XIII deficiency	07
J. Mild Factor VII deficiency	02
K. Combined Factor deficiency	02
	23
3.**Platelet Function Disorder**	
A. Glanzmann Thrombasthenia	18
B. Bernard Soulier Syndrome	02
C. Severe Platelet Function Disorder	03
4. **Von Willebrand Disease**	11
A. Von Willebrand Disease Type 1	1
B. Von Willebrand Disease Type 2A/M	2
C. Von Willebrand Disease Type 2B	1
D. Von Willebrand Disease Type 3	6
5. **Fibrinogen Disorders**	07
A. Afibrinogenemia	03
B. Hypofibrinogenemia	01
C. Hypodysfibrinogenemia	03
6 .Acquired Bleeding Disorder+Improper history	**20**

The average bleeding score for each condition is summarized in the Table No.7 below.

Among 223 patients 75 had negative bleeding score and 143 had bleeding score that was higher than the bleeding score considered normal for that age group which was taken as positive.

For the final analysis we excluded 20 people from our study, 2 on account of incomplete history and 18 as they were patients with acquired bleeding disorders and ISTH BAT is meant for inherited bleeding disorder. After 20 patients were excluded from the study the sensitivity and specificity of the ISTH BAT was 92.2%(85.8%-96.4%) and 65.5% (54.6%-75.4%) respectively while the negative predictive value was 86.4%(75.7%-93.6%).(Table 5(a),(b)).

Table No. 5(a) and (b) Shows the Sensitivity, Specificity,NPV,PPV,LR of Bleeding Score.

Diagnosis	Bleeding Score Positive	Bleeding Score Negative	Total
Abnormal	107	09	116
Normal	30	57	87
Total	137	66	203

Bleeding Score (95% CI)	
Sensitivity	92.2% (85.8-96.4%)
Specificity	65.5% (54.6%-75.4%)
NPV	86.4%(75.7%-93.6%).
PPV	78.1%(75.7%-93.6%)
LR	2.6 (1.99 - 3.59)

We wanted to calculate the specificity and sensitivity of ISTH BAT in mild to moderate bleeding or rare bleeding disorder so we analyzed the result after excluding the cases of Severe Hemophilia A and B , after excluding these cases the sensitivity and specificity of ISTH BAT was found to be 89.1%(81.2%-96.1%) and 63.3%(52.5- 73.2%)with a negative predictive value of 87.6%(78.8%-95.5%).(Table 6(a),(b))

Table No. 6(a) and (b) Shows the Sensitivity, Specificity,NPV,PPV,LR of Bleeding Score after Severe Hemophila patients are excluded.

Diagnosis	BS Positive	BS Negative	Total
Abnormal	66	08	74
Normal	33	57	90
Total	99	65	164

Table 6(b)

Bleeding Score after Severe Hemophilia A and B is excluded	
Sensitivity	89.1%(81.2%-96.1%)
Specificity	63.3%(52.5- 73.2%)
NPV	87.6%(78.8%-95.5%).
PPV	66.7% (56.5%-75.8%)
LR	2.47 (1.86 -3.27)

Table 7:The average bleeding score for each diagnosis.

Diagnosis	Average Bleeding score	Range	No. of patients
No intrinsic hemostatic defect	3.06	0-11	90
Severe Hemophilia A	13.18	0-31	39
Mod. Hemophilia A	8.67	4-12	03
Mild Hemophilia A	5.63	0-14	08
Severe Hemophilia B	11.67	7-18	03
Moderate Hemophilia B	6.50	3-10	02
Factor X deficiency	10.50	4-17	02
Factor VII Deficiency	9.50	9-10	02
Combined Factor deficiency	5	3-7	02
Factor V deficiency	12.33	4-26	03
Factor XIII deficiency	10.15	5-16	07
Glanzmann Thrombasthenia	10.44	2-32	18
Bernard Soulier Syndrome	18.50	18-19	02
Platelet Function Defect	2	0-4	02
Von Willlebrand Disease Type 2A/M	06	2-10	03
Von Willebrand Disease Type 3	9.17	4-20	06
Afibrinogenemia	7	4-9	03
Hypodysfibrinogenemia	12	4-20	03
Acquired bleeding disorders	3.72	2-8	18

Since there were only one patient each for Factor XI deficiency. Prothrombin deficiency ,Von Willebrand DiseaseType 1 and Type 2B and Hypofibrinogenemia the mean bleeding score in these conditions were not calculated.

Thromboelastography:

Of the 205 patients analyzed 104 patients had abnormal TEG who were eventually found to have a bleeding disorder. In another 16 patients who were diagnosed to have a bleeding disorder TEG was normal.

In 71 patients who were found to have no bleeding disorder TEG was also normal. TEG in 13 patients was abnormal who were not diagnosed to have any bleeding disorder. Considering all these values Sensitivity of TEG in diagnosing bleeding disorder was found to be 86.0%(78.5%-91.6%), whereas specificity was found to be 85.7%(76.4-92.4%). The positive predictive value and the negative predictive value were 89.7%(82.6%-94.5%) and 80.9%(71.2%-88.5%) respectively.(Table 8(a), 8(b))

Table No. 8(a) and (b) Shows the Sensitivity, Specificity,NPV,PPV,LR of TEG after Severe Hemophila patients are excluded.

TEG	Diagnosis Abnormal	Diagnosis Normal	Total
Positive	104	12	116
Negative	17	72	89
Total	121	84	205

Table no. 8(b)

TEG PARAMETERS IN BLEEDING DISORDER	
Sensitivity	86.0%(78.5%-91.6%)
Specificity	85.7%(76.4-92.4%).
NPV	80.9%(71.2%-88.5%)
PPV	89.7%(82.6%-94.5%)
LR	6.02 (3.55-10.2)

The K time in TEG represents clot kinetics, we looked for its sensitivity and specificity and found that sensitivity was 57.9%,specificity was 89.9%.

We also calculated the sensitivity and specificity of TEG after the diagnosis was grouped into following groups.

1)Clotting Factor deficiencies(except Fibrinogen and FXIII)

2)Platelet Function disorders

3)Fibrinogen Disorders

For Clotting Factor deficiencies excluding Fibrinogen and Factor XIII deficiency but Von Willebrand Disease included , we looked for the ability of R time and angle to predict the presence of Bleeding disorders. Of the 66 patients in the group

R time:

Of the total 66 patients in this group R time was prolonged in 55 and normal in 9. Among normal controls 11 had prolonged R time and 78 were normal, giving an over- all sensitivity of 86.4%(75.7%-93.6%) and specificity of 87.6%(79%-93.7%). The positive and Negative Predictive value were respectively 83.8%(72.9%-91.6%) and 89.7%.(81.3%-95.2%)(Table 9(a),Table 9(b))

Table No. 9(a) and 9(b) Shows the Sensitivity, Specificity,NPV,PPV,LR of TEG (R time) in coagulation factor deficiencies.

Diagnosis	R Positive	R Negative	Total
Abnormal	57	09	66
Normal	11	78	89
Total	68	87	155

Table 9 (b)

R time in Coagulation Factor deficiencies(Fibrinogen and FXIII deficiencies excluded)	
Sensitivity	86.4%(75.7%-93.6%)
Specificity	87.6%(79%-93.7%).
NPV	89.7%.(81.3%-95.2%)
PPV	83.8%(72.9%-91.6%)
LR	6.02 (3.55-10.2)

The angle in TEG is mainly contributed by fibrinogen and some extent by platelets. Clotting factors also contribute to the angle. We wanted to analyse the angle in the above mentioned group. Of the 66 patients analyzed 46(69.6%) patients had decreased angle.

When R time and angle were taken together the sensitivity and specificity was found to be 87.9% and 78.7% respectively and the positive predictive value was 75.3% and negative predictive value 89.3%.(Table 10(a),10(b))

Table No. 10(a) and 10(b) Shows the Sensitivity, Specificity,NPV,PPV,LR of TEG (R time and angle) in coagulation factor deficiencies.

Diagnosis	R and Angle Positive	Negative	Total
Abnormal	58	8	66
Normal	19	70	89
Total	77	78	155

Table:10(b)

R time and angle in Coagulation Factor deficiencies(Fibrinogen and FXIII deficiencies excluded)	
Sensitivity	87.9 % (77.5%-94.6%)
Specificity	78.7%(68.7%-86.6%)
NPV	89.7%(80.8%-95.5%)
PPV	75.3%(64.2%-84.4%)
LR	4.12 (2.74-6.19)

Platelet Function Defects :

We also analyzed the ability of MA and angle in platelet function disorder and found that of the total 24 patients of Platelet Function disorder MA was decreased in 23 giving it a sensitivity of 95.8%.(Table 11(a) and (b))

Table No. 11(a) and 11(b) Shows the Sensitivity, Specificity,NPV,PPV,LR of TEG (MA) in coagulation factor deficiencies.

Table 11(a)

Diagnosis	MA Positive	Negative	Total
Abnormal	23	1	24
Normal	53	36	89
Total	76	37	113

Table 11(b)

MA in Platelet Function Disorders

Sensitivity	95.8 %(78.9%-99.9%)
Specificity	40.4%(30.2%-51.4%)
NPV	97.3%(85.8%-99.9%)
PPV	30.3%(20.2%-41.9%)
LR	1.61(1.33-1.91)

While the most major contribution to angle is done by Fibrinogen, 25% of it is contributed by Platelets. We found that of 24 patients , 19 had low angle,. Angle in platelet function disorders have a sensitivity, specificity, PPV and NPV of 79.2%,83.1%,72.8%,97.9% respectively. **Table 12(a)**

Table No. 12(a) Shows the Sensitivity, Specificity,of TEG (ALPHA) in coagulation factor deficiencies.

Table 12(a)

Diagnosis	Angle Positive	Angle Negative	Total
Abnormal	19	5	24
Normal	15	74	89
Total	34	79	113

When MA and angle were taken together the findings were as follows.

Table No. 12(b,c) Shows the Sensitivity, Specificity, NPV,PPV,LR of TEG (ALPHA +MA) in coagulation factor deficiencies.

Diagnosis	MA + angle Positive	Negative	Total
Abnormal	23	1	24
Normal	54	35	88
Total	77	36	113

Table no. 12 (c)

MA and Angle in Platelet Function Disorders	
Sensitivity	95.8%(78.9%-99.9%)
Specificity	39.3%(29.1%- 50.3%)
NPV	97.2%(85.5%-99.9%)
PPV	29.9%(20.0%-41.4%)
LR	1.58(1.31-1.9)

When MA and angle were combined the sensitivity was 95.8%. **(Table 12 (b,c))**

There were only 4 patients with Fibrinogen defects, all 4 of them had reduced angle and MA.

For FXIII deficiency out of 7 patients only 2 patients had mildly prolonged TEG, 1 had significant lysis rest were normal.

The third objective of our study was to calculate the sensitivity and specificity of Thromboelastography and Bleeding Score taken together as a screening tool for Inherited bleeding disorders. When that was done the sensitivity,specificity,NPV and PPV were 97.5%,56.5%,94% and 76.3% respectively.

Table No. 13(a) and 13(b) Shows the Sensitivity, Specificity,NPV,PPV,LR of TEG and BS in coagulation factor deficiencies.

Diagnosis	BS +TEG Positive	Negative	Total
Abnormal	116	3	119
Normal	36	47	83
Total	152	50	202

Table 13 (b)

Bleeding Score and TEG Parameters	
Sensitivity	97.5%(92.8%-99.5%)
Specificity	56.6%(45.3%-67.5 %)
NPV	94%(83.5%-98.7%)
PPV	76.3%(68.7%-82.8%)
LR	2.25(1.75-2.88)

While there was not much difference between cases and controls in terms of age, there was difference in bleeding scores, R time, K time, angle and Maximum amplitude which was significant between cases and control. p value being less <0.0001 in case of all the parameters mentioned.

Table 14: Comparison of age, TEG parameters between Cases and Control

PARAMETER	CASES(MEAN)	CONTROL(MEAN)	p Value
Age	18.41	18.68	0.9
R Time	19.49	7.49	<0.0001
K Time	7.09	2.89	<0.0001
Angle	41.66	61.50	<0.0001
MA	57.06	67.21	<0.0001
Ly30	0.24	1.39	0.1

Table 14(b): Compilation of Diagnostic Accuracy of different methods:

METHOD(S)	SENSITIVITY	SPECIFICITY	NPV	PPV
1.BAT	92.2%	65.5%	86.4%	78.1%
2. TEG	86.0%	85.7%	80.9%	89.7%
3. BAT +TEG	97.5%	56.6%	94%	76.3%

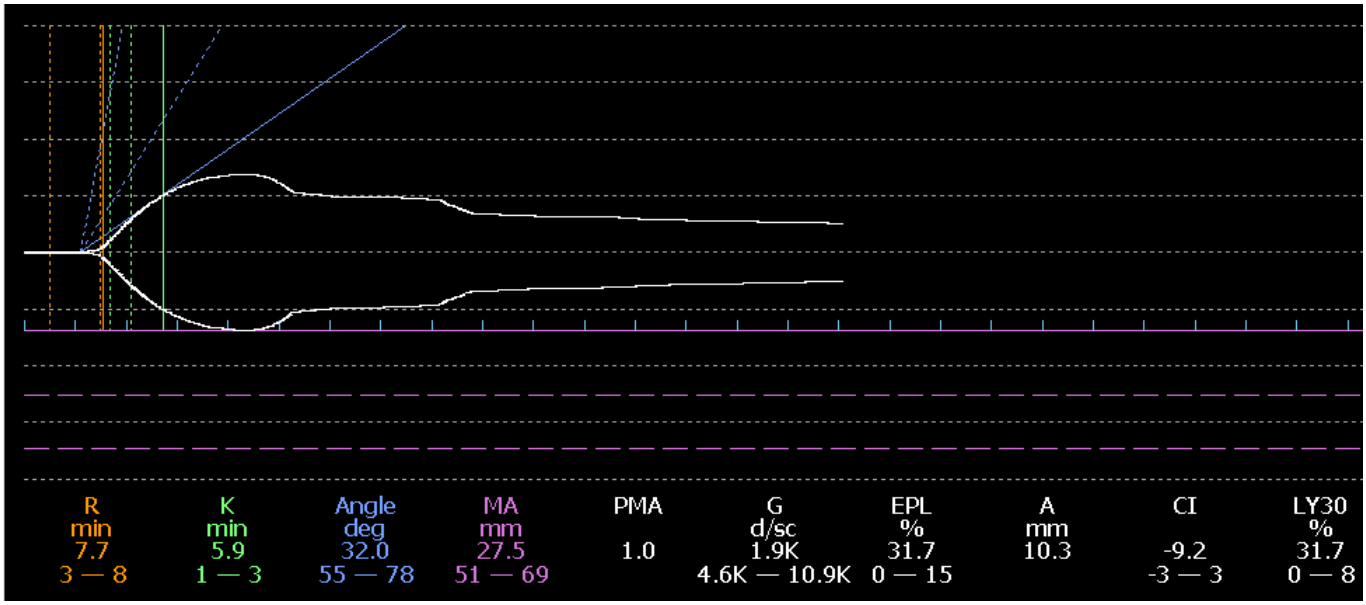

Figure 4 :TEG tracing shows increased K time, decreased angle, decreased MA and increased lysis in Hypodysfibrinogenemia.

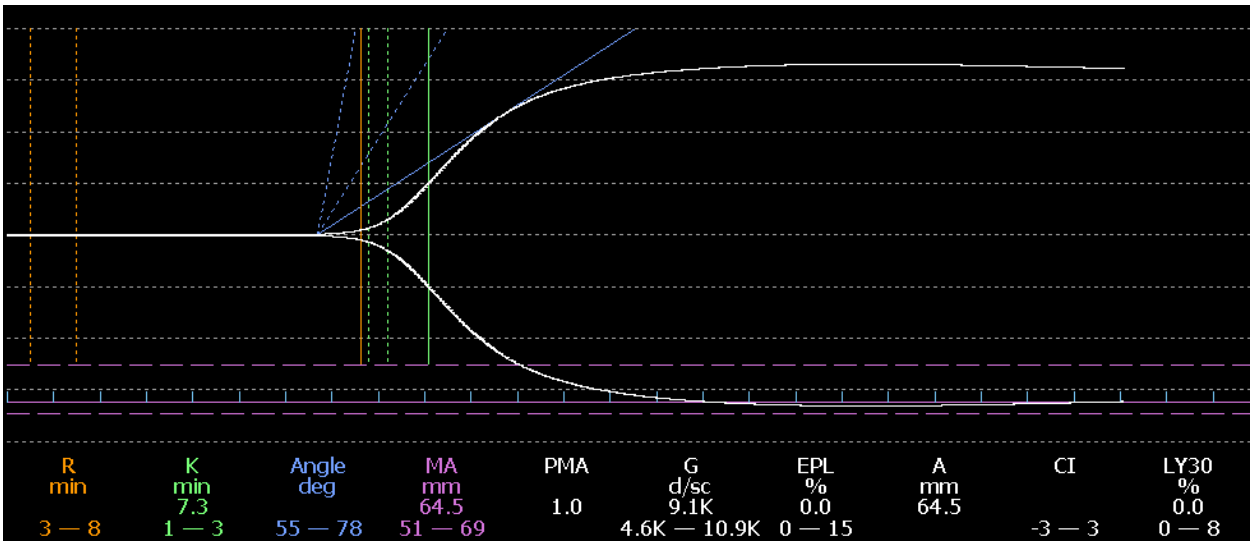

Figure 5:TEG tracing shows increasesd R time, K time , reduced angle in patient with Severe Hemophilia B.

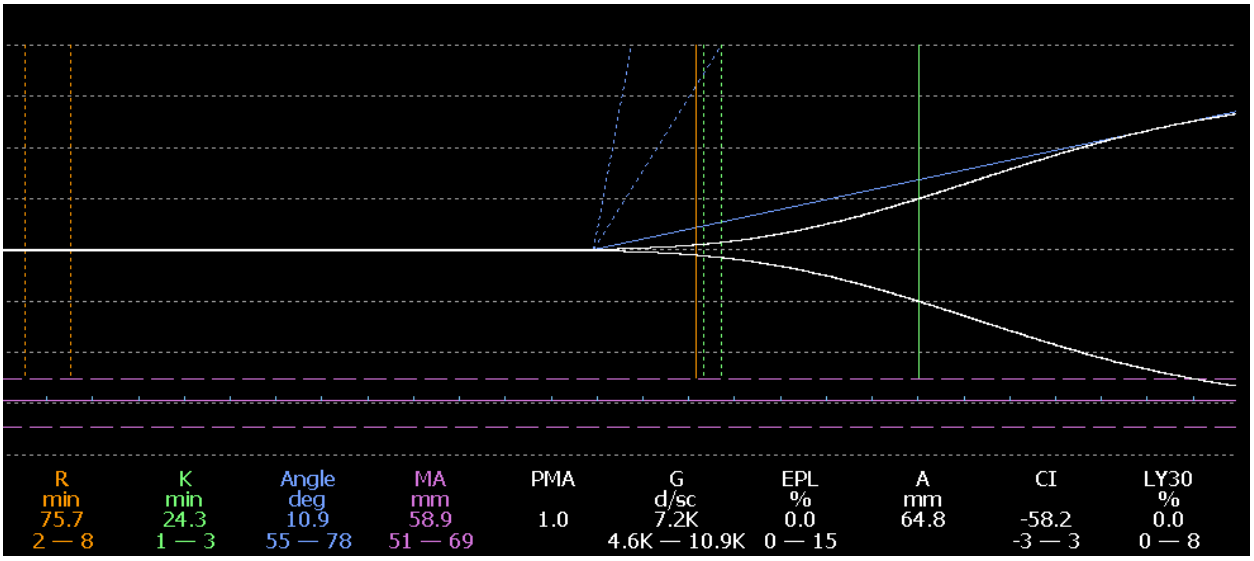

Figure 6: TEG tracing in a patient with Severe Hemophilia A,Prolonged R time, K time and reduced angle can be appreciated.

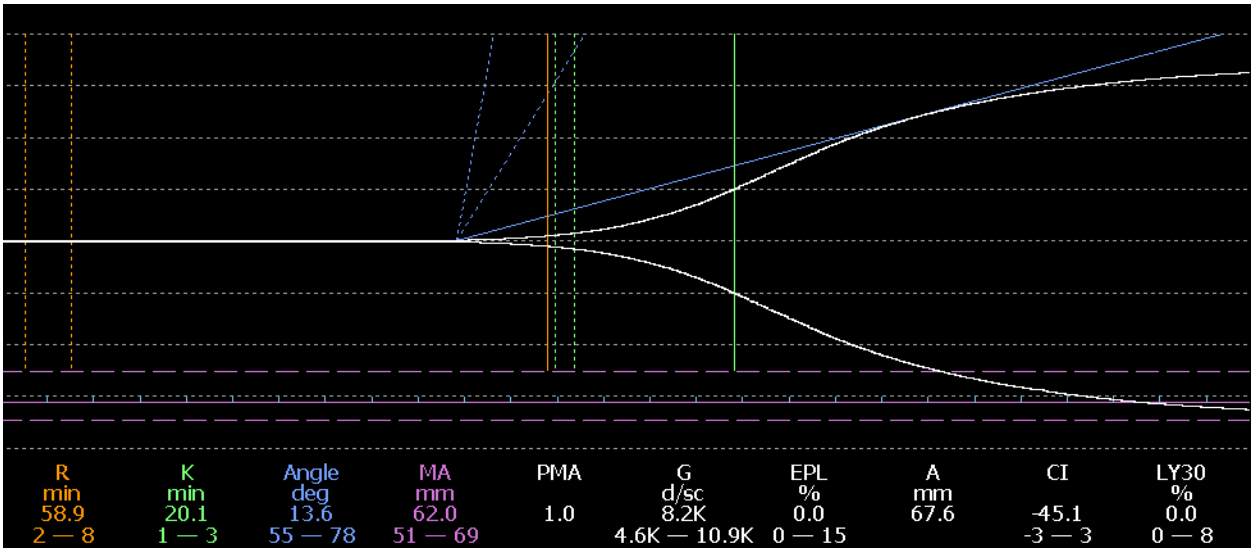

Figure 7 : TEG tracing in a patient with Severe Hemophilia B ,Prolonged R time, K time and reduced angle can be appreciated.

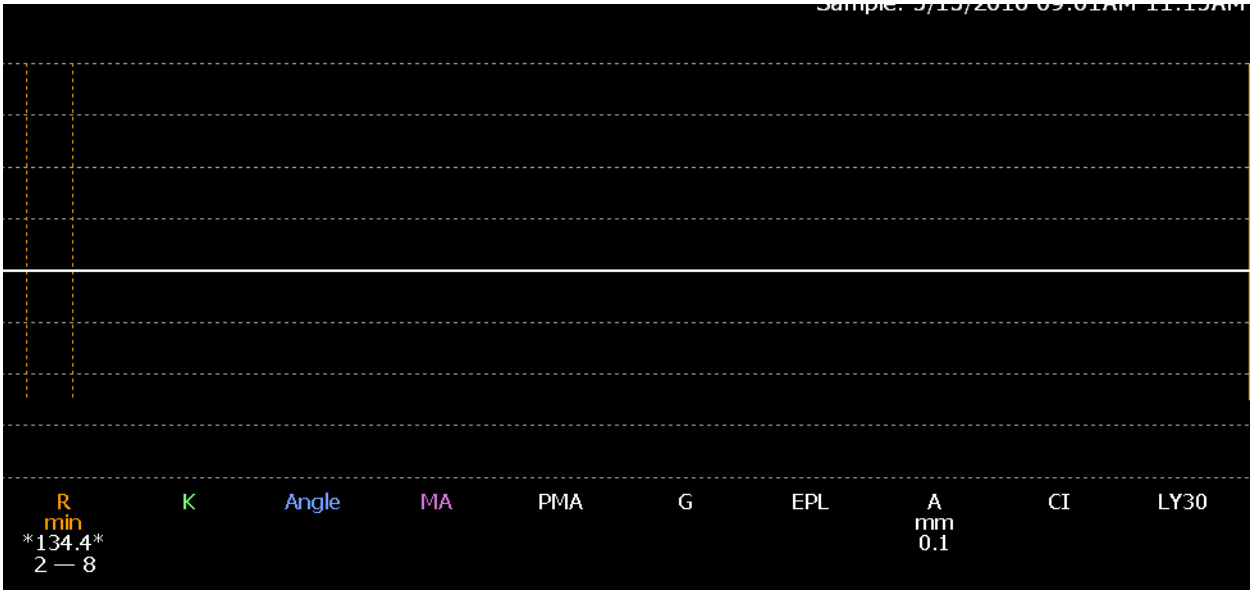

Figure8 : TEG tracing in a patient with Afibrinogenemia shows extremely prolonged R time with no clot formation.

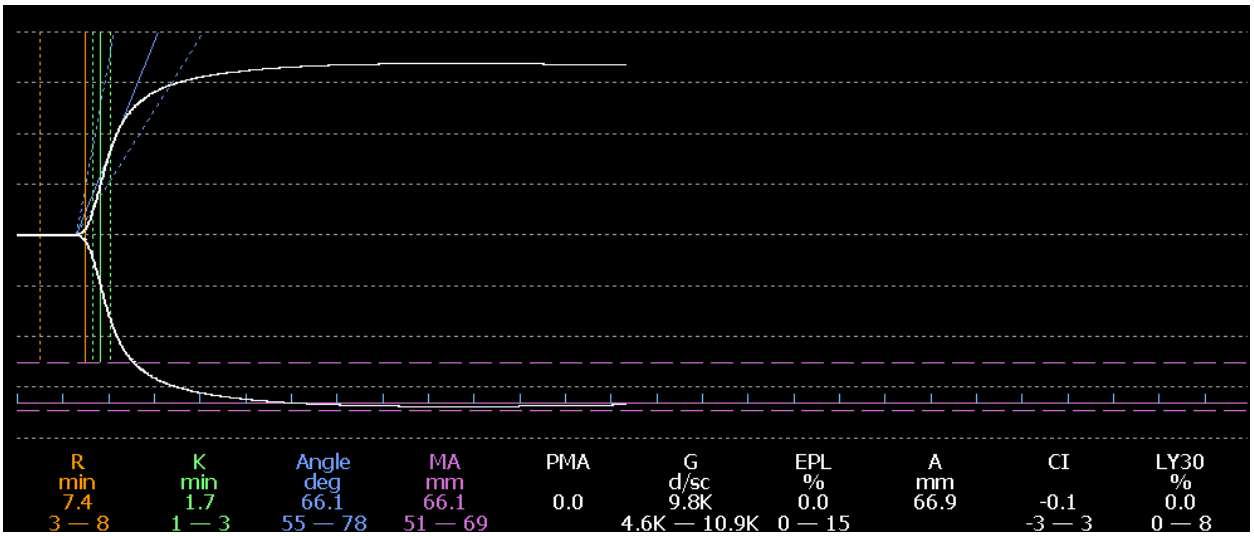

Figure 9: TEG tracing in a patient with Factor XI deficiency shows a normal trace. The patient had no bleeding symptom and was referred for investigation of prolonged APTT.

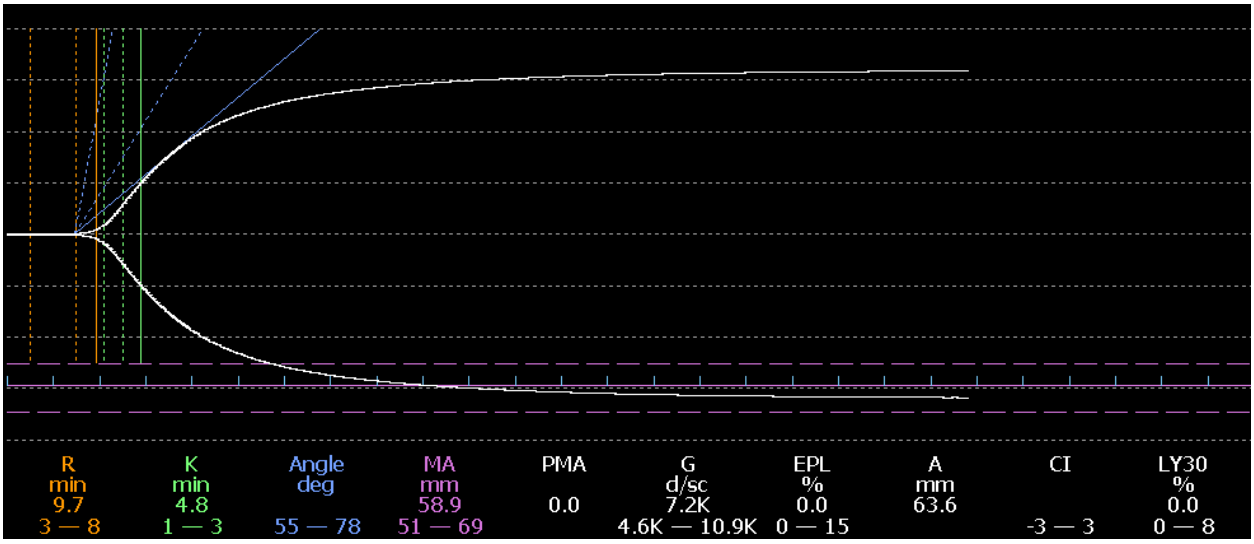

Figure 10: TEG tracing in a patient with Bernard Soulier Syndrome, Prolonged K time and reduced angle can be appreciated.

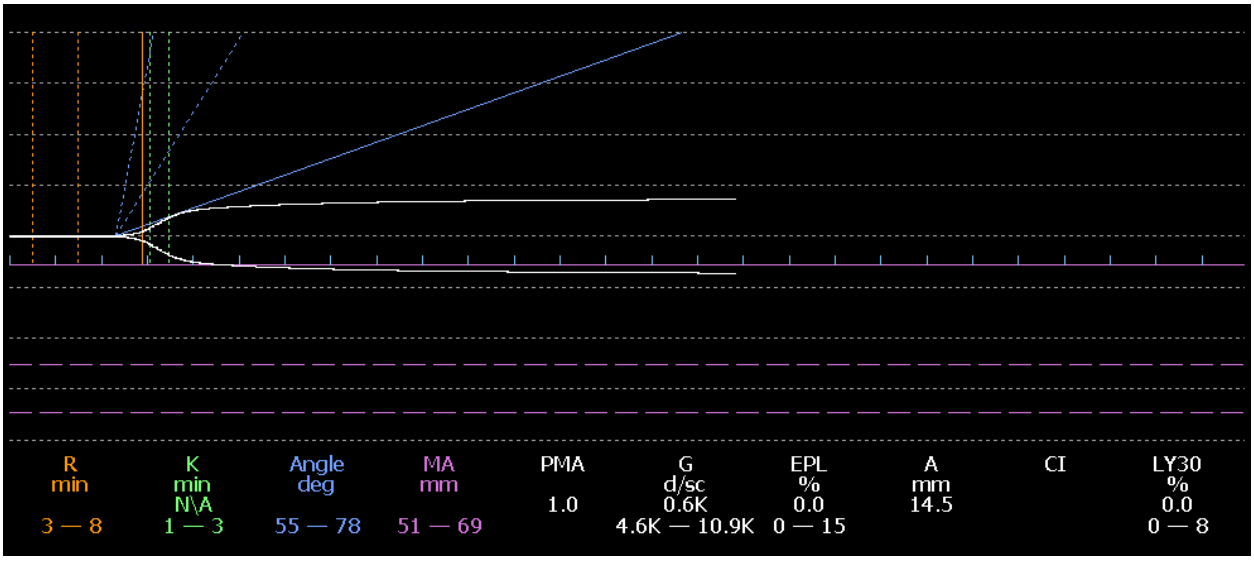

Figure 11: TEG tracing in a patient with Glanzmann Thrombasthenia shows increased K time, increased angle and severely reduced MA.

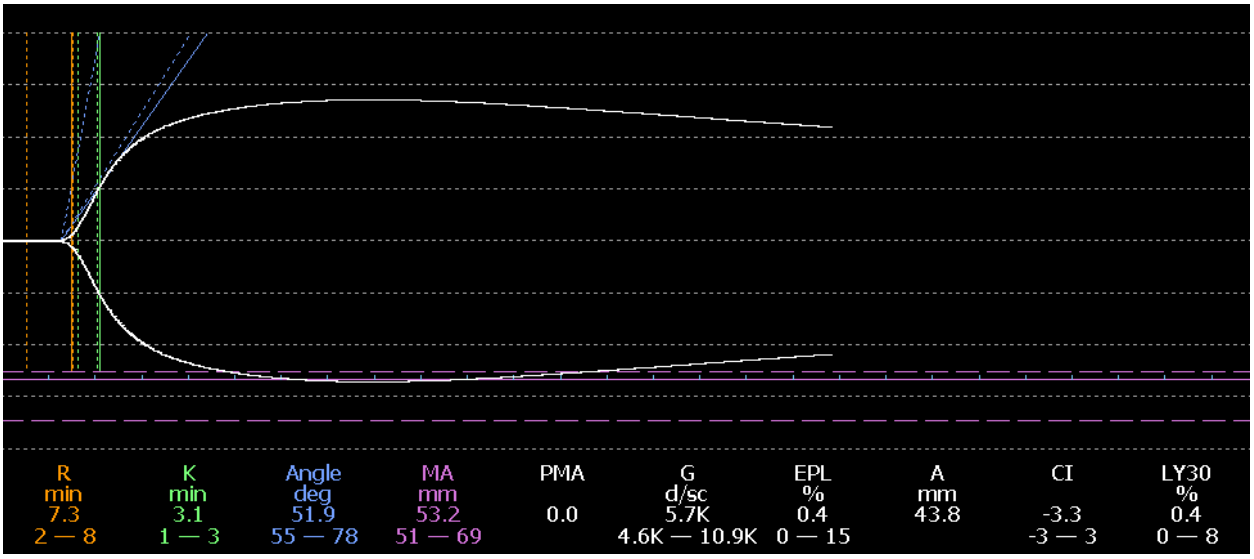

Figure 12: TEG tracing in a patient of Factor XIII deficiency shows decreased angle and increased lysis.

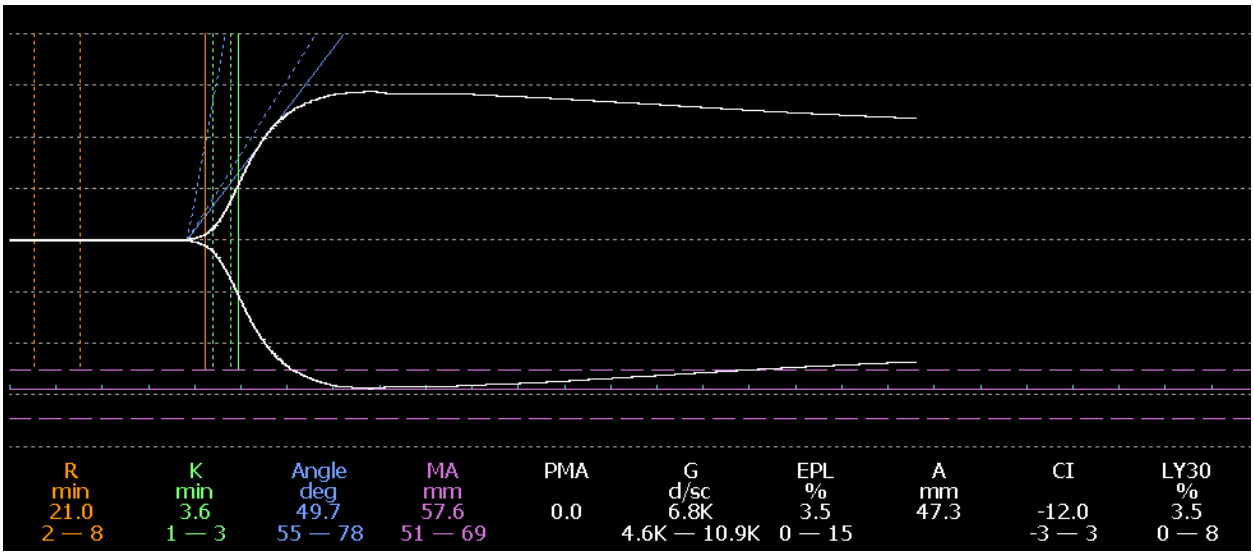

Figure 13: TEG tracing in a patient of Severe Factor V deficiency prolonged R time, K time and reduced angle.

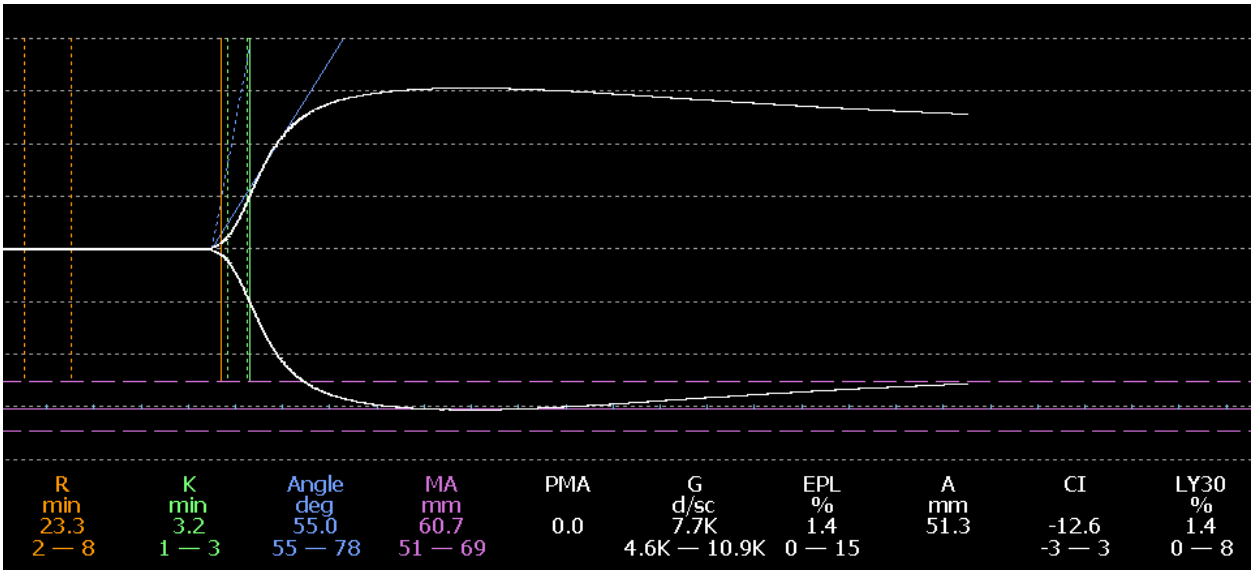

Figure 14: TEG tracing in FXII deficiency shows, prolonged R time, prolonged K time and decreased angle.

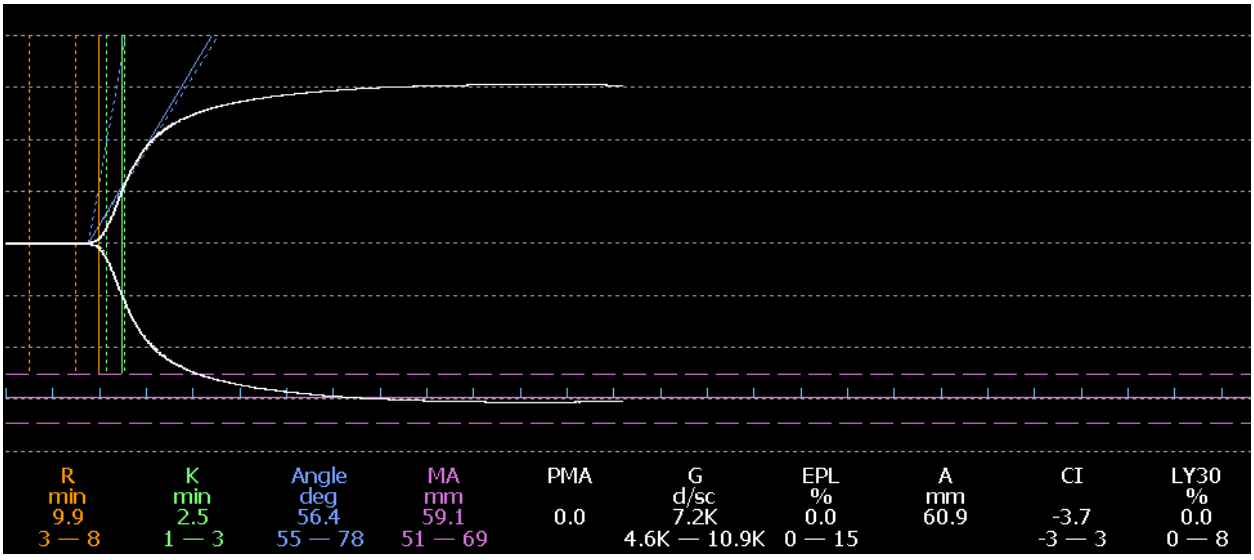

Figure 15: TEG tracing in Von Willebrand Disease type 3, does not show any marked abnormality save for mildly prolonged R time.

Discussion

Discussion

As is known the ususal screening tools like BT, PT, APTT and have a very low sensitivity , specificity and predictive values in diagnosis of bleeding disorders.(53,54) The usefulness of bleeding history in identification of bleeding disorders have been evaluated prospectively by only a few studies.(11)

In this study that was carried over a period of 6 months in between December 2015 – May 2016, 223 patients were studied. These patients are referred to our Laboratory to rule out abnormalities of hemostasis. We divided patients into 3 main groups, they were patients referred for increased bleeding tendency, for incidental finding of deranged lab parameters and those referred for evaluation because of positive Family history. We found that out of 223 patients 201 patients that is 90.1 % of the patients were referred for increased bleeding tendency and a total of 18 patients comprising 8.07 % were referred for coagulation work-up as a result of incidentally detected abnormal coagulation parameters. Only 4 patients were referred for evaluation of hemostasis because of abnormal family history. In a similar study done by Tosetto et al out of Two hundred and fifteen patients studied , 71 were referred for evaluation incidental finding of abnormal test results, 105 for the presence of some bleeding symptoms, and 39 for positive family history (34) .

Out of the 223 patients , 90 patients, on investigation were found to have no abnormality of hemostasis that is almost 40 % of the patients referred. In Clinical practice the utility

of BAT may be either to exclude bleeding disorder in an unselected group or to identify patients who are likely to have a bleeding disorder. In the former case BAT should have a high sensitivity and NPV, hence the need for extensive investigation that usually entails investigation af a bleeding disorder is negated.

On the other hand , if the BAT is intended for the latter, it would need a good specificity and a good PPV.(34)

While the ISTH BAT have been evaluated prospectively for specific bleeding disorders as of now, there is no study where the ISTH BAT has been evaluated for use in all bleeding disorders. The use of BAT has been mainly limited to the diagnosis of Von Willebrand Disease and also in Platelet Function disorder.Tosetto et al evaluated the clinical utility of BAT in mild bleeding disorders, however the BAT used in this particular study was MCMDM BAT (34–36).

In our study we evaluated the utility of ISTH BAT in all patients referred for evaluation of hemostasis, prospectively.We enrolled 223 patients referred to our laboratory for evaluation of hemostasis.

Among 223 patients 75 had negative bleeding score and 143 had bleeding score that was higher than the bleeding score considered normal for that age group which was taken as positive. 20 patients were excluded from the study .

The sensitivity and specificity of the ISTH BAT was 92.2% and 65.5% respectively while the negative predictive value was 86.4%.

Out of the 203 patients who were finally analyzed for the bleeding score versus the diagnosis, 9 patients were patients with negative bleeding score who were diagnosed to have a bleeding disorder. These included 1 patient with Factor XI deficiency, 1 patient of Severe Hemophilia A, 1 patient of Glanzmann Thrombasthenia, 1 patient of platelet function defect, 3 of Mild Hemophilia A and one patient each of Von Willebrand Disease Type 2B/Platelet Type Von willebrand Disease and 1 of Von Willebrand Disease Type 2A/M.

TABLE 15 : COMPARISON OF STUDIES EVALUATING UTILITY OF ISTH BAT.

STUDY	BAT	NPV	PPV
A Tosetto et al,2011	MCMDM-1 VWD For Mild Bleeding Disorders	99.2%	77.5%
Lowe et al,2014	ISTH BAT, for ability to detect Platelet Function defect on Lumiaggregometry	50%	54%
M Bowman et al,2008	MCMDM-1 VWD	1	0.20
M Bowman et al ,2009	Pediatric Bleeding Questionnaire	0.14	0.99
CMC Vellore 2015-2016	**ISTH- BAT**	**86.4%**	**77.1%**
Rashid et al 2016	ISTH-BAT for Platelet Function Disorder	89.5%	49%
Kaur et al 2016	ISTH BAT for Platelet Function Disorder	0.9	1

We attributed the increased sensitivity of BAT in our study to large number of Severe Hemophilia A and B, as these patients have excessive bleeding and the score in them is likely to be higher, so we calculated the specificity and sensitivity of ISTH BAT in mild to moderate bleeding and rare bleeding disorders after excluding the cases of Severe Hemophilia A and B ,sensitivity and specificity of ISTH BAT was found to be 90.4% and 63.3% with a negative predictive value of 89.1% and Positive Predictive value of 66.7%. The advantage of BAT in all the past and the present study appears to be the high negative predictive value, a negative BAT essentially rules out a bleeding disorder. The current study has findings comparable to most of the previous study in terms of Negative predictive value. To our knowledge apart from the ones already mentioned there are no prospective studies that have used ISTH BAT for evaluation of bleeding disorders. The attempts to validate ISTH-BAT are ongoing.

In our study 30 patients had positive BS, and were finally diagnosed to have no bleeding disorder.In the pediatric population there are many patients with pubertal menorrhagia who are treated with hormones and anti-fibrinolytics, the cut-off for pediatric population being low i.e 3, many of the bleeding scores were falsely positive in this group,infact 24/30 cases i.e 80% of the false positive cases were in the pediatric age group,keeping a single cut off in the age-group of 0-18 may help in increasing the sensitivity at the cost of specificity ,as in this age group , with a wide-range , girls attain menarche and also by adolescent age many of the patients would have encountered significant hemostatic challenges so an intermediate category or an adolescent category with a higher cut off if

considered, may help improve the specificity of the tool . Significant false positivity(20% of False Positive cases) were also contributed by females with menorrhagia. In our opinion patients with menorrhagia could be administered the PBAC questionnaire in conjunction with ISTH BAT, this would heighten the specificity of bleeding assessment tool. The low specificity and high false positivity may also be attributed partly to indiscriminate use of anti-fibrinolytics and transfusion in our set-up.

Among the 223 patients enrolled in our study , 90 patients i.e 40 % were found to have no bleeding disorder, in our set-up BAT should have a high sensitivity and NPV, hence the need for extensive investigation that usually entails investigation af a bleeding disorder is negated. With the sensitivity and specificity of the ISTH BAT at 92.2% and 65.5% respectively and negative predictive value of 86.4%, ISTH BAT can be used as a good screening tool, our current practice of using this in conjunction with other screening test is good.

While the initial utility of TEG was meant to reduce transfusion requirement in complicated surgeries but now its utility has expanded to bleeding and thrombotic disorder.(39) TEG and its utility in bleeding disorder has also been studied.

Zia et al studied TEG in the diagnosis of RBDs. This was a retrospective study done on 26 patients with rare bleeding disorder. They had 4 patients of Fibrinogen deficiency, 1 patient of pro-thrombin deficiency, 1 Factor V deficiency,

3 patients of combined Factor V and Factor VIII deficiencies, 1 of Factor XI and 4 of Factor XIII deficiency. Other disorders included in the study were 1patient of PAI 1 deficiency, 1 of Vitamin K dependent clotting factor deficiency, 14 cases of Glanzmann Thrombasthenia and 1 patient of Bernard Soulier Syndrome. Apart from this there were 2 patients, 1 each of High Molecular Kininogen deficiency and 1 of FXII deficiency. (13) In this apart from the usual parameters of TEG they also studied the dynamic property of clot formation by transforming the TEG data into a velocity curve or the V curve. The parameters assessed under V curve were mean rate of thrombus generation (MRTG.(13) This study concluded that when they used all the parameters above they found that 25/26 patients had abnormal TEG giving it a sensitivity of 100%.

In a study done by Chitlur_et al , where they studied 58 children with severe Hemophilia and their TEG profiling was done found that the R time in children with Severe Hemophilia without inhibitors was significantly prolonged than the normal controls ($p<0.001$) and when they compared the time taken for clot formation in Severe Hemophilia A with inhibitors as against those without inhibitors, the R time was significantly prolonged in the former catergory.($p<0.001$)The discrepancy often observed between phenotype and genotype in patients of severe Hemophilia A have been studied and it was seen that patients who had less bleeding had better thrombin generation capacity(46). The utility of TEG in titration of recombinant FVII has also been evaluated.(44)

Zia et al in their retrospective study found TEG to be of limited utility as a screening test for Inherited coagulation disorder. In their retrospective study they found that on 195 patients with 29 cases of diagnosed bleeding disorders that comprised of 16 Von Willebrand DiseaseType 1,

6 patients with Factor VII deficiency, 3 patients of Factor XII deficiency, 2 patients of Factor XI deficiency and One patient of delta storage pool disorder they found TEG to have very poor sensitivity and specificity.(50)

In our study Of the 205 patients analyzed 104 patients had abnormal TEG who were eventually diagnosed to have a bleeding disorder. In another 16 patients who were diagnosed to have a bleeding disorder TEG was normal.

In 71 patients who were found to have no bleeding disorder TEG was also normal. TEG in 13 patients was abnormal who were not diagnosed to have any bleeding disorder. Sensitivity of TEG in diagnosing bleeding disorder was found to be 86.7%, whereas specificity was found to be 84.5%. The positive predictive value and the negative predictive value were 88.9% and 81.6% respectively

Of the 16 patients in whom the TEG was normal and were diagnosed to have bleeding disorder, the distribution were as follows:

Table 16: Patients with False Negative TEG

Diagnosis	No.of patients
Factor XI deficiency	1
Mild Hemophilia A	3
Severe Hemophilia A	1
Bernard Soulier Syndrome	1
Von Willebrand Diisease Type 2A/2M	3
Von Willebrand Disease Type 3	1
Factor XIII deficiency	2
Mild and Severe Factor VII deficiency	2
Mild Platelet Function defect	1
Combined Factor deficiency	1

Among the false negative TEG all the patients of Mild Hemophilia A had Factor levels above 30%. Same was the case with mild deficiency of Factor VII. One case of Severe Factor VII deficiency and one Severe Hemophilia A with normal TEG were patients who received Factor infusions few days prior to the test and the TEG was performed without adequate wash-out period. If these two cases were to be excluded from the study the overall sensitivity would go upto 86.5%.

The TEG parameters we analyzed for this study were R time,K time, Alpha angle, Maximum Amplitude and Lysis. Like the study described earlier by Zia et al where they have derived a different parameter like the V curve , it would probably be worthwhile to look at other parameters for their sensitivity and specificity in a similar setting. Parameters worth looking at would be the G and Coagulation Index or CI and a further study to analyse these parameters would be interesting.

To our knowledge there have been only two studies both by Zia et al that have evaluated the sensitivity and specificity of TEG as a screening tool for bleeding disorders.

In 2011 Zia et al in their retrospective analysis of 195 patients found that the sensitivity of the R time to diagnose a clotting factor (including low factor VIII with vWD) deficiency was only 58% with a specificity of 78%. R time correlated with PTT and PT in up to 50% (vWD: 12%, FVII deficiency: 33%, FXII: 33%, FXI: 50%) of the patients. R time was also prolonged in 46/166 (28%) patients without a definitive bleeding disorder. In this study the TEG performed with low dose tissue factor (1:190 000 concentration).(50)

The same authors in 2015 using 1:10 000 dilution of recombinant human tissue factor in a retrospective analysis of 26 patients obtained a sensitivity of 100%in screening of bleeding disorder.

We also analysed few individual parameters of TEG for specific disorders,the R time and angle in clotting Factor deficiencies excluding the Factor XIII and Fibrinogen disorder patients but we included the patients with Von Willebrand Disease, of the total 66 patients in this group R time was prolonged in 55 and normal in 9. Among normal controls 11 had prolonged R time and 78 were normal, giving an over- all sensitivity of 86.4% and specificity of 87.6%. The positive and Negative Predictive value were respectively 83.8 and 89.7%. This is higher than the sensitivity and specificity obtained by Zia et al with sensitivity of the R time to diagnose a clotting factor (including low factor VIII with vWD) deficiency was only 58% with a specificity of 78%. The difference probably can be attributed to the difference in concentration of tissue factors being used. In our lab use 1 in 2000 dilution. Whether Tissue factor concentration influences the over all sensitivity of TEG needs to be validated by further study.

When R time and angle were taken together the sensitivity and specificity was found to be 87.9% and 78.7% respectively and the positive predictive value was 75.3% and negative predictive value 89.3% in clotting Factor deficiencies.

We also analyzed the ability of MA and angle in platelet function disorder and found that of the total 24 patients of Platelet Function disorder MA was decreased in 23 giving it a sensitivity of 95.8%.

When MA and angle were combined the sensitivity was 95.8%.

There were only 4 patients with Fibrinogen defects, all 4 of them had reduced angle and MA.

In Factor XIII deficiency out of seven patients TEG was abnormal for two parameters in three patients, one patient had higher lysis than normal and two had prolonged R time but due to lesser number of these patients we cannot categorically say if TEG is a good screening tool in these disorders or not. Probably a study with higher number of FXIII deficient patient will be required for a definite conclusion.

The final objective of our study was to look for the specificity and sensitivity of Bleeding Score and TEG together as a screening tool for inherited bleeding disorders, When that was done the sensitivity,specificity,NPV and PPV were 97.5%,56.5%,94% and 76.3% respectively. In this group there were 3 false negative patients 1 patient a 10 year old female patient with Von Willebrand Disease 2A/M , lack of bleeding symptoms in her can be attributed to variable penetrance of VWD and also FVIII levels of over 190%, The other patient was a patient with Mild Hemophilia A with FVIII levels of over 30% and a 60 year old gentleman with FXI deficiency with FVIII levels over 200%. It is understandable that such high levels of FVIII would prevent bleeding thereby having low bleeding score and also would result in normal TEG . To the best of our knowledge there is no study where ISTH BAT and TEG have been used together as a screening tool for bleeding disorders.

The use of these two modalities together as a screening tool gave us a sensitivity and NPV of 97.5% and 94% respectively, and the use of these two together for the same may be considered. We also compared the TEG parameters , BS and age of the patients in between cases and control and we found that there is a significant difference in between the patient and the controls in both BS and TEG parameters. $P < 0.001$.

The major utility of BAT is in distinguishing bleeders from non-bleeders our study had many patients with Severe Hemophilia A and few of B, barring a few most of the Hemophilia patients have excessive bleeding.(55), so a BAT as a screening tool , as sensitive as it may be, can be redundant and the presence of large number of these patients also could have led to heightened sensitivity in our study. However BAT score being a very efficient tool to document bleeding score may be included in a composite score for Hemophilia in the lines of one devised by Schulman et al.(32) The utility of BAT Score in this setting would be worth evaluating. The long administration time of (20 minutes)BAT also is a reason why its acceptability may be lower in busy clinical practice , so an attempt toward patient administered BAT score should be taken. In a multi-lingual,multi-ethnic population like ours this will be a challenge but an attempt in that direction would be a worthwhile endeavour now that the importance of well taken history has been proven. Also a questionnaire in a patient's mother tongue would probably be able to acquire more valid and true information. (56)

This study also has many limitations. Many of these are the inherent limitations of a BAT score. The bleeding score obtained post administration of a BAT depends on history given by the patient , so an improper history will lead to a false bleeding score. To overcome this we used the Thromboelastography in conjunction with BAT.

As we enrolled consecutive patients referred to our lab , we had many patients with Severe Hemophilia and there were few cases of other rarebleeding disorders. Probably a study done over a longer duration with significant numbers in each category of Bleeding disorders can be undertaken for adequate representation of mild, moderate and rare bleeding disorders. Also there is no gold standard reference test for all bleeding disorders so the tests that were done to arrive at a final diagnosis were the ones that is a part of our laboratory protocol.

We also run the Thromboelastography only in the EXTEM mode and we evaluated only R time, K time, Angle, MA and Ly 30 , it remains to be seen if running the TEG in other modes and including other parameters or derivation of other parameters would increase the sensitivity and specificity of the study.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

- Patients referred to our laboratory for evaluation of hemostasis in between November 2015 to May 2016 were enrolled into the study after taking informed consent.

- ISTH BAT questionnaire were administered to each patients who consented to be a part of the study.

- Per Protocol all the tests were done including Thromboelastography and the positivity and negativity of both these modalities were analysed with the final diagnosis.

- Sensitivity and Specificity of Bleeding score , Thromboelastography separately and both taken together in being able to identify Bleeding disorders were studied.

- The TEG parameters and Bleeding scores were compared among cases and controls using independent t-test.

- Based on validated cut-off points for both TEG and BS, diagnostic accuracies were presented with 95% confidence interval.

- The sensitivity and specificity of the ISTH BAT was 92.2%(85.8%-96.4%) and 65.5% (54.6%-75.4%) respectively while the negative predictive value was 86.4%(75.7%-93.6%).

- Sensitivity of TEG in diagnosing bleeding disorder was found to be 86.0%(78.5%-91.6%), whereas specificity was found to be 85.7%(76.4-92.4%). The positive predictive value and the negative predictive value were 89.7%(82.6%-94.5%) and 80.9%(71.2%-88.5%) respectively.

- The sensitivity and specificity of Thromboelastography and Bleeding Score taken together as a screening tool for Inherited bleeding disorders sensitivity,specificity,NPV and PPV were 97.5%(92.8%-99.5%),56.5%(45.3-67.5%),94%(83.5%-98.7%) and 76.3%(68.7%- 82.8%) respectively.

- There was significant difference in between the cases and controls in terms of R time, K time, angle and MA and BS.

Conclusion

- BAT can be used as ascreening tool to distinguish bleeders from none bleeders, given the high sensitivity and NPV , a normal bleeding score would rule out a bleeding disorders.

- TEG can be an effective screening tool in Inherited bleeding disorders however other parameters of TEG needs further evaluation.

- TEG and BS taken together can be an extremely effective screening tool for inherited bleeding disorders.

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DATA COLLECTION SHEET.

NAME:

AGE:

SEX:

HOSPITAL NUMBER:_____

REFERRED FOR:_____

BLEEDING SCORE: _____

Plt count_____

BT_____,

PT_____ INR_____

APTT_____

TT_____

TEG :

R TIME_____,

K_____, ALPHA_____,

MA_____,LY 30_____.

Reference Method_____

DIAGNOSIS_____

ANNEXURE 1

Symptoms	Assigned score
Epistaxis	0 = no or trivial 1 = > 5/year or more than 10 minutes 2 = medical consultation 3 = packing/cauterization or antifibrinolytic 4 = blood transfusion/replacement therapy/desmopressin
Cutaneous	0 = no or trivial 1 = bruises 5 or more (> 1cm) in exposed areas 2 = medical consultation 3 = extensive 4 = spontaneous hematoma requiring blood transfusion
Minor wounds	0 = no or trivial 1 = > 5/year or more than 10 minutes 2 = medical consultation 3 = surgical hemostasis 4 = blood transfusion/replacement therapy/desmopressin
Oral cavity	0 = no or trivial 1 = present 2 = medical consultation 3 = surgical hemostasis or antifibrinolytic 4 = blood transfusion/replacement therapy/desmopressin
Gastrointestinal Bleeding	0 = no or trivial 1 = present 2 = medical consultation 3 = surgical hemostasis or antifibrinolytic 4 = blood transfusion/replacement therapy/desmopressin
Hematuria	0 = no or trivial 1 = present 2 = medical consultation 3 = surgical hemostasis, iron therapy 4 = blood transfusion/replacement therapy/desmopressin
Tooth extraction	0 = no/trivial or none done 1 = bleeding in <25% of all procedures, no intervention 2 = bleeding in >25% of all procedures, no intervention 3 = Resuturing or packing 4 = blood transfusion/replacement therapy/desmopressin
Surgery	0 = no/trivial or none done 1 = bleeding in <25% of all procedures, no intervention 2 = bleeding in >25% of all procedures, no intervention 3 = surgical hemostasis or antifibrinolytic 4 = blood transfusion/replacement therapy/desmopressin

	4= hospitalization/ blood transfusion/replacement therapy/curretage or hysterectomy
Post-partum hemorrhage	0 = no/trivial/no deliveries 1 = consultation /syntocin/lochia > 6 weeks 2 = iron therapy/antifibrinolytics 3 = blood transfusion/replacement therapy/desmopressin 4= surgical intervention
Muscle hematomas	0 = never 1 = post trauma, no therapy 2 = spontaneous, no therapy 3 = replacement therapy/desmopressin 4= surgical intervention/blood transfusion
Hemarthrosis	0 = never 1 = post trauma, no therapy 2 = spontaneous, no therapy 3 = replacement therapy/desmopressin 4= surgical intervention/blood transfusion
Central nervous system bleeding	0 = never 1 = - 2 = - 3 = subdural, intervention 4= intracerebral, intervention
Other bleeding (circumcision/cephalohematoma/ umbilical stump, venipuncture)	0 = no/trivial 1 = present 2 = medical consultation 3 = surgical hemostasis, antifibrinolytics 4= blood transfusion/ replacement therapy/desmopressin

bleedscore	pltcount	bt	pt	inr	aptt	tt	rtime	k	alpha	ma	ly30	reference	diagnosis
0	120000	2.2	10.7	1	61.3	12.3	7.4	1.7	66.6	66.1	0	Factor assay	Factor XI deficiency
17	165000	2.3	10.9	1	151.8	11.7	22.8	7	23.6	59	0	Factor Assay	Severe Hemophilia A
4	158000	3	11.7	1.1	39.5	13.4	6.3	2	60.6	66.4	0		No intrinsic hemostatic defect
6	353000		10.9	1	89.3	13.5	9.9	2.5	56.4	59.1	0	Von willebrand Factor antigen	Von Willebrand Type 3
7	346000	2.3	12.3	1.1	159.6	12.6	38.2	7.3	30.3	64.5	0	Factor Assay	Severe Hemophilia B
4	312000	3	34.9	3.2	42.2	47.2	11.1		11.8	4.8	29.6	Fibrinogen activity and antigen	Hypofibrinogenemis with FXII deficiency
7	390000	2.3	11.4	1	156.9	13.2	40.5	9.3	22.7	50.5	0	Fsctor Assay	Severe Hemophilia A
6	178000	15	11.4	1.1	43.9	13.4	14.5		17.9	11.3	0	LTA/Flow Cytoometry	Glanzmanns Thrombesthenia
4	393000	3.3	12.1	1.1	30.5	12.8	8.3	1.9	63.7	66.5	0		No intrinsic hemostatic defect
2	280000	2.3	11	1	37.5	13	4.7	1.2	55.6	71	0		No intrinsic hemostatic defect
4	327000		29.5	2.7	78.7	13.2	14.3	2.2	62.1	61.4	1	Factor assay	Mild Factor X Deficiency
7	373000	2.3	61.6	3.4	180	14.3	21	3.6	4	57.6	0	Factor assay	Severe Factor V deficiency
2	21000	2	11.9	1.1	36.9	13.5	5.9	2	61.3	66.2	1.2		No intrinsic hemostatic defect
2	123000	3	11	1	38.3	14	4.7	1.8	66.1	70	0		No intrinsic hemostatic defect
0	100000	2.3	12.4	1	35.7	13.7	6.9	2.2	59.8	67.3	0		No intrinsic hemostatic defect
0	454000	3.3	12.2	1.1	44.2	14	9.2	2	65.3	76.9	0		No intrinsic hemostatic defect
8	519000		120	10	180	120						Fibrinogen assay and activity	Afibrinogenemia
1	163000	2	10.1	0.9	29.7	13.9	4.5	1.3	71.9	71.7	0		No intrinsic hemostatic defect
5	228000	3.3	10.1	0.9	34.6	13.6	6.2	1.7	66.4	65.3	0.3		No intrinsic hemostatic defect
12	29000		120	10	180	81.3	9.9		15.3	14.3	0	Fibrinogen assay and activity	Hypodysfibrinogenemia
6	314000	2.3	10.2	0.9	53	13.4	9.7	3	52.1	60.3	0	Von willebrand Factor Antigen assay	Von Willebrand Disease Type 1
0	229000	2.3	10.8	1	55	12.3	5.8	1.3	71.7	70.4	0.3		No intrinsic hemostatic defect
10	173000	2	10.6	1	77.7	15.4	24.2	6.2	33.8	51	0	Factor Assay	Moderate Hemophilia B
0	150000	3.3	10.5	0.9	52.3	13.2	8.4	1.7	62.7	66.8	0	Factor Assay	Mild Hemophilia A
6	120000	2.3	10.4	0.9	52.6	12.4	8.4	1.3	72.7	73.1	0	Factor Assay	Mild Hemophilia A
16	164000	3.3	10.3	0.9	38.4	13.4	7.3	3.1	51.9	53.2	0.4	Factor assay	Factor XIII deficiency
7	351000	2.3	10.7	1	38.5	14	7.6	1.9	65.5	70.4	0		No intrinsic hemostatic defect
6	141000	5.3	10.6	1	33.5	14.1	7.4	1.8	47.1	68.3	0	LTA	Storage pool defect
22	375000	2	10.7	1	165.2	14.9	94.2	4.9	49.7	26.7	0	Factor Assay	Severe Hemophilia A
2	330000	3	10.6	0.9	37.1	13.1	5.8	1.5	69.5	73.9	0		No intrinsic hemostatic defect
8	203000	15	10.3	0.9	25	13.7	5.9		46.2	15.4	0	LTA/Flow cytometry	Glanzmanns Thromosthenia
7	246000		11.5	1.1	27.4	13.4	8.1		46.2	15.1	0	LTA/Flow Cytometry	Glanzmanns Thrombosthenia
0	381000	3	10.3	1	180	13.9	23.3	3.2	55	60.7	1		No intrinsic hemostatic defect
14	207000	2	10.8	1.1	55.5	13.4	10.2	2.3	60.5	60.7	1.4	Factor Assay	Mild Hemophilia A
16	160000	2.3	10.4	0.9	149	12.5						Factor Assay	Severe Hemophilia A
3	326000		10.8	1	166	13.4						Factor Assay	Severe Hemophilia A
2	280000	2.3	11	1	37.5	13	4.7	1.2	55.6	71	0		No intrinsic hemostatic defect
17	279000	3	57.1	5.2	54.3	13.4	9.2	2.8	54.2	67	0.4	Factor Assay	Acquired bleeding disorder
4	403000	3	10.9	1	33.1	12.7	7.1	1.7	70.5	77.5	0		No intrinsic hemostatic defect
8	298000	2.3	11	1.2	48.6	14	7.5	2.1	60.7	67.7	0	Factor assay	Mild Hemophilia A
0	183000	3	12.3	1.1	51.1	14.6	10.4	3.7	46.2	57.1	0		No Intrinsic hemostatic defect
3	290000	2.3	10.3	0.9	30.6	14.5	5.3	1.6	62	62.7	0.8		No intrinsic hemostatic defect
3	228000	2.3	10.7	0.9	80.7	14.2	13.9	3	50.6	60.1	0	Factor assay	Moderate Hemophilia B

6	315000	15	10.3	0.9	39.2	13.2	13.3	3.8	42.8	61.3	0	Von willebrand Factor antigen assay	Von Willebrand Disease Type 3
10	377000		11.7	1	121.5	13.8	44.2	10.8	23.7	60.5	0	Factor assay	Severe Hemophilia B
17	286000	3.3	10.9	1	161.6	14.6	85.9	34.8	7	52.1	0	Factor Assay	Severe Hemophilia A
2	196000	15	10.8	1	38.6	14.9	5.7	1.4	66.1	71.4	0	LTA	Acquired bleeding disorder
4	298000	2.3	10.5	0.9	46.3	13.2	9.2	2.8	53.3	63.2	0		No intrinsic hemostatic defect
12	275000	8	10.8	1	88.8	14	8.5	3.1	43.1	65.6	0	Von willebrand Factor antigen assay	Von Willebrand Disease Type 3
21	265000	2.3	11	1	157.6	14.5	24.8	7.2	28.7	66.1	0.3	Factor Assay	Severe Hemophilia A
0	275000	2.3	10.3	0.9	41.5	13.8	6.2	2	46.4	62.4	0		No Intrinsic hemostatic defect
2	257000	3	11.4	1	30	13.5	6.8	6.4	67.8	67.8	0		No intrinsic hemostatic defect
0	267000	3.3	12.5	1.1	56.3	13.7	14.7	3.7	37.1	56.8	0	Factor Assay	Mild Hemophilia A
2	392000		10.3	0.9	39.1	13.7	4.3	1.3	72.6	71	0		No intrinsic hemostatic defect
2	180000	4.3	10.9	1	36.1	13.9	6.9	1.7	67.6	71.1	0		No intrinsic hemostatic defect
19	217000	2.3	10.2	0.9	38	12.9	10.9	2.7	52.3	66.4	0	Factor assay	Severe Hemophilia A
2	294000	3.3	11.6	1.1	41.6	14.4	7	2	56	66.2	0		No intrinsic hemostatic defect
19	185000	2.3	10.8	1	137	12.5	31.1	7.8	33.4	72	0	Factor Assay	Severe Hemophilia A
22	177000	2.3	10.9	1	143.8	13.7						Factor Assay	Severe Hemophilia A
26	316000	3	12.1	1.1	39.6	12.7	10.2	2.8	52.9	62.9	0	Factor Assay	Factor V deficiency
3	195000	3	11.4	1	52.7	13.5	7.5	2.1	61.5	65.9	0	Factor Assay	Severe Hemophilia A
11	180000	15	10.4	1	33.5	14.1	5.3	5	48.6	29.3	0	LTA/Flow cytometry	Glanzmanns thrombosthenia
18	45000	8	11.1	1	40.4	13.7	6.5	2.3	63.4	63.5	0	LTA/Flow cytometry	Bernnard Soulier Syndrome
17	302000	15	11	1	32.6	13.7	11.8	8.3	29.8	26.7	0	LTA/Flow cytometry	Glanzmanns Thromosthenia
3	202000	3.3	10.9	1	45.2	13.8	6.8	1.7	65.7	60.9	0		No intrinsic hemostatic defect
13	433000	2.3	11.8	1	41.5	12.5	8.2	2.2	62.5	69.4	1.5	Factor Assay	Factor XIII deficiency
9	266000	15	12.6	1.2	38.2	15.1	7.7	2.8	46.6	50.2	0	LTA/Flow cytometry	Glanzmanns Thrombosthenia
6	290000	15	12	1.1	34.5	14.9	8.3		17.4	17	0	LTA/Flow cytometry	Glazmanns Thrombesthenia
4	36000	5.3	11.2	1	40.4	13.8	6.3	2.8	51.4	61	0	LTA	Von willebrand disease type 2B
12	309000		104.3	9.4	180	14.6						Factor Assay	Severe Factor II deficiensy
10	332000	3.3	10.9	1	87.3	15	12.8	3.6	43	59.1	0	Factor Assay	Mild Hemophilia A
20	320000	3	11.1	1	145.5	14.6	52.3	19.7	11.6	44	0	Factor assay	Severe Hemophilia A
20	262000	3	15.6	1.4	36	26.1	7.7	5.9	32	27.5	31.7	Fibrimogen assay and activity	Hypodysfibrinogenemia
0	435000	3	10.3	0.9	36.5	12.9	4.4	1.1	74.3	71.2	0.9		No intrinsic hemostatic defect
17	220000	15	26.9	2.5	54.8	13.2	8.3	1.5	66.8	74	0	LTA/Factor assay	Severe Platelet dysfunction/Factor deficiency
11	223000	15	11.1	1	43.7	14	7.8	6.8	33.8	51.9	0	LTA/Flow Cytometry	Glanzmanns Thrombosthenia
8	172000		10.9	1	43.4	13.2	4.5	1	71.5	68.2	0	Factor assay	Acquired bleeding disorder
5	210000		12	1.1	38	12.3	9	6.9	34.9	24.9	0	LTA/Flow cytometry	Glanzmanns Thrombasthenia
7	370000	2.3	104.4	9.4	180	12.9	49.2	35.8		31	0	Factor Assay	Severe Factor X and mild Factor IX deficiency
3	13000		10.9	1	43.2	14.1	11.9	5.7	34.1	41.8	0		Acquired bleeding disorder
3	464000		11.6	1	75.2	12.4	14.9	2	58.9	69.7	0.8	Factor Assay	Mild Hemophilia A
4	780000		11	1	44.7	14.1	6.3	1.5	68.4	67.5	2.2		No intrinsic hemostatic defect
4	177000	2.3	12.9	1.2	38.1	13.1	6.6	2.2	57.6	67.4	1		No intrinsic Hemostatic defect
5	186000	3	10.6	1	34.3	13	5.4	1.5	69.5	72.8	0		No intrinsic hemostatic defect
2	153000	8	10.9	1	42.1	14.3	9.5	2.2	60.2	63.3	0	Platelet aggregometry	Acquired bleeding disorder
2	196000		10.7	1	33.7	14	6.1	1.8	64.3	66.7	2	Platelet aggregometry	Acquired plateelet dysfunctin with eosinophilia
4	397000	3.3	10.6	1	32.8	13.3	4.8	1.2	73.3	71.8	0		No intrinsic hemostatic defect

4	60000	6	10.6	1	39.8	12.9	6.5	2.1	60.1	64.6	0	LTA	Macrothrombocytopenia
2	306000	3.3	11.1	1	36.4	14.7	4.3	1.3	71	65.6	0.4		No intrinsic hemostatic defect
4	370761	4	10.9	1	35.8	13.6	3.7	1.3	71.2	68.8	0.1		No intrinsic hemostatic defect
32	261000	15	10.2	0.9	32.7	12.4	3.8	6.2	56.3	26.4	0	LTA/Flow Cytometry	Glanzmann Thrombasthenia
2	97000	5	10.8	1	35.4	13	4.5	1.6	67.9	66.5	0		No intrinsic hemostatic defect
4	251000	2.3	10.6	0.9	28.5	13.5	2	2.6	51.6	70.8	0		No intrinsic hemostatic defect
3	369000	5	10.3	0.9	38.6	12.2	5.7	1.1	74.1	76.3	0		No intrinsic hemostatic defect
4	340000	15	12.6	1.2	104.9	13.7	8.8	2.3	60.9	61.6	0	Von Willebrand antigen assay	Von Willebrand disease Type 3
13	219000	15	10.9	1	37.5	13.1	11.8	9.3	27.1	25.5	0	LTA/Flow Cytometry	Glanzmann Thrombasthenia
19	47000	12	10.3	0.9	31.4	13.3	9.7	4.8	37	58.9	0	LTA/Flow Cytometry	Bernard Soulier Syndrome
2	889000	15	11.4	1.1	40.1	12.1	6.3	1.9	60.1	37.4	0	LTA/Flow cytometry	Glanzmann Thrombasthenia
9	177000	15	11	1	35.5	13.3	5.3	4.8	54.9	23.7	0	LTA/Flow Cytometry	Glanzmann Thrombasthenia
6	248000	3.3	10.1	0.9	31.3	13.2	4.8	1.4	66.2	70.5	0		No intrinsic hemostatic defect
3	266000	2.3	10.9	0.9	36.6	13.5	7.3	2.2	54.8	62.8	0		No intrinsic hemostatic defect
4	228000	3.3	10.3	0.9	25	14.1	3.8	1.3	72	73.1	0		No intrinsic hemostatic defect
20	247000	15	11.1	1	93.5	14.6	10.5	2.3	52.6	62.6	0.3	Von Willebrand Factor Antigen	Von Willebrand Disease Type 3
6	232000		10.7	1	42.1	14	6.9	10.6	37.1	23.8	0	LTA/Flow Cytometry	Glanzmann Thromboasthenia
9	283000	2.3	10.8	1	33.6	14.4	5.7	1.8	61.6	66.7	0.1		No intrinsic hemostatic defect
4	494000		120	10	180	120						Fibrinogen antigen and activity	Afibrinogenemia
4	190000	2.3	10.4	0.9	32.5	12.8	4.8	1.4	66.1	69.3	0		No intrinsic hemostatic defect
4	369000		46.6	4.3	133.7	13.5	9	1.3	73	75.2	0	Factor assay	Severe Factor V deficiency
1	358000	3.3	10.4	0.9	35.3	13	5	1.5	69.5	59	1.3		No intrinsic hemostatic defect
4	88000	6	12.1	1.1	133.1	13.9	14.3	3.8	44.2	59	0	LTA	Acquired bleeding disorder
3	481000		18.9	1.7	70.1	14.9	8.8	2.1	62.2	63.3	0	Factor Assay	Acquired bleeding disorder
31	18	3	10.4	1	145	13.3	86.7	32.1	9.6	68.9	0	Factor Assay	Severe Hemophilia A
7	232000	3	10.6	0.9	36.1	13.7	5.3	1.5	67.7	70.9	0		No intrinsic henostatic defect
16	380000	2.3	11	1	37.7	11.2	6.2	2.1	63.6	73.4	0.8	Factor Assay	Factor XIII deficiency
24	247000	2.3	10.5	1	152.1	12.1	89.4	35.8	7.8			Factor Assay	Severe Hemophilia A`
0	180000	3	9.6	0.8	40	12	7.8	1.8	64.5	65.8	0		No intrinsic hemostatic defect
7	528000		10.7	1	151.9	12.9	27	10.3	21.6	57.2	0	Factor Assay	Severe Hemophilia A
14	308000	3	11	1	151.8	13.1	31.5	13.3	19.3	72.6	0	Factor Assay	Severe Hemophilia A
6	450000		12.4	1.1	154.5	15	20.7	5.5	36.4	66.5	0	Factor Assay	Severe Hemophilia A
2	305000	3.3	10.6	0.9	31.7	14.5	3.3	0.9	75.1	72.4	2.3		No intrinsic hemostatic defect
4	236000	5.3	9.8	0.9	62.4	14.5	7.3	2.3	58.7	60.7	0	Factor Assay	Mild Hemophilia A
2	339000		11.4	1	119.8	15	40	14.5	14.1	65.9	0	Factor Assay	Severe Hemophilia A
6	11000		10.4	0.9	39.1	13.7	7.8	9.2	32	24.3	0		Acquired bleeding disorder
2	329000	2.3	12.1	1.1	42.2	14.7	2.4	1.3	72.4	70	0		No intrinsic hemostatic defect
5	236000		10.3	0.9	45.6	12.9	6.7	2.8	52.8	58.2	2.8	Factor Assay	Factor XIII deficiency
3	261000	2.3	11.4	1.1	37.5	14.7							No intrinsic hemostatic defect
28	159000	2.3	10.9	0.9	119.3	14.4	10.2	37.5	7.2	48.9	0	Factor Assay	Severe Hemophilia A
2	330000	2.3	10.8	1	36.8	14.7	7	44	5.5	46.8	0		No intrinsic hemostatic defect
6	404000		11.1	1	155.5	13.7						Factor Assay	Severe Hemophilia A
18	275000	3	11.4	1.1	170.8	13.9						Factor Assay	Severe Hemophilia B
5	398000	2.3	10.6	0.9	42.1	13.1	6.3	1.6	67.9	68.8	0		No Intrinsic hemostatic defect

8	326000	3.3	11	1	139.2	13.3	45	8.7	27.8	57.4	0	Factor Assay	Severe Hemophilia A
2	525000		10.6	0.9	44.5	14.5	6.7	1.6	67.6	67.9	1.9		No Intrinsic hemostatic defect
4	236000	2.3	10.4	1	40	13.7	10.9	2.2	60	62.2	1.1		No intrinsic hemostatic defect
4	313000		10.1	0.9	29.4	12.6	6.5	1.2	73.5	76.7	0		No Intrinsic hemostatic defect
9	253000	3.3	120	10	180	120						Fibrinogen assay and activity	Afibrinogenemia
3	208000	2.3	10.5	0.9	39.1	12.6	7.6	1.9	64	65.8	0		No Intrinsic hemostatic defect
4	447700		10.1	0.9	42.1	14.1	6.8	1.8	65.7	67.5	0.4		No intrinsic hemostatic defect
15	180000	15	10.7	0.9	29.5	12.3	7.5		28.7	19.8	0	LTA/Flow Cytometry	Glanzzmann Thrombasthenia
3	130000	3.3	15.5	1.4	57.2	13.5	7.9	2.8	35.7	60	0		No Intrinsic hemostatic defect
0	297000	3	10.9	1	43.9	13.6	6.3	2.8	55.1	61	0		No Intrinsic Hemostatic defect
3	57000	15	10.9	1	33	13.2	5.8	1.4	75.2	77.2	0		Mild Macrothrombocytopenia/To r/o VWD Plt typ
3	383000	3.3	10.1	0.9	28.4	14.1	4.3	1.1	73.7	74.9	0		No Intrinsic hemostatic defect
15	231000	3.3	10.5	0.9	151.6	13.1	15.9	5	36.6	74.6	0	Factor Assay	Severe Hemophilia A
9	148000	3	12.6	1.2	35.2	13.4	4.7	1.3	72.3	72.9	0	Factor Assay	Mild Factor VII deficiency
2	536000		10.8	1	39.6	13.1	6.1	2	62.4	64.3	0		No Intrinsic hemostatic defect
0	91000	9.3	10	0.9	30.1	12.7	7.4	2.1	59.2	65.8	0		No intrinsic hemostatic defect
3	532000		120	10	153.5	13.1						Fcator Assay	Acquired bleeding disorder
16	411000		10.9	1	146	13.1	33.2	12.7	16.6	70.3	0	Factor Assay	Severe Hemophilia A
0	302000	2.3	11.4	1	121.5	13.1	14.8	3.6	44.2	63.4	0	Factor Assay	Exclude- Improper history
0	405000		11	1	127.1	14.1	15	2.7	56.7	69	0	Factor Assay	Exclude- Improper history
2	181000	3	10.6	0.9	48.3	14.2	10.8	3.9	42.3	62.1	0		No Intrinsic hemostatic defect
3	194000	2.3	10.9	1	39.6	13	5	1.3	68.7	76.3	0.4		No Intrinsic hemostatic defect
2	222000	2.3	13	1.2	44.4	13.7	5	1.3	65.9	72.4	0		No Intrinsic hemostatic defect
6	224000	4	10.2	0.9	30.4	14.4	3.8	1.8	59.7	50.9	2.6	Factor assay	Factor XIII deficiency
12	103000	2.3	14.7	1.4	107	13.6	20.1	8.3	24.4	43.6	0	Factor Assay	Moderate Hemophilia A
2	290000	2.3	11.2	1.1	34.7	14.7	5.7	1.6	68.5	69.6	0.1	Von Willebrand Antigen/Ricof	Von Willebrand Disease Type 2A/2M
2	154000		12.9	1.2	32.9	15.8	4	1.3	68.9	66.7	0.5		No Intrinsic Hemostatic defect
10	450000		120	10	35.7	13.4	7	1.3	71.5	72.3	1.1	Factor Assay	Severe Factor VII deficiency
11	311000	2.3	11	1	142	13.9	49	14.2	14.5	49.4	0	Factor Assay	Severe Hemophilia A
6	339000		11.3	1.1	154.2	14.4	60.9	15.7	14.8	55.6	0	Factor Assay	Severe Hemophilia A
10	141000	3.3	10.3	0.9	32.4	13.8	6.4	1.7	62.7	73.7	0		No Intrinsic hemostatic defect
7	159000	15	10	0.9	68.4	13	11.5	2.7	53.7	61.3	0	Von Willebrand Factor Antigen	Von Willebrand Disease Type 3
19	378000	2.3	11.9	1.1	150.2	13.5	35.8	8.5	26.4	49.1	0.6	Factor Assay	Severe Hemophilia A
0	349000		10.9	1	68.3	13.6	6.2	1.1	74.5	71.4	0.9		No Intrinsic hemostatic defect
8	405000		11.1	1	43.7	13.1	8	1.8	67.3	64.9	2.5	LTA	Acquired bleeding disorder
4	367000	3.3	9.3	0.9	30.1	11.7	3.8	1.3	67.6	72.5	0		No Intrinsic hemostatic defect
11	320000	2	10	0.9	96.7	12.6	8.2	2.3	61.4	60	0	Factor Assay	Severe Hemophilia A
13	147000	4	10.7	1	151	12.8	40.8	23.4	5.8	42.3	0	Factor Assay	Severe Hemophilia A
3	288000	3	9.8	0.9	30.3	12.3	4.7	1.3	72.5	74	0.1		No Intrinsic hemostatic defect
2	35000	3.5	10.9	1	33.1	14.1	8.5	3.2	43.9	52	0		Acquired bleeding disorder
3	397000	2.3	15.9	1.5	78.7	13.9	7.5	1.8	64.1	58	0.6	Factor assay	Combined Factor Deficiency
6	268000	6	12.1	1.1	47.8	13.6	6.5	1.3	66	69.9	0.1	LTA	Von Willebrand disease 2A/M
3	300000	2.3	10.3	1	35.7	13.6	7.4	1.9	57.4	64.1	0.3		No Intrinsic hemostatic defect
3	221000	3.3	12.4	1.5	40.1	14.9	7.4	3.1	50.6	56.6	0	LTA	Acquired bleeding disorder

2	307000	3	10.3	1	36.5	12.8	6.1	1.7	67.3	70	0		No intrinsic hemostatic defect
6	460000		11.7	1	180	12.7	11.7	1.7	68.4	72.8	0	Factor assay	Severe Hemophilia A
6	298000	2.3	11.1	1	37.3	14.7	6.5	2.1	60.3	67.5	0		No Intrinsic hemostatic defect
6	369000	2.3	10.8	1	41.4	13.6	6.5	2.1	60.3	67.5	0		No Intrinsic hemostatic defect

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD
CHRISTIANMEDICALCOLLEGE,
BAGAYAM, VELLORE 632002, TAMIL NADU, INDIA

Ref: FG/9524/07/2015 September 23, 2015

Mr. Robby Pria Sundarsingh
Treasurer
Christian Medical College,
Vellore.

Dear Mr. Robby Pria Sundersingh,

Sub: **Fluid Research Grant NEW PROPOSAL:**

Clinical utility of ROTEM and ISTH bleeding assessment tool in evaluation of patient referred for evaluation of increased bleeding tendency.

.Ref: IRB Min No: 9524 dated 07.07.2015

The Institutional Review Board at its meeting held on July 07th 2015 vide IRB Min. No. **9524** accepted the project for a sum of *Rs. 10,000/- (Rupees Ten Thousand Only) will be granted for 1 year.*

If overspent the excess should be debited from the respective departmental or Special funds.

Kindly arrange to transfer the sanctioned amount to a separate account to be operated by Dr. Pragyal Kafley (pragya.kafley@gmail.com) and Dr.Sukesh C Nair (scnair@cmcvellore.ac.in)

Yours sincerely,

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
Vice - Principal (Research) - Reg. No. 43983
Christian Medical College, Vellore - 632 004.

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Pragyal Kafley, Department of Transfusionmedicine and Immunohaematology, CMC
Dr. Sukesh C Nair, Department of Transfusionmedicine and Immunohaematology,CMC.
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