Original Research Article

Prediction of timely decaying efficiency of Factor VIII replacement therapy using Activated Partial Thromboplastin Time (aPTT) test in Hemophilia A patients undergoing treatment at The National Hospital of Sri Lanka (NHSL)

ABSTRACT

Introduction: Hemophilia A is a genetic disorder caused by Factor VIII deficiency. Prophylactic Factor VIII replacement therapy aims to maintain baseline levels and prevent bleeding. This study aimed to investigate the relationship between Activated Partial Thromboplastin Time (aPTT) and the time since the latest Factor VIII treatment (T) to predict the duration of therapy efficiency.It also aimed to establish a cut-off value for aPTT as an indicator of Elevated Factor VIII Levels (EFL) due to therapy.Patientsattending Hemophilia clinic at NHSL were selected for the study (n=61). The study was conducted over 06 months.
Methodology: The time from the latest Factor treatment to blood collection (T) was recorded, along with age, weight, and Factor VIII dose from patient records. aPTT was measured using the COATRON-M4 coagulation analyzer. EFL was calculated based on the dose taken. Bivariate correlation analysis was performed to establishassociations between aPTT & T and aPTT & EFL. Kaplan-Meier survival analysis was used to determine the maximum recurrence days after the latest Factor VIII dose. Finally, Receiver Operating Characteristic (ROC) curve analysis was conducted to establish aPTT cut-off values as an indicator of EFL in the body.
Results:aPTT showed a significant strong positive correlation with T. The Kaplan–Meier survival analysis estimated the median survival time is about 5days with the initial recurrence of about 2 days, complete recurrence of about 8 days. According to the ROC analysis, the cut-off value of aPTT is estimated as 38.8 s with the sensitivity of 95.2% and specificity of 37.2%.
Conclusion: The results indicated that aPTT could predict the maximum duration of Factor VIII therapy efficiency in Hemophilia A patients. Cut-off values for aPTT as an indicator of EFL, determined using ROC curves, were also obtained. However, these findings need validation with a larger patient sample.

Keywords: Hemophilia A; Activated partial thromboplastin time test; Prophylaxis Factor VIII replacement therapy.

INTRODUCTION

Hemophilia A is the most common hereditary coagulation disorder characterized by a deficiency in clotting Factor VIII[1,2]. According to estimates, Hemophilia A affects 1 in 5000 males worldwide, and it is estimated that the occurrence rate of Hemophilia is 1 in 20,000 cases in Sri Lanka[3].Based on the amount of Factor VIII activity in the blood, Hemophilia A is classified into three categories. Severe Hemophilia A, which has less than 1% Factor VIII activity. Moderate Hemophilia A with Factor VIII activity ranging from 1% to 5%, Mild Hemophilia A with 5–40% activity [4].This is triggered by mutations in the genes that produce clotting Factors, which are proteins vital for blood clotting. These mutations can result in reduced or absent production of clotting factors, leading to excessive bleeding and bruising [5,6].A thorough physical examination is essential for the diagnosis of Hemophilia A. The examination should include an assessment of the patient's medical history, family history, and a physical examination to identify any signs of bleeding or bruising [7].Laboratory diagnosis of Hemophilia A involves testing for abnormalities in clotting Factor levels and coagulation pathways [8Clinical testing for coagulation

measures the speed at which blood clots form after the coagulation cascade starts. Common tests include Bleeding time (BT), Prothrombin time (PT), Activated partial thromboplastin time (aPTT), Thrombin time (TT) to evaluate the extrinsic and intrinsic pathways of coagulation function [9,10].aPTT is a vital laboratory test used to screen Hemophilia A patients as it measures the intrinsic pathway of the coagulation cascade. aPTT is typically prolonged due to the factor VIII deficiency in Hemophilia A patients [11].The confirmatory tests for Hemophilia A include factor assays, mixing studies, genetic testing. In the management of Hemophilia A, several critical facts are taken into consideration to determine the most appropriate method of treatment. Hemophilia A is mostly treated by clotting Factor concentrates infused into the patients [12].Gene therapy is one of the developing treatments for managing Hemophilia A [13].In addition to Factor concentrates and Gene therapy, there are other treatments used in managing Hemophilia A. One type is antifibrinolytics like tranexamic acid, which help stop bleeding. Hormonal therapies, either taken by mouth or applied locally, can be effective for managing heavy menstrual bleeding in Hemophilia patients. New non-replacement therapies, such as Hemlibra, show promise for treating Hemophilia A. They promote thrombin generation by mimicking the activity of certain clotting Factors [14]. Other approaches, like inhibiting natural anticoagulants or using engineered molecules, are being explored. As an inclusive treatment, Factor VIII replacement therapy is given to manage Hemophilia A [15]. Prophylaxis has become the primary treatment, focusing on maintaining a baseline Factor VIII level to prevent bleeding episodes proactively [16,13].Even though a systematic Factor VIII replacement therapy is given, the decaying of the factor levels cannot be controlled. It may not only depend on the way of administering the desired dose but also may vary from person to person. If the Factor VIII levels could be measured in between the therapy intervals the early decaying of Factor VIII levels could well be detected. However, due to the extremely high cost of the Factor VIII assay and the irregular attendance of patients at the clinics, it cannot be performed in routine practice. Since the patients with Hemophilia A are uncertain about the time of the decay of Factor VIII replacement therapy dose, they may encounter several challenges and problems. This uncertainty increases the risk of bleeding episodes as patients may be unaware of when their Factor VIII levels have declined, leaving them vulnerable to spontaneous bleeds or bleeding following minor injuries. It may lead to potentially prolonged bleeding with severe consequences. Moreover, the lack of clarity about when to administer the next dose can result in treatment adherence issues, as patients might miss or delay infusions.On such grounds, predicting the maximum time by employing basic laboratory tests to detect the efficiency of Factor VIII replacement therapy and establishing a cut-off value for aPTT as an indicator of decaying Factor VIII levels in the body would smoothen the way for a new promising diagnosis approach.

METHODOLOGY

Ethical clearance was granted by the Ethical Review Committee of the National Hospital of Sri Lanka (NHSL) (ERC NO: AAJ/ETH/COM/2023).A total of 61 volunteer participants attending the Hemophilia clinic at NHSL were selected for the study.The following statistical calculation will be used to identify the sample size of the study.

$$N = \frac{Z^2 p\,(1 - p)}{d^2}$$

N – Sample size
Z - Standard normal deviate for the chosen confident level. Since the chosen confident level is 95%; Z= +1.96
p – Expected proportion in population P=0.05 [17]
d – Precision

The patient's age, weight, the time from the latest Factor treatment to the collection of blood (T) and the latest Factor VIII therapy dose taken were obtained from the records. A blood sample was collected into a 3.2% Tri sodium citrate tube from each participant for an aPTT test under standard procedure, and the test was performed in the semi-automated method using the coagulation analyser Coatron-M4 (Licon, S.A.). The data and obtained aPTT results were analysed using Microsoft Excel and IBM SPSS version 26. In the first step, Elevated Factor VIII levels (EFL) due to the latest dose taken were

calculated.Correlation bivariate analysis was performed to establish an association between aPTT & T and aPTT & EFL. Then, Kaplan-Meier survival analysis was performed to determine the maximum number of days of recurrence after the latest Factor VIII administration dose. Finally, Operating Characteristic (ROC) curve analysis was performed to set up the cut-off value for aPTT as an indicator of decaying Factor VIII levels.

A total of 61 patients were selected for the study according to the inclusion and exclusion criteria mentioned below. The inclusion criteria for the study consist of patients aged 18-65 years with a confirmed diagnosis of Hemophilia A through medical records and laboratory tests who are undergoing Hemophilia A treatment at the Hematology Clinic of the National Hospital Sri Lanka and have documented records of the last time and amount of the administered Factor VIII replacement therapy. Additionally, patients must be willing to provide blood samples for the aPTT test as part of the study. Exclusion criteria include minors (below 18 years), patients with a history of other bleeding or coagulation disorders, and those with significant medical conditions that may interfere with the assessment of treatment efficiency.

Blood samples were collected from individuals who were attending the Hematology Clinic at NHSL for the Activated Partial Thromboplastin Test (aPTT) test. Samples to be tested were centrifuged at 3000 rpm for 10 minutes to separate platelet-poor plasma from red blood cells. Then aPTT test was performed using semi-automated methods, for each sample and results was recorded.

Statistical analysis

All data was analysed according to the objectives of the study using descriptive and inferential statistics. The records of the time from the latest Factor VIII replacement therapy to the collection of blood (T), patient's age, weight and the amount of Factor VIII replacement therapy doses administered to patients were obtained from individuals attending the Hematology Clinic at NHSL, and the data was directly used for the study. After performing aPTT test for each sample the result was recorded. After compiling the records of Factor VIII replacement therapy doses, time and onset blood samples theentire statistical analysis was performed using IBM SPSS version 26. First the normalization testing was performed and Kaplan-Meier survival analysis, and ROC curve analysis were used in the prediction of the efficiency of the Factor VIII levels.

RESULTS

Testing parameters for normalization

In the first step, the normality of the data was tested separately using SPSS. The Kolmogorov-Smirnov method was used to test the normality as the sample size was above 50. The data was considered in normal distribution when $p>0.05$. It was observed that the distribution of Weight followed a normal distribution. However, the variables aPTT Value, age, T and amount of dose did not exhibit a normal distribution. Accordingly, both the parametric and non-parametric analyses were carried out.

The EFL was calculated using the formula below,

$$EFL = \frac{\text{Amount of dose}}{Plasma\ volume(5\%\ of\ Body\ Weight)} \times 100\%$$

The experimental as well as the calculated data are shown in Table 1.

Table 1: Hemophilia A patient data used in the study.

No	Age	Gender	TFL	aPTT	No	Age	Gender	TFL	aPTT
1	52	M	23.1	67.1	**32**	65	F	22.0	109.3
2	24	M	8.1	86.3	**33**	34	F	8.3	112.3
3	40	M	25	109.5	**34**	26	M	27.7	103.6
4	38	M	7.4	90.7	**35**	29	M	18.1	88.1
5	26	M	18.2	112.2	**36**	41	M	32.7	79.3
6	30	M	11.8	109.7	**37**	61	M	43.9	64.2
7	42	M	20	96.5	**38**	57	M	25.4	68.3
8	36	M	26.6	98.5	**39**	51	M	17.2	70.1
9	28	M	7.4	92.1	**40**	46	M	25	69.3
10	54	M	28.6	84.4	**41**	57	M	23.4	70.3
11	33	M	8.3	105.3	**42**	46	M	8.3	42.4
12	65	M	31.7	83.2	**43**	27	M	8.3	53.9
13	60	M	41.6	130.9	**44**	30	M	8.4	55.5
14	24	M	16.4	90.2	**45**	18	M	28.0	36.2
15	31	M	16.6	116.5	**46**	27	M	18.8	39.3
16	57	M	7.3	79.1	**47**	61	M	29.8	38.3
17	36	M	10	53.6	**48**	32	M	19.6	36.3
18	30	M	29.4	97.1	**49**	45	M	26.3	33.4
19	25	M	24.1	125.6	**50**	32	M	28.8	37.1
20	41	M	28.3	120	**51**	29	M	13.8	43.3
21	35	M	15.3	95.6	**52**	32	M	22.7	41.3
22	28	M	20	90.2	**53**	66	M	34.4	37.2
23	24	M	16.6	80.5	**54**	61	M	42.3	32.3
24	26	M	13.8	82.3	**55**	30	M	19.6	43.2
25	26	M	22.2	57.4	**56**	23	M	15.3	49.2
26	22	M	20.8	112.2	**57**	38	M	18.1	40.6
27	48	M	38.4	65.2	**58**	28	M	19.6	32.7
28	30	M	8.3	115.4	**59**	49	M	28.8	36.4
29	38	M	28.5	87.3	**60**	35	M	15.6	83.1
30	22	M	20	112.4	**61**	22	M	13.3	101.2
31	40	M	23.2	64.6					

Correlation bivariate analysis

Correlation bivariate analysis was performed to establish an association between aPTT and T as well as aPTT and EFL. Pearson Correlation Bivariate analysis was used when the two sets of data were in the normal distribution, and Spearmen Correlation Bivariate analysis was used when the two sets of data or at least one set of data did not follow the normal distribution. Spearmen bivariate analysis was used as one set of data did not follow the normal distribution. The aPTT showed a significant, strong positive correlation with T (r=0.820; p=0.000) and a weak negative correlation with EFL.

Kaplan-Meier survival analysis

The aPTT values were categorized into two groups.

- Group 1- Prolonged values (>42 s),
- Group 2- Normal values (32-42 s).

The corresponding T values were tabulated accordingly. Kaplan-Meier survival analysis was performed to determine the maximum number of days of recurrences as stated in Table 1.

Table 2:Survival Time according to Mean and Median.

Mean				Median			
Estimate	Std Error	95% Confidence Interval		**Estimate**	Std Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
8.772	1.461	5.908	11.636	5.000	1.682	1.704	8.296

Results in Table 1 and Figure 1 reveal that the mean survival time is about 8 days, with an initial recurrence of about 5 Days (lower Bound) and a complete recurrence of about 11 days (upper Bound). The median survival time is about 5 days, with an initial recurrence of about 2 days (lower bound) and a completed recurrence of about 8 days (upper bound).

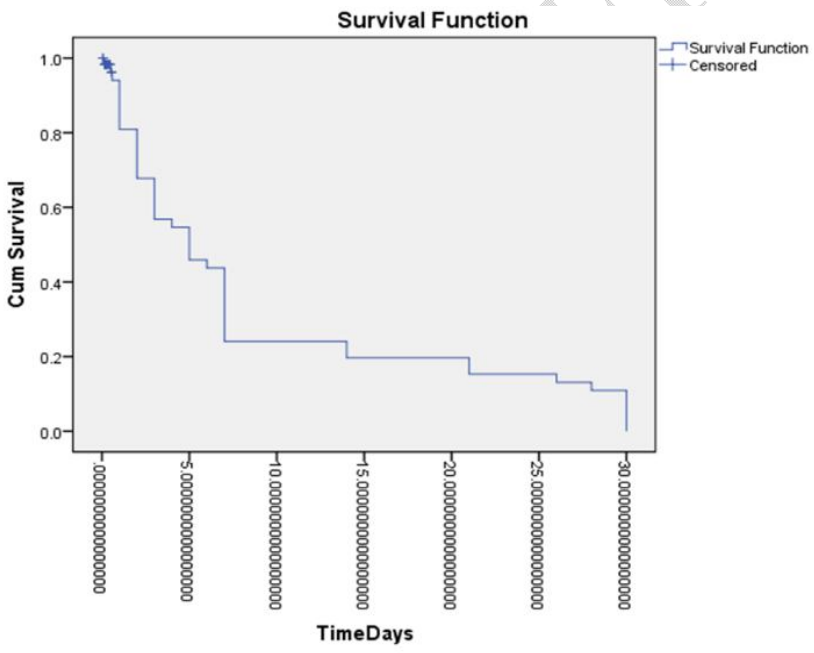

Figure 1: Kaplan-Meier Estimation of Recurrence of Prolonged aPTT.

Since the primary focus was predicting the overall efficacy of Factor VIII replacement therapy in individuals with Hemophilia A, Kaplan–Meier survival analysis was performed to determine the maximum

days of recurrences after latest Factor VIII administration. As the data reveals diverse variations in T, ranging from days to hours median value over the mean value is suggested. It gives the recurrence is around 05 days and it initially starts within 02 days and gives complete recurrence in 08 days. The advantage of this is, by estimating the number of days, patients can anticipate when the therapy will lose efficacy and plan accordingly for the next dose. This helps them avoid uncertainty about when to take the next dose and lowers the risk of bleeding. As a result, patients could carry out their daily activities more confidently and without disruption. Similarly, some research groups used the Kaplan–Meier survival analysis to evaluate the Complete Remission (CR) of therapeutic options such as immune suppressive therapy and targeted therapy in patients with AHA [18,19]. Using the Kaplan–Meier curves, [18] have found that it took 40 to 60 days for complete remission to occur, depending on the type of immunosuppressive therapy used. Another similar study was carried out byKnoebl et al. (2021) [19] to find the use of Emicizumab as a therapeutic option for acquired Hemophilia A used Kaplan–Meier survival analysis, and they observed the CR within a median duration of 115 days for various Factor levels.

Receiver Operating Characteristic (ROC) curve analysis

As the final step, Receiver Operating Characteristic (ROC) curve analysis was performed to set up cut-off values of aPTT as an indicator of decaying factor levels in the body by utilizing information on EFL resulting from administered doses and their corresponding aPTT values.

Table 3: Parameters used for ROC analysis.

No	Age	Gender	TFL	aPTT	No	Age	Gender	TFL	aPTT
1	52	M	23.1	67.1	**32**	65	F	22.0	109.3
2	24	M	8.1	86.3	**33**	34	F	8.3	112.3
3	40	M	25	109.5	**34**	26	M	27.7	103.6
4	38	M	7.4	90.7	**35**	29	M	18.1	88.1
5	26	M	18.2	112.2	**36**	41	M	32.7	79.3
6	30	M	11.8	109.7	**37**	61	M	43.9	64.2
7	42	M	20	96.5	**38**	57	M	25.4	68.3
8	36	M	26.6	98.5	**39**	51	M	17.2	70.1
9	28	M	7.4	92.1	**40**	46	M	25	69.3
10	54	M	28.6	84.4	**41**	57	M	23.4	70.3
11	33	M	8.3	105.3	**42**	46	M	8.3	42.4
12	65	M	31.7	83.2	**43**	27	M	8.3	53.9
13	60	M	41.6	130.9	**44**	30	M	8.4	55.5
14	24	M	16.4	90.2	**45**	18	M	28.0	36.2
15	31	M	16.6	116.5	**46**	27	M	18.8	39.3
16	57	M	7.3	79.1	**47**	61	M	29.8	38.3
17	36	M	10	53.6	**48**	32	M	19.6	36.3
18	30	M	29.4	97.1	**49**	45	M	26.3	33.4
19	25	M	24.1	125.6	**50**	32	M	28.8	37.1

20	41	M	28.3	120	**51**	29	M	13.8	43.3
21	35	M	15.3	95.6	**52**	32	M	22.7	41.3
22	28	M	20	90.2	**53**	66	M	34.4	37.2
23	24	M	16.6	80.5	**54**	61	M	42.3	32.3
24	26	M	13.8	82.3	**55**	30	M	19.6	43.2
25	26	M	22.2	57.4	**56**	23	M	15.3	49.2
26	22	M	20.8	112.2	**57**	38	M	18.1	40.6
27	48	M	38.4	65.2	**58**	28	M	19.6	32.7
28	30	M	8.3	115.4	**59**	49	M	28.8	36.4
29	38	M	28.5	87.3	**60**	35	M	15.6	83.1
30	22	M	20	112.4	**61**	22	M	13.3	101.2
31	40	M	23.2	64.6					

The data was grouped based on Factor levels, creating two distinct groups with one containing Factor levels similar or less than 25% (Group 1) and another comprising Factor levels exceeding 25% (Group 2). As an illustration, the corresponding empirical ROC curve was drawn using SPSS software (Figure 2).

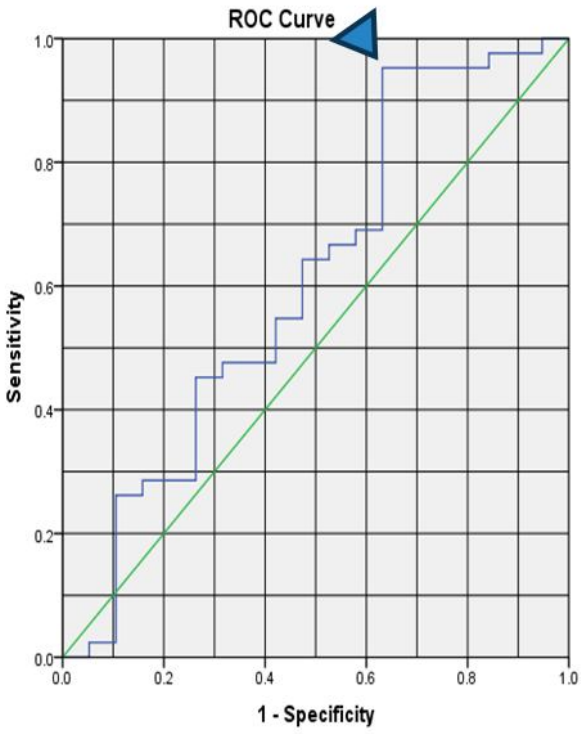

Figure 2: ROC curve analysis of aPTT based on two groups of EFL; EFL=<25% and EFL>25%.

According to the Figure 2, the cut off aPTT value is estimated as 38.8 s with the high sensitivity of 95.2% and specificity of 37.2% (AUC=0.603). Since the aPTT has the weak negative correlation with EFL the result could be interpreted as, Cut-off value of for aPTT =38.8 s (approximately 39 s). Once the aPTT is higher than 39 s; EFL is below than 25%, conversely, aPTT is lesser than 39 s; EFL is higher than 25%. Similar studies were carried out using ROC curve analysis to establish a predictive relationship between Factor XII levels and aPTT [20] and to determine the cut-off value of aPTT for Factor VIII inhibitor [2].We were successful in finding a cut off value with high sensitivity even though it gives low specificity. However, the number of patients should be widened, and accurate monitoring of the patients is required to validate the initial findings before implementing them.

DISCUSSION

This study aimed to explore the relationship between Activated Partial Thromboplastin Time (aPTT) and the duration of efficacy of Factor VIII replacement therapy in Hemophilia A patients. The data analysis incorporated both descriptive and inferential statistics to determine the predictive value of aPTT for therapy efficiency. The findings indicate that aPTT is a useful marker for predicting the time until Factor VIII therapy loses its efficacy, which is essential for optimizing treatment regimens and patient care.

The initial part of the analysis focused on testing the normality of the data, which showed that only the variable for weight followed a normal distribution, while other variables, including aPTT, age, time since last dose (T), and the amount of Factor VIII administered, did not. Consequently, both parametric and non-parametric statistical methods were applied to ensure robustness in the analysis. Bivariate correlation analysis revealed a significant positive correlation between aPTT and T, with a strong correlation coefficient ($r = 0.820$, $p < 0.0001$), indicating that higher aPTT values were associated with longer times since the last Factor VIII dose. However, aPTT showed a weak negative correlation with Elevated Factor VIII Levels (EFL), suggesting that as EFL increases, aPTT decreases.

Kaplan-Meier survival analysis provided further insights into the temporal aspects of Factor VIII replacement therapy efficacy. The analysis demonstrated that the median survival time for therapy efficacy was approximately 5 days, with a recurrence of therapeutic inefficacy beginning around 2 days

post-treatment and reaching complete recurrence at about 8 days. This suggests that the time period during which Factor VIII remains effective after administration is relatively short, which is crucial information for patients and clinicians in planning subsequent treatments. The ability to predict the duration of therapy efficacy can significantly reduce uncertainty in treatment schedules, allowing patients to manage their condition with greater confidence and less risk of bleeding. These findings are consistent with previous studies that used Kaplan-Meier survival analysis to assess the efficacy of other therapeutic options, such as immune-suppressive therapies and targeted treatments for acquired hemophilia A (AHA) [18, 19].

The ROC curve analysis further validated the use of aPTT as a predictor of EFL, with a cut-off value for aPTT determined to be approximately 38.8 seconds. This cut-off yielded a high sensitivity (95.2%) but a relatively low specificity (37.2%), indicating that aPTT is a highly sensitive marker for detecting low EFL, but it may not be as effective at distinguishing between patients with high and low Factor VIII levels. Despite the weak negative correlation with EFL, the cut-off value of 38.8 seconds can still serve as a practical clinical tool. If aPTT exceeds 39 seconds, EFL is likely below 25%, suggesting that the patient may need a dose adjustment or additional monitoring. These findings are consistent with previous research that used ROC curve analysis to establish cut-off values for aPTT in predicting Factor VIII inhibitor development [20, 21].

Although the results show promising potential for using aPTT as an indicator of Factor VIII therapy efficiency, it is important to recognize the limitations of this study. The sample size of 61 patients is relatively small, and a broader patient cohort would be necessary to confirm the findings and increase the generalizability of the results. Moreover, the low specificity of the aPTT cut-off value suggests that additional markers or tests may be required to enhance diagnostic accuracy. Future studies should consider incorporating other laboratory tests, such as Factor VIII activity assays or von Willebrand factor levels, to refine the prediction model and increase its clinical utility.

In conclusion, this study demonstrates that aPTT is a valuable tool for predicting the duration of Factor VIII therapy efficacy in Hemophilia A patients. The findings highlight the potential of using aPTT to guide treatment decisions and improve patient outcomes by providing a more accurate timeline for the effectiveness of therapy. However, further research with a larger patient sample and additional markers is needed to validate these results and refine the clinical application of aPTT in managing Hemophilia A.

CONCLUSION

The prediction of the maximum time that could maintain the efficiency of Factor VIII replacement therapy was successfully achieved, which is essential to overcome the challenges of managing Hemophilia A. Therefore, instead of relying on Factor assay, a simple aPTT test could be used to predict the recurrence: i.e. timeframe during Factor VIII levels boosted by the therapy, return to a state of deficiency. Even though a strong correlation was not established between EFL and the aPTT, A cut-off value for aPTT as an indicator of decaying Factor VIII levels in post-Factor VIII replacement therapy was successfully obtained. Thereby, instead of relying on Factor assay, a simple aPTT test could be used as an indicator for the decaying Factor VIII levels due to the dose taken.This is an initial attempt to predict a day of recurrenceand the Factor VIII levels by APTT. The initial results are promising, and results and the number of patients should be widened. An accurate monitoring of the patients is required to validate the initial findings before implementing them.Future research should expand the prediction of Factor VIII replacement therapy efficacy by incorporating a combination of diagnostic tests, including aPTT, Factor VIII activity assays, Factor VIII concentration, and von Willebrand factor levels. Regular clinical monitoring, including bleeding assessment and inhibitor testing, is essential for evaluating patient response. A multi-laboratory approach could provide a more comprehensive understanding of coagulation status, improving treatment outcomes. Future studies should focus on refining these methods and developing personalized treatment strategies to enhance patient management and clinical outcomes.

CONSENT

All authors declare that ‘written informed consent was obtained from the patients for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office/chief editor/editorial board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that ethical approval was taken from the ethical review committee of The National Hospital of Sri Lanka (Ref AAJ/ETH/COM/2023/SEP). All experiments have been examined and approved by the appropriate ethics committee.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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APPENDIX

Consent Form

If you are voluntarily agreeing to take part in our study, you will be required to answer the questions asked by the investigators about your medical history and a well-trained nursing officer will collect your blood sample under medical supervision. It will employ about 05 minutes to answer the questions.

Any of your questions, expressions, or opinion about our study are accepted. You can give up participation at any point in our study. You can answer the questionnaire according to your will.

To be completed by the participant

1. I read the information sheet……YES / NO
2. I have understood the information included in the information sheet ……YES / NO
3. I had an opportunity to discuss the study and was free to ask any question……...YES / NO
4. I got satisfactory answers to all the questions …..YES / NO
5. I had received enough information about the study……YES/NO
6. I understand that I am free to withdraw from the study at any time, without giving a reason ……..YES / NO
7. I like to participate in this study……YES / NO

Participant Name :……

Signature :……

Investigator's Name :……

Signature : ……..

Witness

I was present when the investigator explained the process and the procedure of the study to the participant and I can confirm that the participant gave their proper consent for taking part in the study.

Name of the witness :……

Signature : ……..

Date : …… / ……. / …….