

Similarities and differences between the immunopathogenesis of COVID-19-related pediatric inflammatory multisystem syndrome and Kawasaki disease

Supplementary material

Index

Supplementary Methods: 2

 Supplementary references: 3

Supplementary Tables..... 4

Supplementary Figures 15

Supplementary Methods:

Patient definitions

Pre-pandemic Kawasaki Disease (KD) patients were diagnosed following the 2017 guidelines of the American Heart Association (1).

Complete KD was defined by the presence of ≥ 5 days of fever and ≥ 4 of the 5 classic criteria for KD. These classic criteria include

- (1) bilateral non-exudative conjunctival injection;
- (2) oral mucosal changes, such as erythema of the lips or strawberry tongue;
- (3) changes of the extremities (edema, erythema and/or desquamation);
- (4) polymorphous rash and
- (5) cervical lymphadenopathy.

In the presence of >4 principal clinical criteria, the diagnosis may be made with only 4 days of fever. Incomplete KD should be considered in any infant or child with prolonged unexplained fever, fewer than 4 of the principal clinical findings, and compatible laboratory or echocardiographic findings.

MIS-C patients were diagnosed according to WHO (2) or Royal College criteria (3):

WHO CRITERIA (8/14)	UK Royal College Criteria (14/14)*
0–19 years Fever > 3 days 2 or more criteria - Rash or conjunctivitis - Hypotension or shock - Cardiac involvement (myocarditis, pericarditis, aneurysms or increased troponin or NT-proBNP) - Coagulopathy (increased PT, APTT or D Dimer) - Intestinal involvement (diarrhea, vomiting or abdominal pain) - Increased CRP, ESR and / or PCT Positive for SARS-CoV-2 infection by RT-PCR, serology, or antigen test, or COVID-19 exposure within 4 weeks prior to the onset of symptoms	A child presenting with persistent fever (> 38.5 °C), inflammation (neutrophilia, elevated CRP, and lymphopenia) and evidence of single or multiorgan dysfunction (shock, cardiac, respiratory, kidney, gastrointestinal, or neurological disorder) with additional features*. This may include children fulfilling full or partial criteria for KD. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus (waiting for results of these investigations should not delay seeking expert advice). SARS-CoV-2 RT-PCR test results may be positive or negative.

APTT activated partial thromboplastin time, CK creatine kinase, COVID-19 coronavirus disease 2019, CRP C-reactive protein, ESR erythrocyte sedimentation rate, KD Kawasaki disease, NT-proBNP N-terminal pro-B-type natriuretic peptide, PT prothrombin time, PCT: pralcalitonin, RT-PCR reverse transcriptase–polymerase chain reaction, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

* Circulating cytokine levels were analyzed in 13/14 patients.

COVID patients were defined as pediatric patients with positive SARS-CoV-2 nasopharyngeal RT-PCR. Finally, **healthy controls** were pediatric patients without immune-related pathologies requiring surgery (e.g. phimosis patients) with negative SARS-CoV-2 nasopharyngeal RT-PCR and SARS-CoV-2 specific serology.

Supplementary references:

1. McCrindle BW et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association [Internet]. *Circulation*. 2017;135(17). doi:10.1161/CIR.0000000000000484
2. World Health Organization. *Multisystem inflammatory syndrome in children and adolescents temporally related to COVID-19* [Internet]. 2020:
3. Royal College of Paediatrics and Child Health. *Guidance: Paediatric multisystem inflammatory syndrome temporally associated with COVID-19* [Internet]. 2020:
4. Kanegaye JT et al. Recognition of a Kawasaki disease shock syndrome [Internet]. *Pediatrics*. 2009;123(5). doi:10.1542/peds.2008-1871

Supplementary Tables

- Supplementary Table 1** Detailed clinical characteristics of each patient by group.
- Supplementary Table 2** Detailed laboratory results of each patient.
- Supplementary Table 3** Circulating cytokine levels expressed as Log2 Fold Change for MIS-C patients, KD, and COVID patients and HC.
- Supplementary Table 4** Circulating cytokine levels expressed as Log2 Fold Change for MIS-C versus MIS-C^{plus} patients.
- Supplementary Table 5** Clinical parameters in MIS-C versus MIS-C^{plus} patients.
- Supplementary Table 6** Laboratory parameters in MIS-C versus MIS-C^{plus} patients.
- Supplementary Table 7** Number of serum and plasma samples among groups:

Supplementary Table 1. Detailed clinical characteristics of each patient by group

<i>Group</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>	<i>KD</i>
<i>ID</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Sex</i>	F	M	M	M	M	F	F	F	M	M	M		M	F
<i>Age (y)</i>	5,00	4,00	4,00	2,00	1,50	4,00	1,50	2,00	6,00	2,00	2,00	0,58	0,50	0,75
<i>Ethnicity</i>	White	White	White	N- African	Latin	White	White	Latin	White	White	Asian	White	N- African	N-African
<i>Overweight</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>Previously existing conditions</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>Fever</i>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<i>Total Fever duration (days)</i>	9	5	4	4	4	15	6	5	8	6	10	4	5	7
<i>Respiratory symptoms</i>	No	Yes	No	No	No	No	Yes	No	No	No	No	No	No	No
<i>Gastrointestinal symptoms</i>	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No	Yes	Yes
<i>Neurological symptoms</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>Bilateral Conjunctivitis</i>	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<i>Erythema of the mouth or pharynx</i>	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<i>Peripheral extremity changes</i>	No	No	No	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No
<i>Polymorphous rash</i>	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No
<i>Lymphadenopathy</i>	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	No	No	No	Yes	No
<i>Diagnosis</i>	IKD	KD	KD	KD shock	KD	IKD	IKD	KD shock	KD	KD	KD shock	KD	KD	IKD
<i>Echo-cardiographic result</i>	LC Ectasia	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Aneurysm
<i>Vascular hyperpermeability</i>	No	No	No	Yes	No	No	No	Yes	No	No	Yes	No	No	No
<i>Shock</i>	No	No	No	Yes	No	No	No	Yes	No	No	Yes	No	No	No
<i>Number of OSIs (total) 0 to 8</i>	2	2	3	4	2	1	3	4	3	1	3	2	3	3
<i>Outcome</i>	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	CA
<i>IVIG</i>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<i>Day of illness on which treatment was given</i>	5	5	5	2	5	5	6	1	8	1	5	5	5	8
<i>Second IVIG dose received</i>	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No
<i>Systemic glucocorticoid</i>	Yes	No	No	Yes	No	No	No	No	Yes	No	No	No	Yes	Yes
<i>IL-6 inhibitor</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>IL-1 inhibitor</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>Anticoagulation therapy</i>	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS
<i>COVID contact</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary material

PCR NF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCR stools	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IgG - SARS-CoV-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IgA - SARS-CoV-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IgM - SARS-CoV-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Group	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C	MIS-C^{plus}	MIS-C^{plus}	MIS-C^{plus}	MIS-C^{plus}	MIS-C^{plus}
ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sex	M	F	F	F	M	F	F	M	M	M	M	F	M	F
Age (y)	2,42	1,50	3,00	3,00	1,83	1,00	11,00	14,00	3,00	0,25	1,67	11,67	7,00	2,75
Ethnicity	White	Latin	White	White	White	N-African	White	Latin	White	Asian	White	White	White	Black
Overweight	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
Previously existing conditions	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total Fever duration (days)	8	5	7	5	5	3	10	6	6	5	6	11	5	10
Respiratory symptoms	No	No	Yes	No	No	No	No	Yes	No	No	No	No	No	Yes
Gastrointestinal symptoms	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neurological symptoms	No	No	No	No	No	No	No	Yes ¹	No	Yes ²	Yes ³	No	Yes ⁴	Yes ⁵
Bilateral Conjunctivitis	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Erythema of the mouth or pharynx	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes	Yes
Peripheral extremity changes	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Polymorphous rash	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Lymphadenopathy	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes	No	Yes	No
Diagnosis	IKD	KD	KD	KD	KD	MIS-C + shock	IKD	MIS-C + shock	KD	IKD	KD	IKD	KD	MIS-C + shock
Echo-cardiographic result	Normal	CA	Normal	Normal	Normal	MD	normal	MD	Normal	CA	Normal	Normal	Normal	CA
Vascular hyperpermeability	No	Yes	No	Yes	No	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Shock	No	No	No	No	No	Yes	No	Yes	No	No	No	No	No	Yes
Number of OSIs (total) 0 to 8	2	3	3	2	1	6	3	4	2	2	5	4	6	6
Outcome	Good	CA	Good	Good	Good	Good	Good	Good	Good	CA	Good	Good	Good	CA
IVIG	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes

Supplementary material

<i>Day of illness on which treatment was given</i>	9	7	7	5	5	3	8	7	5	5	4	No	6	5
<i>Second dose IVIG received</i>	Yes	Yes	No	No	No	No	No	No	No	No	Yes	No	No	No
<i>Systemic glucocorticoid</i>	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes
<i>IL-6 inhibitor</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>IL-1 inhibitor</i>	No	No	No	No	No	No	No	No	No	No	No	No	No	No
<i>Anticoagulation therapy</i>	AAS	AAS	AAS	AAS	AAS	Hep	No	Hep	AAS	AAS + Clopidogrel	AAS	No	AAS	AAS
<i>COVID contact</i>	NO	NO	NO	NO	NO	No	NO	YES	NO	NO	NO	YES	NO	YES
<i>PCR NF</i>	NEG	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS
<i>PCR stools</i>	ND	ND	ND	ND	ND	ND	ND	NEG	ND	NEG	POS	ND	NEG	POS
<i>IgG - SARS-CoV-2</i>	NEG	NEG	ND	NEG	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS
<i>IgA - SARS-CoV-2</i>	NEG	NEG	ND	NEG	NEG	NEG	POS	POS	POS	NEG	Undet	POS	POS	POS
<i>IgM - SARS-CoV-2</i>	NEG	NEG	NR	NEG	NEG	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG

¹Headache; ²Irritability; ³Headache and febrile seizure; ⁴Headache; ⁵Nuchal rigidity

Group	mild COVID	mild COVID	mild COVID	mild COVID	mild COVID	mild COVID	mild COVID	mild COVID	mild COVID
ID	1	2	3	4	5	6	7	8	9
Sex	F	M	F	F	M	M	M	F	F
Age (y)	14,00	11,00	10,00	7,00	9,00	1,58	12,00	0,08	14,00
Ethnicity	White	Latin	Latin	Latin	Latin	White	Latin	Latin	White
Overweight	No	No	No	No	No	No	No	No	No
Previously existing conditions	No	No	No	No	Yes*	No	No	No	No
Fever	39	No	No	No	Yes	Yes	No	Yes	Yes
Total Fever duration (days)	2	No	No	No	2	3	No	2	4
Respiratory symptoms	Yes	No	No	No	No	No	No	No	Yes
Gastrointestinal symptoms	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Neurological symptoms	No	Yes	No	No	No	No	Yes	No	No
Bilateral Conjunctivitis	No	No	No	No	No	No	No	No	No
Erythema of the mouth or pharynx	No	No	No	No	No	No	No	No	No
Peripheral extremity changes	No	No	No	No	No	No	No	No	No
Polymorphous rash	No	No	No	No	Yes	No	No	No	No
Lymphadenopathy	No	No	No	No	No	Yes	No	No	No
Diagnosis	ND	ND	ND	ND	ND	ND	ND	ND	ND
Echo-cardiographic result	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vascular hyperpermeability	No	No	No	No	No	No	No	No	No
Shock	No	No	No	No	No	No	No	No	No
Number of OSIs (total) 0 to 8	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary material

Outcome	Good	Good	Good	Good	Good	Good	Good	Good	Good
IVIIG	No	No	No	No	No	No	No	No	No
Day of illness on which treatment was given	No	No	No	No	No	No	No	No	No
Second dose received	No	No	No	No	No	No	No	No	No
Systemic glucocorticoid	No	No	No	No	No	No	No	No	No
IL6 inhibitor	No	No	No	No	No	No	No	No	No
IL1 inhibitor	No	No	No	No	No	No	No	No	No
Anticoagulation therapy	No	No	No	No	No	No	No	No	No
COVID contact	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
PCR NF	POS	POS	POS	POS	POS	POS	POS	POS	POS
PCR stools	ND	ND	ND	ND	ND	ND	ND	ND	ND
IgG - SARS-CoV-2	POS	POS	POS	POS	POS	POS	POS	NEG	NEG
IgA - SARS-CoV-2	POS	POS	NEG	POS	NEG	POS	POS	NEG	NEG
IgM - SARS-CoV-2	NEG	POS	POS	POS	NEG	POS	POS	NEG	NEG

KD: Kawasaki disease pre-SARS-CoV-2 pandemics; MIS-C: multisystem inflammatory syndrome in children; COVID: pediatric patients with mild COVID-19 disease without MIS-C. N-African: North African; CA: coronary aneurysm; MD: myocardial dysfunction; KD: complete Kawasaki disease; IKD: incomplete Kawasaki disease; LC ectasia: left coronary ectasia; Hep: Heparine; AAS: acetylsalicylic acid; NEG: negative; POS: positive; Indet: indeterminable. ND: not determined. y: years; Hb: hemoglobin; ESR: erythrocyte sedimentation rate; PCT: procalcitonin; CRP: C reactive protein; NT-ProBNP: NT-Pro-brain natriuretic peptide; ALT: alanine transaminase; LDH: lactate dehydrogenase.

Supplementary Table 2. Detailed analytic results of each patient

Group	ID	Age (y)	Hb (g/dL)	Platelets·10 ³ /mm ³ of blood	Lymphocytes /mm ³	Neutrophils /mm ³	Monocytes /mm ³	D-dimer (mg/L)	ESRmm /hour	PCT (ng/ml)	CRP (mg/L)	Ferritin (µg/L)	NTPro-BNP (ng/L)	Na (mmol/L)	ALT (U/L)	LDH (U/L)	Albumin (g/L)
KD	1	5.00	9.6	754	6.400	7100	500	22	ND	0.08	95	124.9	80	135	8.00	289	35
KD	2	4.00	12.0	482	5.800	16400	924	33	ND	0.58	152	176.5	1.345	135	196.00	1144	38
KD	3	4.00	11.6	344	2.700	5600	700	36	ND	0.51	36.9	173.0	411	136	11.00	369	42
KD	4	2.00	11.2	123	1.000	3286	212	2	ND	2.60	224	800.9	552	131	42.00	897	29
KD	5	1.50	9.8	321	8.200	6191	453	33	ND	3.09	47.7	116.0	927	138	12.00	693	40
KD	6	4.00	10.4	798	5.800	5100	600	11	ND	0.18	62	538.0	ND	136	17.00	492	39
KD	7	1.50	10.0	439	6.440	15640	690	47	ND	0.62	407	406.2	1.882	133	18.00	488	36
KD	8	2.00	9.7	294	1.728	10600	600	80	ND	0.37	218.2	206.2	6.714	142	153.00	845	33
KD	9	6.00	10.9	193	1.800	11600	300	12	ND	1.25	110.9	345.7	5.913	137	108.00	541	23
KD	10	2.00	12.1	328	6.200	10528	1504	40	ND	1.63	122.9	173.4	1.307	133	17.00	554	41
KD	11	2.00	11.6	457	7.434	3422	590	55	ND	4.42	67.7	193.6	3.530	ND	38.00	560	26
KD	12	0.58	10.0	586	4.305	14555	615	40	ND	0.27	110.3	136.1	1.445	136	15.00	774	38
KD	13	0.50	10.5	449	2.574	14850	594	54	ND	4.41	301.5	849.4	3.119	136	19.00	576	31
KD	14	0.75	10.5	997	5.300	ND	ND	14	ND	0.20	204	ND	2.100	135	ND	404	28
MIS-C	1	2.42	12.3	456	4.500	2700	2000	46	1.12	0.04	59.9	236.8	131	135	21	661	40
MIS-C	2	1.50	9.8	444	7.300	13500	2700	83	ND	0.57	146.6	96.4	1416	136	25	691	37
MIS-C	3	3.00	8.2	509	2.100	8800	900	23	ND	0.14	33.2	96.1	457	137	28	517	35
MIS-C	4	3.00	10.1	129	1.700	300	500	2	ND	0.21	42.7	450	291	137	34	675	36
MIS-C	5	1.83	11.8	373	3.100	3900	700	43	ND	0.11	46.6	66	178	137	13	891	46
MIS-C	6	1.00	11.8	216	800	700	300	ND	6.3	6.7	364	786	3274	144	10	442	28
MIS-C	7	11.00	12	117	400	400	100	6	4.32	3.97	276.5	>2000	2930	129	92	799	ND
MIS-C	8	14.00	8.5	494	700	16400	1100	ND	1.34	5.4	322.9	393	22668	141	85	671	35
MIS-C	9	3.00	10.9	296	2.200	14000	200	63	ND	6.74	165.9	67	7994	135	14	606	35
MIS-C ^{plus}	10	0.25	7.9	249	4.000	6200	800	2	ND	0.51	128	219	3628	136	7	684	23
MIS-C ^{plus}	11	1.67	12.1	112	1.400	1500	200	14	ND	3.1	73.5	358	647	132	106	754	38
MIS-C ^{plus}	12	11.67	10.8	134	700	4200	200	ND	1.88	2.11	208.4	1380	10165	137	49	402	ND
MIS-C ^{plus}	13	7.00	11.3	188	1800	4300	100	7	9.22	2.45	168.6	604.9	8216	144	32	556	24
MIS-C ^{plus}	14	2.75	7.8	170	10.400	17300	700	ND	6.4	4.65	229.6	399.6	4840	134	22	707	20
COVID	1	14.00	13.3	257	2000	2900	ND	ND	ND	0.02	0.4	51.2	ND	ND	12	308	44
COVID	2	11.00	12.5	301	2700	3600	ND	ND	ND	0.02	0.7	ND	ND	136	45	670	ND
COVID	3	10.00	13.2	338	3200	3200	ND	ND	ND	0.01	0.2	ND	ND	138	19	ND	ND
COVID	4	7.00	13.4	305	3300	7000	ND	ND	ND	ND	ND	ND	ND	139	12	ND	ND
COVID	5	9.00	11.6	251	1500	2400	ND	ND	ND	0.25	12.2	3344	ND	138	126	566	ND
COVID	6	1.58	6.8	316	1900	7300	ND	ND	ND	ND	48.8	39.5	ND	ND	13	564	ND
COVID	7	12.00	13.5	235	2900	4200	ND	ND	ND	0.01	0.2	ND	ND	136.9	16	383	ND
COVID	8	0.08	10	304	3000	500	ND	ND	ND	0.1	1.4	ND	ND	ND	ND	ND	ND
COVID	9	14.00	10.9	310	1200	2000	ND	ND	0.61	0.02	23.1	98.8	ND	140	7	345	ND

KD: Kawasaki disease pre-SARS-CoV-2 pandemics; MIS-C: multisystem inflammatory syndrome in children; COVID: pediatric patients with mild COVID-19 disease without MIS-C. ND: not determined. y: years; Na: sodium Hb: hemoglobin; ESR: erythrocyte sedimentation rate; PCT: procalcitonin; CRP: C reactive protein; NT-ProBNP: NT-Pro-brain natriuretic peptide; ALT: alanine transaminase; LDH: lactate dehydrogenase.

Supplementary Table 3. Circulating cytokine levels expressed as Log2 Fold Change for MIS-C patients, KD, and COVID patients and HC.

	KD		MIS-C		mild COVID		HC		Kruskal-Wallis	HC	HC	HC	KD	MIS-C	KD
	Median	SD	Median	SD	Median	SD	Median	SD		MIS-C	KD	COVID	MIS-C	COVID	COVID
Eotaxin	1.05	1.32	0.96	1.09	1.51	0.95	0.00	1.20	0.030	0.606	0.042	0.006	1.000	0.348	1.000
GM-CSF	1.47	0.87	2.14	1.81	0.77	0.58	0.00	0.45	0.000	0.000	0.000	0.000	1.000	0.246	0.432
GROα	0.69	1.00	0.51	1.22	0.76	0.63	0.00	0.21	0.000	0.001	0.004	0.001	1.000	1.000	1.000
IFN-α	0.46	0.43	0.56	0.55	0.37	0.50	0.00	0.40	0.008	0.002	0.017	0.659	1.000	1.000	1.000
IFN-γ	1.86	0.83	2.77	1.45	0.53	0.66	0.00	0.31	0.000	0.000	0.000	0.003	0.960	0.012	0.018
IL-10	1.84	1.15	0.95	2.24	-0.03	0.73	0.00	0.33	0.000	0.000	0.000	1.000	1.000	0.036	0.042
IL-12p70	0.92	0.95	1.09	0.87	0.75	0.67	0.00	0.33	0.000	0.000	0.000	0.000	1.000	1.000	1.000
IL-13	1.02	0.86	1.12	1.25	0.78	0.53	0.00	0.19	0.000	0.000	0.000	0.000	1.000	0.774	1.000
IL-15	0.89	0.72	1.04	0.87	0.47	0.52	0.00	0.52	0.000	0.000	0.000	0.009	1.000	0.570	0.744
IL-17A	1.50	0.99	1.15	0.98	0.26	0.86	0.00	0.51	0.000	0.000	0.000	0.056	1.000	1.000	0.498
IL-18	2.24	1.20	2.39	2.19	0.24	0.75	0.00	0.60	0.000	0.002	0.000	1.000	1.000	0.138	0.018
IL-1α	1.76	0.92	1.93	0.99	1.80	0.79	0.00	0.85	0.000	0.000	0.000	0.001	1.000	1.000	1.000
IL-1β	1.33	0.98	1.20	1.09	0.46	0.71	0.00	0.38	0.000	0.000	0.000	0.006	1.000	0.492	0.498
IL-1RA	0.92	1.46	0.62	1.97	-0.53	0.68	0.00	0.35	0.003	0.195	0.002	0.073	1.000	0.024	0.006
IL-2	0.63	1.27	1.18	1.42	0.00	0.88	0.00	0.33	0.149	NA	NA	NA	NA	NA	NA
IL-21	1.27	1.28	0.80	1.13	0.38	0.52	0.00	0.66	0.000	0.002	0.000	0.646	1.000	0.348	0.030
IL-22	0.56	1.26	0.30	1.15	-0.37	0.51	0.00	0.85	1.000	NA	NA	NA	NA	NA	NA
IL-23	0.84	1.27	1.29	1.20	0.53	0.51	0.00	0.27	0.000	0.000	0.004	0.003	1.000	0.888	1.000
IL-27	0.83	1.02	0.90	1.09	0.45	0.76	0.00	0.37	0.000	0.003	0.001	0.023	1.000	1.000	1.000
IL-31	1.23	1.38	2.44	1.33	1.06	1.00	0.00	0.19	0.000	0.000	0.000	0.000	1.000	1.000	1.000
IL-4	1.25	0.72	1.38	0.85	0.71	0.52	0.00	0.28	0.000	0.000	0.000	0.000	1.000	0.348	0.654
IL-5	1.00	0.95	1.11	1.27	0.48	0.76	0.00	0.55	0.000	0.000	0.000	0.023	1.000	1.000	0.498
IL-6	1.01	1.02	1.02	1.25	0.54	0.64	0.00	0.42	0.000	0.000	0.000	0.582	1.000	0.294	0.078
IL-7	0.98	1.10	1.06	0.96	0.53	0.90	0.00	0.40	0.000	0.001	0.000	0.189	1.000	1.000	0.498
IL-8	0.64	0.82	1.30	1.72	0.02	0.54	0.00	0.62	0.000	0.000	0.000	1.000	1.000	0.006	0.030
IL-9	1.51	0.90	1.41	1.06	0.96	0.57	0.00	0.35	0.000	0.000	0.000	0.000	1.000	0.072	0.654
IP-10	5.04	1.88	5.33	1.89	1.86	1.18	0.00	0.78	0.000	0.000	0.000	0.028	1.000	0.006	0.006
MCP-1	2.90	2.15	1.82	2.99	-0.05	0.61	0.00	0.77	0.011	0.006	0.007	1.000	1.000	0.072	0.372
MIP-1α	0.98	1.97	0.64	1.63	-1.20	1.63	0.00	1.28	1.000	NA	NA	NA	NA	NA	NA
MIP-1β	-0.29	1.18	0.52	1.72	-0.86	0.93	0.00	0.36	1.000	NA	NA	NA	NA	NA	NA
RANTES	3.02	1.30	3.56	1.85	0.69	1.66	0.00	0.56	0.000	0.000	0.000	0.086	1.000	0.036	0.066
SDF-1	-1.49	0.78	-1.05	0.98	-1.56	0.63	0.00	0.60	0.000	0.004	0.000	0.000	1.000	0.348	1.000
TNF-α	0.55	1.06	0.77	1.40	-0.19	0.77	0.00	0.22	0.158	NA	NA	NA	NA	NA	NA
TNF-β	1.23	1.49	1.56	1.29	0.37	1.01	0.00	0.42	0.002	0.000	0.009	0.672	1.000	0.666	1.000

Log2 Fold Change has been calculated with respect to the median of the HC for each individual. KD: Kawasaki disease pre-SARS-CoV-2 pandemics; MIS-C: multisystem inflammatory syndrome in children; COVID: pediatric patients with mild COVID-19 disease without MIS-C; HC: healthy controls. Cytokine & Chemokine 34-Plex Human ProcartaPlex™ Panel 1A. Bonferroni correction was applied to Kruskal-Wallis test for 34 cytokines comparison. The Mann-Whitney test, with Bonferroni correction for multiple comparisons, was performed when the corrected Kruskal Wallis test was significant. SD: standard deviation

Supplementary Table 4. Circulating cytokine levels expressed as Log2 Fold Change for MIS-C versus MIS-C^{plus} patients.

	MIS-C		MIS-C ^{plus}		MIS-C ^{plus}	MIS-C ^{plus}	MIS-C
	Median	SD	Median	SD	MIS-C	KD	KD
GM-CSF	1.33	0.92	4.68	1.59	0.018	0.007	0.636
GROα	1.14	1.15	0.20	1.42	0.53	0.823	0.585
IFN-γ	1.68	1.12	3.71	0.79	0.01	0.001	0.799
IL-10	0.75	0.95	4.69	2.86	0.202	0.257	0.36
IL-18	0.61	1.61	4.90	1.60	0.018	0.01	0.224
IL-1α	1.80	0.95	2.65	0.39	0.005	0.01	0.689
IL-1RA	0.40	1.24	3.65	2.43	0.202	0.5	0.4
IL-2	1.40	1.43	0.96	1.57	0.876	0.964	0.913
IP-10	3.97	1.92	6.12	0.75	0.018	0.107	0.535
MCP-1	0.53	2.66	6.26	3.04	0.073	0.186	0.799
MIP-1β	-0.02	1.41	1.29	1.39	0.073	0.034	0.743
RANTES	3.10	1.54	6.09	1.23	0.018	0.005	0.856
SDF-1	-1.59	0.70	0.03	0.47	0.003	0.003	0.585

MIS-C^{plus} patients include those differentiated from the remaining MIS-C patients in the linear discriminant analysis. Cytokine & Chemokine 34-Plex Human ProcartaPlex™ Panel 1A (Figure 2D). Pair-wise comparisons were calculated with the Mann-Whitney test. MIS-C: multisystem inflammatory syndrome in children.

Supplementary Table 5. Clinical parameters in MIS-C versus MIS-C^{plus} patients.

		MIS-C (n=9)	MIS-C ^{plus} (n=5)	p
Age	Median (min-max)	3 (1-14)	2.75 (0.25-11.7)	0.9
Days of fever	Median (min-max)	6 (3-10)	6 (5-11)	0.52
Respiratory symptoms	POS (%)	22.2%	20.0%	0.92
Gastrointestinal symptoms	POS (%)	77.8%	100.0%	0.26
Neurological symptoms	POS (%)	11.1%	80.0%	0.02
Bilateral Conjunctivitis	POS (%)	77.8%	60.0%	0.48
Oral mucosal changes	POS (%)	77.8%	60.0%	0.48
Peripheral extremity changes	POS (%)	66.7%	60.0%	0.8
Polymorphous rash	POS (%)	77.8%	80.0%	0.92
Lymphadenopathy	POS (%)	55.6%	80.0%	0.36
Number of OSIS ¹	Median (min-max)	3 (1-6)	5(2-6)	0.09
Aneurysms	POS (%)	11.1%	40.0%	0.21
Cardiac dysfunction	POS (%)	22.2%	0.0%	0.26
Vascular hypermeability	POS (%)	44.4%	60.0%	0.58
Shock ²	POS (%)	22.2%	20.0%	0.92
PCR NF	POS (%)	22.2%	40.0%	0.48
PCR stools	POS (%), n/total)	0.0% (0/1)	50.0% (2/4)	0.36
IgG - SARS-CoV-2	POS (%)	50.0%	80.0%	0.28
IgA - SARS-CoV-2	POS (%)	37.5%	60.0%	0.21
	Indeterminate (%)	0.0%	20.0%	
IgM - SARS-CoV-2	POS (%)	12.5%	0.0%	0.41

¹ Organ systems involvement ² Kanegaye Kawasaki Shock definition criteria (4)

MIS-C: multisystem inflammatory syndrome in children. MIS-C^{plus} patients include those differentiated from the rest of MIS-C patients in the linear discriminant analysis. Cytokine & Chemokine 34-Plex Human ProcartaPlex™ Panel 1A (Figure 2D)

Supplementary Table 6. Laboratory parameters in MIS-C versus MIS-C^{plus} patients.

		MIS-C			MIS-C ^{plus}			p
Hb (g/dL)	Median, min, max	10.9	8.2	12.3	10.8	7.8	12.1	0.52
Platelets·10 ³ /mm ³	Median, min, max	373.0	117.0	509.0	170.0	112.0	249.0	0.08
Low Platelets	%	22.2%			40.0%			0.48
Lymphopenia	%	85.7%			60.0%			0.82
Neutrophils/mm ³	Median, min, max	3900.0	300.0	16400.0	4300.0	1500.0	17300.0	0.61
Neutrophilia	%	44.4%			40.0%			0.87
Monocytes/mm ³	Median, min, max	700.0	100.0	2700.0	200.0	100.0	800.0	0.24
Monocytosis	%	55.6%			40.0%			0.58
PCT (ng/ml)	Median, min, max	.57	0.04	6.74	2.45	0.51	4.65	0.80
Altered PCT	%	55.6%			100.0%			0.08
CRP (mg/L)	Median, min, max	146.6	33.2	364.0	168.6	73.5	229.6	0.70
Altered CRP	%	100.0%			100.0%			NC
Ferritin ug/L	Median, min, max	236.8	66.0	2000.0	399.6	219.0	1380.0	0.36
Altered Ferritin	%	55.6%			100.0%			0.08
NTPro-BNP ng/L	Median, min, max	1416.0	131.0	22668.0	4840.0	647.0	10165.0	0.15
Altered Pro-BNP	%	100.0%			100.0%			NC
ALT	Median, min, max	25.0	10.0	92.0	32.0	7.0	106.0	0.70
Altered ALT	%	33.3%			60.0%			0.33
LDH UI/L at diagnosis	Median, min, max	671.0	442.0	891.0	684.0	402.0	754.0	1.00
Altered LDH	%	44.4%			20.0%			0.36

MIS-C: multisystem inflammatory syndrome in children; Hb: hemoglobin; PCT: procalcitonin; CRP: C reactive protein; NT-ProBNP: NT-Pro-brain natriuretic peptide; ALT: alanine transaminase; LDH: lactate dehydrogenase. MIS-C^{plus} patients include those differentiated from the rest of MIS-C patients in the linear discriminant analysis. Cytokine & Chemokine 34-Plex Human ProcartaPlex™ Panel 1A (Figure 2D)

Supplementary table 7. Number of serum and plasma samples among groups:

	Serum	Plasma
KD	9	5
MIS-C	10	2
COVID	7	3
HC	22	31

KD: Kawasaki disease pre-SARS-CoV-2 pandemics; MIS-C: multisystem inflammatory syndrome in children; COVID: pediatric patients with mild COVID-19 disease without MIS-C; HC: healthy controls

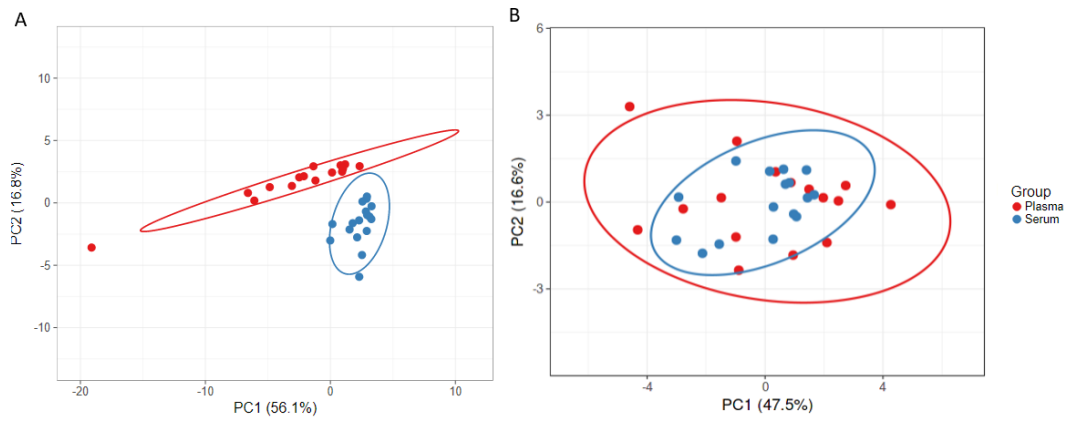
Supplementary Figures

Supplementary Figure 1. Principal component analysis of 34 circulating cytokine levels in serum and plasma pre- and post-normalization

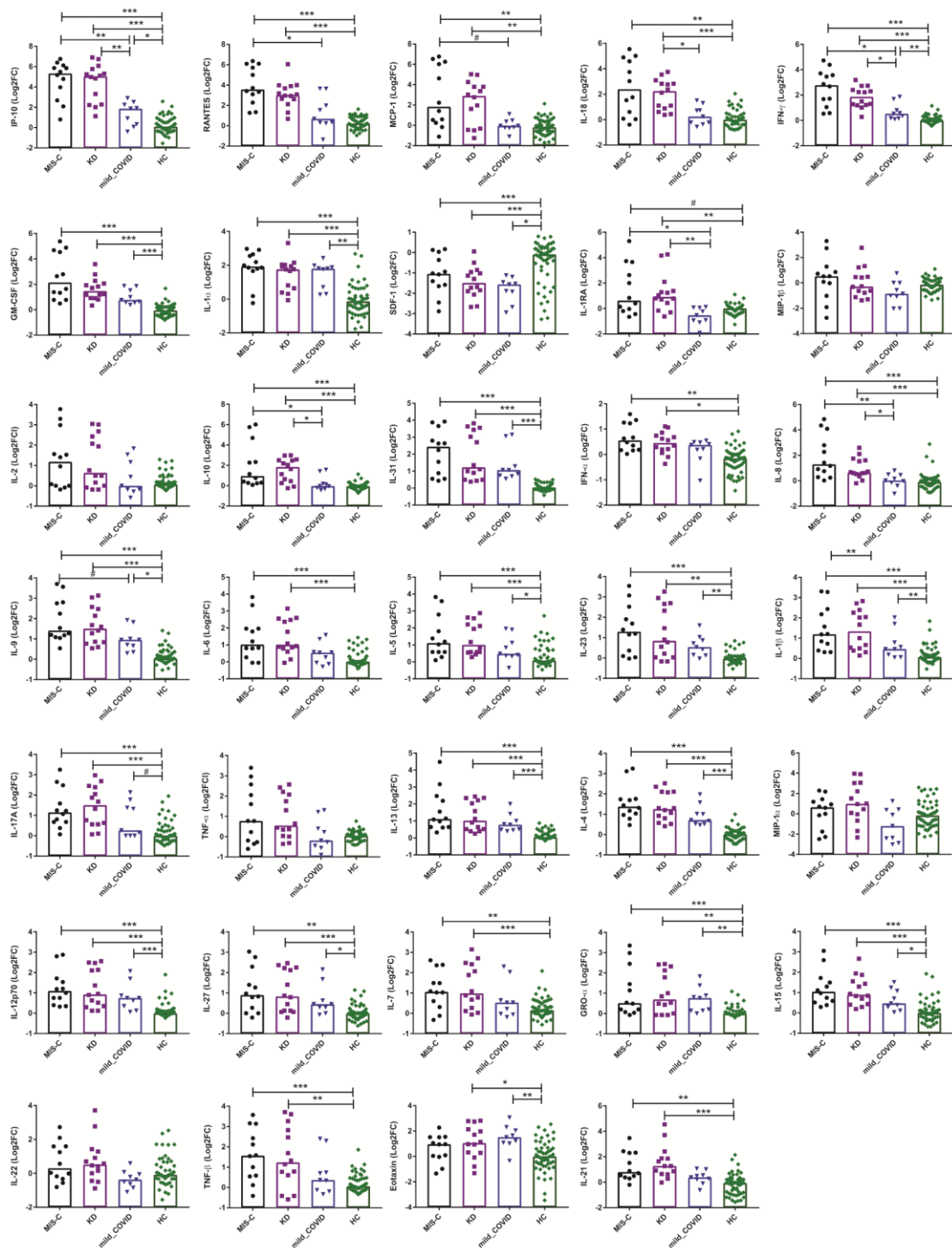
Supplementary Figure 2. Circulating cytokine levels among MIS-C, KD, COVID and HC groups.

Supplementary Figure 3. SARS-CoV-2 specific immune complex detection in MIS-C patients.

Supplementary Figure 4. Quantitative circulating levels of IL-17.

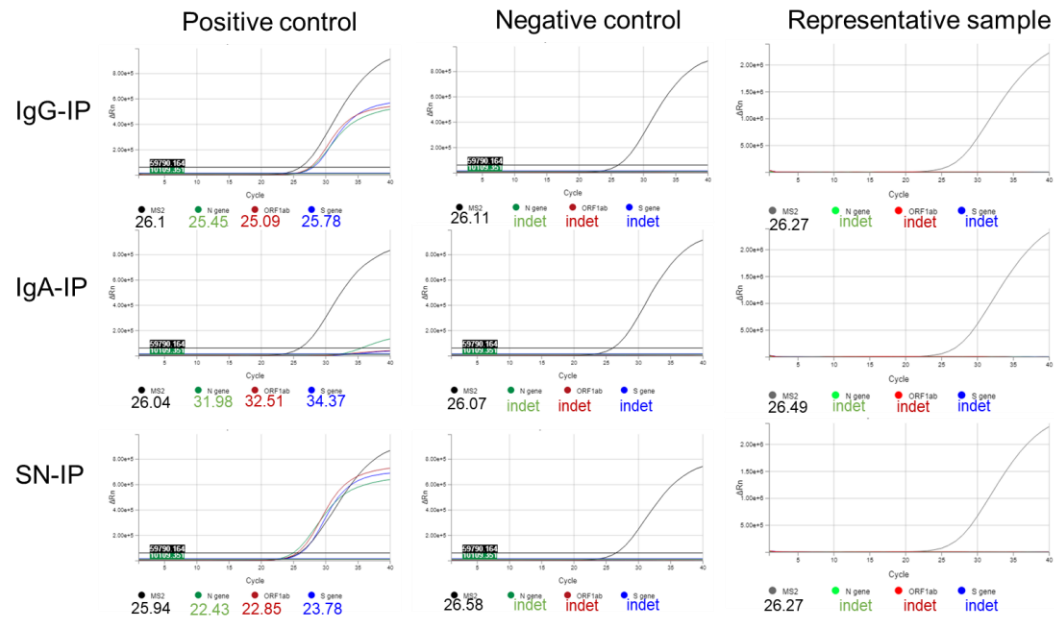


Supplementary Figure 1. Principal component analysis of 34 circulating cytokine levels in serum and plasma pre- and post-normalization. Principal component analysis was performed in serum and plasma samples (A) pre-normalization, consisting of raw median fluorescence intensity and (B) post-normalization after Log2-Fold Change transformation. Performed in serum and plasma samples extracted in parallel from 16 healthy controls.



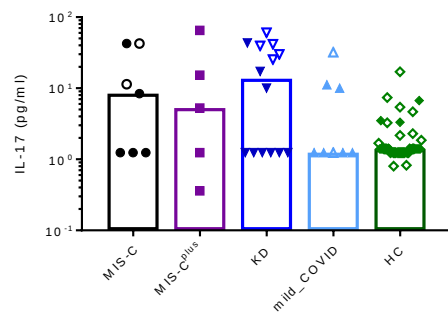
Supplementary Figure 2. Circulating cytokine levels among MIS-C, KD, COVID and HC groups.

Circulating cytokine levels from plasma or serum were evaluated using the Luminex technique and were normalized to HC using to Log2 Fold Change. Bonferroni correction was applied to Kruskal-Wallis test for comparison of 34 cytokines. The Mann-Whitney test, with Bonferroni correction for multiple comparisons, was performed when the corrected Kruskal Wallis test was significant. Bars represent median values. Significant differences among pairs are shown. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. KD: Kawasaki disease pre-SARS-CoV-2 pandemics ($n=14$); MIS-C: multisystem inflammatory syndrome in children ($n=13$); COVID: pediatric patients with mild COVID-19 disease without MIS-C ($n=9$); HC: healthy controls ($n=31$; 53 samples).



Supplementary Figure 3. SARS-CoV-2 specific immune complex detection in MIS-C patients.

Threshold cycle curves presented for a representative example. Negative and positive controls are shown. SARS-CoV-2 detection was performed before and after magnetic immune-precipitation (IP) of immunoglobulin (Ig) G and IgA. Pre-IP: serum or plasma before the IP; IgG-IP: IP of IgG; IgA-IP: IP of IgA of the free fraction resulting from IgG-IP; SN-post-IP: free fraction after IgG and IgA IP MS2: internal quality control for RNA extraction process control; Indet: indetectable.



Supplementary Figure 4. Quantitative circulating levels of IL-17. Open symbols represent plasma samples and filled symbols are serum samples. Bars represent median values. KD: Kawasaki disease pre-SARS-CoV-2 pandemics (n=14); MIS-C: multi inflammatory syndrome in children (n=13); COVID: pediatric patients with mild COVID-19 disease without MIS-C (n=9); HC: healthy controls (n=37, 53 samples)