NEUTROPHIL ACTIVATION AND THEIR-DERIVED NETS CONTRIBUTE TO THROMBOTIC RISK IN PULMONARY PASC

Natalie T. Subia; Dominic Chow, PhD, MD; Cecilia Shikuma, MD; Juwon Park, PhD
Department of Tropical Medicine, Medical Microbiology & Pharmacology;
John A. Burns School of Medicine, University of Hawai'i at Manoa

346

BACKGROUND INFORMATION

Over one-third of all individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) experience persistent residual symptoms. Previously, we found that COVID convalescents with pulmonary symptoms had elevated low-density granulocyte (LDG) levels and enhanced ability to form neutrophil extracellular traps (NETs) and aggregation with platelets. Although emerging evidence supports that immunothrombosis plays a crucial role in COVID-19 pathogenesis, its involvement in long-term pulmonary sequelae of COVID-19 is poorly known

METHODS

To analyze soluble factors linked to thrombosis and inflammation in COVID-19 convalescents, plasma samples collected from participants naïve to SARS-CoV-2 (NP; n=11), infected with SARS-CoV-2 with no residual symptoms (NRS; n=10), and with pulmonary symptoms (PPASC; n=12) were used. We assessed ten analytes (coagulation biomarkers: vWF-a2, Protein C, ADAMTS13, Tissue Factor (TF); inflammation biomarkers: IFN-gamma, MMP-9, MPO; complement pathway: Complement (C)2, C5, and C9) in the plasma samples by Luminex assay. To identify factors involved in neutrophil activation in COVID-19 convalescents, we further assessed associations between LDG parameters (number, activation, NET formation) and soluble factors.

RESULTS

Table 1 Demographics and pre-existing conditions of naïve participants (NP), no residual symptoms (NRS), and pulmonary PASC (PPASC) participants.

Parameters			NP (n=11)	NRS (n=10)	PPASC (n=12)
Demographics	Age (years)		56.9 [53.2-61.6]	55.0 [43.0-60.0]	54.5 [49.8-58.0]
	Male (n, %)		9 (81.8)	7 (70.0)	10 (83.3)
	Caucasian (n, %)		8 (72.7)	3 (30.0)	4 (33.3)
	BMI (kg/m^2)		28.1 [23.8-29.0]	26.3 [20.6-33.0]	30.8 [26.2-34.1]*
Pre-Existing Conditions	Months post-infection		0 [0]	5 [1.5-9.5]	6 [4-14]
	Hospitalized for COVID-19 (n, %)		-	0 (0)	4 (25.0)
	Diabetes (n, %)		1 (8.3)	1 (10)	4 (33.3)
	Hypertension (n, %)		3 (27.3)	2 (20)	5 (41.7)
	History of Smoking (n, %)		5 (45.5)	3 (30)	4 (33.3)
	COPD (n, %)		0 [0]	0 (0)	0 (0)
	Asthma (n, %)		3 (27.3)	1 (10.0)	2 (16.7)
COVID Vaccination Status	Vaccinated for COVID-19 (n, %)		-	7 (70.0)	10 (83.3)
	Vaccinated before COVID-19 Diagnosis		-	7 (70.0)	9 (75.0)
Long-COVID symptoms	Non-Specific	Fatigue	-	-	7 (58.3)
		Hair Loss	-	-	1 (8.3)
		Insomnia	-	-	1 (8.3)
		Anxiety/ Depression	-	-	2 (16.7)
	Neuropsychiatric	Brain Fog	-	-	3 (25.0)
		Headache	-	-	3 (25.0)
	Pain	Joint Pain	-	-	2 (16.7)
	Oropharyngeal	Loss of taste/ smell	-	-	2 (16.7)

Values presented as [median, Q1-Q3] unless otherwise stated.
*p<0.05 compared to NP.

RESULTS

Neutrophils and their **derived NETs contribute** to **PPASC thrombotic risk**

The median age of participants was 57, 55, and 54.5 years for the NP, NRS, and PPASC groups, respectively. Months post-infection did not differ between NRS (5 [IQR;1.5-9.5]) and PPASC (6 [IQR:4-14]). Compared with NP, TF, and ADAMTS13, levels were statistically decreased, and PPASC individuals tended to have higher MPO and vWF-a2 levels. C2a levels were statistically higher in both PPASC and NRS groups than in the NP group, while C5a and C9 expressions were comparable between groups. Further correlation analysis of LDG parameters with soluble factors showed that the percentages of LDGs and NET-forming counts were positively correlated with MMP9, MPO, and vWF-a2 expression but negatively correlated with ADAMTS13 expression. Interestingly, C9 levels were positively associated with the percentages of NET-forming LDGs, vWF-a2, and C5a, but these associations were not shown in the NRS.

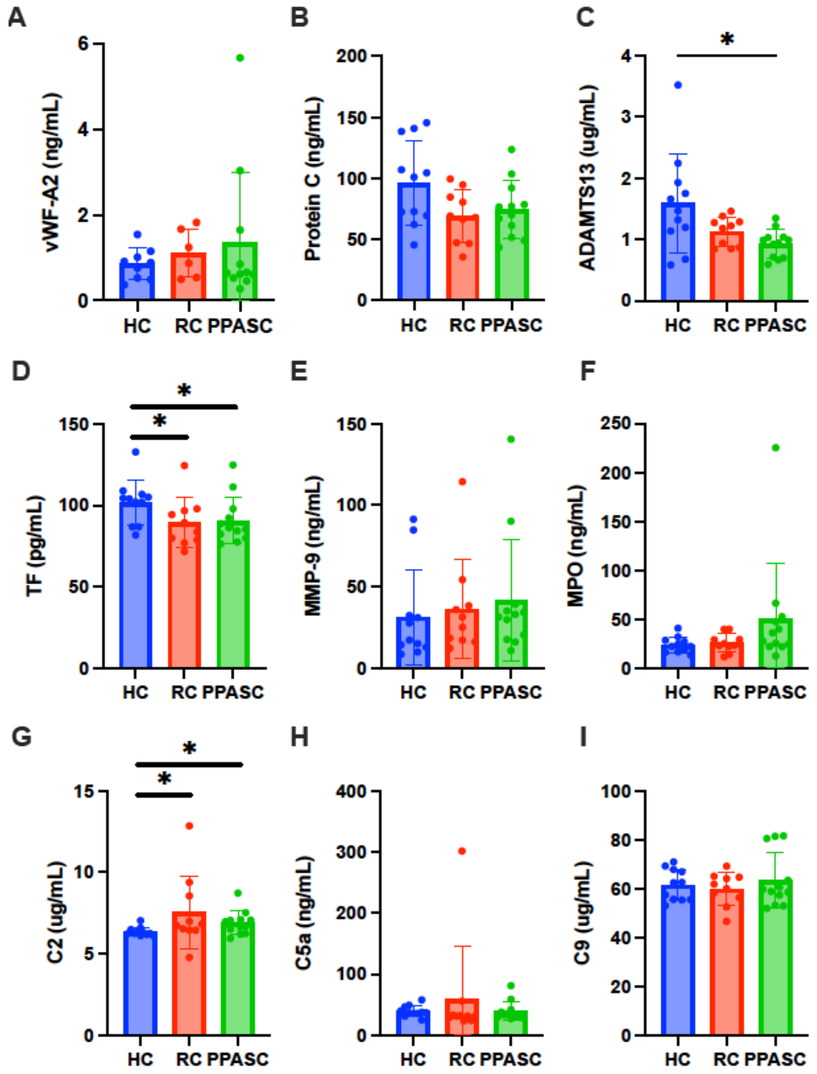

Figure 1 Coagulation and inflammation biomarkers and complement components in PPASC. Mann-Whitney t-test through Prism 9 software was used to calculate the average analyte concentration level for each group showing C2, TF, and ADAMTS13 showing a significant difference (p<0.05)

RESULTS

Table 3 Spearman's correlation of soluble analytes with LDG parameters in PPASC (n=12) & NRS individuals (n=10)

Parameters	Analyte	PPASC (n=12)		NRS (n=10)	
		Rho	P-value	Rho	P-value
LDG %	MMP9	0.699*	0.014	-0.236	0.513
	MPO	0.776*	0.004	-0.503	0.144
	vWF-a2	0.671*	0.020	-0.214	0.662
	ADAMTS13	-0.671*	0.020	-0.613	0.067
	IFN-gamma	0.760*	0.006	-0.030	0.939
	C9	0.413	0.184	-0.794*	0.009
LDG Count	MMP9	0.825*	0.002	0.127	0.733
	C9	0.350	0.266	-0.745*	0.017
	MPO	0.531	0.079	-0.648*	0.049
Net Forming %	MPO	0.734*	0.009	0.674*	0.039
	vWF-a2	0.755*	0.006	-0.071	0.330
	IFN-gamma	0.620*	0.035	-0.128	0.725
	Complement C9	0.685*	0.017	0.578	0.088
Net Forming Count	MMP9	0.755*	0.006	0.139	0.330
	MPO	0.741*	0.008	-0.697	0.031
	vWF-a2	0.650*	0.026	-0.286	0.556
	ADAMTS13	-0.601*	0.043	-0.406	0.247
	IFN-gamma	0.725*	0.010	-0.067	0.858

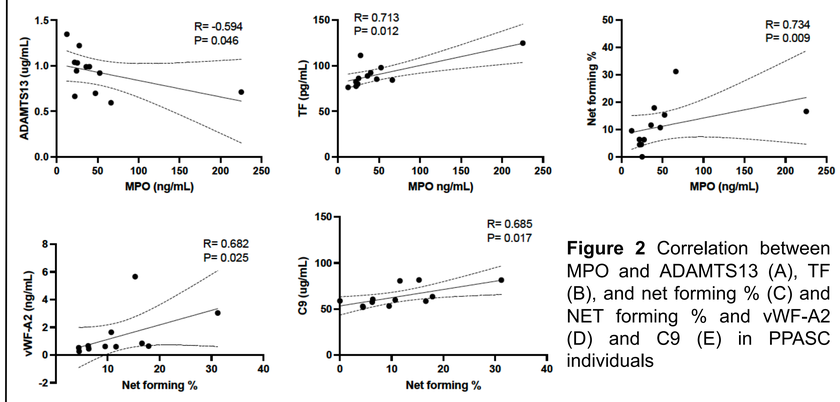

Figure 2 Correlation between MPO and ADAMTS13 (A), TF (B), and net forming % (C) and NET forming % and vWF-A2 (D) and C9 (E) in PPASC individuals

CONCLUSION

The associations between LDG parameters and markers for coagulation and neutrophil activation in PPASC suggest that neutrophils and their derived NETs contribute to thrombotic risk in PPASC. Further studies are necessary to understand the role of sC5b-C9 complex in long-term pulmonary sequelae of COVID-19.

ACKNOWLEDGEMENTS

This work was supported by Myra W. and Jean Kent Angus Foundation, Ola Hawaii (U54MD007601), NIH/NHBLI (K12HL143960), the University of Washington/Fred Hutch Center for AIDS Research, an NIH-funded program under award number P30AI027757, the Molecular and Cellular Immunology Core through the funding of the Centers of Biomedical Research Excellence (COBRE) program (P30GM114737), and the NIH/NIMHD Minority Health Research Training (MHRT) program (T37MD008638).