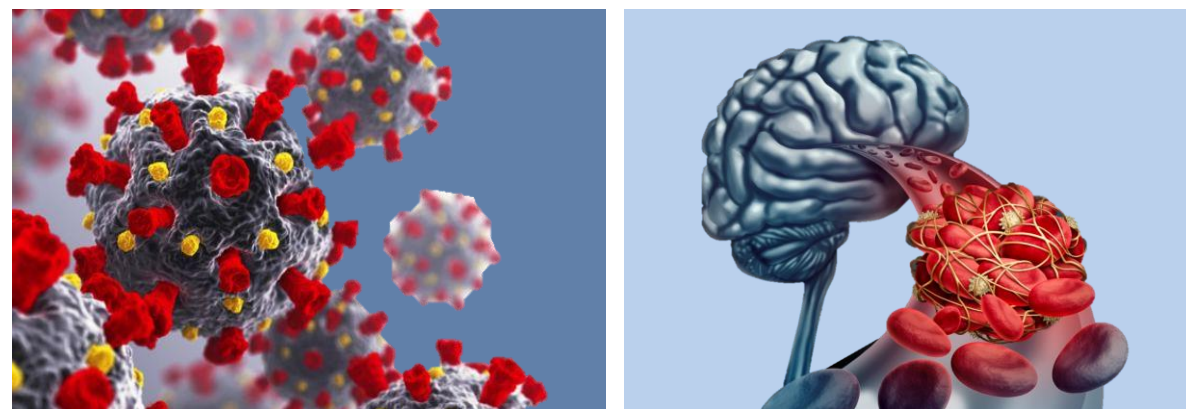


HIGH LEVELS OF CELL-FREE HEMOGLOBIN AND IRON IN CLOTS OF STROKE PATIENTS: NOVEL MECHANISM ASSOCIATED WITH THE SAR-COV2 INDUCED PROTHROMBOTIC STATE

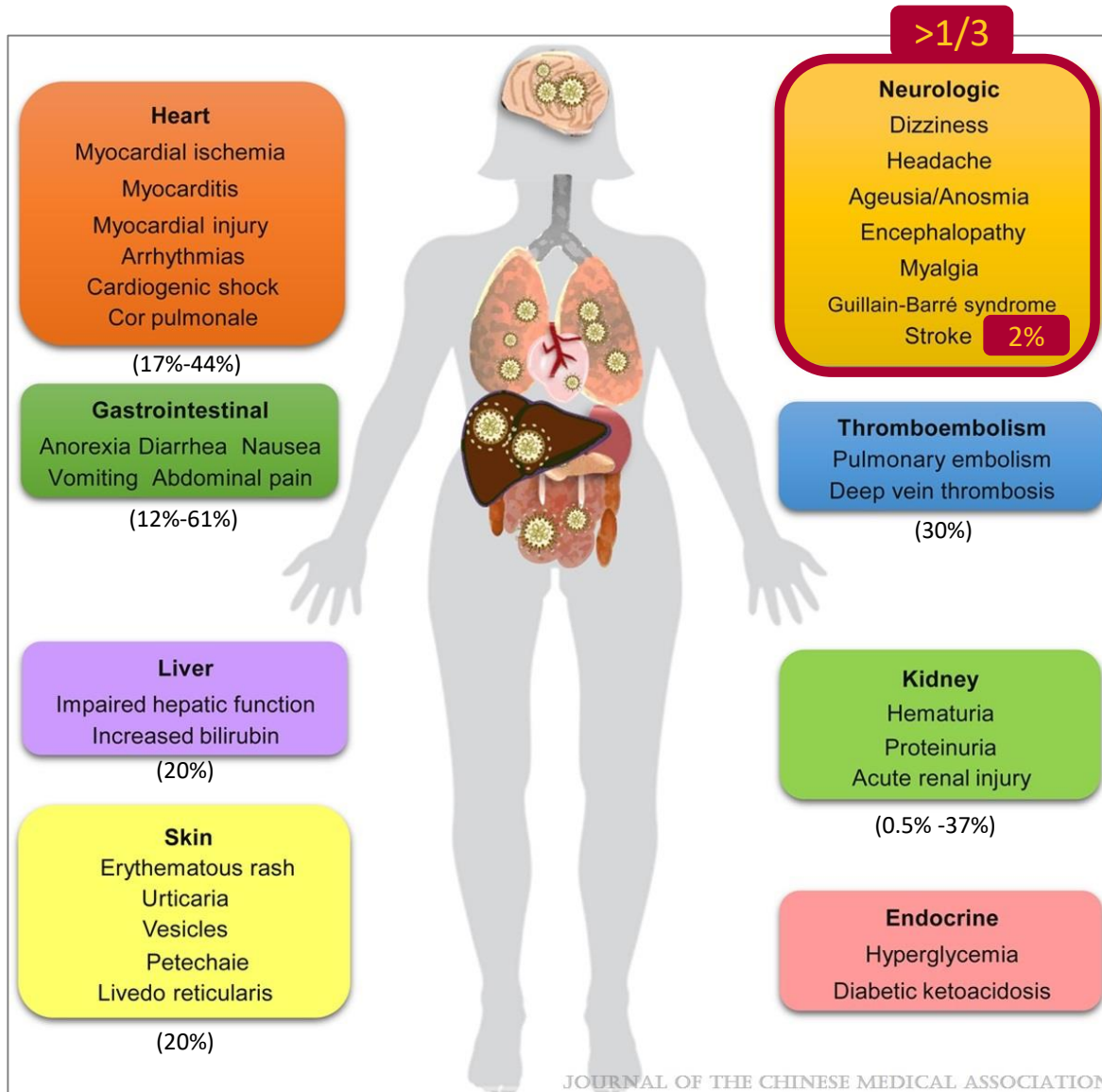
RICORS-ICTUS | Instituto de Salud Carlos III



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BACKGROUND



Extrapulmonary manifestations of COVID-19

ARTICLE

Neurologic manifestations in hospitalized patients with COVID-19

The ALBACOVID registry

Results

Of 841 patients hospitalized with COVID-19 (mean age 66.4 years, 56.2% men), 57.4% developed some form of neurologic symptom. Nonspecific symptoms such as myalgias (17.2%), headache (14.1%), and dizziness (6.1%) were present mostly in the early stages of infection. Anosmia (4.9%) and dysgeusia (6.2%) tended to occur early (60% as the first clinical manifestation) and were more frequent in less severe cases. Disorders of consciousness occurred commonly (19.6%), mostly in older patients and in severe and advanced COVID-19 stages. Myopathy (3.1%), dysautonomia (2.5%), cerebrovascular diseases (1.7%), seizures (0.7%), movement disorders (0.7%), encephalitis (n = 1), Guillain-Barré syndrome (n = 1), and optic neuritis (n = 1) were also reported, but less frequent. Neurologic complications were the main cause of death in 4.1% of all deceased study participants.

BACKGROUND

STROKE

Hypercoagulability
(↑ DD and fibrinogen)

Endothelial cell injury

Hyper-inflammation

Complement system

Coagulation system

Endothelium and platelet activation

Cytokine storms
Macrophages, neutrophils

Ag-Ab complexes
Endothelial cells injury

Secondary hemostasis

Primary hemostasis

vWF, TF, thrombomodulin, PAI-1

NETs
MAC
TF, XIIa, Xa, IIa

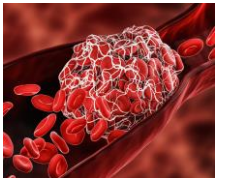
MAC
TF
PAI-1

Coagulation pathway

Hyperimmune platelet response

Coagulation system activation

Fibrinolysis inhibition



OBJETIVES AND METHODS

OBJETIVES

To analyse the histological and ultrastructural characteristics as well as the protein expression and metal composition of clots retrieved during mechanical thrombectomy from COVID-19 infected and non-infected stroke patients

METHODS

- **Data collection:** Demographics, vascular risk factors, TOAST classification, neurological scales, imaging findings, treatment strategies, outcome and laboratory findings
- **Histological examination:** Hematoxylin & eosin and Gomori trichrome staining
- **Immunohistochemistry:** Expression of CD68 (Macrophage marker), CD61 (platelet marker), MPO (Neutrophil and NET marker)
- **Ultrastructural analysis:** Transmission Electron Microscopy (negative staining)
- **Quantitative proteomic analysis:** Tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) using SWATH data-independent acquisition
Hybrid quadrupole-TOF mass spectrometer TripleTOF® 6600
- **Data Analysis:** Peptide and protein identifications were performed using ProteinPilot™ Software V 5.0 (Sciex) and the Paragon algorithm
- **TXRF evaluation :** Total reflection X-Ray fluorescence for detection of 13 metals

RESULTS

I. Clinical, radiological and laboratory characteristics

	COVID-19, N=6	CONTROL, N=6
Demographics		
Age (years, mean \pm SD)	60 \pm 16,4	61 \pm 4,7
Female (n, %)	2 (33,3)	3 (50)
Vascular risk factors		
Atrial fibrillation (n, %)	1 (16,6)	1 (16,6)
Arterial hypertension (n, %)	2 (33,3)	2 (33,3)
Diabetes mellitus (n, %)	2 (33,3)	2 (33,3)
Dyslipidemia (n, %)	0 (0,0)	3 (50)
Smoking (n, %)	2 (33,3)	2 (33,3)
Medications		
Prior antiplatelet therapy (n, %)	0 (0,0)	1 (16,6)
Prior anticoagulant therapy (n, %)	0 (0,0)	0 (0,0)
Oxygen saturation at admission		
Sat O2 <92% (n, %)	3 (50)	1 (16,6)

	COVID-19, N=6	CONTROL, N=6
Index ischemic event		
ASPECTS (median [IQR])	9 (1,3)	9,5 (1,5)
NIHSS score on admission (median [IQR])	17,5 (10,6)	12 (7,9)
NIHSS score after 24 hours (median [IQR])	12 (12,5)	2,5 (5,2)
Occlusion site		
BA (n, %)	1 (16,6)	0 (0,0)
TICA (n, %)	1 (16,6)	1 (16,6)
MCA (n, %)	4 (66,6)	5 (83,3)
Tandem occlusion (n, %)	1 (16,6)	1 (16,6)
TOAST classification		
Cardioembolism (n,%)	2 (33,3)	2 (33,3)
Undetermined etiology (n, %)	3 (50)	4 (66,6)
Other determined etiology (n, %)	1 (16,6)	0 (0,0)
Modified Rankin scale		
90-day mRS > 2 (n, %)	5 (83,3)	1 (16,6)
Acute treatment		
Intravenous Alteplase	4 (66,6)	2 (33,3)

RESULTS

I. Clinical, radiological and laboratory characteristics

	COVID-19, N=6	CONTROL, N=6
Laboratory findings		
Leukocytes (x10 ³ /μL) (mean ± SD)	10,72 ± 2,546	12,5 ± 3,927
Neutrophils (x10 ³ /μL) (mean ± SD)	8,64 ± 2,456	10,5 ± 3,7
Lymphocytes (x10 ³ /μL) (mean ± SD)	1,37 ± 0,565	1,32 ± 0,396
Monocytes (x10 ³ /μL) (mean ± SD)	0,5 1± 0,189	0,3 ± 0,075
Erythrocyte (x10 ⁶ /μL) (mean ± SD)	4,67 ± 0,384	4,65 ± 0,417
Hemoglobin (g/dL) (mean ± SD)	14,2 ± 1,147	13,83 ± 1,823
Hematocrit (%)	41,66 ± 3,012	41,28 ± 4,985
Platelet count (x10 ³ /μL) (mean ± SD)	309,33 ± 192,850	257,66 ± 48,726
Ferritin (ng/mL) (mean ± SD)	398,75 ± 259,732	NA
C-reactive protein (CRP) (mg/L) (mean ± SD)	31,68 ± 32,271	NA
Lactate dehydrogenase, U/L (mean ± SD)	261 ± 82,793	226,83 ± 80,273
Activated partial thromboplastin time (s)	25,83 ± 3,116	29,35 ± 3,024
International normalized ratio (INR) (mean ± SD)	1,27 ± 0,120	1,12 ± 0,084
D-Dimer (μg/mL) (median [IQR])	29399,6 ± 36728,676	997 ± 149,906
Fibrinogen (mg/dL) (mean ± SD)	369,16 ± 149,359	318,83 ± 64,235

RESULTS

II. Macroscopical appearance and histological/ultrastructural features

COVID-19 clots

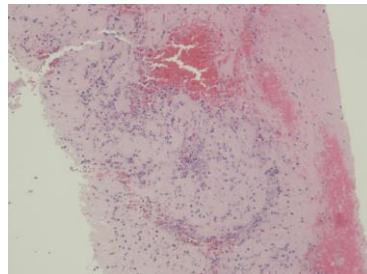
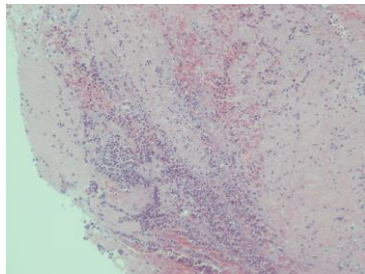
Control clots



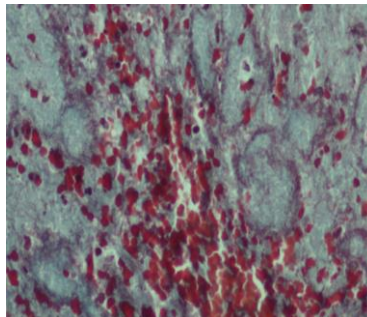
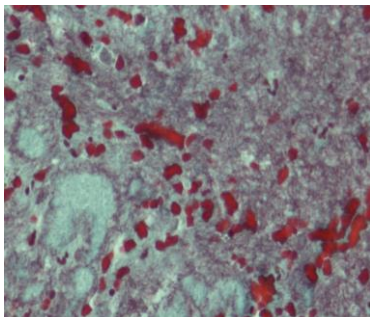
H&E-stained samples

(Fibrin-rich)

(Fibrin-rich)



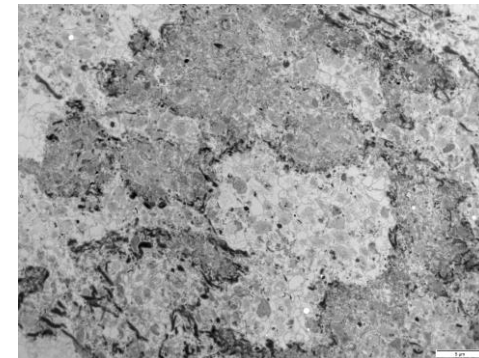
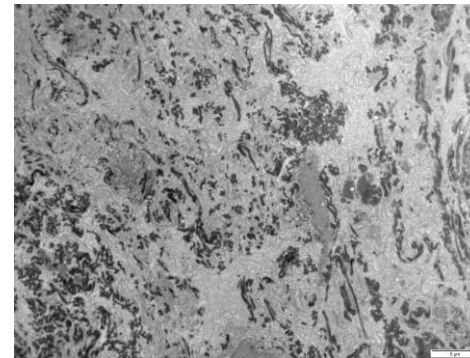
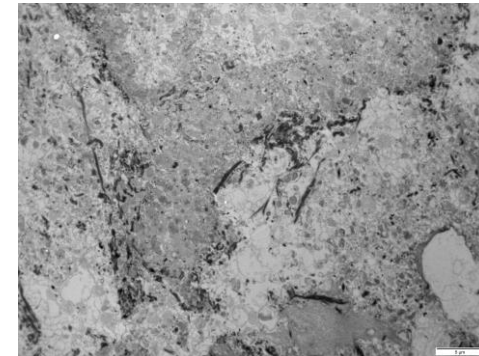
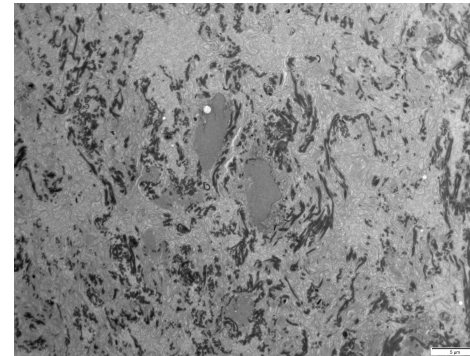
Trichrome-stained samples



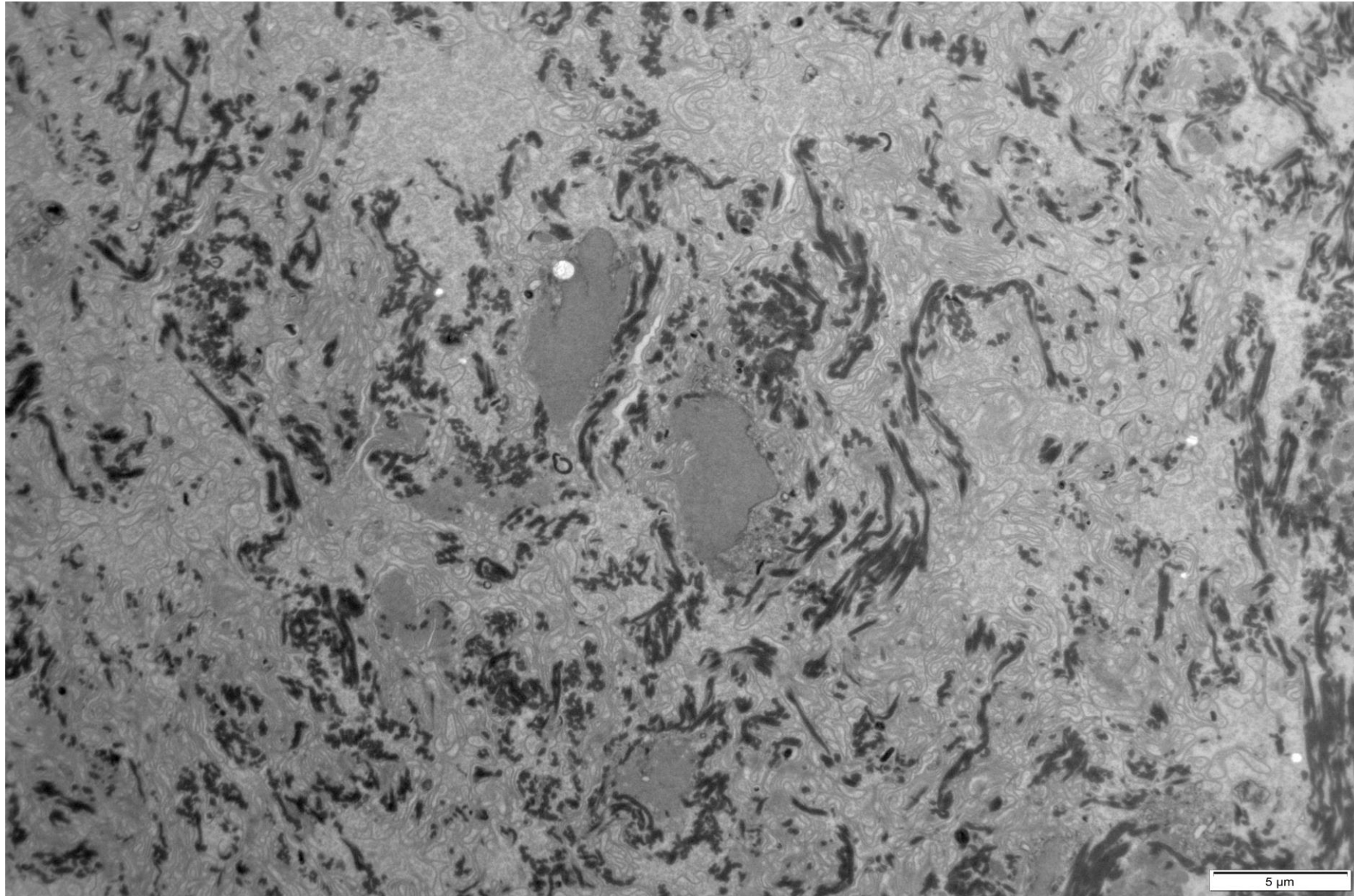
COVID-19 clots

Control clots

Transmission Electron Microscopy
(negative staining)



RESULTS

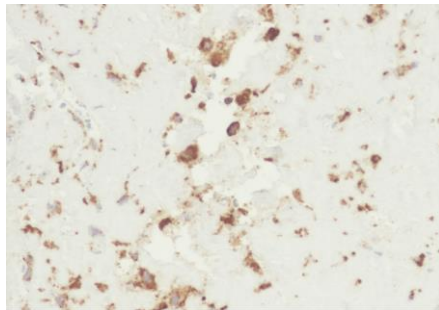


RESULTS

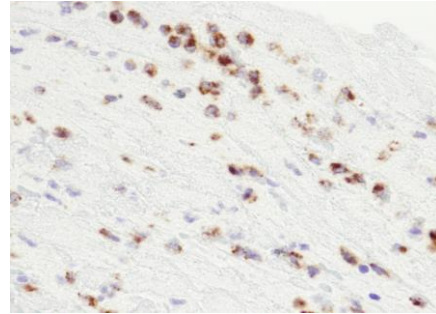
III. Immunohistochemical characterization

Monocytes/Macrophage (CD68⁺)

COVID-19 clots

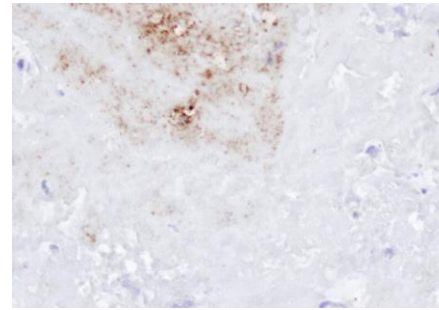


Control clots

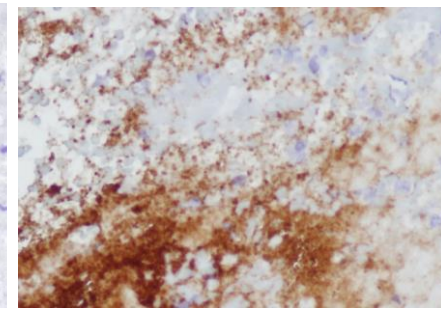


Platelets (CD61⁺)

COVID-19 clots

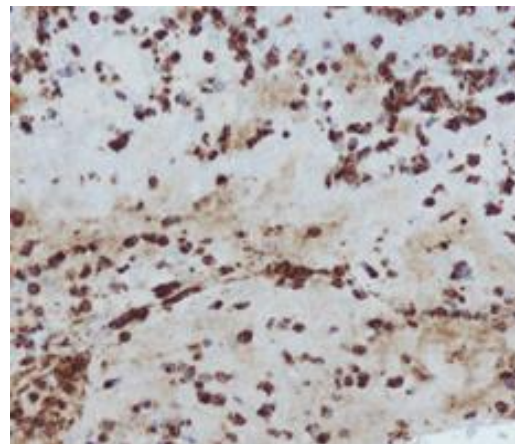


Control clots

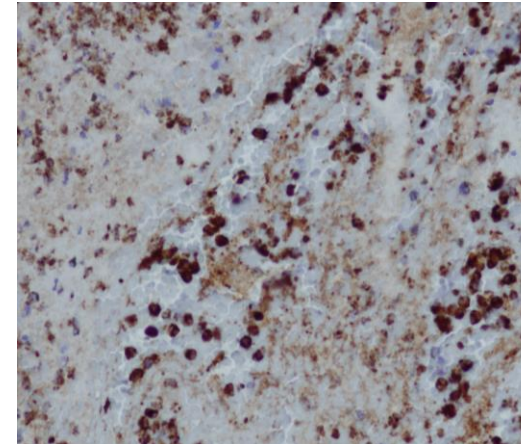


Neutrophils/NETs (MPO⁺)

COVID-19 clots



Control clots



RESULTS

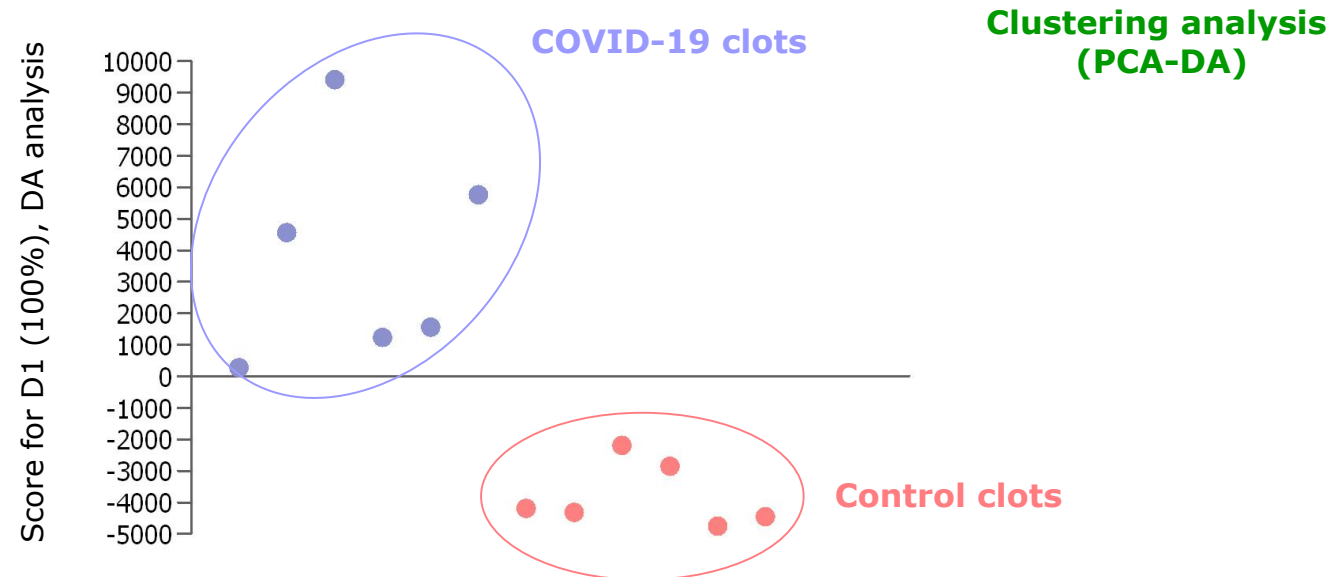
IV. Quantitative Proteomic analysis (LC-MS/MS in SWATH mode)

COVID-19 clots (n=6)

- Characterized proteins/each clot ranged from 265 to 729 proteins
- A total of [904 identified proteins](#)
- 120 (6/6), 137 (5/6) common proteins

Control clots (n=6)

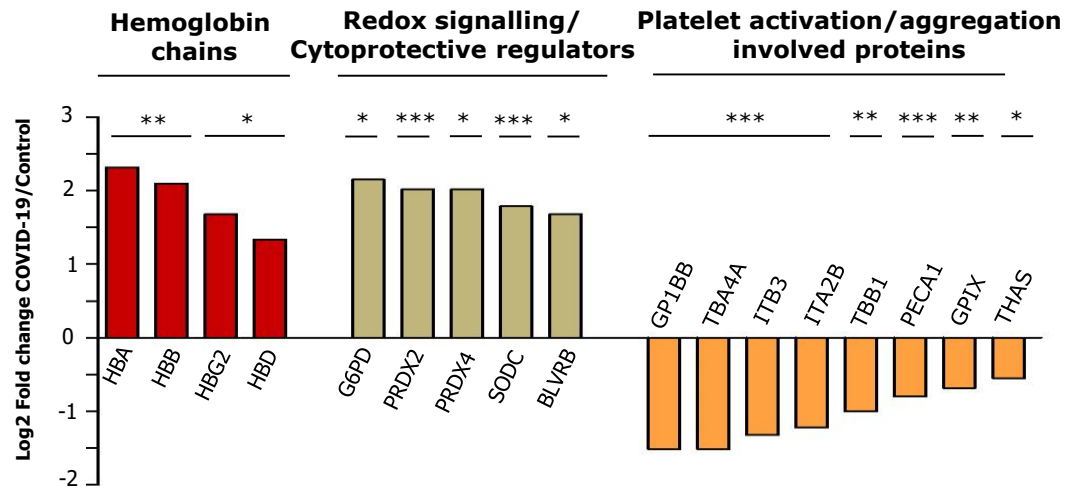
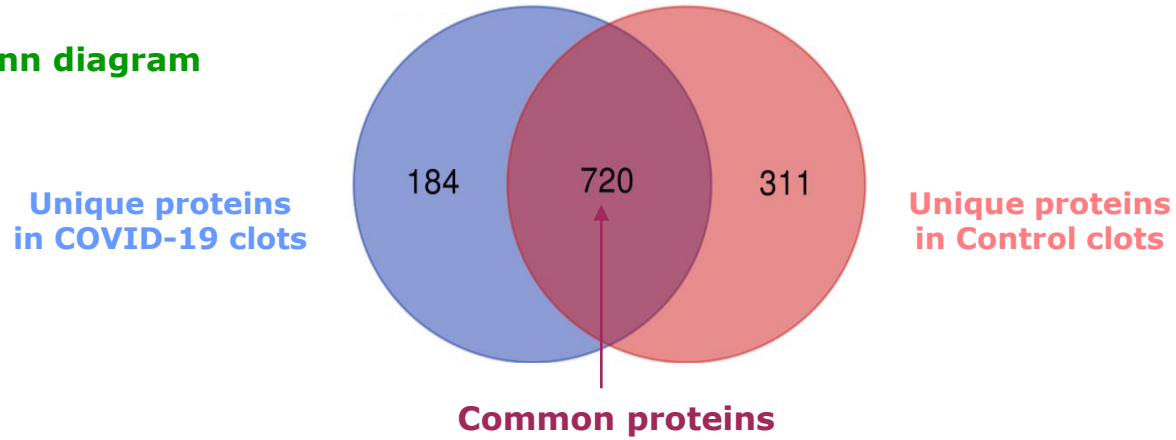
- Characterized proteins/each clot ranged from 305 to 819 proteins
- A total of [1031 identified proteins](#)
- 248 (6/6), 402 (5/6) common proteins



RESULTS

IV. Quantitative Proteomic analysis (LC-MS/MS in SWATH mode)

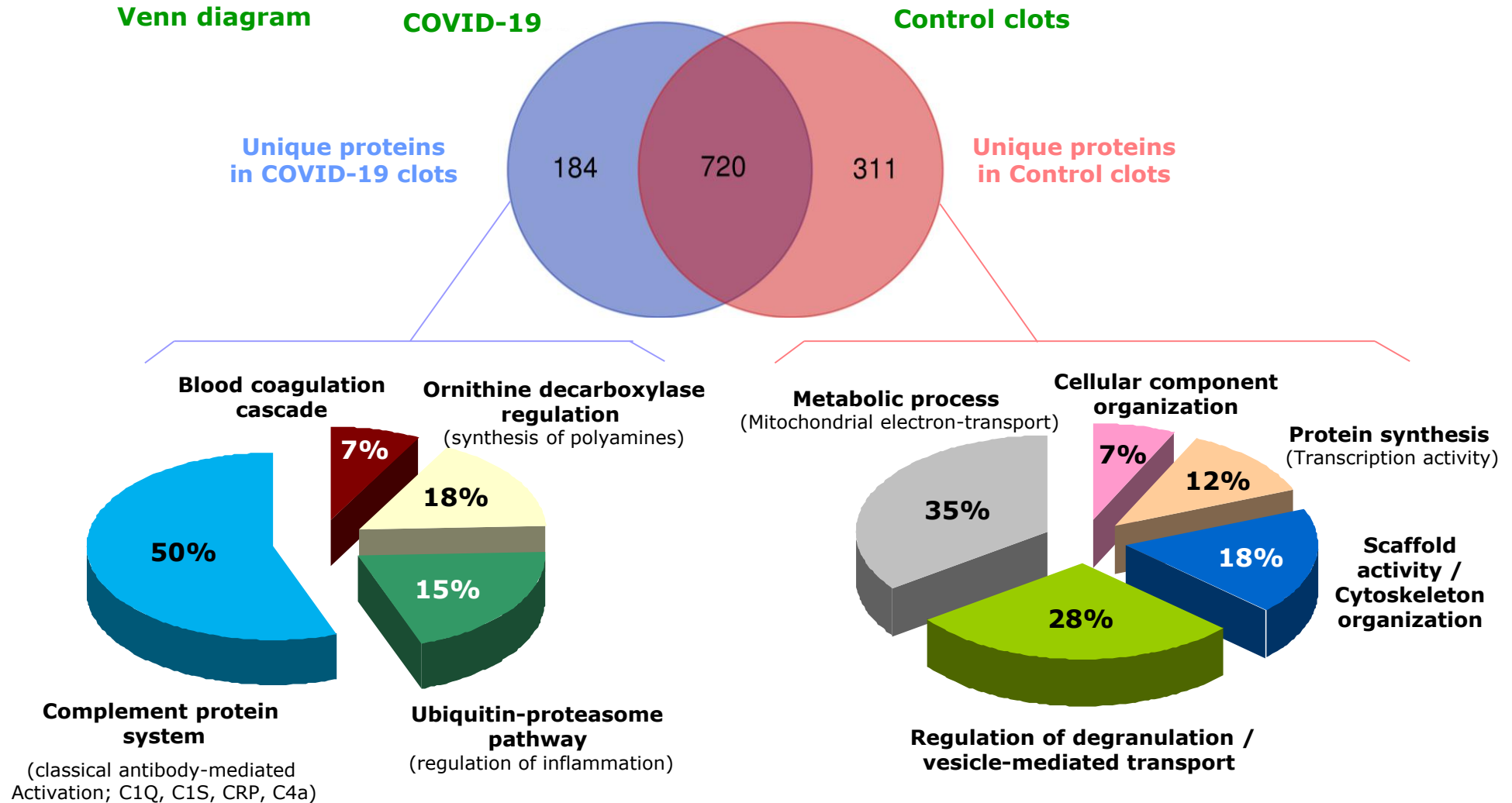
Venn diagram



p-value {
*** ≤ 0,001
** ≤ 0,01
* ≤ 0,05

RESULTS

IV. Quantitative Proteomic analysis (LC-MS/MS in SWATH mode)

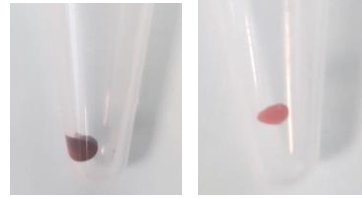


RESULTS

V. Quantitative analysis of metal content

COVID-19

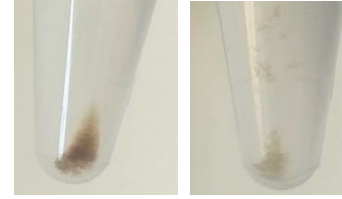
Control



Thrombotic material
(obtained by thrombectomy)

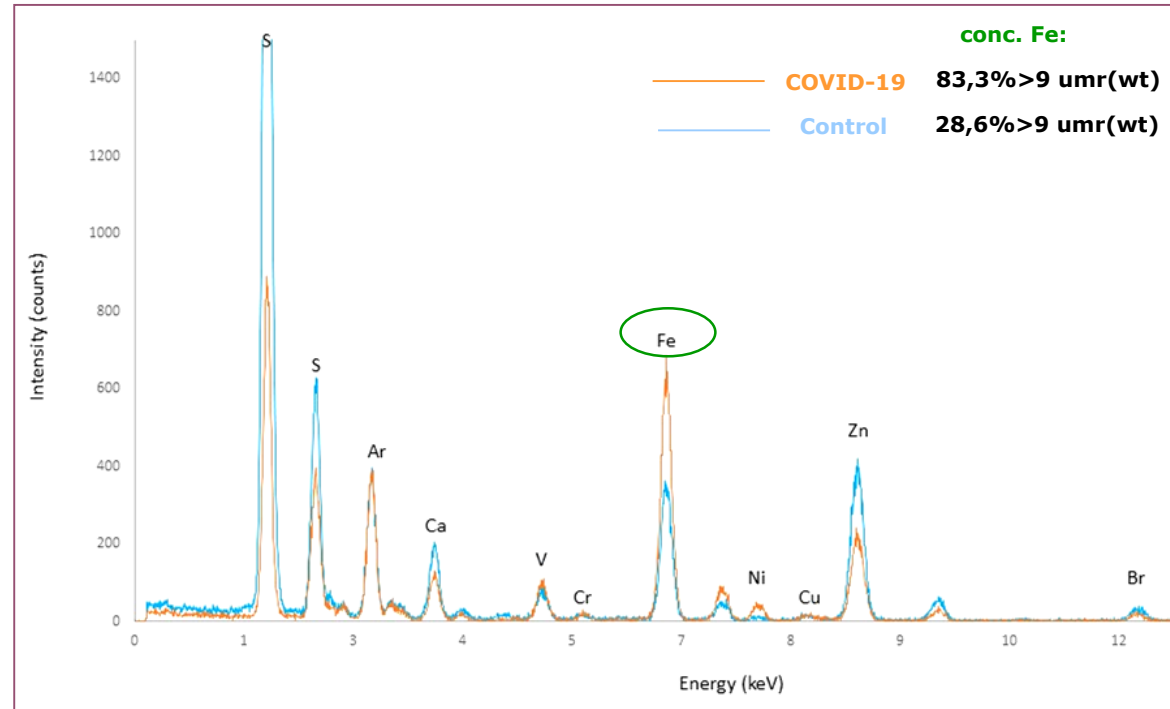
COVID-19

Control

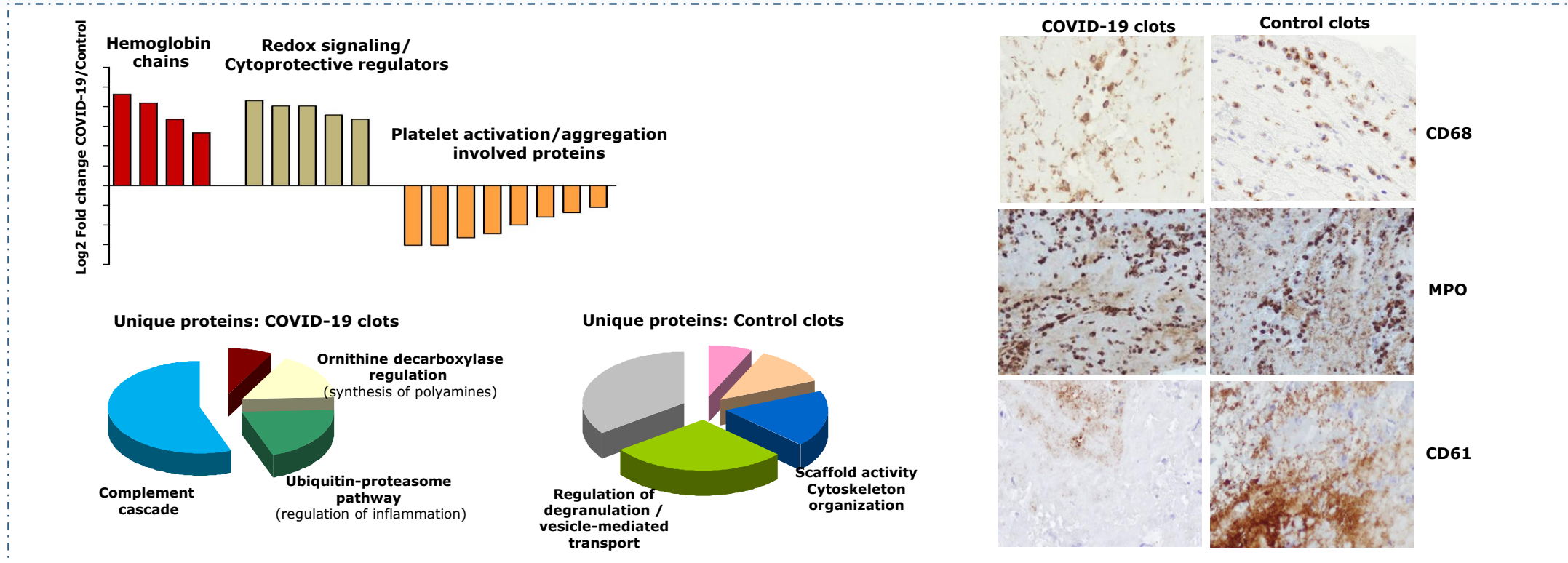


Protein extract
(clot lysis and protein precipitation)

**TXFR
evaluation**



RESULTS: OVERVIEW



- Higher levels of redox signaling enzymes in COVID clots
- Proteasome, ODC proteins, complements found only in COVID
- No differences in macrophage, neutrophil and NETs density

- Lower levels of platelet proteins
- Proteins of degranulation pathway Not found in COVID
- Decreased platelet density
- Random distribution of fibrin

Inflammatory conditions:
Presence of hyperactivated macrophage (M1)
and classical complement cascade

Essential role

Minor role

Cerebral thrombus formation during SARS-Cov-2 infection

Gracias por su atención