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Evaluation of coagulation status using clot waveform analysis in general ward patients with COVID-19

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Abstract

Coronavirus disease (COVID-19)-related systemic cytokine response induces the production of procoagulant factors, which predisposes patients to a prothrombotic state. Viscoelastic testing can identify the degree of hypercoagulability, which is related to outcomes. We aimed to study the changes in clot waveform analysis (CWA) parameters in COVID-19 patients on hospital admission compared to those in a group of healthy individuals. We conducted a retrospective study of COVID-19 patients admitted to general wards and evaluated demographic and clinical parameters as well as laboratory parameters, including coagulation parameters. CWA data from patients (n = 62) with COVID-19 prior to the initiation of anticoagulation therapy were compared with those from healthy controls (n = 67). The measured CWA parameters were min1, min2, max2, and delta change. CWA, fibrinogen, and D-dimer values were higher in COVID-19 patients than in healthy controls ($p < 0.001$). CWA profiles were consistent with hypercoagulability and characterized by an increase in density, velocity, and acceleration of clot formation. Activated partial thromboplastin time, fibrinogen, D-dimer, and C-reactive protein (CRP) values were higher in patients in whom all CWA parameters were raised than in patients with just a few elevated CWA parameters, while Sequential Organ Failure Assessment scores, prothrombin time, fibrin degradation product levels and platelet counts did not differ between the two groups. CWA variables showed hypercoagulopathy on admission in COVID-19 patients who were hospitalized in the general ward, and this pattern was more pronounced in critically ill patients with elevated fibrinogen, D-dimer, and CRP levels. Our results may help identify patients at high risk of thromboembolism.

Keywords COVID-19 · General ward · Clot wave analysis · Hypercoagulability · Fibrinogen

Abbreviations

CWA	Clot wave analysis
aPTT	Activated partial thromboplastin time
CRP	C-reactive protein
VTE	Venous thromboembolic event
DIC	Disseminated intravascular coagulation,
BMI	Body mass index
SOFA	Sequential Organ Failure Assessment
PT	Prothrombin time

Highlights

- CWA can provide more precise information regarding alterations in fibrin clot properties, which might represent risk factors for VTE, than fibrinogen assays.
- CWA is an economically convenient and simple test that can aid clinical stratification and management by implementing an algorithm into the software of an automated coagulometer.

Introduction

Viral infection-associated systemic pro-inflammatory cytokine responses induce the production of procoagulant factors and hemodynamic changes, which predispose patients to prothrombotic states [1]. Studies have reported that patients with COVID-19 who become critically ill present with severe hypercoagulability, which results in

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a cumulative incidence of thrombotic events, mainly pulmonary embolism [2, 3]. Consequently, anticoagulant prophylaxis is recommended in critical care [4, 5]. Clot waveform analysis (CWA) is the global hemostatic assessment that evaluates clot formation kinetics during routine clotting tests. Whenever activated partial thromboplastin time (aPTT) is evaluated, commonly used optical analyzers generate aPTT-CWA data automatically. For practical use, qualitative assessment of the clot waveform pattern could help diagnose and treat disseminated intravascular coagulation, sepsis, and hemophilia. Past studies [6] have shown that higher CWA values are associated with a high Padua Prediction Score, a risk assessment tool for venous thromboembolic events (VTEs), suggesting the utility of CWA parameters as a marker of hypercoagulability. In this study, we evaluated the coagulation state as measured by aPTT-CWA parameters in COVID-19 patients at hospital admission to identify patients at high risk of thromboembolism.

Materials and methods

This retrospective, observational study included 62 COVID-19 patients who were admitted to the internal medicine wards at Tokyo Women's Medical University Hospital Medical Center East between September 29 and November 29, 2020 (COVID-19 group). This group was compared to 67 healthy blood donors (control group). This study was approved by our Institutional Review Board (No. 2020-0029) with informed consent was obtained in the form of an opt-out function on the website.

Patients were identified through a review of electronic medical records. Demographic, clinical, and laboratory data, including aPTT-CWA parameters, were retrieved from our laboratory data storage system retrospectively. Results of complete blood counts and conventional coagulation tests including those for D-dimer, fibrinogen (Clauss method), aPTT, prothrombin time (PT), and International Normalized Ratio, as well as aPTT-CWA parameters, were extracted before anticoagulant treatment on the day of hospital admission.

aPTT-CWA was performed on the CS-5100 (Sysmex, Kobe, Japan) using a Thrombocheck APTT-SLA (Sysmex, Kobe, Japan). CWA is performed using an automated photo-optical detection system to quantify the changes in light transmittance (Fig. 1). The parameters examined by CWA were as follows: (i) the minimum value of the first derivative (min1); (ii) the minimum value of the second derivative (min2); (iii) the maximum peak of the second derivative (max2); and (iv) the maximum density of the clot (Delta).

Data analysis

Variables were tested with Student's t tests or Mann–Whitney U tests for differences in distributions between groups, and the chi-square or Fisher's exact test was used for the analysis of differences in categorical variables between groups. Spearman's correlation coefficients were calculated to assess associations between variables. P values below 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Software, version 22 (IBM Japan, Tokyo, Japan).

Results

Thirty-eight patients (56.7%) were male, the mean age was 61 years, the mean body mass index (BMI) was 23.9 kg/m^2, and the median Sequential Organ Failure Assessment (SOFA) score on admission was 0. Ten (7.5%) patients required oxygen administration via a mask, and three of these patients required intubation a few days after admission. One (1.4%) patient had a VTE, and none had acute renal failure. Anticoagulant treatment was administered to 16 patients after admission, empirically based on the physician's concern for the risk of thrombotic events. Six patients received a low prophylactic dose of unfractionated heparin and 10 patients received both unfractionated heparin and nafamostat mesylate.

COVID-19 patients showed elevated D-dimer (n = 38; 61.3%), FDP (n = 36; 58.1%), and fibrinogen (n = 62; 100%) levels, with normal platelet counts (n = 44; 71.0%). Although the majority of patients showed values in the normal range for PT and aPTT, these values were significantly higher in COVID-19 patients than in healthy controls (Table 1).

The reference ranges for aPTT and CWA parameters were established [7] by our laboratory based on samples from 67 healthy volunteers. The mean and reference intervals were as follows: aPTT, 32.1 s (26.49–37.79 s); min1, 3.46%/s (2.07–4.85); min2, 0.51%/s^2 (0.28–0.75); max2, 0.41%/s^2 (0.28–0.75); and delta change, 37.13% (24.08–50.18). Fifty-seven (91.9%) patients in the COVID-19 group exhibited CWA values above the upper limit of the reference range. Overall, all four CWA parameters were elevated in 43 COVID-19 patients. Consequently, all four CWA parameters in COVID-19 patients were significantly higher than in controls. We observed a significant correlation between fibrinogen levels and each CWA parameters (delta: $r = 0.935$, $p < 0.0001$; min1: $r = 0.865$, $p < 0.0001$; min2: $r = 0.727$, $p < 0.0001$; min3: $r = 0.671$, $p < 0.0001$).

Next, we compared clinical information and coagulation measurements between patients with elevated values for all CWA parameters and patients with elevated values for a few

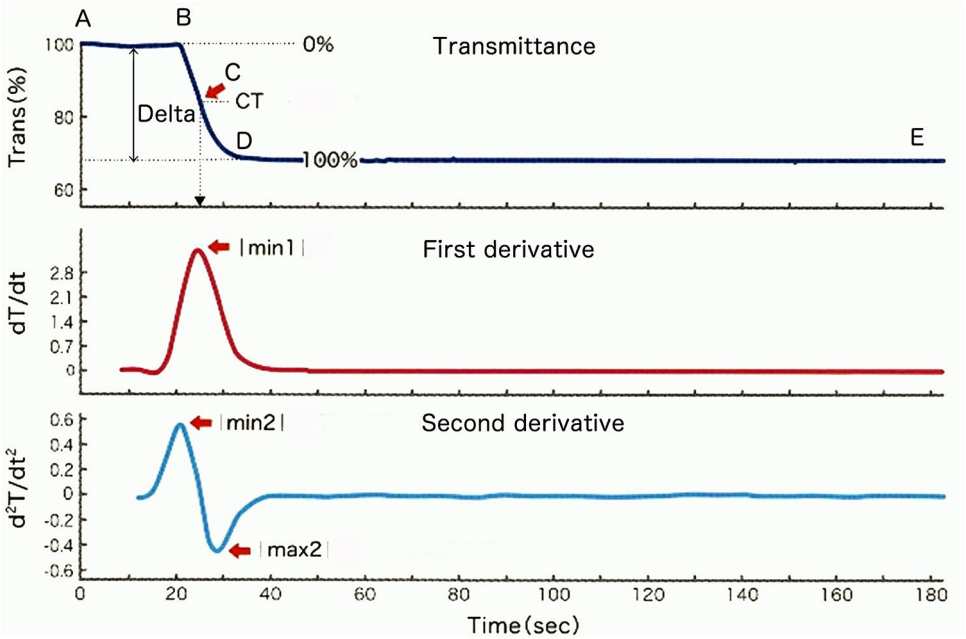

Fig. 1 Normal clot waveform and derived parameters. The upper waveform shows the recording of changes in transmitted light intensity (T) over time (t). The middle waveform shows the first derivative of transmittance (dT/dt) reflecting the coagulation velocity, which indicates the conversion of fibrinogen to a fibrin clot. The lower waveform shows the second derivative of transmittance data (d^2T/dt^2) reflecting the acceleration and deceleration of the reaction. Events during coagulation are indicated by **A** (beginning of the signal), **B** (the index of the second derivative representing the onset of coagulation), **C** (the index of the first derivative representing the midpoint of coagulation), **D** (end of coagulation), and **E** (end of signal). The transmittance data is separated into the pre-coagulation phase (**A**, **B**), the coagulation phase (**B**–**D**), and the post-coagulation phase (**D**, **E**). Common parameters that were analyzed included: 1) the time of the coagulation onset, midpoint C (clotting time [CT]); 2) the slope of the mid-point of coagulation (maximum coagulation velocity [min1]); 3) terms for coagulation acceleration and deceleration (maximum coagulation acceleration [min2], maximum coagulation deceleration [max2]); and 4) the magnitude of the signal change during coagulation (delta)

CWA parameters. Patients with elevated values for all CWA parameters showed significantly elevated values for aPTT, fibrinogen, D-dimer, and C-reactive protein (CRP).

Discussion

In our study, we aimed to evaluate the changes in aPTT-CWA parameters in COVID-19 patients on hospital admission compared to those in healthy individuals. We found that CWA profiles showed a significantly higher mean aPTT, min1, min2, max2, and median delta change in COVID-19 patients than in healthy controls. Although the majority of patients showed normal values of aPTT, a higher proportion of COVID-19 patients showed values closer to the upper limit of the reference range of CWA parameters early on admission, characterized by increases in the density, velocity, and acceleration of clot formation. This means that the patients' clots began to form as usual, but once initiated, the clots rapidly increased in strength and ultimately had greater density than that of the clots in healthy controls. Moreover, in these patients, there was a significant increase in fibrinogen, CRP, and D-dimer levels. Taken together, these findings suggest a hypercoagulative state in hospitalized COVID-19 patients, even those in the general ward, and this could possibly justify antithrombotic prophylaxis for patients not admitted to the intensive care unit (ICU) [8].

In the COVID-19 group, fibrinogen levels were universally elevated regardless of the thrombotic potential. In contrast, five patients did not demonstrate a hypercoagulable profile on their CWA. Hyperfibrinogenemia has not been unequivocally demonstrated as a strong risk factor for VTEs. In contrast, alterations of fibrin clot structure/function appear to be more associated with VTE [9, 10]. Considering that CWA parameters were strongly correlated with plasma fibrinogen levels, which is consistent with a previous report [11], and that fibrinogen theoretically enhances the speed of clot formation, CWA can provide more precise information regarding alterations in fibrin clot properties, which might represent risk factors for VTE, than fibrinogen assays.

All the patients who showed CWA values closer to the upper limit of the reference range exhibited raised delta and min1 [12] on admission. It seemed that delta and min1 were raised first, followed by min2, and finally max2. Higher

Table 1 Comparison of the conventional coagulation and CWA parameters between patients with COVID-19 and healthy controls

	Patients with COVID-19 N = 62	Healthy controls N = 67	P
aPTT, s, mean ± SD	33.9 ± 4.2	32.5 ± 2.7	0.013
aPTT ratio, mean ± SD	1.22 ± 0.15	1.17 ± 0.09	0.012
aPTT ratio ≦0.9, n (%)	0	0	
CWA parameters			
Min1, %s, mean ± SD	6.12 ± 1.26	3.26 ± 0.63	0.001
Min2, %/S2, mean ± SD	0.89 ± 0.20	0.48 ± 0.10	0.001
Max2, %/S3, mean ± SD	0.69 ± 0.17	0.38 ± 0.09	0.0001
Delta change, %, mean ± SD	69.6 ± 14.2	39.8 ± 16.3	0.0001
CWA > ULRR, n (%)	62(100%)	0	
Min1 > 3.46	50 (80.6%)	0	
Min2 > 0.51	43(69.4%)	0	
Max2 > 0.41	62(100%)		
Delata change > 37.13			
Fibrinogen, g/dl, mean ± SD	443.8 ± 108.9	226.2 ± 40.4	0.0001
FDP, median (IQR)	2.7 (1.25–4.1)	< 2.5	0.0001
D-dimer, nmol/L, median (IQR)	0.6 (0.25–1.3)	< 0.5	0.0001
PT, s, mean ± SD	12.0 ± 0.80	11.4 ± 7.3	0.001
INR, mean ± SD	1.04 ± 0.08	0.98 ± 0.07	0.0001
Platelet counts, 10^9/L, mean ± SD	19.5 ± 5.5	25.9 ± 6.4	0.001
Hemoglobin, g/L, mean ± SD	13.9 ± 2.0	13.4 ± 1.7	0.62

aPTT activated partial thromboplastin time, *CWA* clot waveform analysis; *Min1* the minimum value of the first derivative, *Min2*, the minimum value of the second derivative, *Max2*, maximum peaks of the second derivative, *ULRR* upper limit of reference range, *FDP* fibrin degradation products, *PT* prothrombin time, *INR* International Normalized Ratio, *SD* standard deviation, *IQR* interquartile range

delta and min1 values could possibly suggest initiation of the progression of coagulopathy. CWA is based on dynamic changes in light transmittance caused by fibrin formation and delta derived from the total difference of transmittance, which is specifically dependent on the fibrinogen concentration. An overall elevated CWA profile, in comparison to only a few raised parameters (delta, min1, and min2), is more common in critically ill patients with elevated fibrinogen, D-dimer, and CRP levels than in others. A past study [13] investigated the behavior of CWA in patients with VTE providing values exceeding the upper limit of normal, yielding a much higher odds ratio for acute VTE. Compared with previous studies that stratified risk factors [6, 13] and those proposing algorithms for VTE prophylaxis, when all CWA parameters are elevated on admission, this might represent a ''very high risk'' of developing a VTE. aPTT values in patients with overall elevated CWA profiles were significantly higher than those in patients with just a few elevated CWA parameters, which indicates the possible association between a prolonged aPTT and an increase in disease severity.

Of note, early in admission, COVID-19 patients were clearly hypercoagulable, despite the average PT, aPTT, and SOFA scores being within normal reference limits. The results demonstrated the importance of using global hemostatic tests for improved risk stratification, even if carried out using one point [14]. CWA is an economically convenient and simple test that can aid clinical stratification and management by implementing an algorithm into the software of an automated coagulometer [15].

Our study had several limitations. First, this study was conducted in a small, single center, and the findings can only be generalized following the confirmation of our results in larger multi-center cohort studies. Second, this was a retrospective study, and as no routine imaging surveillance was performed for deep vein thrombosis during hospitalization, we included only one patient who developed a VTE. Lastly, the healthy control group used as a reference included younger individuals and more women, which may have influenced the results.

In summary, COVID-19 patients hospitalized in general wards presented with a hypercoagulable state early in the disease course, as shown by significantly higher values for aPTT-CWA parameters and elevated D-dimer and fibrinogen levels.

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Declarations

Conflicts of interest All authors declare that they have no conflict of interest.

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