Association Between Mitochondrial Gene Expression & Metabolomic Alterations in Patients with Severe COVID-19 Pneumonia Treated with Cytokine Adsorption Therapy

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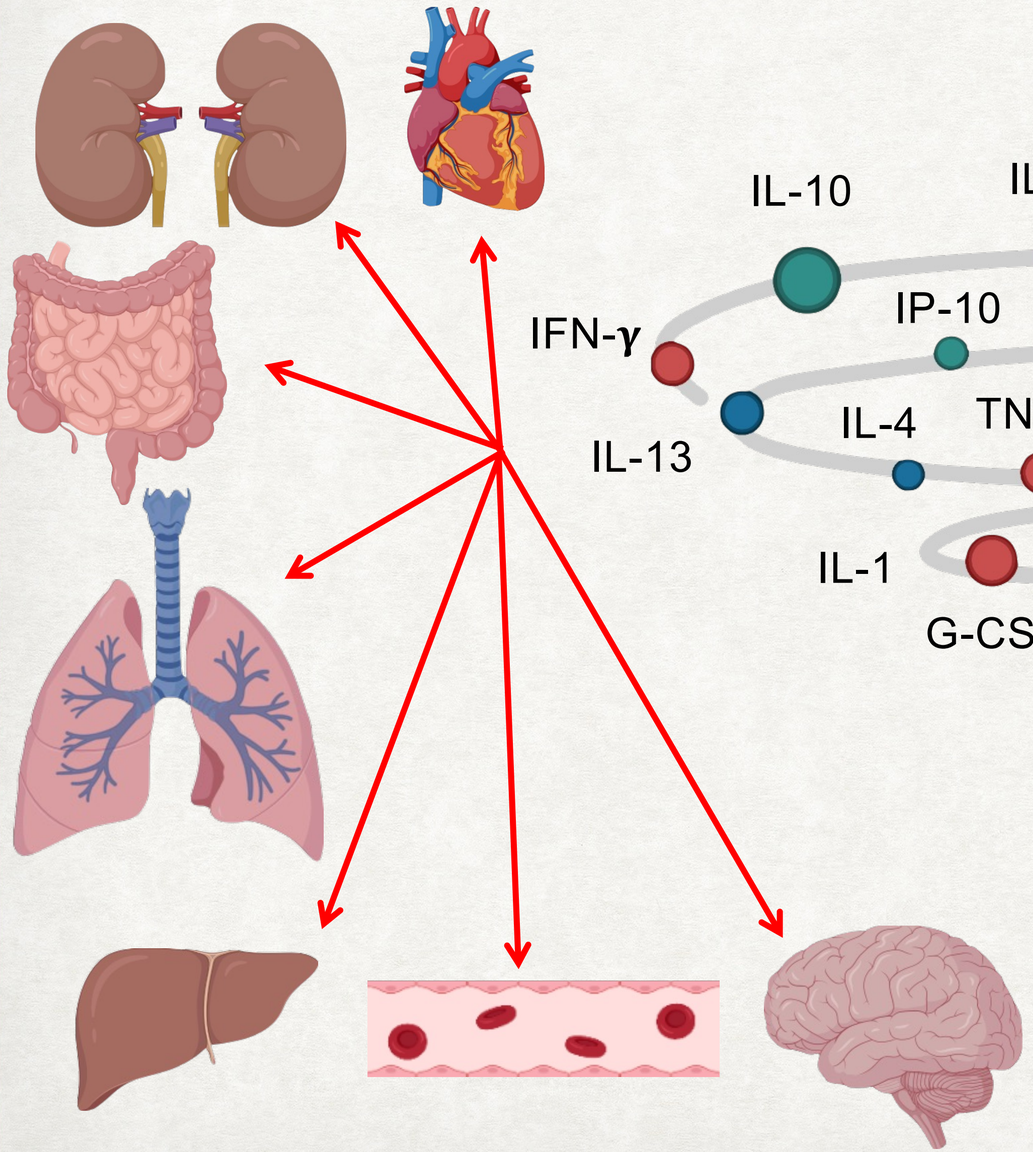
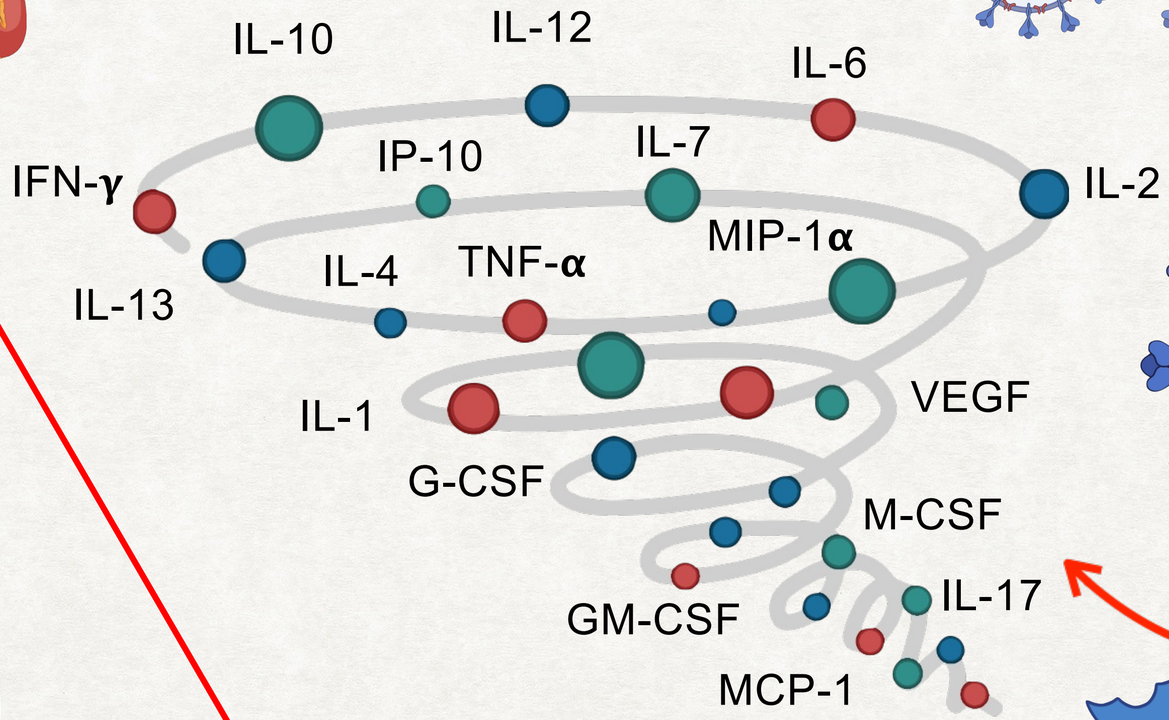
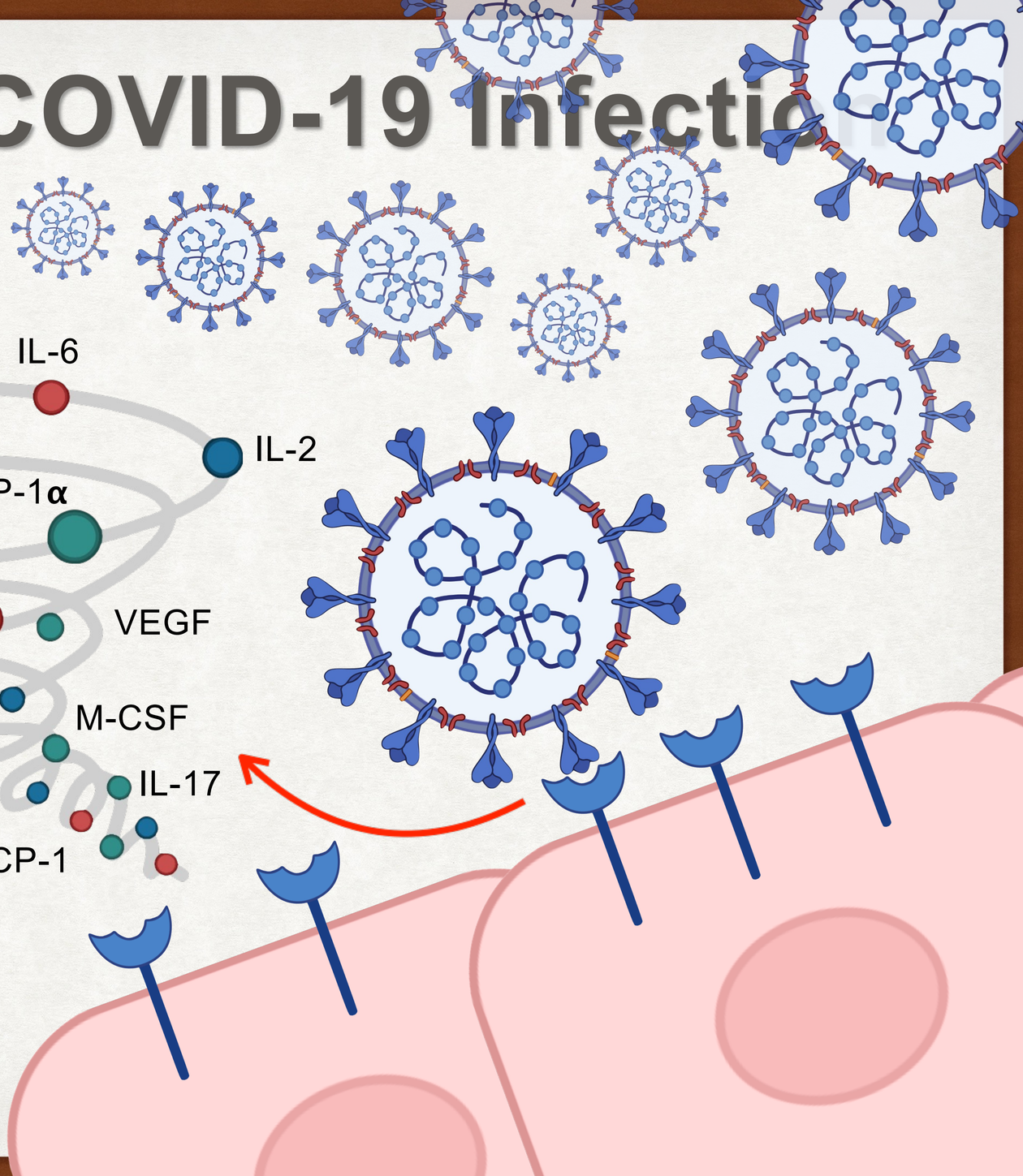
Cytokine Storm in COVID-19 Infection



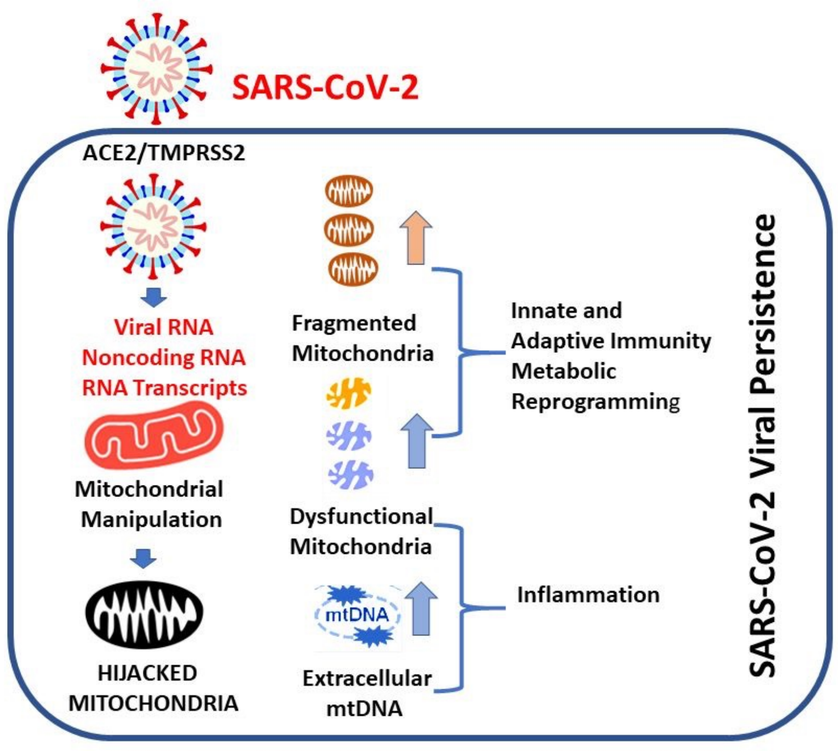




Costela-Ruiz VJ, et al. Cytokine Growth Factor Rev. 2020;54:62-75.

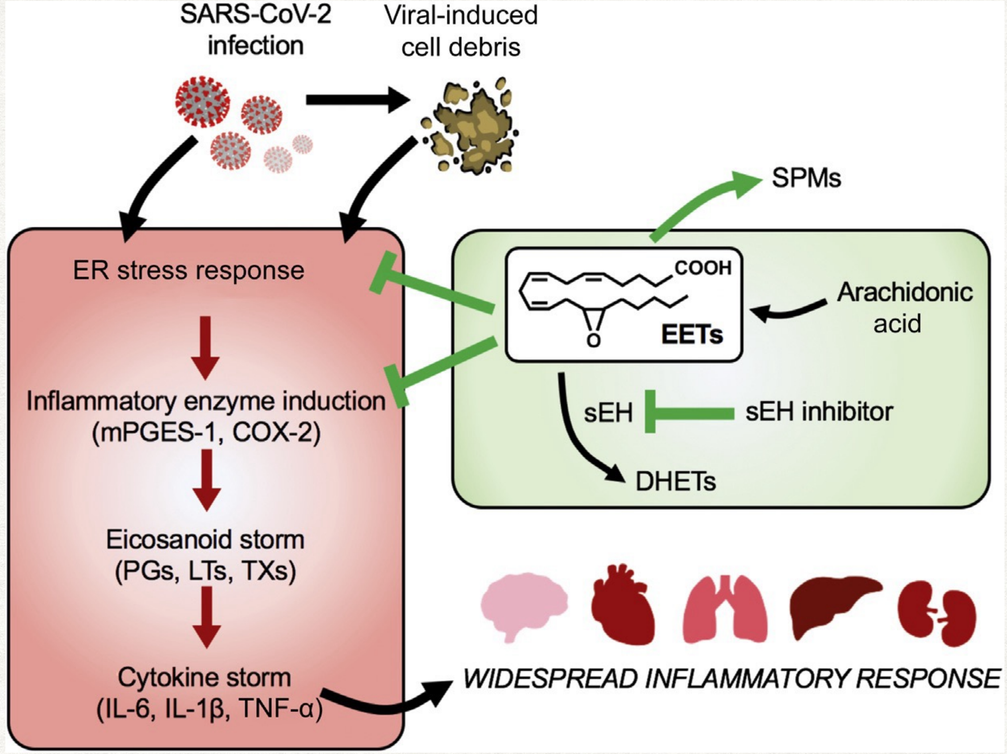
Mitochondrial & Metabolomic Changes During COVID-19 Infection

Mitochondrial function



An investigation into the SARS-CoV-2 hijacking of mitochondria should lead to novel approaches to prevent & treat COVID-19.

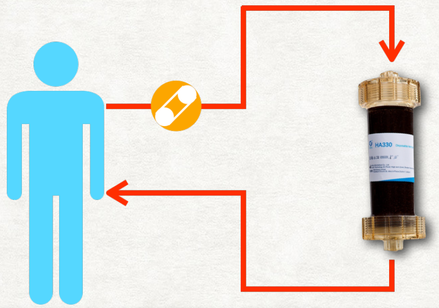
Metabolomics changes

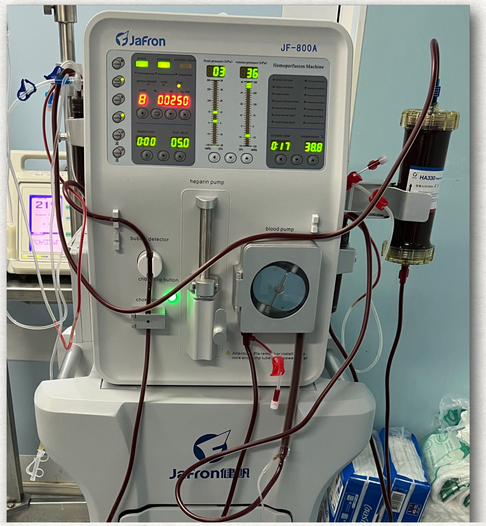


Eicosanoids (prostaglandins, thromboxane & leukotrienes) are critical mediators of inflammation.

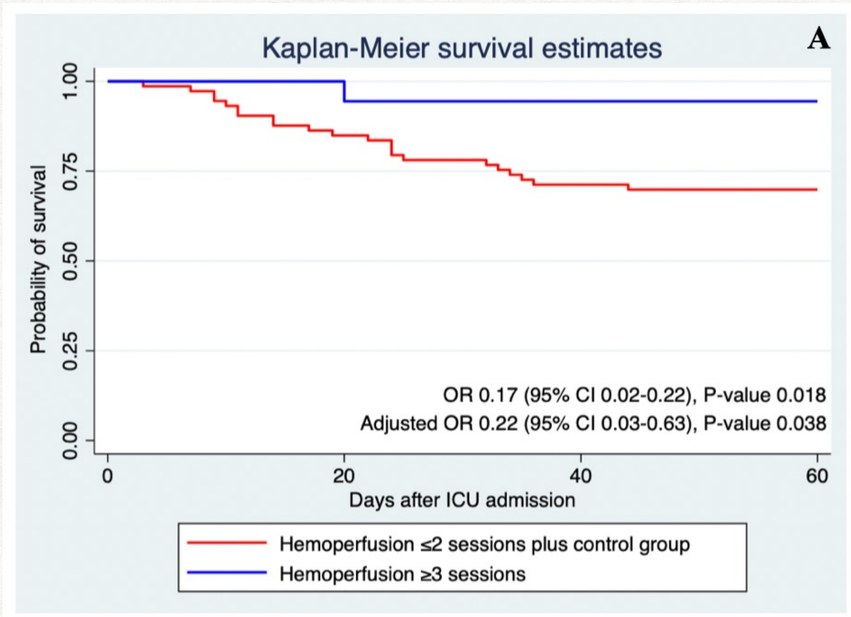
Singh KK, et al. Am J Physiol Cell Physiol. 2020;319(2):C258-67, Hammock BD, et al. Am J Pathol. 2020;190(9):1782-8.

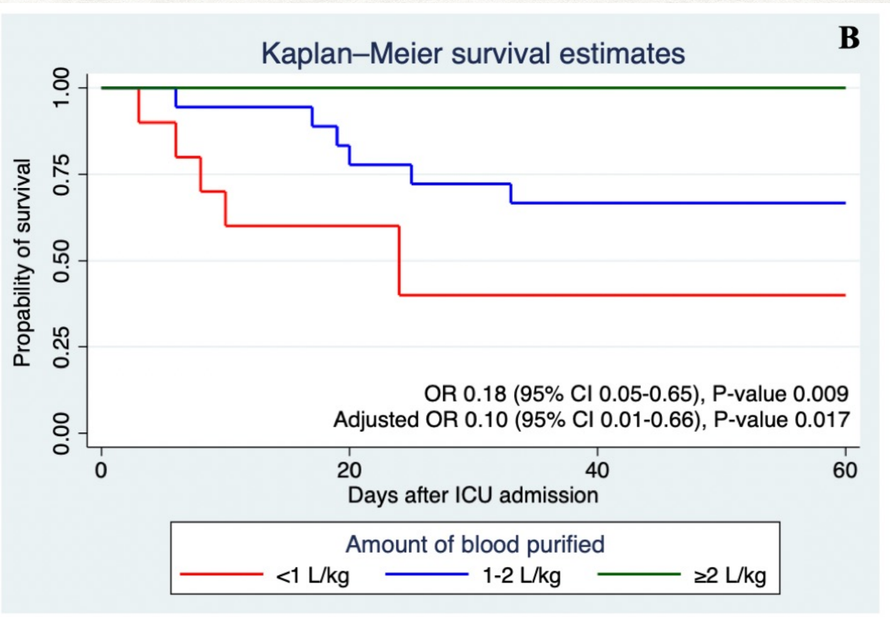
Cytokine Adsorption Therapy During COVID-19-Associated Pneumonia





Prospective observational study of 112 patients





The optimal dose of at ≥3 sessions or the amount of blood purified ≥1 L/kg was associated with a reduction in 60-day mortality.

Knowledge gap

Scarce data on changes of mitochondrial function & metabolomic profiles after hemoperfusion therapy

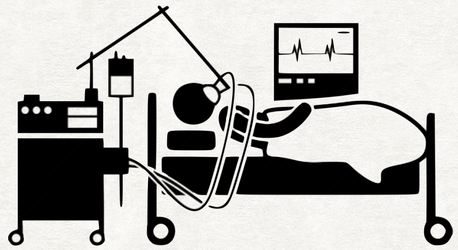
Objective

To investigate benefits of cytokine adsorption by evaluating changes in inflammation, metabolomic profiles, and circulating mitochondrial function in patients with COVID-19-associated pneumonia

Kusirisin P, et al. Blood Purif. 2024;53(1):10-22.

Methods

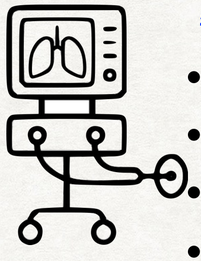
Single-center prospective observational study



Adult severe COVID-19 pneumonia admitted in ICU, Chiang Mai University Hospital, Thailand

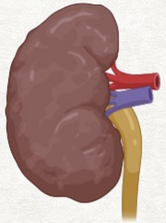
Between April 2021 - January 2022

Patients with ≥1 of these cytokine storm parameters



Acute respiratory failure

- Respiratory rate >24/min
- Heart rate >125/min
- Oxygen saturation <94%
- ARDS (PaO_2/FiO_2 ≤300)



or Acute kidney injury

- ↑ SCr ≥0.3 mg/dL in 48 h
- ↑ SCr >1.5 times in 7 days
- Urine <0.5 mL/kg/h for 6 h

+

High inflammatory markers

- D-dimer >1,000 mg/mL
- CPK >2 times of upper limit
- hs-CRP >75 mg/L
- LDH >245 U/L
- Total lymphocyte count <1,000
- Ferritin >500 mg/mL

Hemoperfusion (HP) group

VS

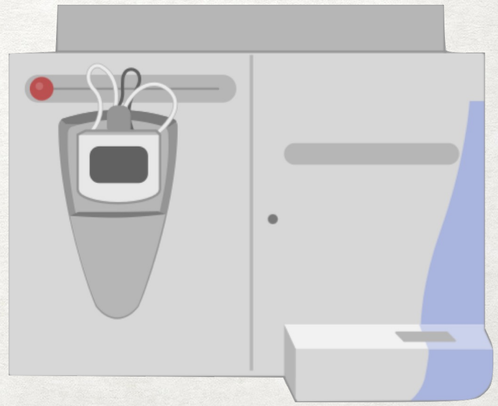
Control group

Outcomes:

- Comparison between baseline & day 7 after treatment
  - Clinical severity: APACHE II, SOFA score, PaO_2/FiO_2
  - Lab: inflammatory cytokines, metabolomic, & mitochondrial profiles

Laboratory Protocol

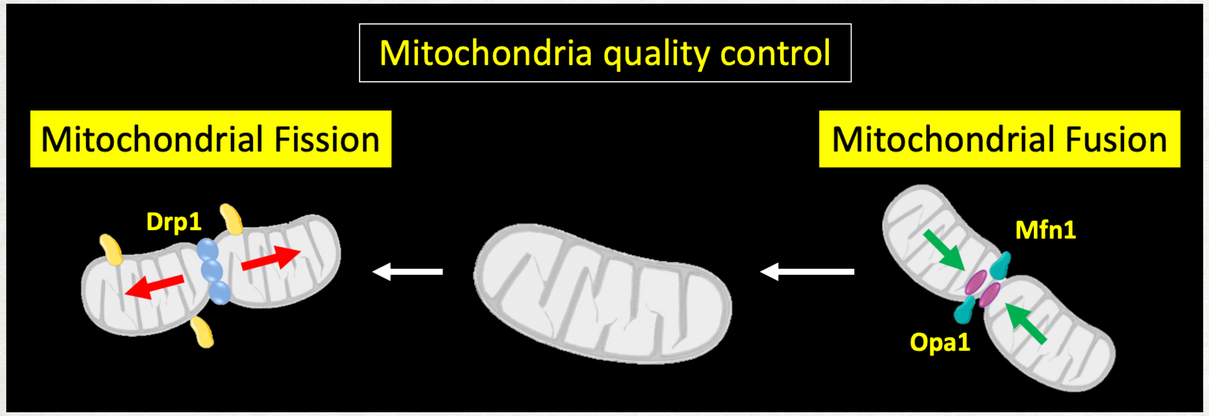
- **Clinical severity:** CBC, Cr, hs-CRP, D-dimer, CPK, LDH, ferritin, lactate
- **Inflammatory markers:** IL-1β, IL-6, IL-8, IFN-γ (MILLIPLEX® cytokine assay kit)
- **Plasma metabolomic studies:** organic acids, amino acids, free fatty acids, phospholipids, glycerols, steroids, eicosanoids, acylcarnitines (liquid chromatography-mass spectrometry & analysis with HILIC-ESI-MS & RPLC-ESI-MS)
- **Mitochondrial function** (real-time quantitative RT-PCR)
  - Mitochondrial dynamics: fission & fusion
  - Mitochondrial respiration: complex I-V



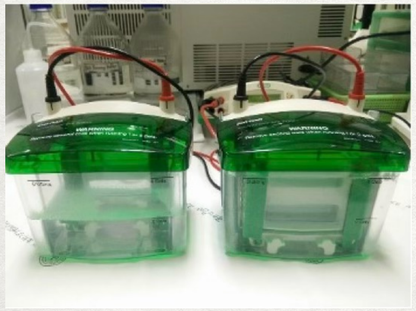
CPK, creatine phosphokinase; hs-CRP, high sensitivity C-reactive protein; IFN, interferon; IL, interleukin; LDH, lactate dehydrogenase

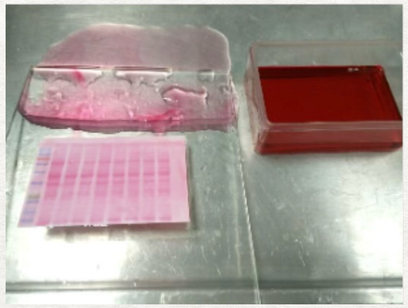
Mitochondrial Function

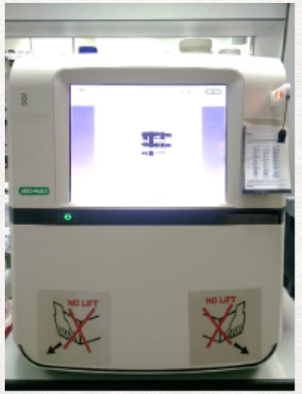
Mitochondrial dynamics



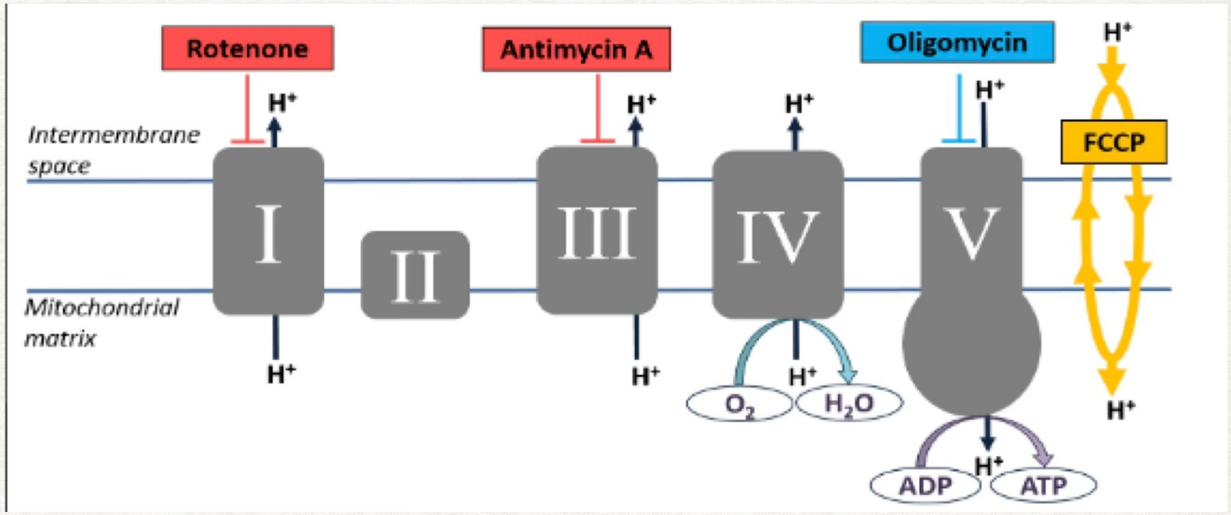
Western blot







Mitochondrial respiration



Cell Mito Stress Test Kit with extracellular flux analyzer (Agilent Seahorse XFe96)

Geto Z, et al. J Inflamm Res. 2020;13:97-107, Chang X, et al. Theranostics. 2021;11(14):6766-85.

Results

Patients with COVID-19 pneumonia admitted to ICU

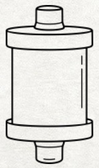
(n = 165)

Excluded

- Non-severe COVID-19 pneumonia (n = 53)
- Insufficient blood samples for laboratory tests (n = 95)

Eligible patients

(n = 17)



Hemoperfusion group

(n = 8)

Control group

(n = 9)

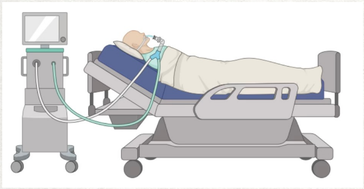
Baseline Characteristics

Clinical parameters	HP (n = 8)	Control (n = 9)	P
Age, years	56.8 ± 17.7	54.3 ± 15.5	0.768
Male, n (%)	7 (87.5)	3 (33.3)	**0.024**
BMI, kg/m^2	27.0 ± 8.0	29.3 ± 6.9	0.549
Comorbidities, n (%)			
• Hypertension	4 (50.0)	3 (33.3)	0.486
• Obesity	3 (37.5)	5 (55.6)	0.457
• CKD	3 (37.5)	0 (0.0)	**0.043**
PaO_2/FiO_2	125.2 ± 51.4	181.7 ± 76.5	0.099
APACHE II score	19 (5, 27)	8 (6, 9)	0.243
SOFA score	9 (3, 15)	3 (2, 4)	0.118
Methylprednisolone, n (%)	3 (37.5)	7 (77.8)	0.200
Tocilizumab, n (%)	3 (37.5)	4 (44.4)	0.772
Inotropic drugs, n (%)	4 (50.0)	1 (11.1)	0.079
Prone position, n (%)	3 (37.5)	4 (44.4)	0.772
HP session	3.3 ± 0.7	0	-
Time to first HP, h	70.5 (39, 142)	-	-
Death, n (%)	2 (25.0)	1 (11.1)	0.453
Length of ICU stay, day	24.3 ± 9.7	14.8 ± 17.1	0.189
Ventilator-free day, day	11.1 ± 6.9	8.1 ± 3.9	0.277

Laboratory parameters	HP (n = 8)	Control (n = 9)	P
Cytokine storm parameters			
• D-dimer, ng/mL	750.5 (270, 1866)	522 (293, 732)	0.637
• CPK, U/L	161.0 (47, 1016)	82 (35, 222)	0.224
• hs-CRP, mg/L	117.8 (45.2, 236.6)	103.6 (67.8, 171.1)	0.886
• LDH, U/L	349.14 ± 152.27	410.44 ± 170.74	0.468
• TLC, cells/mm^3	1066 (559, 1499)	920 (811, 1360)	0.923
• Ferritin, ng/mL	612 (382, 802)	1263 (928, 1504)	0.125
Renal function			
• Serum creatinine, mg/dL	0.99 (0.79, 1.55)	0.72 (0.65, 0.85)	**0.034**
• eGFR, mL/min/1.73 m^2	73.54 ± 33.63	95.60 ± 16.77	0.102
Lactate, mmol/L	3.06 ± 1.37	1.84 ± 0.54	**0.026**
Inflammatory markers			
• IL-1β, pg/mL	29.01 (1.77, 42.9)	3.76 (1.01, 6.50)	0.248
• IL-6, pg/mL	73.5 (54.5, 128.4)	28.79 (15.02, 57.7)	**0.027**
• IL-8, pg/mL	8.60 (2.51, 33.38)	4.36 (2.4, 5.48)	0.248
• IFN-γ, pg/mL	1.43 (0.87, 4.71)	2.0 (1.88, 2.16)	0.855
Metabolomes			
• Thromboxane B3, nM	1.28 (1.15, 4.74)	1.24 (1.11, 1.31)	0.427
• Leukotriene E4, pM	184.5 (170, 380.6)	100.6 (33.3, 238.2)	0.081
• Prostaglandin B2, pM	175.77 ± 76.34	174.49 ± 60.32	0.971
• HETE, nM	19.05 (6.2, 104.1)	33.39 (15.6, 42.9)	0.560

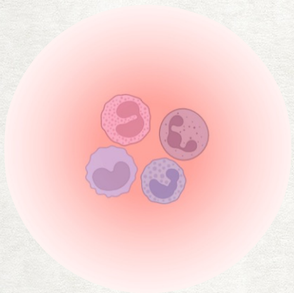
Clinical & Laboratory Outcomes Compared with Baseline

Clinical outcomes



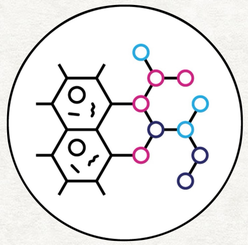
APACHE II	↓ 36.4% **(p = 0.028)**	↓ 12.5% (p = 0.093)
SOFA score	↓ 66.7% **(p = 0.017)**	↓ 33.3% (p = 0.063)
PaO_2/FiO_2	↑ 139.3% **(p = 0.002)**	↑ 86.1% (p = 0.064)

Inflammatory markers



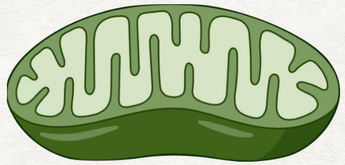
hs-CRP	↓ **84.6%** **(p = 0.012)**	↓ 74.2% (p = 0.317)
IL-1β	↓ 60.8% (p = 0.180)	↑ 222.4% (p = 0.655)
IL-6	↓ **67.6%** **(p = 0.012)**	↓ 15.0% (p = 0.586)
IL-8	↓ **43.6%** **(p = 0.012)**	↑ 33.5% (p = 0.161)
IFN-γ	↓ **50.9%** **(p = 0.027)**	↑ 26.4% (p = 1.000)

Metabolomes



Thromboxane B3	↓ **19.9%** **(p = 0.018)**	↓ 21.5% (p = 0.263)
Leukotriene E4	↓ **19.7%** **(p = 0.018)**	↓ 9.7% (p = 0.327)
Prostaglandin B2	↓ **12.2%** **(p = 0.035)**	↓ 4.0% (p = 0.899)
HETE	↓ **68.3%** **(p = 0.018)**	↓ 26.7% (p = 0.899)

Mitochondrial function-related gene

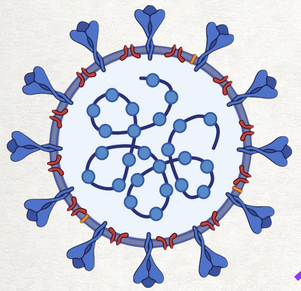


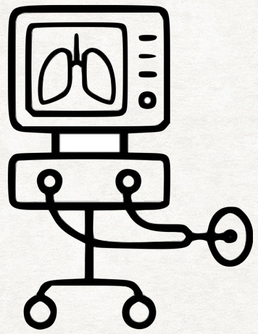
	Before HP	After HP	P
Drp1	30.07 ± 1.85	29.48 ± 1.13	0.452
Mfn1	31.93 ± 2.33	31.22 ± 2.82	0.596
Complex I	27.91 ± 2.25	27.04 ± 2.68	0.548
Complex II	29.08 ± 2.44	28.20 ± 3.30	0.571
Complex III	29.25 ± 2.77	28.31 ± 2.80	0.469
Complex IV	31.57 ± 1.42	29.46 ± 2.51	**0.025**
Complex V	28.96 ± 2.58	27.48 ± 2.44	0.283

Correlation between Clinical & Laboratory Changes after HP

Outcomes	APACHE II score		SOFA score		PaO_2/FiO_2	
	β coefficient	P	β coefficient	P	β coefficient	P
hs-CRP	0.3571	0.432	-0.5509	0.157	0.6333	0.067
IL-1β	0.8000	0.200	-0.6325	0.368	0.8000	0.200
IL-6	0.3077	0.331	-0.1261	0.696	0.8317	**0.020**
IL-8	0.5714	0.180	0.7860	**0.036**	0.0875	0.311
IFN-γ	0.7381	**0.037**	0.7857	**0.021**	0.0857	0.077
Thromboxane B3	0.2913	0.292	0.9712	**0.001**	0.5000	0.328
Leukotriene E4	0.2377	**0.014**	0.1773	0.527	0.4286	0.880
Prostaglandin B2	0.8345	**0.039**	0.1289	0.647	0.8129	**0.049**
HETE	-0.3414	0.213	0.2077	0.458	0.2143	0.428

Discussion





Improve severity score

↓ APACHE-II score

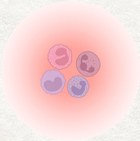
↓ SOFA score

↑ oxygenation (PaO_2/FiO_2)

Lab

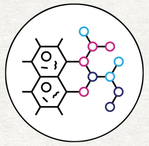
↓ hs-CRP

↓ IL-6 ↓ IL-8 ↓ IFN-γ



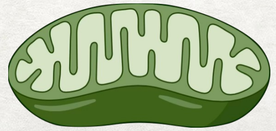
↓ inflammatory markers

↓ thromboxane B3 ↓ leukotriene E4 ↓ prostaglandin B2 ↓ HETE



↓ metabolomic profiles

↓ mitochondrial function (complex IV)



Discussion

- **Strength**
  - First study in mechanism of amelioration in hemoperfusion therapy through inflammatory, metabolomic, & mitochondrial process
  - Same concept as other sepsis
  - Serve as potential adjunctive treatment to timely appropriate antimicrobials & other medical therapy
- **Limitation**
  - Limited patients due to end of pandemic
  - Critical COVID-19 patients usually need prompt decision with early treatment to cease disease progression → not conducted in randomized trial

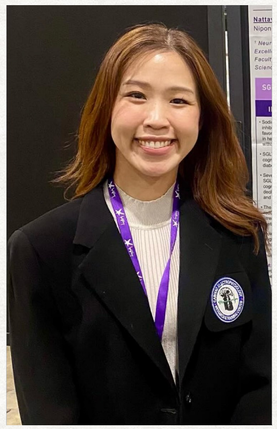
Conclusion

- Hemoperfusion therapy could mitigate the severity of COVID-19-associated ARDS via the amelioration of inflammatory markers, metabolomic profiles, and mitochondrial function.
- Further prospective randomized-controlled trial in patient with resemblant setting such as sepsis

Acknowledgements

Cardiac Electrophysiology Research & Training (CERT) Center Team



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