Extreme γ' fibrinogen levels in COVID-19 patients

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Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) 2 causing coronavirus disease 2019 (COVID-19) has infected more than 220 million individuals worldwide and caused more than 4.5 million deaths. COVID-19 disease progression can be accompanied by a "cytokine storm" that leads to secondary sequelae such as thrombosis and acute respiratory distress syndrome (ARDS). Several inflammatory cytokines, including high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6), have been associated with COVID-19 disease progression, but have far too much daily intra-individual variability to be useful in tracking the course of the disease. In contrast, we have shown that the inflammatory biomarker gamma prime fibrinogen (γ' Fbg) has a 6-fold lower coefficient of variability compared to other inflammatory markers such as hs-CRP. The mRNA for the γ chain of γ' Fbg is upregulated 8.3-fold by IL-6 *in vitro*, so that γ' fibrinogen is likely to be a more stable and therefore superior surrogate marker to IL-6 as well.

Because of this, we measured γ' Fbg in serial blood samples from COVID-19 patients at a tertiary care center in order to investigate its association with clinical measures of disease progression. **Our hypothesis was that γ' Fbg levels would be elevated in COVID-19 patients compared to historical controls, and that the degree of elevation would be associated with disease severity.**

Methods

Hospitalized COVID-19 patients at Oregon Health & Science University (OHSU) Hospital were retrospectively enrolled between 3/16/2020 and 8/1/2020. Samples were collected earlier, stored frozen in a biological repository and then analyzed anonymously. This study was approved by the OHSU IRB, STUDY00021409, and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Patients who had at least two EDTA plasma draws were randomly selected from this time frame.

γ' Fbg was measured using the GammaCoeur ELISA according to the manufacturer's instructions (Zeus Scientific, Branchburg NJ/Gamma Diagnostics, Portland, OR; patent pending) and measured at 450 nm in a BioTek ELx808 (BioTek Instruments, Winooski, VT).

Standard descriptive statistics were used to summarize patient demographic and clinical characteristics. The mean and median γ' Fbg in COVID-19 patients was compared to the mean and median γ' Fbg in the ARIC study using z-test. Histograms were constructed to visually inspect the distribution of γ' Fbg in both cohorts. Within the COVID-19 cohort, γ' Fbg was evaluated for associations with ARDS and ECMO using Wilcoxon Rank-Sum tests, and after testing both the mean and median, Holm's correction for multiple comparisons was used to identify significant associations. Analysis was conducted in JMP 15.2 (SAS Inc., Cary, NC). Significance was assessed at $p<0.05$.

Results

Hospitalized COVID-19 patients at Oregon Health & Science University (OHSU) Hospital were retrospectively enrolled between 3/16/2020 and 8/1/2020 (Table 1).

The γ' Fbg levels in the COVID-19 patients were compared to historical controls (Fig. 1) from the Atherosclerosis Risk In Communities (ARIC) study. The 10,601 ARIC participants had their γ' Fbg levels measured in plasma samples from drawn between 1993-1995. Participants with prevalent cardiovascular disease (coronary heart disease, heart failure, peripheral artery disease, or stroke) were excluded. The mean γ' Fbg levels in ARIC were 30.9 ± 9.0 mg/dL (mean ± SD) compared to 84.2 ± 38.5 mg/dL in the COVID-19 patients ($p<0.0001$). Our results showed that ten out of the eighteen patients with COVID-19 that we tested had the highest levels of γ' Fbg that we believe have ever been recorded, from 81.4 to 260.5 mg/dL. The previous highest γ' Fbg level in the ARIC study was 80.3 mg/dL.

We then investigated the association between γ' Fbg levels and several clinical outcomes, including the development of ARDS, the need for extracorporeal membrane oxygenation (ECMO), and mortality (Table 2). Average and median daily γ' Fbg levels by in-hospital outcomes in COVID-19 patients. There was a non-significant trend towards higher levels of γ' Fbg in patients who developed ARDS. However, in patients who progressed to ECMO, there was a significant association with the median average daily γ' Fbg level. Furthermore, in the two patients who died, there was a significant association with both the mean and median average daily γ' Fbg level compared to the patients who lived.

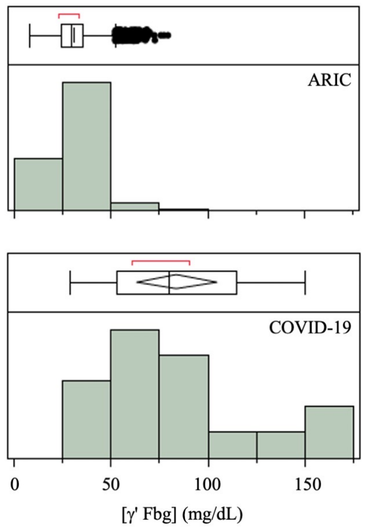

Fig. 1. Comparison Between γ' Fbg Levels in the Atherosclerosis Risk In Communities (ARIC) Cohort and COVID-19 Patients.

Variable	Mean (±SD)	Median (range)
Age (years)	45.8 ± 14.0	46 (22-71)
Female (%)	35.3	n/a
[γ' Fbg] (mg/dL)	51.6 ± 19.9	40.3 (11.5-88.3)
ARDS (%)	52.9	n/a
ECMO (%)	35.5	n/a
Deaths (%)	11.8	n/a

Table 1 - Patient characteristics.

In-hospital Outcomes	N	Average Daily γ' Fbg		Median Daily γ' Fbg	
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
Lived	15	46.8 (15.5) *	48.4 (24.9) *	38.4 (16.3)	40.1 (20.5)
Died	2	87.8 (0.6) *	87.8 (0.9) *	87.8 (0.6)	87.8 (0.9)
No ECMO	11	45.2 (15.9)	45.1 (24.9) *	35.6 (17.1)	35.4 (21.6)
ECMO	6	63.5 (22.4)	62.4 (43.1) *	60.0 (23.6)	55.9 (49.4)
No ARDS	8	45.2 (18.9)	45.0 (30.3)	34.6 (19.5)	36.0 (28.1)
ARDS	9	57.3 (20.0)	48.8 (35.0)	52.8 (22.2)	50.2 (40.1)

Table 2 - *unadjusted p-value <0.05. No comparisons were statistically significant after adjusting for multiple comparisons using Holm's correction.

Discussion

We found that COVID-19 patients can develop extraordinarily high levels of γ' Fbg. This has several important clinical implications. γ' Fbg contains a high affinity binding site for thrombin that binds to anion-binding exosite II on thrombin and protects it from inactivation by heparin. High levels of γ' Fbg therefore provide a reservoir of heparin-resistant clot-bound thrombin when the γ' Fbg is clotted (Figure 2)

These findings have potential clinical implications regarding prophylactic anticoagulation of COVID-19 patients. The resistance of γ' Fbg-bound thrombin to heparin suggests that heparin prophylaxis may be less effective than treatment with other anticoagulants, particularly direct thrombin inhibitors. It is possible that inhibition of factor Xa by current DOACs may also reduce the levels of active thrombin and thereby prevent activation of thrombin substrates by γ' Fbg-bound thrombin, including factor V, factor VIII, factor XI, factor XIII, and fibrinogen, as well as platelet substrates such as PAR-1 and PAR-4. In addition, it is also possible that warfarin anticoagulation may be effective at preventing thrombosis due to γ' Fbg-bound thrombin by reducing the levels of active vitamin K-dependent coagulation factors, including thrombin itself. Enrollment of COVID-19 patients in anticoagulation studies to investigate these hypotheses is currently ongoing.

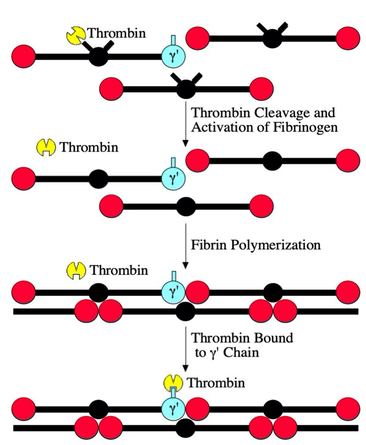

Figure 2. Model of γ' Fbg mechanism of thrombosis

In summary, we suggest that γ' Fbg levels may be an important marker of disease progression in patients with COVID-19, and γ' Fbg may play a role in the coagulation abnormalities noted in these patients.