In vitro comparative study of activated clotting time in fresh human whole blood with various levels of coagulability using two devices

Nobuo Watanabe1,2,3,*, Masataka Inoue3, Masaki Honda1, Kriengsak Masnok3, Teruhiko Negishi4

1Dept. of Bio-Science and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, Japan
2Dept. of Life Sciences, Systems Engineering and Science, Graduate School of Engineering and Science, Shibaura Institute of Technology, Japan
3Biofluid Science and Engineering Laboratory, Functional Control Systems, Graduate School of Engineering and Science, Shibaura Institute of Technology, Japan
4Negishi Internal Medicine and Neurology Clinic, Saitama, Japan

Received: 27 September 2021 / Accepted: 24 January 2022
© Japanese Society of Biorheology 2022

Abstract With increasing demand for a variety of clinical diagnostics related to blood coagulability such as activated clotting time (ACT), several measurement devices have been developed. Recently, a new ACT measurement device, the Coagulometer CA-200, has become available. One issue is that there will inevitably be differences in measured ACT values among different devices. In addition, as ACT measurement becomes more widely used in clinical practice, measurement of blood coagulation will be required in patients with a wider range of blood coagulability. Consequently, the purpose of this study was to investigate blood coagulability as the function of protamine dose in heparin sodium-treated fresh human blood and to examine the correlation between measured ACT values from two devices, namely, the Coagulometer CA-200 and Hemochron Jr. Signature+.

The results showed similar ACT curves as the dose of protamine was increased, becoming asymptotic at higher coagulability conditions. Under the theoretical maximal procoagulant condition, ACT values were 108 ± 22.5 and 122 ± 11 s for the CA-200 and Hemochron devices, respectively. In blood with the maximum heparin sodium content of 7 U/mL, the measured ACT was 800 and 600 s for the respective devices. Furthermore, there was a linear relationship between the ACT measurements of the two devices. This study clearly showed the feasibility of measurement with the CA-200 and its relatively higher resolution in low coagulability conditions.

Keywords Activated Clotting Time, Coagulometer CA-200, heparin-treated blood, protamine.

1. Introduction

The anticoagulant medicine heparin and its chelating agent protamine have been used during open-heart surgery to prevent blood clots in extracorporeal circulation [1, 2]. While coagulation is being controlled, several diagnostic parameters such as activated clotting time (ACT), prothrombin time (PT), and activated partial thromboplastin time (aPTT) are often measured [3, 4]. Among these parameters, ACT has the longest history of more than 40 years, and it remains one of standard methods for evaluating blood coagulant capacity. In the past, instruments for ACT measurement required 2 mL of blood, but today only 1 drop (50 μL) of blood is necessary. Such a small sample of blood has the advantage of being less invasive with respect to the patients’ blood circulation.

The ACT is maintained at approximately 300 s or longer during long open-heart surgeries with extracorporeal circulation [5, 6], 180–220 s during extracorporeal membrane oxygenation (ECMO) therapy [7–9], 300–350 s during catheter cardiac ablation therapy [10], and 350–375 s during percutaneous coronary intervention [11]. In pediatric treatment, ECMO therapy should have a longer ACT value compared with that in adults [12]. Given the variety of ACT management strategies, there is a wide range of ACT targets depending on the patient’s treatment.

*E-mail: nobuo@sic.shibaura-it.ac.jp
In addition, there is increasing demand for a method to effectively check that patients have taken their anticoagulant medicine [3]. From these perspectives, it would be very useful to understand coagulation ability as a function of protamine dose in heparin sodium-treated blood. Furthermore, several devices for measuring ACT have recently begun to be used in clinical practice, thanks to advances in medical technology [4]; however, the output ACT values differ slightly within a certain range based on differences among these devices [13–16].

To lessen variation in outcomes due to differences among devices, appropriate correlations should be clearly demonstrated to ensure that clinicians can perform diagnostics reliably and precisely. The Coagulometer CA-200 (APEL Co., Ltd., Japan) is a relatively new ACT device that has been approved for clinical use in Japan since December 2018. It is important to perform a comparative study using the new device and the conventional standard device for diagnostics of patient’s blood coagulability. However, to date, there has been no scientific publication on the Coagulometer CA-200.

In consideration of these points, the purpose of this study was to investigate the coagulation capability of fresh human blood samples as a function of protamine dose in heparin sodium-treated fresh blood, and the correlation between the ACT value of the Coagulometer CA-200 and that of the widely used Hemochron Jr. Signature+.

2. ACT Measurement device Coagulometer CA-200 and its measurement principle

As shown in Figure 1, the CA-200 uses a special cartridge, which is similar in size to that of the Hemochron Jr., but it contains a stainless screw shaft in the narrow flow path (Figure 2).

The ACT measurement procedure for the CA-200 is as follows. When the measurement is started, whole blood is mixed with coagulation accelerators, such as kaolin and celite, and repeatedly cycled through the narrow flow path of the cartridge. In this narrow flow path, there is a small stainless screw shaft that disturbs the flow of the oscillating blood. As a result, rheological changes due to the coagulation process can be detected using light transmission. The time taken for such rheological change to occur is measured as the ACT.

3. Methods

3.1 Blood sample preparation:

Participants were 10 students from the Shibaura Institute of Technology (8 men and 2 women; mean age 23 ± 1.7 years). This study design was in accordance with the Declaration of Helsinki and its later amendments, and all participants provided written informed consent prior to donating blood in this study.

Blood (10 mL) was collected through venipuncture into a syringe containing 70 μL of anticoagulant heparin sodium solution containing 70 U of heparin sodium (Novo-Heparin 5,000 U/5 mL for Injection; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). The acquired blood samples were delivered to our laboratory and used for the following experiment.

3.2 Experimental procedure

A disposable cartridge (JACT+; Accriva Diagnostics, Inc., San Diego, CA) for the Hemochron Jr. Signature+ (Instrumentation Laboratory Co., Bedford, MA) was removed from the refrigerator and allowed to sit at room temperature for 1 h before the start of the experiment. The cartridge contains silica, kaolin, and phospholipid as activating agents.

For the Coagulometer, on the other hand, the disposable cartridge (CA-200 test cartridge; Apel Co., Ltd., Japan) can be stored at room temperature and does not require refrigeration. The cartridge contains kaolin and celite as activating agents.

Then, the respective cartridges of the Hemochron Jr.
Signature+ and the Coagulometer CA-200 were inserted in each device. Once inserted, part of the cartridge protrudes forward from the device and is properly positioned to obtain a blood sample for ACT measurement.

The obtained blood samples were divided into 1-mL cuvettes, theoretically giving each blood sample 7 U of heparin sodium (7 μL of heparin sodium solution). One of nine additions of protamine sulfate (0, 1, 2, 3, 4, 5, 6, 7, and 8 μL) (Protamine Sulfate 100 mg for I.V. Inj.; Mochida Pharmaceutical Co., Ltd.), was pipetted into each cuvette and agitated by gently stirring. Then, 50 μL of the blood sample was transferred to the cartridge, which was already inserted in the device, and the ACT measurement was initiated. Of the nine blood sample preparations, the one containing 7 μL of protamine sulfate solution should completely chelate the amount heparin sodium already present, and this represents the condition closest to in vivo coagulability.

Finally, we used the two ACT devices to investigate the variation in ACT values of blood samples having various degrees of coagulability and then evaluated the correlation between the measured ACT values. This study was conducted with the approval of the Ethics Committee, Shibaura Institute of Technology (#19-003).

3.3 Statistical analysis

Pearson’s coefficients ($r$) were calculated to assess the correlation between the two sets of data. A $P$ value < 0.05 was considered to indicate statistical significance. Comparisons were made using multiple $t$-tests, and significant differences were defined as a $P$ value < 0.05 (95% confidence interval). All statistical analyses were performed using GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA).

4. Results

As shown in Fig. 3, both devices showed their highest ACT value when no protamine sulfate was added, and the anticoagulant effect of heparin sodium was strongest (when the horizontal axis is zero as shown in the Figure): 802 ± 155 s for the Coagulometer and 586 ± 145 s for the Hemochron. The ACT value of the Coagulometer was approximately 200 s longer than the Hemochron (with a significant differ-

Figure 2 Side view of the Coagulometer CA-200 cartridge (right) and an expanded cross-sectional schematic drawing of the cartridge (left); the narrow flow path contains a stainless screw shaft, through which blood is forced into an oscillating motion during the ACT measurement.

Figure 3 ACT values measured by the Coagulometer and the Hemochron as a function of protamine dose.
ence at $P = 0.0425$). However, there were no significant differences in the ACT values of protamine sulfate between 1 and 7 μL, as shown in Table 1. Moreover, both devices showed a strong negative correlation between ACT values and the amount of protamine sulfate (Coagulometer, $r = -0.8426$; Hemochron, $r = -0.8151$).

The protamine sulfate dose increased as the ACT values decreased significantly (Coagulometer, $P = 0.004$; Hemochron, $P = 0.0036$), converging asymptotically. In the theoretically complete chelation condition with a protamine sulfate addition of 7 μL, the ACT values were 108 ± 22.5 and 122 ± 11 s for the Coagulometer and the Hemochron, respectively.

Fig. 4 shows the positive correlation between the ACT values measured by the Coagulometer (x-axis) and the Hemochron (y-axis). There was also a very strong positive correlation ($r = 0.9088$), and the behavior of the Coagulometer and Hemochron showed a linear relationship with a coefficient of determination of 0.826 and $P < 0.001$. A total of 90 samples were collected from 10 volunteers.

### 5. Discussion

In this study, we used a conventional ACT device, the Hemochron Jr. Signature+, and the more recent Coagulometer CA-200, to compare the ACT values of various protamine sulfate doses in heparin sodium-treated blood against a variety of fresh human blood samples. This paper is the first to report the device performance of the Coagulometer CA-200. The Hemochron Jr. was used as a reference device in the assessment of device performance.

In both devices, there was a clear tendency for heparin sodium-treated blood to be chelated by protamine sulfate. However, previous studies conducted to investigate blood anticoagulant treatment in clinical practice such as open heart surgery have shown a slight tendency toward more deviation compared with the expected linear relationship [12]. Such a tendency is considered to occur because heparin sodium will be metabolized or will diffuse through the vascular wall into the extracellular fluid or tissue, resulting in a slightly decreased correlation between the amount of heparin sodium and the protamine sulfate dose in vivo com-

### Table 1

Comparison of activated clotting times as a function of protamine sulfate dose in 1 mL of fresh human blood containing 7 U of heparin sodium

| Protamine sulfate (μL) | Mean Coagulometer time (s) | Mean Hemochron time (s) | Difference of mean | Standard error of difference | t ratio* | $P$ Value | Significantly different ($P < 0.05$)? |
|------------------------|-----------------------------|-------------------------|--------------------|-----------------------------|---------|-----------|------------------------------------|
| 0                      | 801.8                       | 586.2                   | 215.6              | 58.06                       | 3.714   | 0.0425    | Yes                                |
| 1                      | 544.2                       | 409.1                   | 135.1              | 54.72                       | 2.469   | 0.1956    | No                                 |
| 2                      | 339.3                       | 264.1                   | 75.20              | 38.19                       | 1.969   | 0.3097    | No                                 |
| 3                      | 182.4                       | 193.5                   | -11.10             | 25.43                       | 0.436   | 0.8929    | No                                 |
| 4                      | 99.20                       | 136.3                   | -37.10             | 12.70                       | 2.922   | 0.1129    | No                                 |
| 5                      | 127.2                       | 126.6                   | 0.6000             | 19.28                       | 0.031   | 0.9758    | No                                 |
| 6                      | 103.2                       | 125.9                   | -22.70             | 11.11                       | 2.043   | 0.3097    | No                                 |
| 7                      | 108.4                       | 123.1                   | -14.70             | 7.262                       | 2.024   | 0.3097    | No                                 |
| 8                      | 105.6                       | 122.4                   | -16.80             | 5.515                       | 3.046   | 0.1058    | No                                 |

*t ratio = Difference of mean/SE of difference
pared with that in vitro.

In addition, as shown in Fig. 4, there was a very strong positive correlation \((r = 0.9088)\), and the behavior of the Coagulometer and Hemochron showed a linear relationship with a coefficient of determination of 0.826 and \(P < 0.001\). Both devices showed asymptotic agreement with ACT values as the protamine sulfate dosage was increased, especially when the protamine dose was \(>3\) μL, and the curvature of ACT values in Fig. 3 showed a similar trend. Such evidence shows the feasibility of the Coagulometer CA-200 as well as the conventional Hemochron Jr. Signature+.

Regarding the measured results at lower protamine doses, the Coagulometer CA-200 had a longer time, indicating a finer resolution in coagulability measurement that should satisfy the needs in long open-heart surgeries to more precisely administer the appropriate anticoagulant treatment.

Furthermore, we consider the Coagulometer CA-200 to be superior to the Hemochron Jr. Signature because it is simple and easy to handle, does not require a refrigerator for cartridge storage, and has a lower operating cost, all of which make it more attractive to physicians and clinicians.

Historically, Rudolf Virchow is responsible for substantial contributions to the study of thrombus formation and its underlying mechanisms, known today as Virchow’s triad [17–19], which comprises “vessel wall condition,” “blood components,” and “flow.” Among these three factors, flow is still a hot topic in the understanding of the thrombus-formation mechanism [20–23].

A recent publication by Chan et al. [22] suggested shear stress over 10 Pa might be a trigger for platelet activation [24, 25], which would in turn accelerate coagulation cascade. In addition to the shear stress magnitude, the shear stress gradient (the time derivative of shear stress) might also regulate platelet activation, and this topic is now receiving a lot of attention in the field of thrombus research [23, 25, 26]. Both of the ACT devices used in this study have a cartridge containing a narrow flow path. The shear stress condition during ACT measurement is not publicly available. However, the generated flow in the Hemochron Jr. can be speculated as laminar because of the simple rectangular cross-section of the flow path, and the turbulent flow in the Coagulometer CA-200 can be speculated as laminar because of the complex flow path containing a stainless screw shaft (Fig. 2). Thus, the resulting generated shear stress would differ between the two devices. Both of these stresses have the potential to induce a procoagulant effect, namely, platelet activation [24, 25]. The complete elucidation of shear stress-induced platelet activation for most procoagulant effects will enable the minimize the time required for blood coagulation measurements, thereby optimizing future blood coagulative diagnostics.

6. Conclusion

This study involved in vitro ACT measurement experiments using the Hemochron Jr. Signature+ and Coagulometer CA-200 in which coagulability was incrementally adjusting by adding protamine sulfate to heparin sodium-treated fresh human blood. Then, the biorheological aspects of ACT measurement were discussed.

The ACT values of both devices showed a clear curvature as a function of protamine dose, and the results showed a linear correlation, suggesting the feasibility of using these devices as well as a possible correlation among ACT devices that can contribute to point-of-care cases by defining a certain definition of blood coagulability normalized to differences between devices.

Acknowledgements This study was financially supported by grants from JSPS KAKENHI (#20K12609, to N.W.) and APEL Co., Ltd., Japan. In addition, APEL CO., LTD., Japan temporarily provided the ACT devices (Coagulometer CA-200 and Hemochron Jr. Signature+) for this study. It should be noted that the Coagulometer CA-200 was recently replaced by the CA-300. The authors thank Mr. Mitsuru Kashiwada, Mr. Yasuichi Haga, and Mr. Akira Takayama of APEL Co., Ltd., Saitama, Japan for their advisory support in this study. T.N. supported scientific discussion at the initial phase of this study and contributed to the blood sampling procedure. M.H. performed the experiments as part of his Bachelor thesis research at the Shibaura Institute of Technology (Saitama, Japan). M.I. assisted with his experiments. K.M. contributed to the statistical analysis. N.W. provided supervision throughout this research project.

References

1. Dutton DA, Hothersall AP, McLaren AD, Taylor KM, Turner MA. Protamin titration after cardipulmonary bypass. Anaesthesia. 1983; 38: 264–8.
2. Keeler JF, Shah MV, Hansbro SD. Protamine-the need determine the dose. Anaesthesia. 1991; 46: 925–8.
3. Smythe MA, Caffée A. Anticoagulation monitoring. Journal of Pharmacy Practice. 2004; 17(5): 217–26.
4. Prisco D, Paniciotta R. Point-of-care testing of hemostasis in cardiac surgery. Thrombosis Journal. 2003; 1: 1. https://doi.org/10.1186/1477-9560-1-1.
5. Bull BS, Korpman RA, Huse WM, Briggs BD. Heparin therapy during extracorporeal circulation I. Problems inherent in existing heparin protocols. Journal of Thoracic Cardiovascular Surgery. 1975; 69: 674–84.
6. Bull BS, Huse WM, Brauer FS, Korpman RA, Calif LL. Heparin therapy during extracorporeal circulation II. The use of a dose-response curve to individualize heparin and protamine dosage. Journal of Thoracic Cardiovascular Surgery. 1975; 69: 685–9.
7. Lawson DS, Waleczak R, Lawson AF, et al. North American neonatal extracorporeal membrane oxygenation (ECMO) devices: 2002 survey results. J Extra Corp Technol. 2004; 36: 16–21.
8. Lawson DS, Lawson AF, Waleczak R, McBobb C, McDermott P, Shearer IR, Lodge A, Jaggers J. North American neonatal extracorporeal membrane oxygenation (ECMO) devices and team roles: 2008 survey results of Extracorporeal Life Support Organization (ELSO) centers. J Extra Corp Technol. 2008; 40(3): 166–74. PMID: 18853828; PMCID: PMC4680642.
9. Delmas C, Jacquemin A, Vardon-Bounes F, et al. Anticoagulation
Monitoring Under ECMO Support: A Comparative Study Between the Activated Coagulation Time and the Anti-Xa Activity Assay. J Intensive Care Med. 2020; 35(7): 679–86. doi: 10.1177/0885066618776937.

10. Kaseno K, Naito S, Nakamura K, Sakamoto T, Sasaki T, Tsukada N, Hayano M, Nishiuchi S, Fuke E, Miki Y, Nakamura K, Yamashita E, Kumagai K, Oshima S, Tada H. Efficacy and Safety of Periprocedural Diabigatran in Patients Undergoing Catheter Ablation of Atrial Fibrillation. Circulation Journal. 2012; 76(10): 2337–42.

11. Chew DP, Bhatt DL, Lincoff AM, Moliterno DJ, Brener SJ, Wolski KE, Topol EJ. Defining the optimal activated clotting time during percutaneous coronary intervention—Aggregate results from 6 randomized, controlled trials—. Circulation. 2001; 103: 961–6.

12. Baird CW, Zurakowski D, Gandhi S, Burdis-Koch L, Tamblyn J, Munoz R, Fortich K, Pigula FA. Anticoagulation and pediatric extracorporeal membrane oxygenation: impact of activated clotting time and heparin dose on survival. Ann Thorac Surg. 2007; 83: 912–20.

13. Colby CE, Sheehan A, Benitz W, Meurs KV, Halamek LP, Moss RL. Maintaining adequate anticoagulation on extracorporeal membrane oxygenation therapy: Hemochron Junior low range versus hemochron400. J Extra Corpor Technol. 2003; 35(1): 35–8.

14. Lewandrowski EL, Van Cott EM, Gregory K, Jang I, Lewandrowski KB. Clinical evaluation of the i-STAT kaolin activated clotting time (ACT) test in different clinical settings in a large academic urban medical center: comparison with the Medtronic ACT Plus. Am J Clin Pathol. 2011; 135(5): 741–8. doi: 10.1309/AJCPFSF8ASGONQNM6. PMID: 21502428.

15. Dirkmann D, Nagy E, Britten MW, Peters J. Point-of-Care measurement of activated clotting time for cardiac surgery as measured by the hemochron signature elite and the Aboot i-STAT: agreement, concordance, and clinical reliability. BMC Anesthesiol. 2019 Sep 6; 19(1): 174. doi: 10.1186/s12871-019-0846-z.

16. Jaryno SA, Zucker ML, Rouby SEI, Franks BLM, Nichols J, Anestis K, McRae K, McDuffie S, Marcello D, Jacobs E, LaDuca FM. Validation of the hemochron Jr. Signature+ Microcoagulation System and the Hemochron Configuration Manager. Point of Care: The Journal of Near-Patient Testing & Technology. 2003; 2(2): 129–34.

17. Virchow R, Über den Faserstoff V . Phlogose und Thrombose im Gefäbsystem. Frankfurt./M: Verl. v. Meidinger&Sohn&Corp; 1856. p. 458.

18. Chung I, Lip GYH. Virchow’s triad revised: blood constituents. Phathophysiol Haemost Thromb. 2003; 33: 449–54.

19. Wolberg AS, Aleman MM, Leidemier K, Machlus KR. Pro-coagulant activity in hemostasis and thrombosis: Virchow’s triad revisited. Anesth Analg. 2012; 114(2): 75–85. doi: 10.1213/ANE.0b013e31823a088c.

20. Watanabe N, Affeld K, Schaller J, Schmitteier S, Reiningier AJ, Goubergrits L, Kerteszcher U. Investigation of the human platelets’ adhesion under low shear condition in a rotational flow chamber. Journal of Biomechanics. 2011; 25: 64–70.

21. Affeld K, Goubergrits L, Watanabe N, Kerteszcher U. Platelet deposition to collagen-coated surface at low shear rates—experimental results and a numerical Monte Carlo Model. Journal of Biomechanics. 2013; 46(2): 430–6.

22. Chan CHH, Simmonds MJ, Fraser KH, Igarashi K, Ki KK, Murashige T, Joseph MT, Fraser JF, Tansley GD, Watanabe N. Discrete responses of erythrocytes, platelets, and von Willebrand factor to shear. Journal of Biomechanics. 2022; 130: 110898. ISSN 0021-9290, https://doi.org/10.1016/j.jbiomech.2021.110898.

23. Rana A, Westein E, Niego B, Hagemeyer CE. Shear-dependent platelet aggregation: Mechanisms and therapeutic opportunities. Front Cardiovasc Med. 2019; 6: 141. doi: 10.3389/fcvm.2019.00141.

24. Hellums JD, Peterson DM, Stathopoulos NA, Moake JL, Giorgio TD. Studies on the mechanisms of shear-induced platelet activation. In: Hartmann A., Kuschinsky W. (eds) Cerebral Ischemia and Hemorheology. Springer, Berlin, Heidelberg. (1987) https://doi.org/10.1007/978-3-642-71787-1_8.

25. Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, Carberry J, Fours A, Jackson SP. A shear gradient–dependent platelet aggregation mechanism drives thrombus formation. Nat Med. 2009; 15: 665–73. https://doi.org/10.1038/nm.1955.

26. Kanda H, Yamakuchi M, Matsumoto K, Mukaihara K, Shigehisa Y, Tachioka S, Okawa M, Takenouchi K, Oyama Y, Hashiguchi T, Imoto Y. Dynamic changes in platelets caused by shear stress in aortic valve stenosis. Clin Hemorheol Microcirc. 2021; 77(1): 71–81. doi: 10.3233/CH-2009928.