Demonstration of Prothrombotic Status in Patients with COVID-19 Using Thrombelastography and a Novel T-TAS Assay

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INTRODUCTION

- COVID-19 is characterized as a prothrombotic state with elevated levels of fibrinogen and D-dimer.[1]
- Prothrombotic state may contribute to thromboembolic events and mortality by triggering cardiovascular events.[1]
- Laboratory assessment of prothrombotic state include platelet function tests and viscoelastic haemostatic assays.[2]
- Thromboelastography provides a holistic view of haemostasis with detailed information on dynamic changes in clot characteristics from initiation of clot formation to platelet-fibrin clot generation, stability, and lysis.[3]
- T-TAS (total Thrombus-formation Analysis System), a state-of-the-art assay, provides quantitative assessment of the thrombus formation in the presence of different shear conditions.
- Here, we describe the utility of TEG6s, a point-of-care thromboelastography assay and T-TAS to assess prothrombotic state in patients with COVID-19.

1.Gurbel PA, et al. *J Thromb Thrombolysis*. 2021;52:992-998. 2. Gorog DA, et al. *Nat Rev Cardiol*. 2022 Jan 13:1-21.
3. Gurbel PA, et al. *Platelets*. 2016;27:642-649. 4. Tantry US, et al. *Platelets*. 2022;33:520-530.

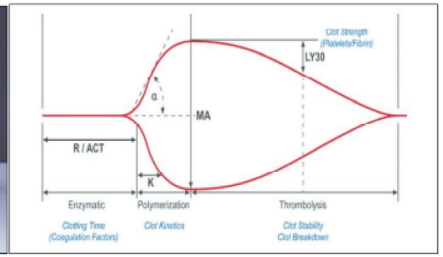
AIM

- To assess the prothrombotic status in patients with COVID-19 using TEG6s and T-TAS.

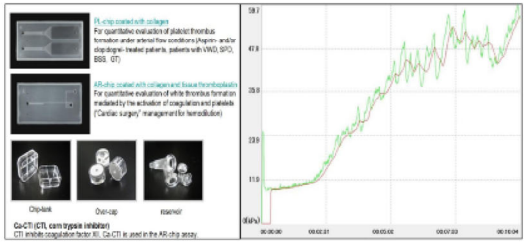
METHODS

- Hospitalized COVID-19 patients (n = 111) and healthy subjects (n = 155) were included.
- Blood was collected from the antecubital vein into a vacutainer tube containing 3.2% trisodium citrate and Benylsulfonyl-D-argininyl-prolyl-4-amidinobenzylamide at the time of hospital administration in patients with COVID-19.

- **TEG6s:** TEG6s is a fully automated microfluidic cartridge-based device. The standard hemostasis assay cartridge (Citrated Multi-Channel) has 4 channels, each with calcium chloride (to reverse the sodium citrate) and: (1) kaolin, (2) kaolin + tissue factor activated (RapidTEG), (3) kaolin + heparinase, and (4) kaolin + abciximab (functional fibrinogen).
- The TEG parameters measured include maximum amplitude of platelet-fibrin clot (MA) and fibrin clot, time to initial platelet-fibrin clot formation (R), fibrinogen level.



- **T-TAS:** T-TAS uses a disposable microchip to analyze thrombus formation in whole blood under different shear conditions.
- An atheroma (AR) chip is coated with collagen and tissue thromboplastin to measure thrombus formation as platelet aggregation and fibrin deposition (primary and secondary hemostasis) under low shear conditions (600/s).
- A platelet (PL) chip is coated with collagen to measure platelet aggregation (primary hemostasis) under high shear conditions (1,500, or 2,000/s).
- The process of thrombus formation inside the chip is analyzed by monitoring the change in flow pressure. The area under the flow pressure curve (AUC) was computed to assess the total thrombus formation. The thrombus formation is quantitated by start time of occlusion (T10, time to reach 10kPa pressure), total time required to thrombus occlusion (Occlusion Time), and total thrombus formation as AUC.

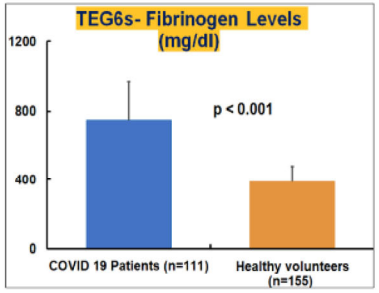


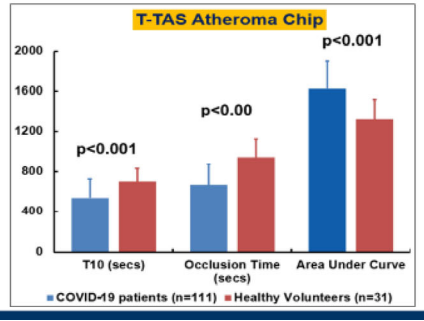
RESULTS

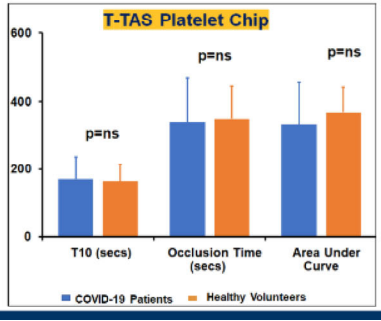
	Patients with COVID-19	Normal Range from healthy subjects	p-value
T-TAS: Atheroma Chip	n=111	n=31	
Occlusion Start Time (T10, secs)	537 ± 191	699 ± 135	p<0.001
Occlusion Time (secs)	666 ± 212	948 ± 176	p<0.001
Area Under Curve	1625 ± 279	1318 ± 203	p<0.001
T-TAS: Platelet Chip			
T10 (secs)	170 ± 67	163 ± 51	ns
Occlusion Time (secs)	337 ±130	347 ± 96	ns
Area Under Curve	331 ± 124	369 ± 72	ns
Thromboelastography (TEG6s)	n=111	n=155	
Reaction Time (min)	6.3 ± 2.0	6.3 ± 1.1	ns
Platelet-fibrin Clot Strength (mm)	67.9 ± 5.7	61 ± 4.3	p<0.001
Fibrin Clot Strength (mm)	41.1 ± 12.7	21 ±5	p<0.001
Fibrinogen Levels (mg/dl)	749 ± 222	388 ± 89	p<0.001

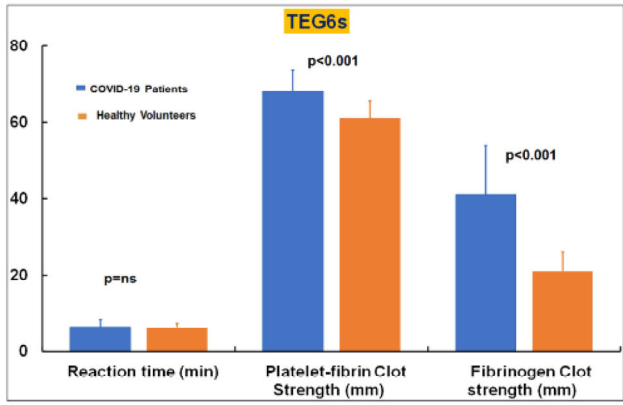
Demographics

	n = 111		
Male, n	67	Medical History, n (%)	
Female, n	47	Cardiovascular disease	23 (21)
Age, years	59.6 ± 17.9	Diabetes mellitus	46 (41)
Body Mass index	34.4±11.7	Respiratory disease	28 (25)
Hypertension, n (%)	81 (73)	Human immunodeficiency virus infection	4 (3.6)
Hypercholesterolemia, n (%)	48 (43)	Renal Disease	15 (14)
Current Smoker, n (%)	9 (8)	Liver Disease	4 (3.6)
Medications, n 9%)		Cancer	7 (6.3)
Metformin	24 (22)	Neurological Disease	19 (17)
Insulin	46 (41)	Other Autoimmune Disease	7 (6)









CONCLUSIONS

- **Significantly higher platelet-fibrin clot strength, fibrin clot strength and fibrinogen levels in the TEG6s assay indicate a prothrombotic state in patients with COVID-19.**
- **The prothrombotic state in COVID-19 patients may be driven by significantly higher fibrinogen levels in the presence of normal platelet function.**
- **Significantly lower T10 and occlusion time, and higher AUC in the T-TAS assay with atheroma chip further indicate a prothrombotic state.**
- **Our data indicates that a novel T-TAS assay and TEG6s can be used to assess the prothrombotic state in COVID-19 patients.**

CONFLICT OF INTEREST

Dr. Gurbel has received consulting fees and/or honoraria from Bayer, Otitopic, Janssen, UpToDate, Cleveland Clinic, Adeno, Wolters Kluwer Pharma, Web MD Medscape, Baron and Budd, North American Thrombosis Forum, Innovative Sciences; institutional research grants from the Haemonetics, Janssen, Bayer, Instrumentation Laboratories, Amgen, Idorsia, Otitopic, Hikari Dx, Novartis, Precision Biologic, Nirmidas Biotech, and R-Pharma International; in addition, Dr. Gurbel has two patents, Detection of restenosis risk in patients issued and Assessment of cardiac health and thrombotic risk in a patient.

Other authors report no conflict of interest.