Dielectric Blood Coagulometry as a Novel Coagulation Test

Sahoko Chiba1, Yuki Sumi1 *, Ken Uchihori2, Takasato Fujiwara3, Tomoyuki Ogata4, Shuta Yamauchi5, Tsukasa Okamoto6, Tomoya Tateishi1, Haruhiko Furusawa1, Toshihide Fujie1, Hiroyuki Sakashita1, Kimitake Tsuchiya1, Meiyo Tamaoka1, Yasunari Miyazaki1 and Naohiko Inase1

1 Department of Respiratory Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
2 Department of Thoracic Oncology, Hyogo Cancer Center, 13-70 Kitaojicho, Akashi, Hyogo 673-8558, Japan
3 Department of Respiratory Medicine, Yokosuka Kyosai Hospital, 1-16 Yonegahamadori, Yokosuka, Kanagawa 238-8558, Japan
4 Department of Respiratory Medicine, JA Toride Medical Center, 2-1-1 Hongo, Toride, Ibaraki 302-0022, Japan
5 Department of Respiratory Medicine, Hiratsuka Kyosai Hospital, 9-11 Oi awake, Hiratsuka, Kanagawa 254-8502, Japan

* Tel.: +81-3-5803-5954; fax: +81-3-5803-0260
E-mail address: sumi-alg@umin.ac.jp
Sahoko Chiba schipulm@tmd.ac.jp

ABSTRACT

Background: Dielectric blood coagulometry (DBCM) has been presented as a new test for estimating the risk of venous thromboembolism (VTE) by measuring the permittivity change associated with blood coagulation. Yet no earlier studies have investigated the clinical utility of DBCM.

Objective: We aimed to estimate the clinical utilities of DBCM.

Methods: Our group prospectively measured DCT, the clotting time estimated by DBCM, in 192 inpatients at the Department of Respiratory Medicine of Tokyo Medical and Dental University Hospital between May 2011 and January 2013. We also measured in 28 healthy volunteers as control group. Additionally, other laboratory findings were evaluated for both patients and controls.

Results: The normal range of DCT was estimated at between 21.0 and 54.8 minutes based on measurements of healthy volunteer controls. Coagulation was significantly accelerated in patients with interstitial pneumonitis, lung cancer, chronic obstructive pulmonary diseases and sleep apnea syndrome, when measured by DBCM. There was little correlation between DCT and conventional coagulation test results.

Conclusions: DBCM is a novel tool for estimating blood coagulation and may be useful for
identifying patients at high risk of thrombosis. However a single DCT measurement would be insufficient for a definite diagnosis of thrombosis. Further long-term observation is needed to evaluate the usefulness of DBCM.

Keywords: DBCM (dielectric blood coagulometry), DCT (clotting time estimated with dielectric blood coagulometry), thrombosis, clotting time, pulmonary thromboembolism, venous thromboembolism

1. INTRODUCTION

Blood clotting diseases like myocardial infarction, stroke, deep vein thrombosis (DVT) and pulmonary embolism (PE) account for a substantial percentage of morbidities. Cardiovascular diseases are the main cause of mortality in most OECD countries, and accounted for 33% of all deaths in 2011. They cover a range of diseases related to the circulatory system, including ischemic heart disease (often referred to as heart attack) and cerebrovascular diseases such as stroke[1]. In 2010, the 4 leading causes of death in USA were, in rank order: Diseases of heart; Malignant neoplasms; Chronic lower respiratory diseases; Cerebrovascular diseases [2]. Diseases of heart and Cerebrovascular diseases accounted for 30% of all deaths. Venous thromboembolism (VTE), comprising DVT and pulmonary embolism (PE), is the third most common cardiovascular disease after acute ischemic syndromes and stroke [3]. The mortality rate of VTE at 3 months is 8.6-17% [4,5,6]. Therefore, researches on identifying subjects at high risk of thrombosis and prophylaxis for them are needed. About 80% of patients with DVT are clinically silent [7], and fewer than half of all fatal PE cases are detected before death [8]. The more sensitive and specific test to detect thrombosis are required. Thrombosis is caused by abnormalities in one or more of the following (Virchow's triad): hypercoagulability, endothelial cell injury, and disturbed blood flow[9]. Aggregation of erythrocytes was shown to change the dielectric response in both the model system of animal erythrocytes and the human whole blood [10]. Dielectric blood coagulometry (DBCM) is a new technique designed to estimate blood cell coagulation process by measuring the permittivity change in the whole blood [10]. The faster blood cells agglutinate, the shorter DCT get in theory. Yet little has been done so far to estimate the clinical utilities of DBCM. In this study we used DBCM to estimate the dielectric clotting time (DCT: clotting time estimated by DBCM) and determined the correlations between DCT and patient profiles or commonly used laboratory tests.

2. MATERIAL AND METHODS

2.1 Subject recruitment

We prospectively recruited inpatients treated at the Department of Respiratory Medicine of Tokyo Medical and Dental University Hospital between May 2011 to January 2013. Patients who agreed to participate in the study after receiving informed consent fulfilled the eligibility criterion for enrollment. In the analysis, we excluded subjects with poor DCT measurements due to inappropriate sample handling procedures. In the further analysis we selected 97 patients with following respiratory diseases; interstitial pneumonia, lung cancer, sleep apnea syndrome, chronic obstructive pulmonary disease (COPD), mycobacterium infection, and PE. We excluded patients taking antiplatelet drugs or anticoagulation drugs and patients who had two or more diseases. We also measured DCT among 28 normal healthy volunteers. The protocol for the study was reviewed and approved by the Ethics Committees of the Tokyo Medical and Dental University.

2.2 DCT measurement
Whole blood samples (1.8 mL) mixed with 0.2 mL of 3.8% trisodium citrate were incubated at 37°C for 10 min, and dispensed into 350 µL aliquots that had been prepared with 29.8 µL of 250 mM CaCl₂ aqueous solution just before the dielectric blood coagulometry measurement. The coagulation process of each sample was monitored by dielectric blood coagulometry at 1 minute intervals for 60 minutes at 37°C in a frequency range from 100 Hz to 40 MHz using a prototype dielectric coagulation analyzer developed by Sony Corporation (Prototype model: DiCA-R). The samples were held in a capacitor-type sample holder consisting of a polypropylene cylinder tube with two titanium electrodes, one squeezed into the top end of the tube and the other squeezed into the bottom. The empty capacitance (i.e., the cell constant) and the volume of the sample holder were set at 0.1 pF and 320 µL, respectively. The dielectric permittivity of blood increases with blood coagulation in MHz region according to Hayashi et al. [10]. On this basis, we defined the dielectric clotting time obtained for this study as the saturation time of the dielectric permittivity at 10 MHz.

2.3 Data collection

The following clinical laboratory parameters were evaluated: C-reactive protein (CRP), hematology, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, fibrin/fibrinogen degradation products (FDP), D-dimer, antithrombin (antithrombin III, AT III), thrombin-antithrombin complex (TAT), alpha 2 plasmin inhibitor - plasmin complex (PIC), partial pressure of oxygen in alveoli (PaO₂), and arterial oxygen saturation measured by a pulse oximeter (SpO₂) with fraction of inspired oxygen. CRP, FDP, and PIC were measured by latex agglutination test. The reagents for measurement were as follows: CRP (N-Assay LA CRP-T Nittobo; Nittobo medical, Fuku shima, Japan), FDP (LPIA FDP-P; LSI Medience Corporation, Tokyo, Japan), PIC (LPIA-ACE PPI; LSI Medience Corporation). Hb was measured by Sodium Lauryl Sulfate (SLS)-Hb detection using SULFOLYSER (Sysmex Corporation, Hyogo, Japan). PT and APTT were determined by coagulation method using following reagents; PT (HemosIL RecombiPlasTin; Instrumentation Laboratory, MA, U.S.A.), APTT (Thrombocheck APTT-SLA; Sysmex Corporation). TAT was measured by chemiluminescent enzyme immunoassay using STACIACLEIA TAT (LSI Medience Corporation). Data were taken from the patient sheet such as diagnosis, age, sex, and smoking history.

2.4 Statistics

The correlations of the clinical parameters with DCT were determined by statistical analysis. The robustness of our results was calculated using GraphPad PRISM5 (GraphPad Software, Inc.). The distribution of DCT in healthy control subjects was estimated by the D’Agostino and Pearson omnibus normality test. Differences in DCT distributions among subjects with given diagnoses were estimated by the Kruskal-Wallis test followed by Dunn’s Multiple Comparison Test. Correlation coefficients between paired groups between DCT and the results of conventional laboratory tests were calculated by Spearman’s correlation, a method that makes no assumptions about Gaussian-like distributions. A p value of less than 0.05 was considered significant.

3. RESULTS

3.1 Patient characteristics

We recruited 192 patients. We excluded 18 patients because we could not measure DCT due to inappropriate sample handling procedures. We analyzed characteristics, comparison and correlations between DCT and conventional clinical parameters about 174 patients (Figure 1). The patients characteristics were shown in figure 2. The age range was
19 - 86 years old (male; 19-85, female; 19-86). In the further analysis, we excluded patients taking antiplatelet drugs or anticoagulation drugs and patients who had two or more diseases, eventually, we analyzed 97 patients with following respiratory diseases; interstitial pneumonia (n = 30), lung cancer (n = 28), sleep apnea syndrome (n = 28), COPD (n = 5), mycobacterium infection (n = 6). Among them, four patients developed PE after administration (n = 4). We also measured DCT among 28 normal healthy volunteers (Figure 3).

3.2 DCT and patients profiles

We found no significant correlations between smoking history and DCT (r = 0.10, p = 0.18). No significant correlation between these parameters was observed between DCT and age or sex.

3.3 DCT and conventional laboratory parameters

Figure 4a shows the relation between the DCT and D-dimer in the 140 patients in whom D-dimer was estimated. No significant correlation between these parameters was observed. Likewise, we found no significant correlations between DCT and PT (r = -0.08, p = 0.40), APTT (r = 0.13, p = 0.20), FDP (r = 0.10, p = 0.39), fibrinogen (r = 0.09, p = 0.33), Antithrombin (ATIII) (r = 0.09, p = 0.45), TAT (r = -0.26, p = 0.43), PIC (r = 0.38, p = 0.25), PaO₂ (r = -0.06, p = 0.65), SpO₂ (r = -0.02, p = 0.83) [data not shown]. We did, however, find a significant relation between DCT and hemoglobin (Hb) (r = 0.33, p = 0.0001), age (r = -0.18, p = 0.02), and CRP (r = -0.19, p = 0.02) represented in Figure 4b-4d. We also carried out statistical analysis of correlation between DCT and conventional laboratory tests in individual subgroups according to diagnosis. We found significant difference between DCT and D-dimer, Hb, or BMI among the patients with lung cancer (Figure 5a), and with age among patients with COPD (Figure 5b).

3.4 DCT and diseases

The mean DCT measured in normal healthy volunteers was 37.9 ± 8.5 minutes (Figure 6). The reference values for DCT were based on test results for 95% of the healthy population. The distribution of DCT in healthy control subjects followed a Gaussian distribution in the D'Agostino and Pearson omnibus normality test using GraphPad PRISM5. The reference range of DCT was therefore defined as between 21.0 (average value minus standard deviation multiplied by 2) minutes and 54.8 (average value plus standard deviation multiplied by 2) minutes. In the thrombosis group, DCT was a mean of 25.3 ± 15.9 minutes and was below the reference range for DCT in 75% (3 out of 4) of the patients. One patient who developed a PE the day after the DBCM examination had a DCT of 46 minutes. With this patient excluded, the mean DCT for the patients with thrombosis was reduced to 17.3 ± 2.1 minutes. In the interstitial pneumonia group the DCT was a mean of 23.7 ± 8.0 minutes and was below the reference range for DCT in 40% (12 out of 30) of the patients. Among the patients with acute exacerbation, the DCT was a mean of 19.0 ± 4.5 minutes and was below the reference range for DCT in 75% (3 out of 4) of the patients. In the lung cancer group, the DCT was a mean of 25.9 ± 9.8 minutes and was below the reference range in 32% (9 out of 28) of the patients. In the patients with sleep apnea syndrome, the DCT was a mean of 28.9 ± 9.1 minutes and was below the reference range in 21% (6 out of 28) of the patients. In the patients with COPD, the DCT was a mean of 25.4 ± 8.2 minutes and was below the reference range in 20% (1 out of 5) of the patients. In the group with mycobacterium infection the DCT was a mean of 29.5 ± 12.0 minutes and was below the reference range in 33% (2 out of 6) of the patients. Of the 6 patients in this group, 4 were
diagnosed with tuberculosis and 2 were diagnosed with nontuberculous mycobacterium. In the whole patient population, the DCT was a mean of 26.3 ± 9.2 minutes and was abnormal in 31 % (30 out of 97) of the patients. Significant differences were observed in the following pairs: "healthy control vs. interstitial pneumonia", "healthy control vs. lung cancer", "healthy control vs. sleep apnea syndrome" and "healthy control vs. COPD".

4. DISCUSSION

This is the first report to describe the clinical application of DBCM. In healthy volunteer controls, the clotting time measured by DBCM (DCT) was a mean of 37.9 ± 8.5 minutes and followed a Gaussian distribution. We therefore defined the reference range for DCT as between 21.0 and 54.8 minutes. DBCM is expected to serve as a new modality for estimating the risk of thrombosis [10]. DCTs were out of the reference range in many patients examined by DBCM who manifested no evidence of thrombosis. This validates the sensitivity of DCT estimation and implies that the admitted patients may have been in poor condition and a hypercoagulable state. About 30 % of the inpatients were likely to have a hypercoagulability estimated by DCT. Age and CRP were negatively correlated with DCT. Aging is one of the strongest and most prevalent risk factor for venous thrombotic disease [11]. Infection and inflammatory diseases were also identified as risk factors for venous thrombosis [12]. Our result was consistent with these past reports. In the DBCM, shortening of DCT means hypercoagulability. In our study, the degrees of severity of anemia correlated with shortening of DCT. Generally, polycythemia exaggerates blood coagulability [13]. Otherwise, our study showed that anemia accelerated blood coagulability. It might be attributed to “Anemia of chronic disease” (ACD) [14], or “Anemia of inflammatory response”, seen in chronic infection, chronic immune activation, or malignancy. In response to inflammatory cytokine IL-6, the liver produces increased the amounts of CRP and hepcidin which blunt erythropoiesis. It has been theorized that limiting a pathogen’s access to iron can reduce its virulence because iron is needed by infectious organisms such as bacteria [15]. The association between chronic infectious, inflammatory, or neoplastic disorders and thrombosis has also been well established [12,16].

DBCM is a novel technique for estimating blood coagulation and nobody had ever estimated human blood samples using DBCM. DCT, the clotting time estimated by DBCM, showed little correlation to the conventional laboratory tests like d-dimer. On that point we got new apparatus for estimating blood clotting. A single DCT measurement would be insufficient for a definite diagnosis of thrombosis. This might be explained that DCT indicates the tendency to form blood clot but is not acute phase indicator like d-dimer during thromboregulation. Applying DBCM had limitations in the clinical setting so far, but may be useful as a screening for hypercoagulable state. We are looking at long term prognosis of the patients.

Thromboelastograph (TEG) machine, which records the process of whole blood coagulation to screen for hypercoagulability [17], is similar to DBCM in principle. TEG also can assess platelet function, clot strength, and fibrinolysis which PT or APTT cannot MEASURE. It would be interesting to compare DBCM and TEG.

5. CONCLUSION

The utility of the DBCM is limited to crude screening to identify hypercoagulability and insufficient for a definite diagnosis of thrombosis. Longer-term follow-up study is needed to test whether subjects with a shorter DCT face a high risk of developing thrombotic diseases.
COMPETING INTERESTS
This study was funded by Research Support from Sony Corporation.

CONSENT
All authors declare that written informed consent was obtained from the patients for publication of this report.

ETHICAL APPROVAL
All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCES


ABBREVIATIONS

DCT clotting time estimated with dielectric blood coagulometry
PE pulmonary embolism
CRP C-reactive protein
PT prothrombin time
APTT activated partial thromboplastin time
FDP fibrin/fibrinogen degradation products
ATIII antithrombin, antithrombin III
PaO2 partial pressure of oxygen in alveoli
SpO2 arterial oxygen saturation measured with pulse oximeter
TAT thrombin-antithrombin complex
PIC alpha 2 plasmin inhibitor – plasmin complex
COPD chronic obstructive pulmonary disease
Hb hemoglobin
Figure 1. Study flow chart to analyze correlation between dielectric clotting time (DCT) and clinical laboratory parameters.
Figure 2. Characteristics of the patients in whom correlation between DCT and conventional laboratory tests were examined. There were 174 patients after exclusion of subjects with unable DCT measurements due to inappropriate sample handling procedures.

Figure 2a. Sex ratio.

Figure 2b. Age distribution. Years is an abbreviation for "years old".

Figure 2c. The proportion of smoking history.

The number of subjects is indicated.
Figure 3. Study flow chart to analyze DCT in each disease.
Figure 4. Correlation between DCT and conventional laboratory tests.

Figure 4a. Correlation between DCT and D-dimer. The shaded area indicates the reference range for DCT and D-dimer.

Figure 4b. Correlation between DCT and hemoglobin concentration. The shaded area indicates the reference range for DCT.

Figure 4c. Correlation between DCT and age. The shaded area indicates the reference range for DCT.

Figure 4d. Correlation between DCT and CRP. The shaded area indicates the reference range for DCT and CRP.
Figure 5. Correlation between DCT and conventional laboratory tests in individual subgroups.

Figure 5a. Correlation between DCT and D-dimer, Hb, or BMI among the patients with lung cancer. The shaded area indicates the reference range.

Figure 5b. Correlation between DCT and age among patients with COPD. The shaded area indicates the reference range.
Figure 6. DCT and diseases. The shaded area indicates the reference range for DCT. Bars indicate average values with standard deviation. DCT; dielectric clotting time, PE; pulmonary embolism, COPD, chronic obstructive pulmonary disease; Mycobacterium; mycobacterium infection. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. 