Comparison between clot waveform analysis (CWA) of normal and abnormal aPTT of sepsis patients in Dr. Hasan Sadikin Hospital Bandung

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Abstract. Sepsis is a clinical syndrome caused by the entry of harmful pathogens toxins into the blood vessels, which will cause the immune system response and ultimately will also interfere with the hemostasis system including coagulation system which can end up with DIC. Therefore, monitoring of coagulation in sepsis becomes very important. The examination of coagulation such as PT and aPTT by mechanical methods only provide information about the timing of the coagulation and completely unknown how the coagulation process occurs. The currently available coagulometer tool that checks the coagulation system with photonic method to visualize the waveforms clot (Clot Waveform Analysis). This study was to determine the difference between CWA of normal and abnormal aPTT in sepsis patients. It was conducted at the Laboratory of Clinical Pathology, RSUP Dr. Hasan Sadikin Bandung. The subjects were all patients diagnosed with sepsis based on SOFA sepsis criteria, in the period of May 1, 2016, until August 31, 2016, were examined aPTT by CS2100i. The CWA were obtained from the examination of aPTT with the tool. Waveform of aPTT in sepsis was a biphasic waveform if the value of slope _1_ > -0.25% T / sec. The waveform were found in normal aPTT (83.33%) and prolonged aPTT group (100%). It were occurred in the most normal aPTT groups and in all subjects with prolonged aPTT. This finding was useful for the treatment of sepsis patients when aPTT is normal because it proved that in sepsis patients with normal aPTT shows a biphasic waveform.

1. Introduction
Sepsis is a clinical syndrome caused by the host response to infection and a dangerous medical condition with a high mortality rate and common in the developing countries. The incidence of sepsis in the world continues to increase, in the United States as many as 22,992 cases in 2000 to 2002 with 21.92% mortality and the hospital burden of more than $ 20 million in 2011. Based on data from 1999 to 2014 there were 2,470,666 deaths in the United States and 22% were caused by sepsis [1]. In Indonesia, in the Department of Prof. Dr. RD Kandou Manado, sepsis patients in the period November 2014 to November 2015, there were 35 people [2]. In Dr. Hasan Sadikin Hospital from 2013 to 2015...
respectively of 668, 1011, and 917 patients [3]. Some factors contribute to the increased of sepsis incidences such as increasing of ability to diagnose sepsis, the use of invasive devices such as central venous catheters, and increased incidence of HIV (Human Immunodeficiency Virus) / AIDS (Acquired Immunodeficiency Syndrome) [4].

The human body has ability to maintain hemostasis systems that maintain blood components remain in the liquid state (fluid state) so that the body in a physiological state capable of maintaining the flow of blood in the blood vessels. If there is a blood vessels damage, the system of hemostasis will respond to such defects through a mechanism of interaction of the blood vessels and tissues supporting the interaction of platelets and blood vessels. The formation of fibrin due to the coagulation system, regulation of blood clots by the inhibitor factors of coagulation, fibrinolytic system, remodeling and repair of damaged blood vessels [5,6].

One routine coagulation examination conducted to determine an activation of coagulation is aPTT test. However, if it is done by mechanical methods, the information obtained only describe the timing of the clot. We do not know how the coagulation process occurs. This process must be known because patients who have normal aPTT wave does not always have a normal clot wave. Currently, aPTT examination that uses a photo-optical method is available With this method how the process of light transmission during the process of coagulation, can be visualized and analyzed and are commonly called clot waveform analysis (CWA).

Clot waveform analysis is a rapid method, inexpensive and is part of a routine aPTT examination, which can be seen as a biphasic waveform in the sepsis patients. Biphasic wave is if the slope of the precoagulase phase is more than -0.25% T/sec. This wave is characterized by low-gradient slope and slope followed by a sharp decline. This biphasic pattern can be seen in sepsis despite in the normal aPTT [7].

Test value of aPTT in sepsis patients can be vary as normal or abnormal depending on the stage of the ongoing sepsis. Patients will have a prolonged aPTT when sepsis has progressed to the next stage. Attention will be higher if its already prolonged aPTT, whereas CWA can show abnormalities in the normal aPTT. By knowing the comparison of CWA between normal and abnormal aPTT of sepsis patients, it is expected to help clinicians to diagnose at early stage coagulation abnormalities in sepsis patients so the management of sepsis patients can be done faster and more precise [7].

2. Methods
This study conducted in the Division of Hematology, Department of Clinical Pathology, Dr. Hasan Sadikin Hospital in Bandung, start on May 1, 2016, until August 31, 2016. All patients who had been diagnosed as sepsis by SOFA criteria SOFA on May 1, 2016, until August 31, 2016, were examined by CS2100i for aPTT test at the Clinical Pathology Laboratories that included by the inclusion and exclusion criteria put in the study subjects. The data retrieved from the LIS (Laboratory Information System) computer and the examination of aPTT taken from the CS2100i Sysmex and data of subject characteristics obtained from Medical Records Section.

The inclusions criteria of this study were all patients with sepsis examined aPTT by CS2100i tool in Clinical Pathology Laboratotium Hasan Sadikin Hospital, aged 18 to 70 years old. The exclusion criteria were patients with liver function abnormalities, bleeding disorders or got anticoagulant therapy.

This study design was observational descriptive cross-sectional study (cross-sectional). The picture of CWA was a biphasic waveform by calculating each patient's chart slope _1_. The graph declared having biphasic waveform if the value of slope _1_ > -0.25% T / sec. The first step was determining of the coordinates of two points connected by a straight line on a slope _1_ as (x1; y1) and (x2; y2), then the magnitude of the slope is calculated by the following formula [8]:

\[ \frac{y2 - y1}{x2 - x1} \]

All data was recorded in a form and then it did coding and entry for analysis. Subjects were divided into 2 groups: normal and prolonged aPTT groups.
3. Results
We obtained 35 subjects during the study period and who meet the inclusion and exclusion criteria were 30 people.

Characteristics of research subjects can be seen in table 1. The data normality test calculated by Smirnow Kolmogorov test and the normal distribution of age data was expressed with mean ± SD.

| Variables     | n (%)     | The mean ± SD |
|---------------|-----------|---------------|
| Gender        |           |               |
| - Man         | 17 (56.7) |               |
| - Women       | 13 (43.3) |               |
| Age (years)   |           | 47.6 ± 1.6    |
| - 15-24       | 2 (6.7)   |               |
| - 25-34       | 5 (16.7)  |               |
| - 35-44       | 5 (16.7)  |               |
| - 45-54       | 6 (16.7)  |               |
| - 55-64       | 8 (26.7)  |               |
| - 65-75       | 4 (13.3)  |               |

The male subjects were 17 (56.7%) and the female were 13 people (43.3%). The mean age of subjects were 47.6 ± 1.6 years. The APTT test results on the subject can be seen in table 2.

| Parameter | Normal | Abnormal | Total |
|-----------|--------|----------|-------|
|           | N      | %        | N     | %     | N     | %     |
| aPTT      | 24     | 80       | 6     | 20    | 30    | 100   |

Subjects had a normal and prolonged aPTT were 80% and 20% respectively. The CWA picture of normal and prolonged aPTT can be seen in figure 1.

The biphasic waveform of CWA aPTT can be seen in table 2. Biphasic waveform was visible in the most patients with either normal or prolonged aPTT group. The APTT normal group was 24 subjects, there was 83.33% subjects with biphasic waveform, and all (100%) of 6 subject of prolonged aPTT group had biphasic waveform (Table 3).

4. Discussions
Among the 30 of sepsis patients, male was more than female. It was consistent with the study by Kaukonen, 2015, the male sepsis patients as much as 60,485 people (55.2%) and more than 49,179 people were female (44.8%). Wang, et al (2014) showed the same thing that the male sepsis patients as many as 95 people (65.1%) more than female (51 people, 34.9%) [9]. The incidence of sepsis was not
influenced by gender but age and type of the underlying disease. Several studies were conducted regarding the relationship of sex with sepsis, there were found that male was more susceptible to sepsis. Other studies showed that gender did not affect on significantly against sepsis [10].

Table 3. Biphasic waveform of CWA aPTT.

|                | Normal aPTT | Prolonged aPTT |
|----------------|-------------|---------------|
| Biphasic waveform (Slope 1 > -0.25% T/sec) | N % | N % |
| No biphasic waveform (Slope 1 ≤ -0.25% T/sec) | 20 83.33 | 4 16.67 |
| Biphasic wave (Slope 1 > -0.25% T/sec) | 6 100 | 0 0 |
| No biphasic waveform (Slope 1 ≤ -0.25% T/sec) | 0 0 | 0 0 |

In this study, it was found that the mean age of subjects was 47.6 ± 1.55 years, and the most patients with sepsis was over 55 years as many as 12 people (40%). Sepsis incidence increases by age as the elderly and the mortality rate has also increased. Based on the United States data, more than half the patients in the ICU was 65 years and the diagnosis of a critical illness such as sepsis [11]. Sepsis proved to be a threat to geriatric patients. Based on an existing literature mentioned the immune system will be decreased so that the infection or sepsis circumstances can be more easily occured [12]. There were 43% of sepsis patients were 60-90 years old in the Prof. Dr. RD Kandou Manado Hospital in December 2014 to November 2015 [2].

Among 30 sepsis patients, who had a normal aPTT were 24 patients (80%) and prolonged aPTT were 6 patients (20%). This indicates that the majority of sepsis patients had a normal aPTT.

Most patients in both groups had biphasic waveform slope_1 whose value is greater than -0.25% T/sec. The biphasic waveform at 83.33% of sepsis patients with normal aPTT and 100% of patients with prolonged aPTT. These waves dominate on sepsis patients CWA either on normal or prolonged aPTT.

Abnormalities of the transmission wave when measuring the aPTT was first discovered by Downey et al in 1997 [13]. This disorder is a biphasic waveform (BPW) which is caused by the calcium-induced formation of complexes between the Very-Low-Density Lipoproteins (VLDL) and CRP. Biphasic waveform is known as a marker of sepsis and DIC[14]. Normal sigmoid wave in transmittance aPTT turned into a biphasic pattern in patients with sepsis and DIC. These waves are important for predicting major complications of septicemia. Biphasic waveform is characterized by the low-gradient slope on precoagulase phase followed by a sharp decrease in slope in the phase of coagulation. This biphasic pattern appears in sepsis despite the normal aPTT. Some researchers define this biphasic waveform was if the slope of precoagulase phase was more than -0.25% T/sec.

The existence of a biphasic waveform indicates a poor prognosis in patients with sepsis. Smith et al, 2004, in the United States found in connection with clinical outcomes in patients who were treated in the ICU. A total of 24 patients were selected consecutively either coming from the Emergency room or and treated not in the ICU compared with 24 patients who did not have a biphasic waveform aPTT. Patients with biphasic waveform aPTT had a time of hospital stay longer (mean [median], 16.9 [11] vs 4.9 [2.5] days; p = 0.011), had a positive bacterial culture results (16/24 [67%] vs 3/24 [13%]; P <0.001), more often transferred to the ICU (6/24 [25%] vs 0/24 [0%]; P = 0.010), and more receiving transfusions PRC (11/24 [46%] vs 5/24 [21%]; P = 0.047) or the transfusion of fresh frozen plasma (5/24 [21%] vs 0/24 [0%]; p = 0.025) [15].

In the UK Toh et al, 2002, examined the role of the biphasic waveform aPTT in 1187 patients admitted to the ICU. It was evident that the biphasic waveform may predict a diagnosis of DIC with an average of 18 hours compared with D dimer (56% of 362 patients with DIC) [13].

Toh et al, 2002, examined 1187 patients in the unit ITU (Intensive Therapy Unit) which examined the aPTT in the first hour and compares the biphasic waveform and not biphasic to predict mortality and sepsis. The aPTT biphasic waveform found in 346 patients and as many as 44% were death, while
not biphasic death was 26%. A biphasic waveform of aPTT had OR (odds ratio) was 4.5 to predict death and 11 to predict sepsis. While CRP (C-Reactive Protein) can predict death with an OR was 2.3 and 6.4 for sepsis. The CWA examination of aPTT in patients admitted to the ITU could be a single parameter, simple and quick to predict mortality and sepsis compared to CRP [13].

Some advantages of CWA in this study is able to detect an abnormality of coagulation in sepsis patients with normal aPTT, easy to handle and efficient because it was a routine aPTT test without adding reagents.

Limitations of this study were from the beginning of the study, the subjects are not grouped into a normal and prolonged aPTT so that the number of subjects of both groups disproportionately.

5. Conclusions
The conclusion of this study was CWA in sepsis patients with normal aPTT almost like as abnormal aPTT, they showed a biphasic waveform.

It is advisable for sepsis patients with normal aPTT were necessary to check clot waveform analysis (CWA) to determine whether there are abnormalities of hemostasis system for the handling of patients can be early performed. So far, this aPTT CWA examination has not been applied to the patients due to there is no standardized way of reporting the CWA examination. Further studies should be done with prospective cohort study design to determine the outcome of patients with biphasic waveform CWA aPTT.

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