Hypercoagulability in COVID-19: Is there an Antiphospholipid Syndrome?

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INTRODUCTION

- Patients hospitalized for COVID-19 severe infection are more prone to heightened inflammation and coagulation activation leading to thrombotic events.[1,2]
- Hypercoagulability and coagulation abnormalities in COVID-19 patients as detected by D-dimer, CRP, aPTT, fibrinogen levels, platelet-fibrin clot strength by thrombelastography, and coagulation assays have been associated with increased severity and poor prognosis.[1,2]
- Lupus anticoagulant (LA) interferes and prolongs the clotting process, which is a risk factor for arterial/and venous thrombosis. LA positivity may be a chronic or a transient condition in the setting of certain infections and medications.[3]
- Antiphospholipid antibodies (aPLs) are produced as an autoimmune response to phospholipids present on cell membranes and are associated with increased risk of thrombosis.[4]
- Testing for LA is essential in patients with hypercoagulable states and antiphospholipid syndromes.
- The existence of an antiphospholipid syndrome (APS) determined by results of LA/aPL testing and their relation to measures of hypercoagulability and thrombotic events in COVID-19 patients remains controversial.

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. Rasool ZS, Tiwari V. Biochemistry, Lupus Anticoagulant. 2021 Jul 22. In: StatPearls
4. Zhu R et al. Thromb Res. 2022 Thromb Res. 2022;214:106-114..

AIM

- To determine if markers of APS are elevated in patients with COVID-19 and are associated with hypercoagulability and in-hospital clinical events.

METHODS

- This is a sub-analysis of the evaluation of hemostasis in hospitalized COVID-19 pts study (TARGET-COVID study, NCT04493307).
- Hospitalized patients diagnosed with COVID-19 by RT-PCR assay (n=100) within 48 hours of hospitalization between April and December 2020 were included.
- Blood samples obtained in 100 pts at baseline and in a subset of patients at day 3 and 5.
- APS laboratory detection was based on the recommendations of ISTH methods:

- Lupus Anticoagulant Testing (Precision BioLogic Inc. Dartmouth, Canada)
dilute Russell's Viper Venom Time (dRVVT) assay: LA screening (CRYO*check* LA Check™) and LA confirmatory (CRYO*check* LA Sure™) assays

- aPTT-based integrated LA assay: hexagonal-phased phospholipid (CRYO*check* Hex LA™).

- LA testing: Automated coagulometer (Stago's STA-R Evolution®). LA positive: >44 sec, 1.19 ratio and ≥6 sec for LA Check, dRVVT (LA-Check/LA Sure), and Hex LA tests, respectively. LA positive by LA confirmatory test if its corresponding screening result was also positive.

- aPL antibody profiling (IgA,IgM,IgG) (Corgenix, Broomfield, CO, USA) against aβ2GP1, anticardiolipin (aCL), and anti-phosphotidyl serine (aPS) assays were performed by using Corgenix REAADS ELISA kits assay and manufacturers suggestive interpretive ranges

- Hypercoagulability/Coagulation:

- Factor Activity Levels (Factor-V, VIII, XII, and Prekalikrein) (Precision BioLogic Inc). CRYO*check*™ Factor Deficient Plasma and Chromogenic FVIII assay was used. Coagulation Factor activity levels was determined in healthy donors and compared with COVID-19 patients.

- Whole blood thrombogenicity: TEG-6S (Haemonetics Corp., Braintree, MS, USA) with citrated multichannel cartridge. Hypercoagulability = platelet-fibrin clot strength (MA≥68mm).

- D-Dimer, PT/PTT, and hsCRP (Pathology Lab, Sinai Hospital, Baltimore, MD, USA).

- Demographics, medical HX, medications, and in-hospital clinical outcome data were collected from electronic health records.

RESULTS

Demographics

	Total Group (n=100)	LA/aPL Negative (n=56)	LA/aPL Positive (n=44)	p-value
Age (yrs)	59±19	56±19	63±18	**0.05**
Male, n (%)	52 (53.0)	32 (57.1)	20 (45.4)	0.18
Ethnicity (n, %)				0.32
African American	65 (65.0)	32 (49.3)	33 (50.7)	
Caucasian	22 (22.0)	15 (68.2)	7 (31.8)	
Hispanic	9 (9.0)	6 (66.7)	3 (33.3)	
Asian	4 (4.0)	3 (75.0)	1 (25.0)	
Body mass index (kg/m²)	34.1±12.4	33.8±12.1	34.4±13	0.84
Medical History (n, %)				
Hypertension	74 (74.0)	36 (64.2)	38 (86.4)	**0.02**
Autoimmune disease	52 (52.0)	24 (42.9)	28 (63.6)	**0.04**
Diabetes mellitus	45 (45.0)	19 (33.9)	26 (59.1)	**0.01**
Hyperlipidemia	47 (47.0)	21 (37.5)	26 (59.1)	**0.03**
Obesity	54 (54.0)	30 (53.7)	24 (54.5)	0.62
Cardiovascular disease	25 (25.0)	11 (19.6)	14 (31.8)	0.30
Respiratory disease	29 (29.0)	19 (33.9)	10 (22.7)	0.35
Neurological disease	26 (26.0)	16 (28.6)	10 (22.7)	0.57
Renal disease	13 (13.0)	6 (10.7)	7 (15.9)	0.32
Liver disease	7 (7.0)	5 (8.9)	2 (4.5)	0.36
Cancer	7 (7.0)	4 (7.1)	3 (6.8)	0.91

Medications

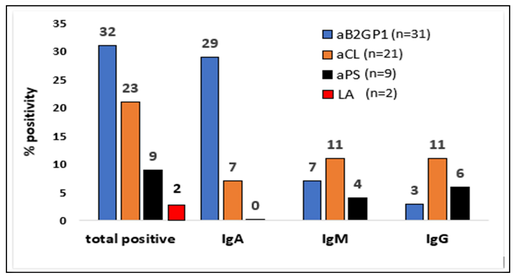
	Total Group (n=100)	LA/aPL Negative (n=56)	LA/aPL Positive (n=44)	p-value
Antiviral, n (%)				
Convalescent plasma	27 (27.0)	16 (28.6)	11 (25.0)	0.69
Remdesivir	33 (33.0)	20 (35.7)	13 (29.5)	0.52
Others	4 (4.0)	4 (7.1)	0 (0)	0.07
Antithrombotic, n (%)				
Enoxaparin	55 (55)	29 (51.7)	26 (59.1)	0.46
Heparin	28 (28.0)	17 (30.4)	11(25.0)	0.55
Oral anticoagulants	9 (9.0)	3 (5.4)	6 (13.6)	0.16
Aspirin	32 (32.0)	17 (30.4)	15 (34.1)	0.70
$P2Y_{12}$ Inhibitors	6 (6.0)	1 (1.8)	5 (11.4)	**0.046**
Antibiotics	79 (79.0)	45 (80.4)	34 (77.3)	0.71
Steroids	73 (73.0)	41 (73.2)	32 (72.3)	0.92
Lipid Lowering	35 (35.0)	16 (28.6)	19 (43.2)	0.13
Beta Blockers	29 (29.0)	15 (26.8)	14 (31.8)	0.59
ACE Inhibitors	19 (19.0)	9 (16.1)	10 (22.7)	0.41
PPI/ H_2 blockers	39 (39.0)	21 (37.5)	18(40.9)	0.73
Diabetes Mellitus				
Insulin	42 (42.0)	17 (30.4)	25 (56.8)	**0.008**
Metformin	20 (20.0)	10 (17.9)	10 (22.7)	0.55

Baseline Laboratory Measurements

	Total Group (n=100)	LA/aPL Negative (n=56)	LA/aPL Positive (n=44)	p-value
Platelet (x1000/ mm³)	277±130	282±119	270±143	0.67
Hematocrit (%)	36.3± 6.2	37.2± 6.0	35.0± 6.2	0.08
Hemoglobin (g/dL)	11.6± 2.3	12.1± 2.2	10.9± 2.3	**0.01**
White blood cells (K/mm^3)	9.3± 4.5	9.4±4.4	9.2±4.5	0.88
Neutrophil/Leukocyte ratio	10.0±10.1	10.4±10.8	9.6±9.3	0.72
Creatinine (mg/dL)	1.1±1.6	0.92± 0.6	1.5± 2.3	0.11
Albumin (g/dL)	3.7± 0.6	3.7±0.6	3.6± 0.6	0.20
Glucose (mg/dL)	160±87	155±91	167±81	0.51
Hemoglobin A1c	7.2±2.1	7.2±2.5	7.3±1.6	0.86
Aspartate transaminase (u/L)	58±82	51±56	68±107	0.31
Alanine phosphatase (u/L)	54 ±72	51±63	58±83	0.66
Alkaline Phosphatase (u/L)	88± 63	89± 28	86± 32	0.77
D-Dimer (mg/L, FEU)	2.5±3.6	1.8±2.9	3.5±4.2	**0.04**
C-Reactive protein (mg/L)	93±82	89±81	97±83	0.66
Ferritin (ng/mL)	682±804	691±667	671±540	0.90
Procalcitonin (ug/L)	1.2±4.0	0.7±2.1	1.9±5.4	0.73
Lactate dehydrogenase (u/L)	449±335	478±386	445±263	0.39
Prothrombin time (secs)	14.7±3.3	14.6±2.2	14.9±4.3	0.73
INR	1.4±1.7	1.1±0.2	1.7±2.5	0.17

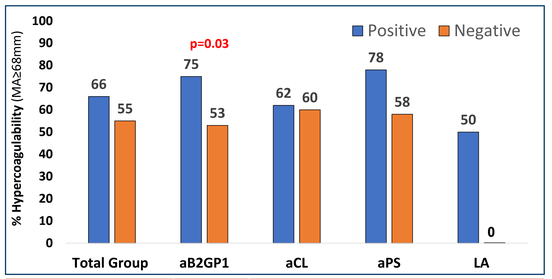
- Mean age was 59±19 yrs; predominately African American with a high prevalence of hypertension, obesity, autoimmune disease, hyperlipidemia, and diabetes.
- Antiviral, antithrombotic, antibiotic, and steroid use were common.
- Most widely used anticoagulant was Enoxaparin
- Mean Glucose, HGBA1C, D-Dimer, CRP, Ferritin, Procalcitonin, and LDH levels was above upper limits normal for the total group.
- Patients in LA/aPL positive group were significantly
  - Older
  - Had a higher incidence of hypertension, autoimmune dx, diabetes, and hyperlipidemia
  - More often treated with insulin, and P2Y12 inh.
  - Had a higher D-Dimer and lower hemoglobin levels

Frequency of LA/aPL Positivity



- LA/aPL positivity was observed in 44% of patients.
- LA positive (n=2) patients were antibody positive for aCL
- IgA aB2GP1 which is known to regulate the clotting cascade and the clearance of inflammatory and prothrombotic cells was the most frequently observed aPL among the total study group.

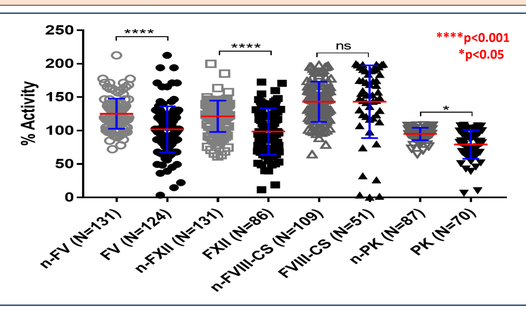
Frequency of Hypercoagulability as Measured by TEG



		Negative	Positive			
	Ref. Range	LA/APA (n=56)	LA (n=2)	aB2GPI (n=31)	aCL (n=21)	aPS (n=9)
TEG						
CK-R	4.6-9.1 min	7.3±3.5	11.9±6.7	6.9± 2.9	7.4±3.5	6.4±1.7
CKH-R	4.3-8.3 min	6.3±1.6	8.6±5.3	6.2±2.5	6.4±2.8	5.9±1.6
MA-FF	15-32 min	39.0±12.7	20.1±1.0	39.6±14.0	43.1±14.8	46.8± 13.0
FF level	278-581mm	721± 216	365± 19	722± 255	786±268	835±236
MA	52-68 mm	67.7±5.2	61.6± 5.4	68.0± 5.6	68.2± 6.7	68.7± 5.1
Clot lysis	0-2.2 (%)	0.7± 1.2	0.5± 0.7	0.9± 1.3	0.4± 0.5	0.7± 1.6
D-DIMER	≥0.5mg/L, FEU	1.8± 2.9	1.4± 1.4	3.3± 4.6	3.6± 4.3	3.2± 3.6

- Hypercoagulability was observed in 66% of the total group with the highest rates observed in aB2GP1+ and aPS+ pts.
- Frequency of hypercoagulability was significantly higher in aB2GP1 positive vs. negative pts.
- Mean MA-FF, Functional Fibrinogen, and MA levels were elevated in both positive and negative LA/aPL patients with no significant differences observed between the groups.
- D-Dimer was significantly higher in LA/aPL positive versus negative patients (p=0.04)

Factor Activity Levels in Control vs COVID-19 Patients



- COVID-19 patients had significantly lower FV, FXII, PK activity compared to normal subjects which may be related to the use of anticoagulation therapy.
- There was no relation between factor activity levels, LA/aPL positivity, hypercoagulability and In-hospital clinical events.

In-Hospital Clinical Outcomes Between LA/aPL Positive and Negative Patients

	Negative	Positive				
	LA/APA (n=56)	Total (n=44)	LA (n=2)	aB2GPI (n=31)	aCL (n=21)	aPS (n=9)
SOFA Score	2.5± 2.2	2.7±1.7	2.5±0.7	2.4± 1.4	2.9± 1.8	3.4±1.9
Days in Hospital	11.6± 16.5	12.6±10.3	7.0± 1.0	12.4± 10.5	13.5± 10.4	15.6± 6.6
MACE (n,%)	15 (26.8)	11 (25.0)	1 (50.0)	7 (22.6)	5 (23.8)	6 (66.7)
Myocardial Infarction (n,%)	1(1.7)	5 (11.4)	0 (0)	4 (12.9)	3 (14.3)	3 (33.3)
Stroke	2 (3.6)	1 (50.0)	1 (50)	(0)	1 (4.7)	0 (0)
Pulmonary Embolism	3 (5.4)	3 (6.8)	0 (0)	2 (6.5)	0 (0)	1 (11.1)
Mortality	10 (17.8)	4 (9.1)	0 (0)	2 (6.5)	2 (9.5)	4 (44.4)

- In hospital MACE and Mortality was observed in 26% and 14% of the total group, respectively.
- Patients with positive aPS antibodies had the highest mean SOFA score, days in hospital and significantly (p<0.05) higher MACE and mortality rates than LA/APA negative; aCL, and aB2GP1 positive patients.

CONCLUSIONS

- Based on LA assay, aPL syndrome is infrequent in COVID-19 however there is a high prevalence of aPL antibodies that correlate with D-dimer with the greatest prevalence observed for aβ2GP1 (IgA).
- Anti-phosphotidyl serine antibody positivity was associated with higher in-hospital MACE and mortality.
- These observations deserve further investigation.

CONFLICT OF INTEREST

- Dr. Gurbel reports grants and personal fees from Bayer HealthCare LLC, Otitopic Inc, Amgen, Janssen, and US WorldMeds LLC; grants from Instrumentation Laboratory, Haemonetics, Medicure Inc, Idorsia Pharmaceuticals, and Hikari Dx; personal fees from UpToDate; Dr Gurbel is a relator and expert witness in litigation involving clopidogrel; 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.
- Dr. Tantry reports receiving honoraria from UptoDate and Aggredyne.
- Other author reports no disclosures.