Sustained endothelial, coagulation and inflammatory cytokine activation without macrovascular dysfunction at 3 months after COVID-19: a reflection on SARS-CoV-2 induced thrombo-inflammation

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Background: Endothelial damage caused by COVID-19 may imperil the cardiovascular health of millions. More than a year since WHO declared the COVID-19 pandemic, information on the lasting effects of this infection on the cardiovascular system beyond the acute phase is still lacking.

Purpose: To study macrovascular endothelial dysfunction and activation, coagulation and inflammation, 3 months after resolution of acute COVID-19 symptoms.

Methods: A cross-sectional observational cohort study was conducted including 203 patients with PCR confirmed COVID-19 disease, 6–20 weeks after acute COVID-19. The primary endpoint was macrovascular endothelial function, assessed by the carotid artery reactivity (CAR) test. The CAR measures the carotid artery diameter in response to hand in ice-water immersion. A historic cohort of 313 subjects served as controls. Propensity score matching was used to correct for baseline differences. Plasma endothelin-1 (ET-1), interleukin (IL)-1ra, IL-6, IL-18 were measured by ELISA. ET-1 levels were also measured in a partially overlapping COVID-19 cohort of which plasma samples were available during the acute phase. Coagulation enzyme:inhibitor complexes for thrombin:antithrombin (TAT), factor (F) IXa:AT, FVIIa:AT, FXIa:AT, FXIa:alpha 1 antitrypsin (a1AT), FXIa:C1 esterase inhibitor (C1inh), kallikrein(PKa):C1inh and von Willebrand Factor:antigen (vWF:Ag), were assessed by in house developed ELISA.

Results: After propensity score matching, the prevalence of macrovascular dysfunction did not differ between the COVID-19 (22.5%) versus the historical control cohort (18.6%, RD –3.92%, 95%-CI –15 to 7.19, p=0.49). Plasma concentrations of markers for endothelial activation were elevated (>1 SD above normal); ET-1 (64.9%), and vWF:Ag (80.8%). In controls, ET-1 levels were significantly lower as compared to COVID-19 patients during the acute phase and after 3 months. ET-1 levels were significantly higher 3 months after COVID-19 as compared to the acute phase. Cytokines were high in a majority of patients: IL-18 (73.9%), IL-6 (51.2%), and IL-1ra (48.9%). TAT and FIXa:AT, reflecting a prothrombotic state, were high in 48.3% and 29.6% of the patients, respectively. FVIIa:AT, as marker of the extrinsic pathway, was elevated (35%). Markers of contact activation were also increased: PKa:C1inh (16.3%), FXIa:AT (16.3%), FXIa:a1AT (20.7%), and FXIa:C1inh (17.7%) (picture 1).

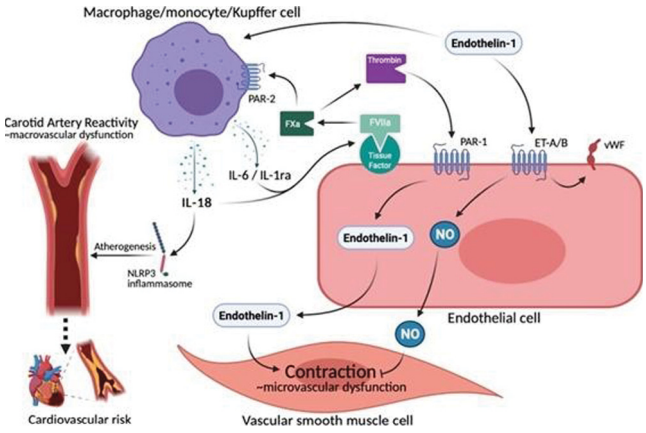
Conclusions: At 3 months after acute COVID-19 there was no indication of macrovascular dysfunction as compared to matched historic controls; there was evidence, however, of sustained thrombo-inflammation, indicated by high circulating concentrations of ET-1, vWF:Ag, proinflammatory cytokines, and markers of coagulation (picture 2). Elevated IL-18 levels could potentially induce arterial inflammation and subsequent atherogenesis. Our data highlight the importance of further studies on SARS-CoV-2 related thrombo-inflammation, as well as longer follow-ups in recovered patients.

Table. Elevated levels of markers of endothelial activation, coagulation and inflammation

Markers of endothelial dysfunction	Normal range	High (> mean +1 SD), %	Very high (> mean +2 SD), %
CAR reaction	≥0%	16.6	
Endothelin-1	1.24±0.37 pg/ml*	64.9	46.0%
VWF:Ag	≤160%	80.8	
Pro-inflammatory cytokines	**Normal range**	**High, %**	
IL-1ra	100-400 pg/ml	48.9	
IL-6	≤ 1.8 pg/ml	51.2	
IL-18	37-215 pg/ml	73.9	
Coagulation enzyme:inhibitor complexes	**Normal range (mean ± 1 SD)**	**High (> mean +1 SD), %**	**Very high (> mean +2 SD), %**
TAT	≤ 4 ug/ml	48.3	
Pka:C1inh	1.68±0.51 ng/ml*	16.3	15.3
FVIIa:AT	306.1±68.45 pg/ml*	35.0	25.6
FIXa:AT	226.60±39.33 pg/ml*	29.6	10.8
FXIa:AT	9.77±2.75 pg/ml*	16.3	15.3
FXIa:α1AT	99.35±20.77 pg/ml*	20.7	17.2
FXIa:C1inh	286.7±110 pg/ml*	17.7	14.8

* mean±SD

Sustained thrombo-inflammation markers



Thrombo-inflammation after COVID-19