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Analysis of APTT Based Clot Waveform Parameters in Various Clinical Conditions – A Study at A Tertiary Care Center

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Abstract

Various coagulation tests like Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) are estimated by automated coagulation analyzers. The newer fully automated analyzers generate clot wave forms aPTT-CWA for these parameters are derived. In this study, the objective was to analyze clot wave form characteristics morphology and its first and second derivative values in cases with abnormal APTT. ACL TOP 300 generated curves for APTT in a total 125 patients with 20 normal controls are included. First derivative, second derivative, morphology of curve: sigmoid, biphasic, prolonged pre-coagulation phase, second derivative morphology like early and late shoulder, biphasic peak, delayed deceleration were the analyzed parameters. Wave clot forms of 125 patients were included in this study. Patients (M:F - 2.2:1, mean age: 46.9 ± 20 years). A spectrum of clinical conditions was Covid (20%), liver disease (23%), polytrauma (10.4%), cardiac diseases (8.8%), sepsis/DIC (7.2%), thromboembolism (7.2%), renal diseases (6.4%), bacterial infections (4%), dengue (4%), snake bite (1.6%) and factor deficiency (1.6%). Liver and heart disease showed a significant difference in acceleration and deceleration peaks followed by sepsis, dengue, polytrauma and sepsis/DIC. Deceleration peak was prolonged in patients of Covid ($p<0.05$). Sepsis and liver diseases showed prolonged first derivative peak ($p<0.05$). CWA is very easily available on all automated coagulation analyzers. It is inexpensive with fast turn round time. Both quantitative as well as qualitative informations such as velocity, acceleration of clot formation and wave pattern details were recorded. Our study highlights importance of quantitative and qualitative CWA parameters acquired by performing APTT test for the automated analyzers.

Keywords

APTT, Clot Waveform, Velocity Acceleration.

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INTRODUCTION

Clot Waveform Analysis (CWA) is an extended interrogation of the curve generated by an optical detection system during the measurement of coagulation assays such as Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT). It detects light transmittance basd on its absorbance. This is a global hemostatic assay, that reflects the overall hemostatic factor (1).

The automated photo-optical coagulation analyzers used for the estimation of PT and APTT display the clot reaction curves along with the first and second derivative curves (first and second DCs) (2).

The height of the first DC in the APTT-CWA is used to reflect to the "thrombin burst" as a hemostatic ability. The low height of the first DC in APTT-CWA suggests a risk of bleeding. The height of the second DC in APTT-CWA is useful for detecting coagulation factor deficiency (3).

Thromboelastography (TEG) also shows a different pattern of information, but is slightly expensive and time-consuming (4). The coagulation system has a specific mechanism that includes the cascade system, thrombin burst and enhancement of clotting activation by phospholipids (PLs) (5).

The various available assays which evaluate the coagulation system these days are activated partial thromboplastin time (aPTT), PT, thromboelastography (TEG), and thrombin generation test (TGT) (6).

CWA is a global coagulation assay that evaluates the kinetics of fibrin formation during testing of aPTT or PT. Clot waveforms provide information on light transmittance during clot formation (7). Automatic optical end-point coagulation analyzers have the ability to show the clot reaction curve of the PT and APTT and reflect the "thrombin burst" with "enhancement of clotting activation by PLs (8).

The plot waveform analyses the slope generated by optical detection during routine coagulation tests, such as aPTT or PT. The optical detection system generates a clot formation process with respect to the change in transmittance and absorbance of the light beam through the sample (9).

Continuous measurement of the change in light transmission or absorbance during the PT and APTT assays is performed and the data are given in the form of a wave. This generated clot wave has three phases: 1) Pre-coagulation, 2) Coagulation: Either decreased light transmittance or increased absorbance along with formation of fibrin is seen in the clotting process which is seen as slope on waveform, 3) Post coagulation: Towards the end of coagulation, the light transmittance or absorbance stabilizes, which is seen as a linear segment on the waveform (10).

TGT and CWA provide similar information, as both correlate with the rate

and velocity of thrombin formation and reflect the entire process of thrombin generation. Clinical conditions such as DIC/Sepsis and prediction of bleeding risk in DIC (3), Factor VIII deficiency (3), bacterial infections (11), Covid 19 (12), patients on anticoagulant therapy exhibit different clot waveform morphology. The aim of the present study was to analyze and compare clot waveform characteristics, such as morphology and first- and second-derivative values, in cases with abnormal APTT.

MATERIALS AND METHODS

It is a prospective cross-sectional study with a duration of 6 months. A total of 125 patients and 20 age matched controls were included in the study. All the abnormal APTT samples were thoroughly selected through the analyzer. Patients receiving unfractionated heparin (UFH) or low-molecular-weight heparin (LWMH) were excluded from the study. Blood samples were collected in anticoagulant tubes containing 1:9 volumes of 3.2% trisodium citrate. For obtaining platelet poor plasma: platelet-poor plasma was obtained by centrifugation of the blood samples at 3000 rpm for 15 min. The plasma is analyzed for platelet count on a cell counter, which should be less than 10000/ per microliter. PT/APTT was performed using an ACL Top 300 CTS Coagulation Analyzer. The morphology of clot waveform in all the conditions with abnormal APTT was studied.

The parameters studied were the first derivative (maximum velocity of clot formation), second derivative (maximum and minimum acceleration and deceleration during clot wave formation). Statistical analysis was performed using SPS15 software. The mean and standard deviation were calculated for the APTT, First and second derivatives. Mann Whitney U test was performed and p value was determined. Statisctical significance was set at $P<0.05$.

RESULTS

A total of 145 patients were included in the study, with 20 control samples. The mean age group in the present study was 46.9 ± 20 years with male to female ratio of 2.2:1. The ranges of APTT, first, and second derivatives in controls and cases were determined (Table 1).

The morphology of the clot waveforms was studied in all 125 cases. The various clot wave patterns studied were sigmoid, biphasic, prolonged pre-coagulation phase, slow or steep slope, second-derivative morphology, biphasic peak, and delayed deceleration. The liver disease and Corona Virus Disease 2019 (Covid-19) were the two most prevalent conditions among the 125 cases in the current investigation. (Figure 1).

The most common pattern was a sigmoid pattern and prolonged pre-coagulation phase. Various morphologies of the clot waveforms in different clinical conditions are shown in

Figure 2. The mean values of APTT and the first and second derivatives in each clinical condition were calculated and compared with those of the controls (Table 2).

A significant association was found between cases of liver disease and sepsis with respect to the first derivative ($p<0.05$). A significant association was found in cases of Covid-19, sepsis, heart disease, and liver disease with respect to the second derivative ($p<0.05$) (Table 3).

Table 1. Range of APTT, First and Second Derivatives in Cases and Controls

Parameters	Controls	Cases
APTT	29.7 to 36.5 seconds	-
First derivative	98.08 - 354.87 TU/L	11.43 - 344.81 TU/L
Second derivative (Acceleration)	376.1 - 1212.81 TU/L	21.29 - 865.02 TU/L
Second derivative (Deceleration)	225.52 - 563.867 TU/L	8.95 - 270.29 TU/L

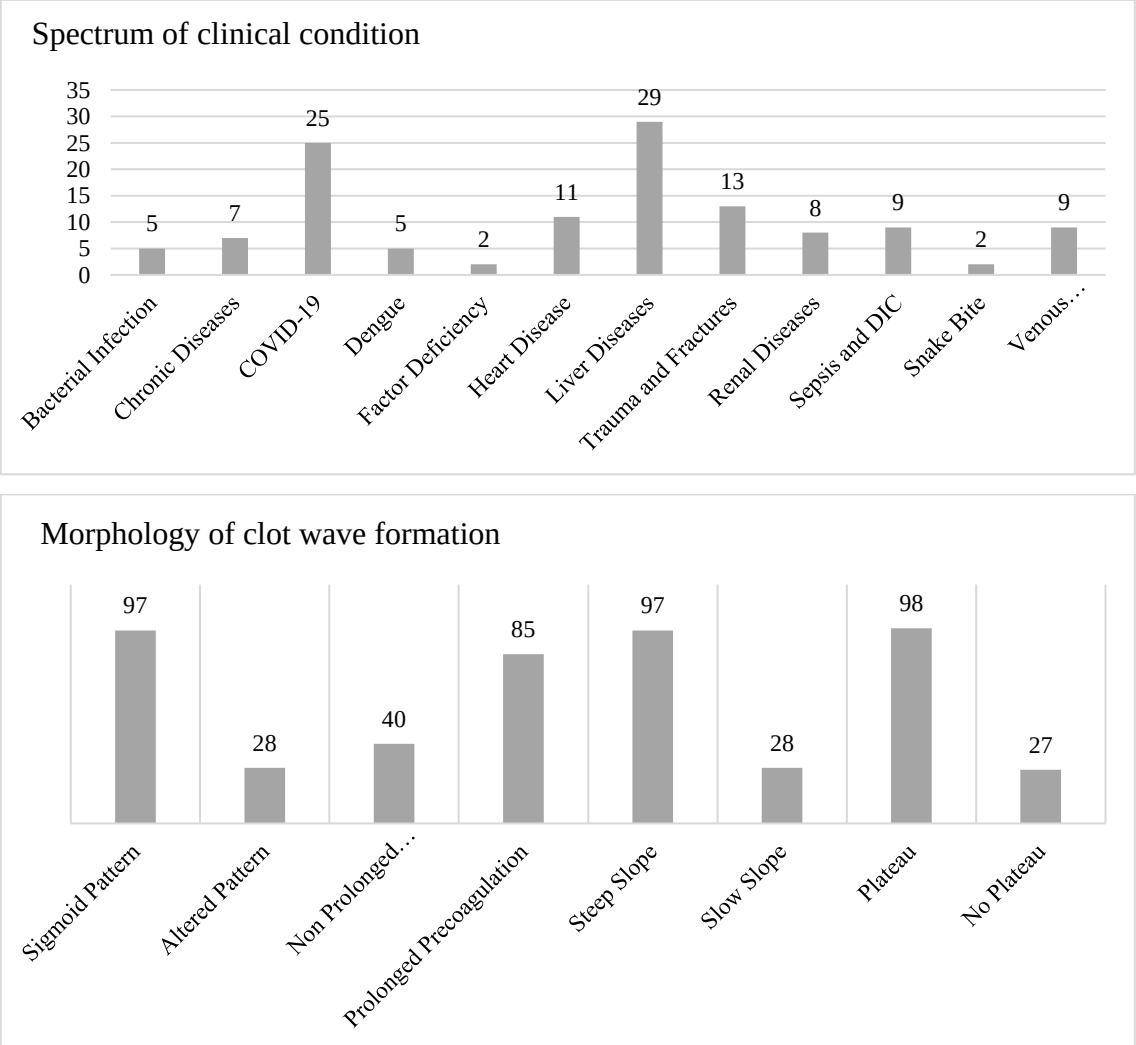

Figure 1. Spectrum of clinical cases and various morphology patterns of clot wave

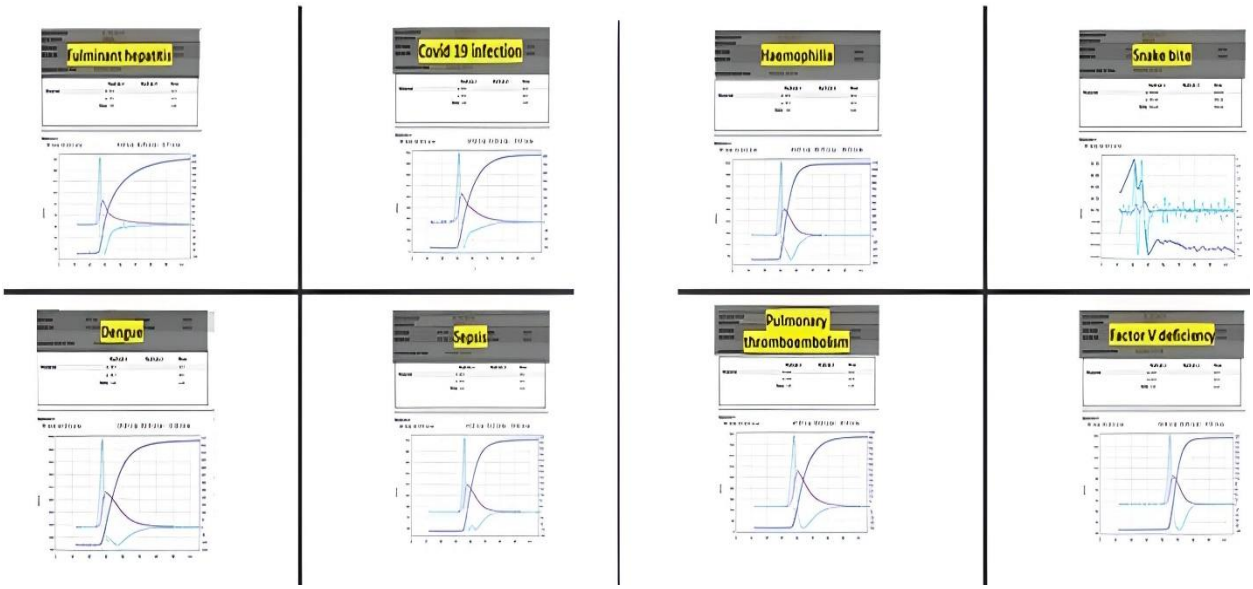

Figure 2. Waveforms of normal and biphasic aPTT clots. On the MDA System, photo-optical monitoring of clot formation yields a sigmoid pattern that looks like a wave.

Table 2. Mean Values of APTT, First and Second Derivative in Various Clinical Condition

	Mean APTT	Mean Velocity (First derivative)	Mean acceleration (Second derivative)	Mean deceleration (Second derivative)
Controls	33.3	217.95	742.05	362.38
Covid-19	57.3	257.78	624.72	253.05
Heart disease	45.3	200.34	513.29	229.30
Chronic diseases	49.9	209.07	569.16	264.75
Bacterial infections	47.5	344.81	865.02	270.29
Liver Diseases	42.6	166.62	430.30	202.71
Dengue	42.5	151.39	385.33	146.68
Factor deficiency	52.6	256.20	667.98	232.26
Renal diseases	62.5	283.29	640.51	263.04
Sepsis/DIC	73.6	144.24	295.31	121.39
Snake bite	117.4	11.43	21.29	8.95
Venous thromboembolism	55.3	254.34	452.47	199.88

Table 3. Association of APTT, First and Second Derivatives in Various Clinical Conditions

Clinical condition		APTT	First derivative	Second derivative (+)	Second derivative (-)
Covid	Mann Whitney U test	14.000	207.000	186.000	128.000
	P value	0.000*	0.575	0.284	0.013*
Sepsis	Mann Whitney U test	0.000	41.000	16.000	12.000
	P value	0.000*	0.047*	0.001*	0.001*
Heart disease	Mann Whitney U test	0.000	88.000	58.000	53.000
	P value	0.000*	0.364	0.032*	0.019*
Liver disease	Mann Whitney U test	6.000	185.000	113.000	90.000
	P value	0.000*	0.033*	0.000*	0.000*

*P value < 0.05

There were two cases of snakebite with abnormally raised APTT and abnormal morphology of the clot waveform. The precoagulation phase was entirely distorted and the first and second-derivatives were suggestive of consumptive coagulopathy (DIC). In addition, two cases of factor deficiency (Factor V and Factor VIII) showed corresponding changes in the clot wave pattern with early and late shoulders in the second derivative curves.

DISCUSSION

Multiple factors, such as the blood vessel wall, plasma proteins, platelets and coagulation factors are involved in the coagulation cascade. PT and aPTT are the routinely performed tests that give information regarding hemostasis. Coagulation assays are of utmost importance in this new era. (13).

CWA is based on APTT and PT tests, which are global coagulation tests and is studied on the principle of optical detection system through an automated coagulation analyzer. Various ACL series are widely available and use an automated coagulation analyzer that works on the light absorbance principle and helps in studying the entire process of hemostasis depicted in the form of waves as described above (14).

In the present study, clot waveforms detected hemostatic alterations and abnormal patterns in various clinical cases like Covid-19, bacterial infection, liver diseases, DIC/sepsis, hemophilia, venous thromboembolism. A rise in the incidence of thrombotic events, such as pulmonary embolism, has been observed in critical ill cases of Covid-19 with severe hypercoagulability is been seen in various studies (15).

M.F Rubereto et al., (16) studied CWA in 191 patients with liver cirrhosis and found values of maximum acceleration and deceleration were lower in the cirrhotic patients as compared to the control groups which correlate with our study. Takuya et al., (12) studied the clot wave forms of APTT in 26 patients with Covid-19 and the results showed abnormal patterns of second derivative morphology (early shoulder type and late shoulder type), which were similar to those found in our study.

In a study by Tan et al., (1) of 101 patients, it was concluded that patients with bacterial infections showed significantly higher CWA parameters than controls. In contrast, patients with dengue infection had significantly lower CWA parameters. This similar observation was also seen in our study.

Kei et al., (3) studied the clot wave form in 211 patients of sepsis and showed first and second derivatives curves were useful in diagnosis and prediction of bleeding risks. There was a significant association between the second derivative and the disease condition in this study.

A study by Dave et al., (17) showed that the risk of severe bleeding in patients with Hemophilia A invariably accompanied by an aberrant clot waveform and thrombin generation test, while the Factor VIII level did not always reflect the actual bleeding severity. Similarly, we had two cases of factor deficiency in our study with abnormal clot wave formation, thus providing new insight into the potential utility of CWA in detecting hypercoagulability or risk of bleeding in various clinical conditions (17).

A study by Kanouchi et al., (18) showed significant atypical peak and deceleration/acceleration ratio extension using clotting waveforms, specifically in patients with LA-positive APS. Oka et al., (19) studied the CWA for the assessment of DOAC effects and provided valuable insights into the relevance of anticoagulation to therapeutic efficacy and bleeding risk from the perspective of fibrinolysis.

CWA is an extended study of the routine aPTT test that utilizes pre-existing test protocol and equipment assay. Many automated analyzers use changes in light transmittance or absorbance to measure clotting times, and these optical changes over time as clot forms are captured and presented as a clot waveform curve in the software of the analyzers (20).

As more studies showing the correlation between the clot wave patterns have surfaced, the importance of full utilization of the data provided by the automation machines can be interpreted without any additional cost and turn over time (14).

Clot waveform analysis provides similar information to TGT, as it correlates with the rate and velocity of thrombin formation, and reflects the whole process of thrombin

generation. This contrasts with routine coagulation assays, in which clotting time only reflects coagulation initiation (14, 16).

CONCLUSIONS

CWA is readily available in newer coagulation analyzers. In addition to routine coagulation tests, CWA, which is readily available without any extra cost, helps study the process of hemostasis in normal as well as abnormal patients without any additional turnaround time. Quantitative as well as qualitative information was obtained from the clot waveform analysis, which can be used for clinical decisions. Our study highlights the importance of quantitative and qualitative CWA parameters obtained using a simple APTT test.

AUTHOR CONTRIBUTIONS

Rachana Lakhe and Preeti Doshi: Design, acquisition and analysis of data and drafting of manuscript, statistical analysis with critical revision. Amit Nisal and Ravindra Nimbargi: The project was supervised.

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CONFLICT OF INTEREST

There is no financial relationship between them. The authors declare that there are no conflicts of interest.

REFERENCES

1. Tan CW, Wong WH, Cheen MH, Chu YM, Lim SS, Ng LC et al. Assessment of aPTT-based clot waveform analysis for the detection of haemostatic changes in different types of infections. Scientific reports. 2020; 10(1):1-7. DOI: 10.1038/s41598-020-71063-1
2. Wada H, Matsumoto T, Ohishi K, Shiraki K, Shimaoka M. Update on the clot waveform analysis. Clinical and Applied Thrombosis/Hemostasis. 2020; 26:1076029620912027. DOI: 10.1177/1076029620912027
3. Suzuki K, Wada H, Matsumoto T, Ikejiri M, Ohishi K, Yamashita Y et al. Usefulness of the APTT waveform for the diagnosis of DIC and prediction of the outcome or bleeding risk. Thrombosis Journal. 2019; 17(1):1-8. DOI: 10.1186/s12959-019-0201-0
4. Hartmann J, Mason D, Achneck H. Thromboelastography (TEG) point-of-care diagnostic for hemostasis management. Point of Care. 2018; 17(1):15-22. DOI: 10.1097/POC.0000000000000156
5. Sachetto ATA, Mackman N. Modulation of the mammalian coagulation system by venoms and other proteins from snakes, arthropods, nematodes and insects. Thromb Res. 2019; 178:145-154. DOI: 10.1016/j.thromres.2019.04.019.
6. Duarte RC, Ferreira CN, Rios DR, Reis HJ, Carvalho MD. Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations. Revista brasileira de hematologia e hemoterapia. 2017; 39:259-65. DOI: 10.1016/j.bjhh.2017.03.009
7. PO, Depasse F. Clot waveform analysis: Where do we stand in 2017? Int J Lab Hematol. 2017; 39(6):561-568. DOI: 10.1111/ijlh.12724.
8. Wada H, Shiraki K, Matsumoto T, Ohishi K, Shimpo H, Sakano Y et al. The Evaluation of APTT Reagents in Reference Plasma, Recombinant FVIII Products; Kovaltry® and Jivi® Using CWA, Including sTF/7FIX Assay. Clin Appl Thromb Hemost. 2021; 27:1076029620976913. DOI: 0.1177/1076029620976913.
9. Shima M, Thachil J, Nair SC, Srivastava A; Scientific and Standardization Committee.

Towards standardization of clot waveform analysis and recommendations for its clinical applications. J Thromb Haemost. 2013; 11(7):1417-20. DOI: 10.1111/jth.12287.

10. Mohammadi AM, Erten A, Yalcin O. Technology advancements in blood coagulation measurements for point-of-care diagnostic testing. Frontiers in Bioengineering and Biotechnology. 2019; 7:395. DOI: 10.3389/fbioe.2019.00395
11. Toh CH, Giles AR. Waveform analysis of clotting test optical profiles in the diagnosis and management of disseminated intravascular coagulation (DIC). Clin Lab Haematol. 2002; 24(6):321-7. DOI: 10.1046/j.1365-2257.2002.00457.x.
12. Shimura T, Kurano M, Kanno Y, Ikeda M, Okamoto K, Jubishi D et al. Clot waveform of APTT has abnormal patterns in subjects with COVID-19. Scientific reports. 2021; 11(1):1-1. DOI: 10.1038/s41598-021-84776-8
13. Negrier C, Shima M, Hoffman M. The central role of thrombin in bleeding disorders. Blood reviews. 2019; 38:100582. DOI: 10.1016/j.blre.2019.05.006
14. Abraham SV, Rafi AM, Krishnan SV, Palatty BU, Innah SJ, Johny J et al. Utility of clot waveform analysis in Russell's viper bite victims with hematotoxicity. J Emerg Trauma Shock. 2018; 11(3):211-216. DOI: 10.4103/JETS.JETS_43_17.
15. Ichikawa J, Okazaki R, Fukuda T, Ono T, Ishikawa M, Komori M. Evaluation of coagulation status using clot waveform analysis in general ward patients with COVID-19. Journal of Thrombosis and Thrombolysis. 2022; 53(1):118-22. DOI: 10.1007/s11239-021-02499-z
16. Ruberto MF, Sorbello O, Civolani A, Barcellona D, Demelia L, Marongiu F. Clot wave analysis and thromboembolic score in liver cirrhosis: two opposing phenomena. International Journal of Laboratory Hematology. 2017; 39(4):369-74. DOI: 10.1111/ijlh.12635
17. Dave RG, Geevar T, Mammen JJ, Vijayan R, Mahasampath G, Nair SC. Clinical utility of activated partial thromboplastin time clot waveform analysis and thrombin generation test in the evaluation of bleeding phenotype in Hemophilia A. Indian Journal of Pathology and Microbiology. 2021; 64(1):117. DOI: 10.4103/IJPM.IJPM_336_19
18. Kanouchi K, Narimatsu H, Shirata T, Morikane K. Diagnostic analysis of lupus anticoagulant using clot waveform analysis in activated partial thromboplastin time prolonged cases: A retrospective analysis. Health Science Reports. 2021; 4(2):e258. DOI: 10.1002/hsr2.258
19. Oka S, Wakui M, Fujimori Y, Kuroda Y, Nakamura S, Kondo Y et al. Application of clot-fibrinolysis waveform analysis to assessment of in vitro effects of direct oral anticoagulants on fibrinolysis. International Journal of Laboratory Hematology. 2020; 42(3):292-8. DOI: 10.1111/ijlh.13168
20. Cheong MA, Tan CW, Wong WH, Kong MC, See E, Yeang SH et al. A correlation of thrombin generation assay and clot waveform analysis in patients on warfarin. Hematology. 2022; 27(1):337-42. DOI: 10.1080/16078454.2022.2043573