Comparison of Whole Blood Coagulation Profiles in COVID-19 and Sepsis Patients Using a Handheld Dielectric Coagulometer

Sina Pourang[1], Michael A. Suster[1], Asha Thomas[2], Stephanie D. Lapping[2], Lalitha V. Nayak[2], and Pedram Mohseni[1]

[1]Dept. of Electrical, Computer, and Systems Engineering, Case Western Reserve University, Cleveland, OH, USA
[2]Division of Hematology/Oncology, University Hospitals Cleveland Medical Center, Cleveland, OH, USA
{pedram.mohseni@case.edu}

***Abstract*—Rapid point-of-care (POC) assessment of thrombosis is clinically important in patients who develop significant blood coagulation abnormalities such as noted with sepsis or COVID-19. In this work, we compare the coagulation profiles of whole blood in sepsis and COVID-19 patients using a handheld dielectric coagulometer termed ClotChip®. ClotChip® is a three-dimensional, parallel-plate, capacitive sensor integrated into a single-use microfluidic channel with a total volume of <20 µL for sample analysis. The readout curve is defined as the temporal variation in the real part of dielectric permittivity of whole blood at 1 MHz. ClotChip® is sensitized towards detecting altered coagulation states by adding recombinant thrombomodulin (rTM) or activated protein C (APC). Using a handheld ClotChip® device, we measure the coagulation status in whole blood samples from hospitalized patients with sepsis and COVID-19 (both regular floor and intensive care unit) and compare it to samples from healthy donors. The coagulation profiles show a difference between COVID-19 and sepsis patients when samples are treated with rTM, as well as a difference between moderate and severe COVID-19 infections when samples are treated with APC. This study demonstrates that ClotChip® measures a coagulation profile in COVID-19 that is different from that in sepsis, highlighting its future potential as a POC diagnostic/prognostic tool in COVID-19-associated coagulopathy.**

***Index Terms*–Blood coagulation, COVID-19, dielectric spectroscopy, microfluidics, point-of-care testing.**

I. Introduction

Caused by a novel coronavirus, COVID-19 was initially reported to present mainly as a mild infection [1], with pneumonia/acute respiratory distress syndrome (ARDS) as the cause for morbidity and mortality in only specific at-risk populations. However, rapidly evolving data from this pandemic demonstrated that patients develop a significant hypercoagulable state leading to arterial, venous, and generalized microvascular thrombosis, which is now recognized as a hallmark of the disease and a frequent cause of morbidity and mortality [2]-[7].

There is an intimate connection between inflammation and coagulation, and dysregulation of either one is associated with significant derangement in the other [8]. Thus, as part of the natural immune response, infection leading to inflammation is commonly associated with activation of the coagulation system [9]. The coagulopathic process in COVID-19 suggests a generalized thrombotic microangiopathic process combined with low-grade disseminated intravascular coagulation (DIC), which is especially noted in patients with severe disease [10]-[12]. Patients critically ill with sepsis also demonstrate activation of the coagulation system and an increased risk of thrombosis [13]. However, several studies highlight clear differences in the coagulopathy associated with COVID-19 versus sepsis, suggesting that while the mechanisms that portend the prothrombotic risks are not fully understood, they are clearly complex and unique to this disease [14], [15]. Importantly, these studies examine the blood coagulation status of patient plasma or serum and do not assess the contribution of cellular elements that participate in hemostasis *in vivo*.

There are also several shortcomings with the current coagulation assays, including high inter-laboratory variability, large blood volumes, and an increased time interval between blood collection and testing [16]. Viscoelastic studies with thromboelastography (TEG) and rotational thromboelastometry (ROTEM) have examined whole blood in COVID-19 patients and shown a hypercoagulable profile [17]-[19]. However, these assays are rarely available outside the operating theater of well-equipped hospitals and generally unavailable in rural/community hospitals or clinical laboratories where most coagulation assays are performed.

We have previously reported a novel microfluidic sensor – termed ClotChip® – that employs dielectric spectroscopy to provide a rapid, comprehensive assessment of whole blood coagulation *ex vivo* [20]-[24]. In this study, we utilize the ClotChip® sensor as part of a handheld, point-of-care (POC) device to compare the coagulation profiles in sepsis and COVID-19.

II. Methods

A. Study Participants

All patients or their legally authorized representatives provided written informed consent for enrollment in this study in accordance with the Declaration of Helsinki. The protocol and informed consent forms were approved by the Institutional Review Board (IRB) at the University Hospitals Cleveland Medical Center (UHCMC). All patients were enrolled, and a blood sample was collected, within 72 hours of admission to the regular floor or admission/transfer to the intensive care unit (ICU) under IRB-approved protocol No. 20200993.

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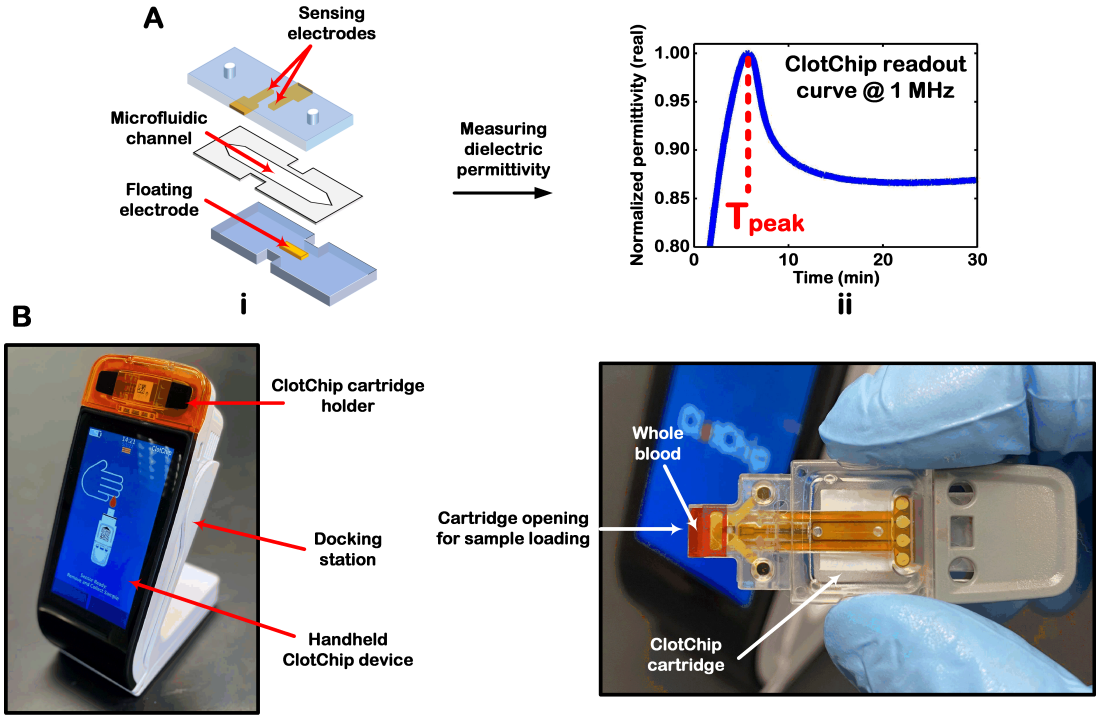

Fig. 1. ClotChip® working principle. A) (i) The ClotChip® sensor consisted of two PMMA plastic substrates with gold sensing and floating electrodes that formed a 3D, parallel-plate, capacitive sensing area with a gap of 250 µm. (ii) Example of the ClotChip® readout curve for a human whole blood sample undergoing coagulation. The readout was taken as the temporal variation in the normalized real part of blood dielectric permittivity at 1 MHz. The time-to-permittivity peak (T_{peak}) parameter has previously been established to indicate the time that it takes for a fibrin clot to start forming. B) Photographs of the handheld ClotChip® device and its single-use, disposable cartridge designed for testing whole blood samples at the POC.

Whole blood was drawn from either a peripheral vein or a central venous catheter (when available) into 3-mL vacutainer tubes containing 3.2% sodium-citrate anticoagulant (ratio of blood to anticoagulant = 9:1). The anticoagulant concentration was 109 mM. Blood was collected once from each subject.

Hospitalized patients with acute COVID-19 infection were recruited from UHCMC (n = 33; 17 from ICU and 16 from regular floor). Enrollment criteria were a positive COVID-19 test confirmed by real-time polymerase chain reaction (PCR), respiratory symptoms (e.g., cough and shortness of breath), hospital admission, ≥18 years of age, and informed consent. The primary criteria to admit COVID-19 patients to the ICU were a need for advanced oxygen therapies (e.g., nasal flow cannulas), noninvasive ventilation (continuous or bilevel positive airway pressure), or mechanical ventilation. Consequently, ARDS accounted for most ICU patients. Furthermore, all 16 regular floor and 14 (of 17) ICU patients were on anticoagulation therapy. Specifically, 7 patients had received unfractionated heparin and 23 patients had received low-molecular-weight heparin (Enoxaparin).

Patients with sepsis (n = 10) were also recruited from UHCMC. Enrollment criteria were SEPSIS-3, ICU admission, ≥18 years of age, and informed consent. The ten sepsis patients were tested with the handheld ClotChip® device and were all on anticoagulation therapy. Specifically, 7 patients had received unfractionated heparin and 3 patients had received low-molecular-weight heparin (Enoxaparin). Finally, de-identified, healthy, human whole blood samples (n = 19) collected into 3-mL vacutainer tubes were purchased from the Hematopoietic Biorepository and Cellular Therapy Core at Case Western Reserve University under a separate IRB-approved protocol. The donors had no known bleeding disorders, hepatic or renal disease, or cancer, and were not on anticoagulant/antiplatelet medications.

B. ClotChip® Measurements

As stated previously, ClotChip® adopts the electronic measurement technique of dielectric spectroscopy to monitor whole blood coagulation in a single-use, disposable sensor. The sensor comprised two polymethyl methacrylate (PMMA) plastic substrates with gold sensing and floating electrodes to form a three-dimensional (3D), parallel-plate, capacitive sensing area with a gap of 250 µm within a microfluidic channel (see Fig. 1A) [24]. The sensor's capacitive structure extracted the dielectric permittivity of a coagulating blood sample placed within the microchannel. The ClotChip® readout curve was defined as the temporal variation in the normalized real part of whole blood permittivity at 1 MHz as shown in Fig. 1A. Based on our previous studies [20], [23], the time-to-permittivity peak (T_{peak}) parameter was taken to indicate the time that it takes for a fibrin clot to start forming. We have previously shown that T_{peak} is sensitive to the detection of clotting defects at the non-cellular level (i.e., coagulation factor) and that it exhibits a very strong positive correlation (r = 0.99, P < 0.0001) with the clotting time (CT) parameter of the ROTEM NATEM assay [20].

All patient samples and healthy (normal) samples were tested within 2 hours of blood collection [23], using a

handheld ClotChip® device designed for POC testing that employed a disposable cartridge with a total volume of <20 µL for sample analysis as shown in Fig. 1B. The single-use, injection-molded, PMMA cartridge contained gold electrodes in the sensing area and a heating element to keep the blood sample at 37°C during the measurement. The cartridge was placed in the handheld device, and the device measured the ClotChip® readout curve at 1 MHz and displayed the T_{peak} value at the conclusion of the test.

C. *Blood Sample In Vitro Treatment*

To reverse the effect of heparin (or Enoxaparin) used in sepsis and COVID-19 patients, 300 µL of whole blood was pretreated with hepzyme at a final concentration of 2 IU/mL and preheated at 37°C for 10 min in an incubator. To maintain consistency in our methods for sample preparation, the healthy samples were similarly pretreated with hepzyme. This would also alleviate the need for *a priori* knowledge of heparin presence in the blood sample in future clinical studies in which the user will be blinded to the nature of the sample.

To sensitize the ClotChip® T_{peak} parameter for detecting whole blood coagulation abnormalities such as noted in sepsis and COVID-19, two naturally occurring antithrombogenic agents – recombinant thrombomodulin (rTM) and activated protein C (APC) – were used based on previous studies that examined their effects in coagulation assays [25]-[27]. As shown in Fig. 2, a series of *in vitro* studies involving lipopolysaccharide (LPS) to mimic a procoagulant state of blood [28], [29] revealed that a concentration of 5 µg/mL for rTM and 10 µg/mL for APC would result in a sufficient change in T_{peak} for detecting the procoagulant state [30]. These agents were next added to incubated blood samples. The ratio of whole blood to rTM and APC was 19:1 and 9:1, respectively. Before conducting measurements and to induce coagulation, 25.6 µL of $CaCl_2$ (250 mM) was pipetted into the citrated whole blood sample containing hepzyme and rTM or APC. Finally, a pipette was used to apply a drop of blood (<20 µL) to the ClotChip® cartridge opening for sample loading, and a measurement was initiated within 1 min from the time of $CaCl_2$ addition.

D. *Statistical Methods*

Data obtained in this study are reported as mean ± standard deviation (SD), unless stated otherwise. In box-and-whiskers plots (Fig. 3), the box represents the range from the first to the third quartile, the horizontal line represents the median, plus sign (+) represents mean of the data; whiskers extend to the maximum and minimum data values, and dots represent individual subject data shown as the mean of duplicate measurements of each sample. Comparisons between groups in ClotChip® measurements were analyzed using the Mann–Whitney *U*-test. The statistical significance threshold was set at the 95% confidence level for all tests ($P < 0.05$). Statistical data analysis was performed using GraphPad Prism software suite (GraphPad Software, San Diego, CA).

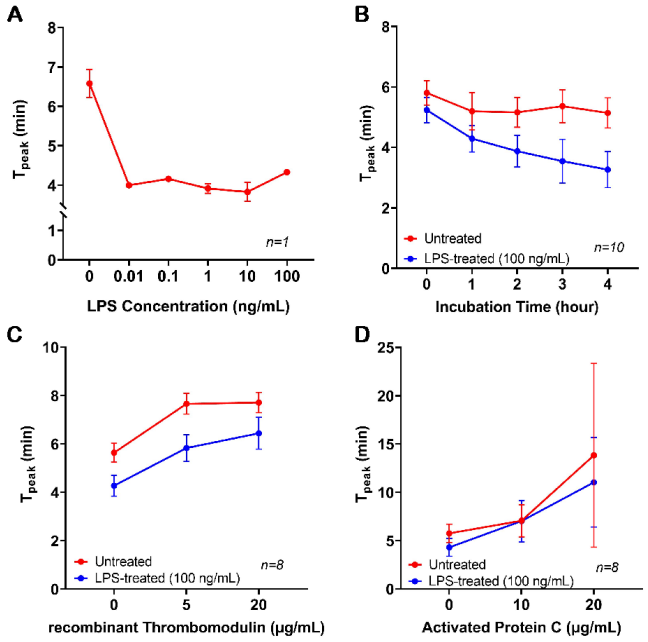

Fig. 2. Optimization of ClotChip® sensor for detecting a procoagulant state in human whole blood. A) Effect of different concentrations of LPS (0–100 ng/mL) on ClotChip® T_{peak} parameter after 4 hours of incubation time at 37°C. B) Effect of incubation time at 37°C on T_{peak} with untreated and LPS (100 ng/mL)-treated samples (n = 10). C) Effect of two different concentrations of rTM on T_{peak} with untreated and LPS (100 ng/mL)-treated samples (n = 8). The LPS-treated samples were incubated for 2 hours at 37°C prior to the addition of rTM. D) Effect of two different concentrations of APC on T_{peak} with untreated and LPS (100 ng/mL)-treated samples (n = 8). The LPS-treated samples were incubated for 2 hours at 37°C prior to the addition of APC. Error bars indicate duplicate measurements and are presented as mean ± SD.

III. Results

Figure 3 shows a comparison of T_{peak} between COVID-19 (regular floor, n = 16; ICU, n = 17), sepsis (n = 10), and healthy (normal, n = 19) whole blood samples tested at the POC using the handheld ClotChip® device in Fig. 1B. No significant difference in T_{peak} was noted in hepzyme only-treated samples (considered as baseline T_{peak} values) in the four groups (Fig. 3A). Comparison of T_{peak} for the groups after *in vitro* treatment with 5 µg/mL of rTM showed significant prolongation of T_{peak} for sepsis ($P < 0.01$) and both COVID-19 groups (regular floor, $P < 0.0001$; ICU, $P < 0.0001$) as compared to normal samples (Fig. 3B). Similarly, comparison of T_{peak} for the groups after *in vitro* treatment with 10 µg/mL of APC also showed significant prolongation of T_{peak} for sepsis ($P < 0.0001$) and both COVID-19 groups (regular floor, $P < 0.01$; ICU, $P < 0.05$) as compared to normal samples (Fig. 3C).

Next, the extent of T_{peak} prolongation with rTM or APC from baseline values was compared between the four groups. Difference in T_{peak} of rTM- and hepzyme only-treated samples showed significant T_{peak} prolongation for both COVID-19 groups (regular floor, $P < 0.0001$; ICU, $P < 0.05$) as compared to normal samples. Such T_{peak} prolongation for the sepsis samples was not significant (Fig. 3D). Difference in T_{peak} of APC- and hepzyme only-treated samples showed significant

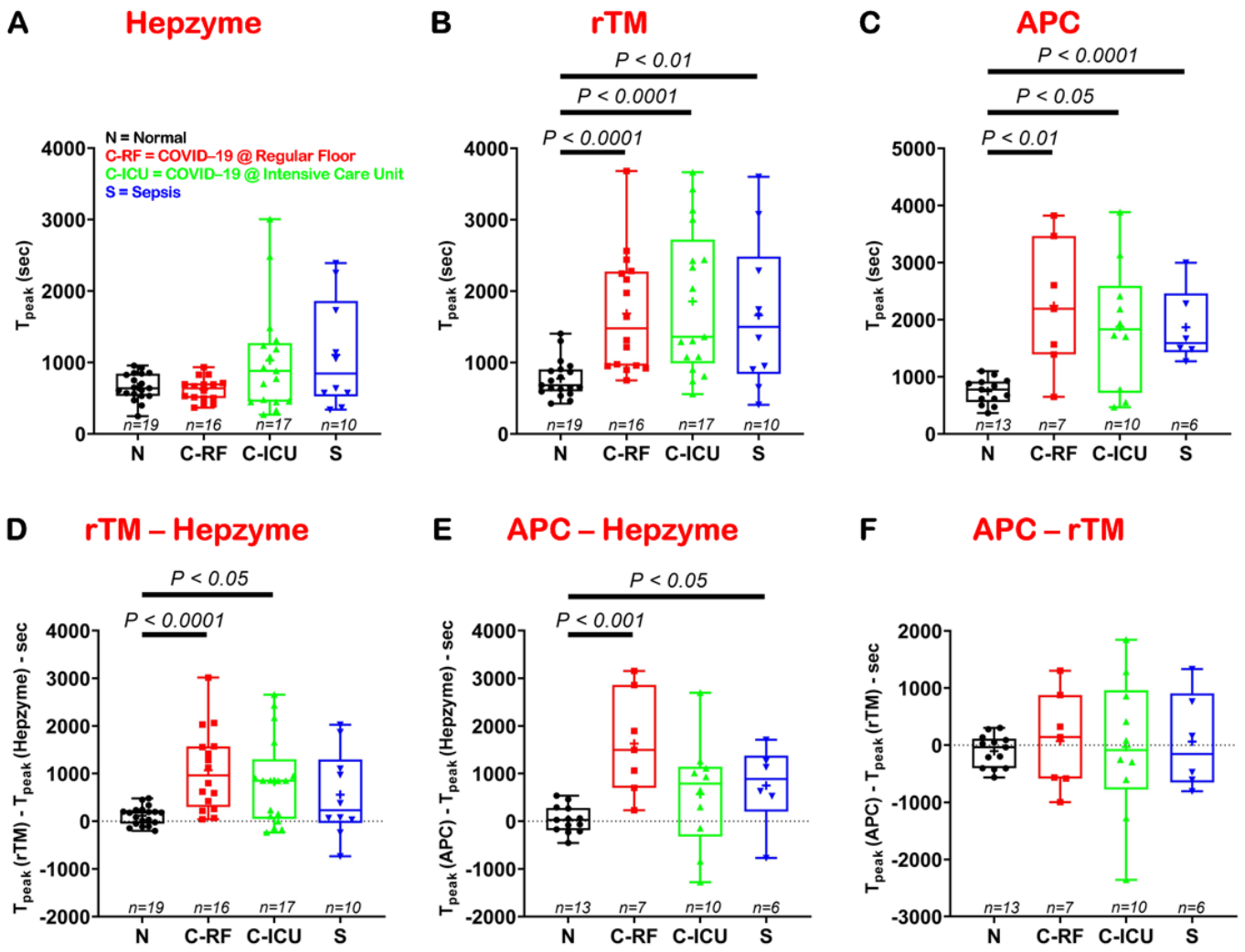

Fig. 3. Comparison of the ClotChip® T_{peak} parameter between sepsis (n = 10), COVID-19 (regular floor, n = 16; ICU, n = 17), and healthy (normal, n = 19) whole blood samples measured using the handheld ClotChip® device shown in Fig. 1B.

T_{peak} prolongation for sepsis ($P < 0.05$) and regular-floor COVID-19 ($P < 0.001$) groups as compared to normal samples. Such T_{peak} prolongation for the ICU COVID-19 group was not significant (Fig. 3E). Finally, the difference in T_{peak} of APC- and rTM-treated samples showed no further prolongation of T_{peak} in sepsis or COVID-19 groups as compared to normal samples (Fig. 3F).

IV. Discussion & Conclusion

We have previously shown the ClotChip® utility in assessing hypocoagulable states in whole blood such as noted in hemophilia [31]. With rTM or APC addition in this study, the ClotChip® readout exhibited increased sensitivity to detecting altered coagulation states in both COVID-19 and sepsis patients. The whole blood coagulation profiles obtained with the handheld ClotChip® device showed a difference between COVID-19 and sepsis patients when samples were treated with rTM (Fig. 3D), as well as a difference between moderate (regular floor) and severe (ICU) COVID-19 infections when samples were treated with APC (Fig. 3E).

Presently, the interpretations of our results are limited by the fact that each patient's assessments were performed only once during their hospital stay. Also, our sample size is not large enough to correlate the ClotChip® results to clinical outcomes. Thromboelastography studies in critically ill COVID-19 patients have shown the ability to identify patients with increased thrombosis rates [18]. Furthermore, in another study reported by Sehgal et al. [17], abnormal coagulation profiles captured by the TEG assay could identify a group of patients with worse outcomes. Thus, we believe there is significant merit in our work and plan to pursue ClotChip® studies with longitudinal samples involving larger patient groups.

In summary, using our handheld ClotChip® device, we demonstrated that the whole blood coagulation profile in COVID-19 infection is different from that in sepsis. Future studies will investigate the underlying mechanisms involved in generating the altered coagulation profile noted in COVID-19 as captured by the handheld ClotChip® device and the potential use of this device as a diagnostic/prognostic tool in COVID-19-associated coagulopathy.

REFERENCES

[1] Z. Wu and J. M. McGoogan, "Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention," *JAMA*, vol. 323, pp. 1239–1242, 2020.

[2] F. B. Belen-Apak and F. Sarialioğlu, "Pulmonary intravascular coagulation in COVID-19: Possible pathogenesis and recommendations on anticoagulant/thrombolytic therapy," *J. Thromb. Thrombolysis*, vol. 50, pp. 278–280, 2020.

[3] J. Helms, et al., "High risk of thrombosis in patients with severe SARS-CoV-2 infection: A multicenter prospective cohort study," *Intensive Care Med.*, vol. 46, pp. 1089–1098, 2020.

[4] F. A. Klok, et al., "Incidence of thrombotic complications in critically ill ICU patients with COVID-19," *Thromb. Res.*, vol. 191, pp. 145–147, 2020.

[5] S. Middeldorp, et al., "Incidence of venous thromboembolism in hospitalized patients with COVID-19," *J. Thromb. Haemost.*, vol. 18, pp. 1995–2002, 2020.

[6] M. Oudkerk, et al., "Diagnosis, prevention and treatment of thromboembolic complications in COVID-19: Report of the National Institute for Public Health of the Netherlands," *Radiology*, vol. 297, pp. E216–E222, 2020.

[7] S. F. Lax, K. Skok, and M. Trauner, "Pulmonary arterial thrombosis as an important complication of COVID-19 pulmonary disease: Letter to the editor," *Virchows. Arch.*, vol. 477, pp. 467–468, 2020.

[8] J. H. Foley and E. M. Conway, "Cross talk pathways between coagulation and inflammation," *Circ. Res.*, vol. 118, pp. 1392–1408, 2016.

[9] S. Antoniak, "The coagulation system in host defense," *Res. Pract. Thromb. Haemost.*, vol. 2, pp. 549–557, 2018.

[10] M. Levi, J. Thachil, T. Iba, and J. H. Levy, "Coagulation abnormalities and thrombosis in patients with COVID-19," *Lancet Haematol.*, vol. 7, pp. e438–e440, 2020.

[11] J. E. Gómez-Mesa, S. Galindo-Coral, M. C. Montes, and A. J. Muñoz Martin, "Thrombosis and coagulopathy in COVID-19," *Curr. Probl. Cardiol.*, vol. 46, p. 100742, 2021.

[12] B. J. Hunt and M. Levi, "Re The source of elevated plasma D-dimer levels in COVID-19 infection," *Br. J. Haematol.*, vol. 190, pp. e133–e134, 2020.

[13] D. Kaplan, et al., "VTE incidence and risk factors in patients with severe sepsis and septic shock," *Chest*, vol. 148, pp. 1224–1230, 2015.

[14] E. G. Bouck, et al., "COVID-19 and sepsis are associated with different abnormalities in plasma procoagulant and fibrinolytic activity," *Arterioscler. Thromb. Vasc. Biol.*, vol. 41, pp. 401–414, 2021.

[15] R. A. Campbell, et al., "Comparison of the coagulopathies associated with COVID-19 and sepsis," *Res. Pract. Thromb. Haemost.*, vol. 5, p. e12525, 2021.

[16] M. Hardy, et al., "Management of the thrombotic risk associated with COVID-19: Guidance for the hemostasis laboratory," *Throm. J.*, vol. 18, pp. 1–16, 2020.

[17] T. Sehgal, et al., "Thromboelastography determined dynamics of blood coagulation and its correlation with complications and outcomes in patients with coronavirus disease 2019," *Res. Pract. Thromb. Haemost.*, vol. 6, p. e12645, 2022.

[18] J. Hartmann, A. Ergang, D. Mason, and J. D. Dias, "The role of TEG analysis in patients with COVID-19-associated coagulopathy: A systematic review," *Diagnostics*, vol. 11, pp. 1–13, 2021.

[19] J. R. Mortus, et al., "Thromboelastographic results and hypercoagulability syndrome in patients with coronavirus disease 2019 who are critically ill," *JAMA Netw. Open*, vol. 3, p. e2011192, 2020.

[20] D. Maji, M. A. Suster, E. Kucukal, U. D. S. Sekhon, A. Sen Gupta, U. A. Gurkan, E. X. Stavrou, and P. Mohseni, "ClotChip: A microfluidic dielectric sensor for point-of-care assessment of hemostasis," *IEEE Trans. Biomed. Circ. Syst.*, vol. 11, no. 6, pp. 1459–1469, Dec. 2017.

[21] D. Maji, S. Pourang, U. D. S. Sekhon, A. Sen Gupta, M. A. Suster, and P. Mohseni, "Toward diagnosis of platelet loss in trauma injury using a microfluidic dielectric sensor," in *Proc. IEEE Sensors Conf.*, Montreal, Canada, Oct. 27-30, 2019.

[22] S. Pourang, D. Maji, U. D. S. Sekhon, A. Sen Gupta, M. A. Suster, and P. Mohseni, "Monitoring fibrin polymerization effects on whole blood coagulation using a microfluidic dielectric sensor," in *Proc. IEEE Sensors Conf.*, Oct. 25-28, 2020.

[23] D. Maji, M. De La Fuente, E. Kucukal, U. D. S. Sekhon, A. H. Schmaier, A. Sen Gupta, U. A. Gurkan, M. T. Nieman, E. X. Stavrou, P. Mohseni, and M. A. Suster, "Assessment of whole blood coagulation with a microfluidic dielectric sensor," *J. Thromb. Haemost.*, vol. 16, pp. 2050–2056, Oct. 2018.

[24] S. Pourang, U. D. S. Sekhon, D. Disharoon, S. P. Ahuja, M. A. Suster, A. Sen Gupta, and P. Mohseni, "Assessment of fibrinolytic status in whole blood using a dielectric coagulometry microsensor," *Biosens. Bioelectr.*, vol. 210, pp. 1–10, Aug. 2022.

[25] K. Tanaka, S. Tawara, K. Tsuruta, D. Hoppensteadt, and J. Fareed, " Pharmacological differentiation of thrombomodulin alfa and activated protein C on coagulation and fibrinolysis in vitro," *Clin. Appl. Thromb. Hemost.*, vol. 24, pp. 859–866, 2018.

[26] L. de Saint Martin, et al., "Increased thrombin generation measured in the presence of thrombomodulin in women with early pregnancy loss," *Fertil. Steril.*, vol. 95, pp. 1813–1815, 2011.

[27] P. Aggarwal, et al., "Comparative studies on the anticoagulant actions of recombinant thrombomodulin and heparin and their neutralization by FEIBA as measured by thromboelastography," *Blood*, vol. 128, p. 2608, 2016.

[28] L. Koch, S. Hofer, M. A. Weigand, D. Frommhold, and J. Poeschl, " Lipopolysaccharide-induced activation of coagulation in neonatal cord and adult blood monitored by thrombelastography," *Thromb. Res.*, vol. 124, pp. 463–467, 2009.

[29] L. Koch, S. Hofer, M. A. Weigand, D. Frommhold, J. Poeschl, and P. Ruef, "Inhibition of LPS-induced activation of coagulation by p38 MAPK inhibitor," *ISRN Hematol.*, vol. 2012, p. 762614, 2012.

[30] S. Pourang, M. A. Suster, P. Mohseni, and L. V. Nayak, "Assessment of hypercoagulable state in whole blood in sepsis patients using a novel microfluidic dielectric sensor," *Blood*, vol. 138, suppl. 1, p. 1882, Nov. 2021.

[31] D. Maji, L. Nayak, J. Martin, U. D. S. Sekhon, A. Sen Gupta, P. Mohseni, M. A. Suster, and S. P. Ahuja, "A novel, point-of-care, whole-blood assay utilizing dielectric spectroscopy is sensitive to coagulation factor replacement therapy in hemophilia A patients," *Haemophilia*, vol. 25, pp. 885–892, 2019.