THE LANCET
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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Rodriguez-Smith J J, Verweyen E L, Clay G M, et al. Inflammatory biomarkers in COVID-19-associated multisystem inflammatory syndrome in children, Kawasaki disease, and macrophage activation syndrome: a cohort study. *Lancet Rheumatol* 2021; published online June 8. https://doi.org/10.1016/S2665-9913(21)00139-9.

Appendix Table 1: Clinical manifestations, hospitalization details, and treatments by disease groups separated by sex

									Stratification of MIS-C group[Ω]			
	SJIA (N=11)		MAS (N=9)		KD (N=9)		MIS-C (N=19)		Low CXCL9 (N=5)		High CXCL9 (N=10)	
	Male (n=5)	Female (n=6)	Male (n=5)	Female (n=4)	Male (n=2)	Female (n=7)	Male (n=11)	Female (n=8)	Male (n=1)	Female (n=4)	Male (n=5)	Female (n=5)
Fever	5 (100%)	6 (100%)	5 (100%)	4 (100%)	2 (100%)	7 (100%)	11 (100%)	8 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
Rash	3 (60%)	5 (83%)	5 (100%)	4 (100%)	2 (100%)	5 (71%)	7 (64%)	5 (63%)	0 (0%)	3 (75%)	3 (60%)	3 (60%)
Conjunctival injection	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (50%)	5 (71%)	7 (64%)	4 (50%)	0 (0%)	4 (100%)	3 (60%)	3 (60%)
Mucositis	0 (0%)	1 (17%)	1 (20%)	0 (0%)	1 (50%)	6 (86%)	3 (27%)	4 (50%)	0 (0%)	1 (25%)	2 (40%)	4 (80%)
Swelling of hands/feet	0 (0%)	2 (33%)	0 (0%)	0 (0%)	1 (50%)	4 (57%)	3 (27%)	4 (50%)	0 (0%)	1 (25%)	2 (40%)	2 (40%)
Adenopathy	2 (40%)	2 (33%)	4 (80%)	0 (0%)	1 (50%)	5 (71%)	5 (45%)	3 (38%)	0 (0%)	3 (75%)	1 (20%)	1 (20%)
Abdominal pain	0 (0%)	1 (17%)	3 (60%)	2 (50%)	0 (0%)	0 (0%)	8 (73%)	5 (63%)	1 (100%)	3 (75%)	3 (60%)	3 (60%)
Diarrhea	0 (0%)	1 (17%)	1 (20%)	0 (0%)	0 (0%)	3 (43%)	6 (55%)	4 (50%)	0 (0%)	3 (75%)	1 (20%)	4 (80%)
Headache	1 (20%)	3 (50%)	1 (20%)	0 (0%)	0 (0%)	2 (29%)	8 (73%)	4 (50%)	1 (100%)	3 (75%)	4 (80%)	3 (60%)
Respiratory symptoms	1 (20%)	2 (33%)	1 (20%)	1 (25%)	1 (50%)	2 (29%)	6 (55%)	4 (50%)	1 (100%)	3 (75%)	1 (20%)	3 (60%)
Pharyngitis	1 (20%)	4 (67%)	0 (0%)	1 (25%)	0 (0%)	1 (14%)	5 (45%)	5 (63%)	0 (0%)	1 (25%)	3 (60%)	3 (60%)
Arthritis	3 (60%)	3 (50%)	4 (80%)	2 (50%)	0 (0%)	2 (29%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hepatomegaly	1 (20%)	0 (0%)	2 (40%)	1 (25%)	0 (0%)	1 (14%)	1 (9%)	1 (13%)	0 (0%)	0 (0%)	1 (20%)	1 (20%)
Splenomegaly	1 (20%)	0 (0%)	2 (40%)	1 (25%)	0 (0%)	0 (0%)	1 (9%)	1 (13%)	0 (0%)	0 (0%)	1 (20%)	1 (20%)
Acute kidney injury	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (27%)	3 (38%)	0 (0%)	0 (0%)	3 (60%)	3 (60%)
Shock*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (57%)	7 (64%)	6 (75%)	1 (100%)	1 (25%)	5 (100%)	4 (80%)
requiring fluid bolus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (57%)	7 (64%)	6 (75%)	1 (100%)	1 (25%)	5 (100%)	4 (80%)
requiring inotropes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14%)	3 (27%)	2 (25%)	1 (100%)	0 (0%)	2 (40%)	1 (20%)
Coronary changes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (57%)	2 (18%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)
Myocardial dysfunction	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (43%)	4 (36%)	3 (38%)	0 (0%)	1 (25%)	3 (60%)	2 (40%)
Respiratory support	1 (20%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	3 (43%)	7 (64%)	4 (50%)	1 (100%)	2 (50%)	4 (80%)	2 (40%)
Oxygen only	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (29%)	7 (64%)	3 (38%)	1 (100%)	2 (50%)	4 (80%)	1 (20%)
HFNC	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14%)	2 (18%)	1 (13%)	0 (0%)	0 (0%)	2 (40%)	1 (20%)
CPAP or BiPAP	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Intubation ventilation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)

Mental status changes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (27%)	1 (13%)	0 (0%)	0 (0%)	3 (60%)	1 (20%)
Treatments[¥]	0 (0%)	2 (33%)	5 (100%)	4 (100%)	2 (100%)	7 (100%)	9 (82%)	8 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
ICU care	0 (0%)	0 (0%)	1 (20%)	1 (25%)	0 (0%)	3 (43%)	6 (55%)	6 (75%)	1 (100%)	2 (50%)	3 (60%)	3 (60%)
IVIG	0 (0%)	1 (17%)	0 (0%)	0 (0%)	2 (100%)	7 (100%)	9 (82%)	8 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
corticosteroids	0 (0%)	2 (33%)	4 (80%)	4 (100%)	0 (0%)	5 (71%)	8 (73%)	8 (100%)	1 (100%)	4 (100%)	4 (80%)	5 (100%)
IL-1 inhibitor	0 (0%)	0 (0%)	5 (100%)	4 (100%)	0 (0%)	0 (0%)	3 (27%)	2 (25%)	0 (0%)	1 (25%)	2 (40%)	1 (20%)
Infliximab	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (29%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
IL-6 inhibitor	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	1 (20%)	0 (0%)	2 (40%)[ǂ]	3 (75%)[ǂ]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
antibiotics	1 (20%)	3 (50%)	1 (20%)	0 (0%)	1 (50%)	6 (86%)	9 (82%)	5 (63%)	0 (0%)	4 (100%)	4 (80%)	3 (60%)
antiviral	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)

SJIA=systemic juvenile idiopathic arthritis, MAS=macrophage activation syndrome, KD=Kawasaki Disease, MIS-C=Multisystem inflammatory syndrome in Children

Biological female and male sex

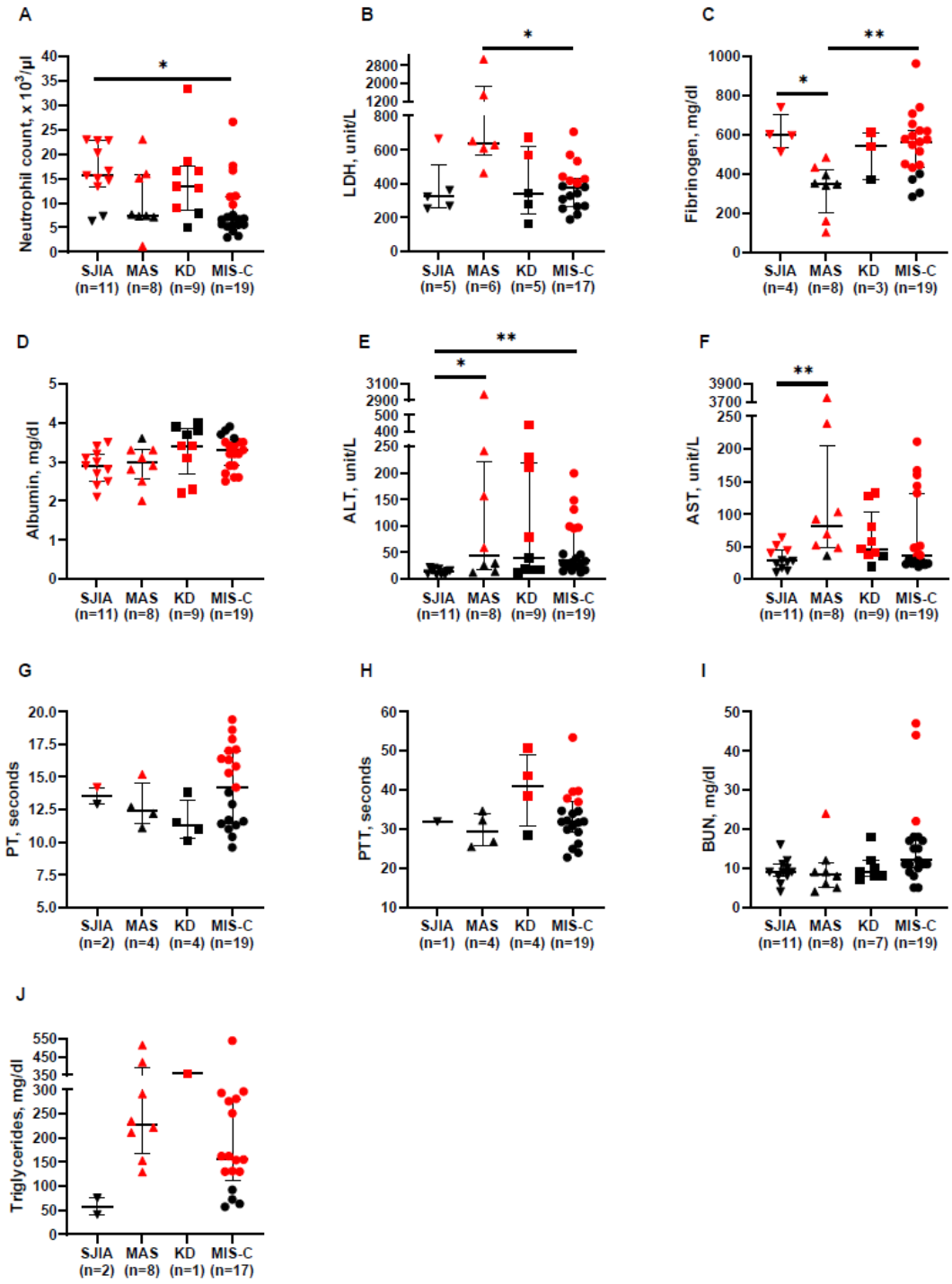
Ω 15 of the 19 MIS-C patients who had CXCL9 measured were furthered stratified based on CXCL9 levels below and above 739pg/ml.

Data are n (%), unless noted otherwise, all patients were included in percentage calculations.

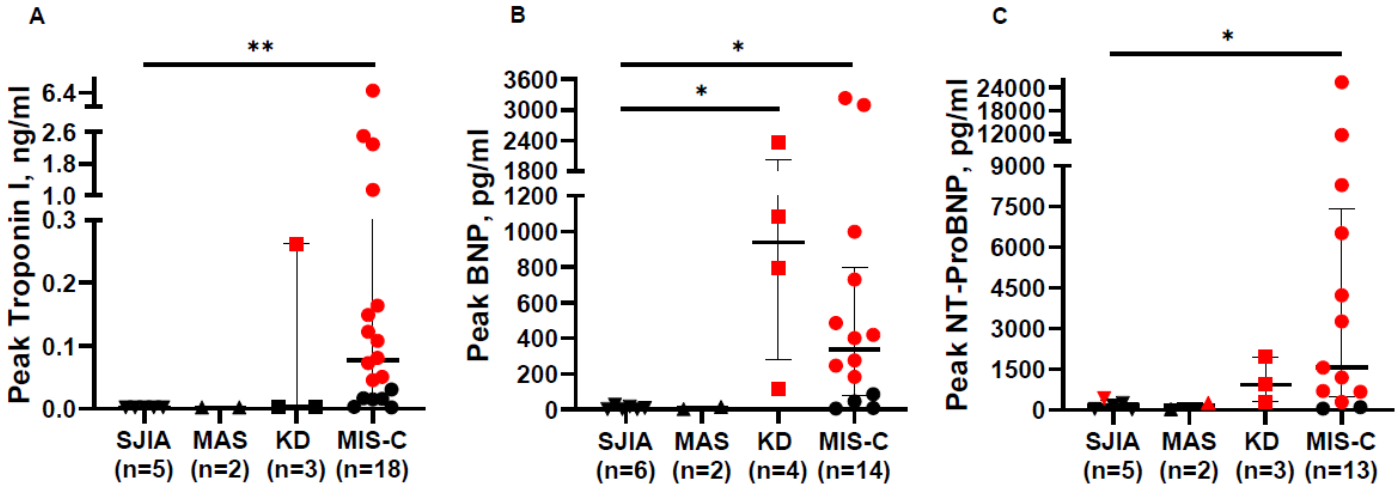
* Shock is needing inotrope support or fluid resuscitation >20ml/kg;

¥ refers to IVIG, steroids, IL-1 inhibitor, infliximab, or IL-6 inhibitor

ǂ other treatments refers cyclosporine (n=2) and emapulumab (n=3) ϵ Before admission, some MAS patients received following: tofacitinib and cyclophosphamide n=1; etoposide n=1; tocilizumab n=1



Appendix Figure 1: Comparison of hematology and laboratory results by disease groups. All data are first sets of labs obtained during admission. Red data points indicate outside the normal range for specific study. Data analyzed between SJIA vs MAS, MIS-C vs SJIA, MIS-C vs MAS, MIS-C vs KD using one-way ANOVA if data was parametric or Kruskal-Wallis if nonparametric and post hoc multiple comparison correction were made. Reported are median with 25 and 75% interquartile ranges (IQR) * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$



Appendix Figure 2: Comparison of cardiac markers by disease group. All data are peak values reported during admission. Red data points indicate outside the normal range for specific study. Data analyzed between SJIA vs MAS, SJIA vs KD, MIS-C vs SJIA, MIS-C vs MAS, MIS-C vs KD using one-way ANOVA if data was parametric or Kruskal-Wallis if nonparametric and post hoc multiple comparison correction were made. Reported are median with 25 and 75% interquartile ranges (IQR) * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

Appendix Table 2. Cut-off values of CXCL9 for the differentiation between MISC and KD and their coordinates on ROC analysis

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 54.00	100.0	79.61% to 100.0%	20.00	1.026% to 62.45%	1.250
> 147.0	100.0	79.61% to 100.0%	40.00	7.107% to 76.93%	1.667
> 256.0	93.33	70.18% to 99.66%	40.00	7.107% to 76.93%	1.556
> 350.5	93.33	70.18% to 99.66%	60.00	23.07% to 92.89%	2.333
> 477.0	93.33	70.18% to 99.66%	80.00	37.55% to 98.97%	4.667
> 535.0	93.33	70.18% to 99.66%	100.0	56.55% to 100.0%	
> 558.0	86.67	62.12% to 97.63%	100.0	56.55% to 100.0%	
> 590.5	80.00	54.81% to 92.95%	100.0	56.55% to 100.0%	
> 738.5	66.67	41.71% to 84.82%	100.0	56.55% to 100.0%	
> 1040	60.00	35.75% to 80.18%	100.0	56.55% to 100.0%	
> 1469	53.33	30.12% to 75.19%	100.0	56.55% to 100.0%	
> 2534	46.67	24.81% to 69.88%	100.0	56.55% to 100.0%	
> 3460	40.00	19.82% to 64.25%	100.0	56.55% to 100.0%	
> 4547	33.33	15.18% to 58.29%	100.0	56.55% to 100.0%	
> 5905	26.67	10.90% to 51.95%	100.0	56.55% to 100.0%	
> 6877	20.00	7.048% to 45.19%	100.0	56.55% to 100.0%	
> 7650	13.33	2.369% to 37.88%	100.0	56.55% to 100.0%	

Appendix Table 3. Cut-off values of S100A8/A9 for the differentiation between SJIA and KD and their coordinates on ROC analysis

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 6007	100	72.2% to 100%	12.5	0.641% to 47.1%	1.14
> 6881	100	72.2% to 100%	25.0	4.44% to 59.1%	1.33
> 10389	100	72.2% to 100%	37.5	13.7% to 69.4%	1.60
> 16504	100	72.2% to 100%	50.0	21.5% to 78.5%	2.00
> 21823	100	72.2% to 100%	62.5	30.6% to 86.3%	2.67
> 25124	100	72.2% to 100%	75.0	40.9% to 95.6%	4.00
> 27673	100	72.2% to 100%	87.5	52.9% to 99.4%	8.00
> 29577	100	72.2% to 100%	100	67.6% to 100%	
> 32701	90.0	59.6% to 99.5%	100	67.6% to 100%	
> 37592	80.0	49.0% to 96.4%	100	67.6% to 100%	
> 43414	70.0	39.7% to 89.2%	100	67.6% to 100%	
> 56879	60.0	31.3% to 83.2%	100	67.6% to 100%	
> 76983	50.0	23.7% to 76.3%	100	67.6% to 100%	
> 94003	40.0	16.8% to 68.7%	100	67.6% to 100%	
> 101581	30.0	10.8% to 60.3%	100	67.6% to 100%	
> 113583	20.0	3.55% to 51.0%	100	67.6% to 100%	
> 162248	10.0	0.513% to 40.4%	100	67.6% to 100%	

Appendix Table 4. Cut off values of S100A12 for the differentiation between SJIA and KD and their Coordinates on ROC analysis

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 171.0	100.0	72.25% to 100.0%	12.50	0.6412% to 47.09%	1.143
> 346.5	100.0	72.25% to 100.0%	25.00	4.442% to 59.07%	1.333
> 560.5	100.0	72.25% to 100.0%	37.50	13.68% to 69.43%	1.600
> 926.0	100.0	72.25% to 100.0%	50.00	21.52% to 78.48%	2.000
> 1458	100.0	72.25% to 100.0%	62.50	30.57% to 86.32%	2.667
> 1762	100.0	72.25% to 100.0%	75.00	40.93% to 95.56%	4.000
> 1827	100.0	72.25% to 100.0%	87.50	52.91% to 99.36%	8.000
> 1984	100.0	72.25% to 100.0%	100.0	67.56% to 100.0%	
> 2325	90.00	59.58% to 99.49%	100.0	67.56% to 100.0%	
> 2864	80.00	49.02% to 96.45%	100.0	67.56% to 100.0%	
> 4522	70.00	39.68% to 89.22%	100.0	67.56% to 100.0%	
> 6719	60.00	31.27% to 83.18%	100.0	67.56% to 100.0%	
> 7562	50.00	23.66% to 76.34%	100.0	67.56% to 100.0%	
> 9700	40.00	16.82% to 68.73%	100.0	67.56% to 100.0%	
> 12591	30.00	10.78% to 60.32%	100.0	67.56% to 100.0%	
> 14464	20.00	3.554% to 50.98%	100.0	67.56% to 100.0%	
> 17133	10.00	0.5129% to 40.42%	100.0	67.56% to 100.0%	

Appendix Table 5. Cut off values of IL-18 for the differentiation between SJIA and KD and their Coordinates on ROC analysis

	Sensitivity %	**95% CI**	**Specificity %**	**95% CI**	**Likelihood ratio**
> 242.5	100.0	74.12% to 100.0%	14.29	0.7328% to 51.31%	1.167
> 310.0	100.0	74.12% to 100.0%	28.57	5.077% to 64.11%	1.400
> 466.5	100.0	74.12% to 100.0%	42.86	15.82% to 74.95%	1.750
> 837.5	100.0	74.12% to 100.0%	57.14	25.05% to 84.18%	2.333
> 1315	100.0	74.12% to 100.0%	71.43	35.89% to 94.92%	3.500
> 1593	100.0	74.12% to 100.0%	85.71	48.69% to 99.27%	7.000
> 2632	100.0	74.12% to 100.0%	100.0	64.57% to 100.0%	
> 5413	90.91	62.26% to 99.53%	100.0	64.57% to 100.0%	
> 7757	81.82	52.30% to 96.77%	100.0	64.57% to 100.0%	
> 13100	72.73	43.44% to 90.25%	100.0	64.57% to 100.0%	
> 18643	63.64	35.38% to 84.83%	100.0	64.57% to 100.0%	
> 27347	54.55	28.01% to 78.73%	100.0	64.57% to 100.0%	
> 51909	45.45	21.27% to 71.99%	100.0	64.57% to 100.0%	
> 70469	36.36	15.17% to 64.62%	100.0	64.57% to 100.0%	
> 82115	27.27	9.746% to 56.56%	100.0	64.57% to 100.0%	
> 109455	18.18	3.231% to 47.70%	100.0	64.57% to 100.0%	
> 136128	9.091	0.4663% to 37.74%	100.0	64.57% to 100.0%	

Appendix Table 6 . Cut off values of CXCL9 for the differentiation between MIS-C patients with strict MAS versus MIS-C patients in no MAS and their Coordinates on ROC analysis

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 386.6	100.0	60.97% to 100.0%	11.11	0.5699% to 43.50%	1.125
> 558.1	100.0	60.97% to 100.0%	22.22	3.948% to 54.74%	1.286
> 590.5	100.0	60.97% to 100.0%	33.33	12.06% to 64.58%	1.500
> 604.5	100.0	60.97% to 100.0%	44.44	18.88% to 73.33%	1.800
> 739.0	100.0	60.97% to 100.0%	55.56	26.67% to 81.12%	2.250
> 1040	83.33	43.65% to 99.15%	55.56	26.67% to 81.12%	1.875
> 1469	83.33	43.65% to 99.15%	66.67	35.42% to 87.94%	2.500
> 2534	83.33	43.65% to 99.15%	77.78	45.26% to 96.05%	3.750
> 3460	83.33	43.65% to 99.15%	88.89	56.50% to 99.43%	7.500
> 4547	66.67	30.00% to 94.08%	88.89	56.50% to 99.43%	6.000
> 5905	50.00	18.76% to 81.24%	88.89	56.50% to 99.43%	4.500
> 6877	33.33	5.923% to 70.00%	88.89	56.50% to 99.43%	3.000
> 7650	16.67	0.8549% to 56.35%	88.89	56.50% to 99.43%	1.500
> 9451	16.67	0.8549% to 56.35%	100.0	70.09% to 100.0%	

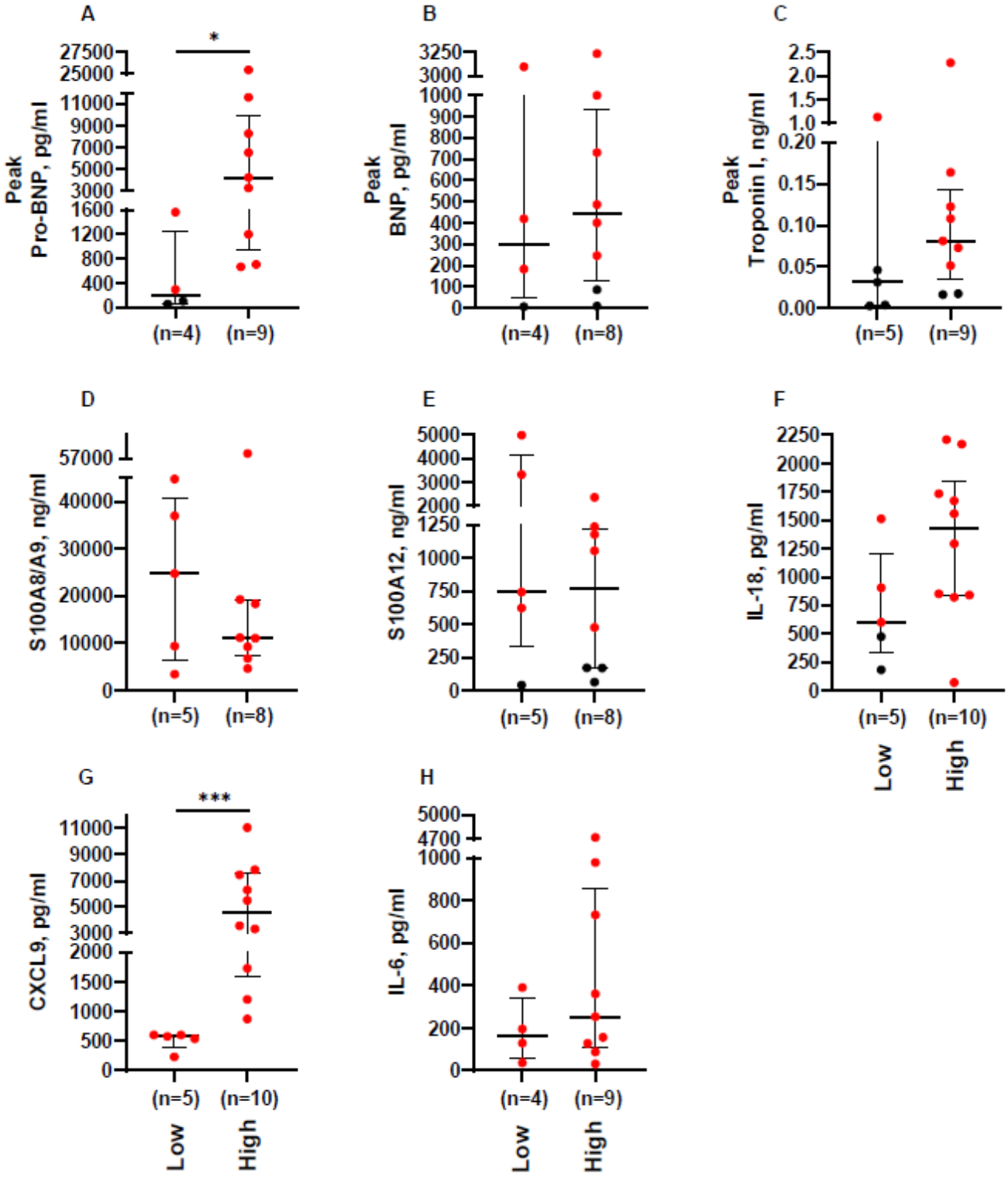
Appendix Table 7. Cut off values of CXCL9 for the differentiation between MIS-C patients with strict MAS and subclinical MAS versus MIS-C patients with no MAS

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 386.6	100.0	70.09% to 100.0%	16.67	0.8549% to 56.35%	1.200
> 558.1	100.0	70.09% to 100.0%	33.33	5.923% to 70.00%	1.500
> 590.5	100.0	70.09% to 100.0%	50.00	18.76% to 81.24%	2.000
> 604.5	100.0	70.09% to 100.0%	66.67	30.00% to 94.08%	3.000
> 739.0	100.0	70.09% to 100.0%	83.33	43.65% to 99.15%	6.000
> 1040	88.89	56.50% to 99.43%	83.33	43.65% to 99.15%	5.333
> 1469	77.78	45.26% to 96.05%	83.33	43.65% to 99.15%	4.667
> 2534	66.67	35.42% to 87.94%	83.33	43.65% to 99.15%	4.000
> 3460	66.67	35.42% to 87.94%	100.0	60.97% to 100.0%	
> 4547	55.56	26.67% to 81.12%	100.0	60.97% to 100.0%	
> 5905	44.44	18.88% to 73.33%	100.0	60.97% to 100.0%	
> 6877	33.33	12.06% to 64.58%	100.0	60.97% to 100.0%	
> 7650	22.22	3.948% to 54.74%	100.0	60.97% to 100.0%	
> 9451	11.11	0.5699% to 43.50%	100.0	60.97% to 100.0%	

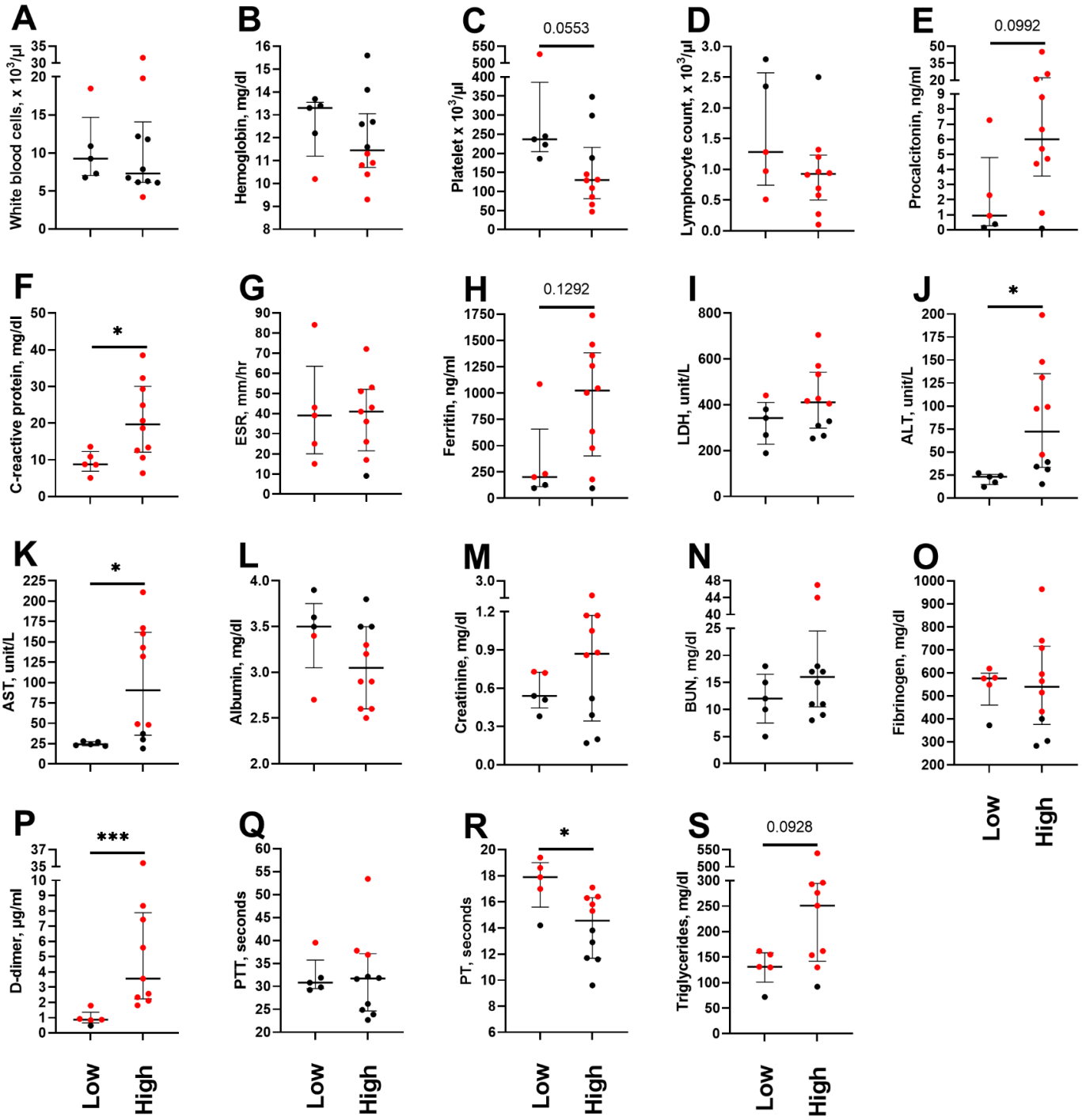
Appendix Table 8 . Demographics of MIS-C patient with CXCL9 levels below and above 739pg/ml

	Lower CXCL9 (N=5)	**Higher CXCL9 (N=10)**
	n (%)	**n (%)**
Female[§]	1 (20%)	5 (50%)
Male[§]	4 (80%)	5 (50%)
Age, median (IQR), years*	9 (7-11)	9 (4.5-15)
Hispanic	0 (0%)	3 (30%)
White	3 (60%)	3 (30%)
Black	2 (40%)	4 (40%)
Asian	0 (0%)	0 (0%)
Native American	0 (0%)	0 (0%)
Pre-existing conditions	2 (40%)	5 (50%)
Days of symptoms until admission, median (IQR)*	6 (5-19)	5.5 (4-8)
Reported COVID19 exposure	2 (40%)	7 (70%)
SARS-COV-2 serology (+)	3 (60%)	6 (60%)
Nasopharyngeal swab PCR (+)	1 (20%)	4 (40%)
Reported COVID19 exposure or/and (+) testing	4 (80%)	9 (90%)
Met complete Kawasaki criteria	1 (20%)	3 (30%)
Met macrophage activation syndrome (MAS) criteria [ǂ]	0 (0%)	6 (60%)

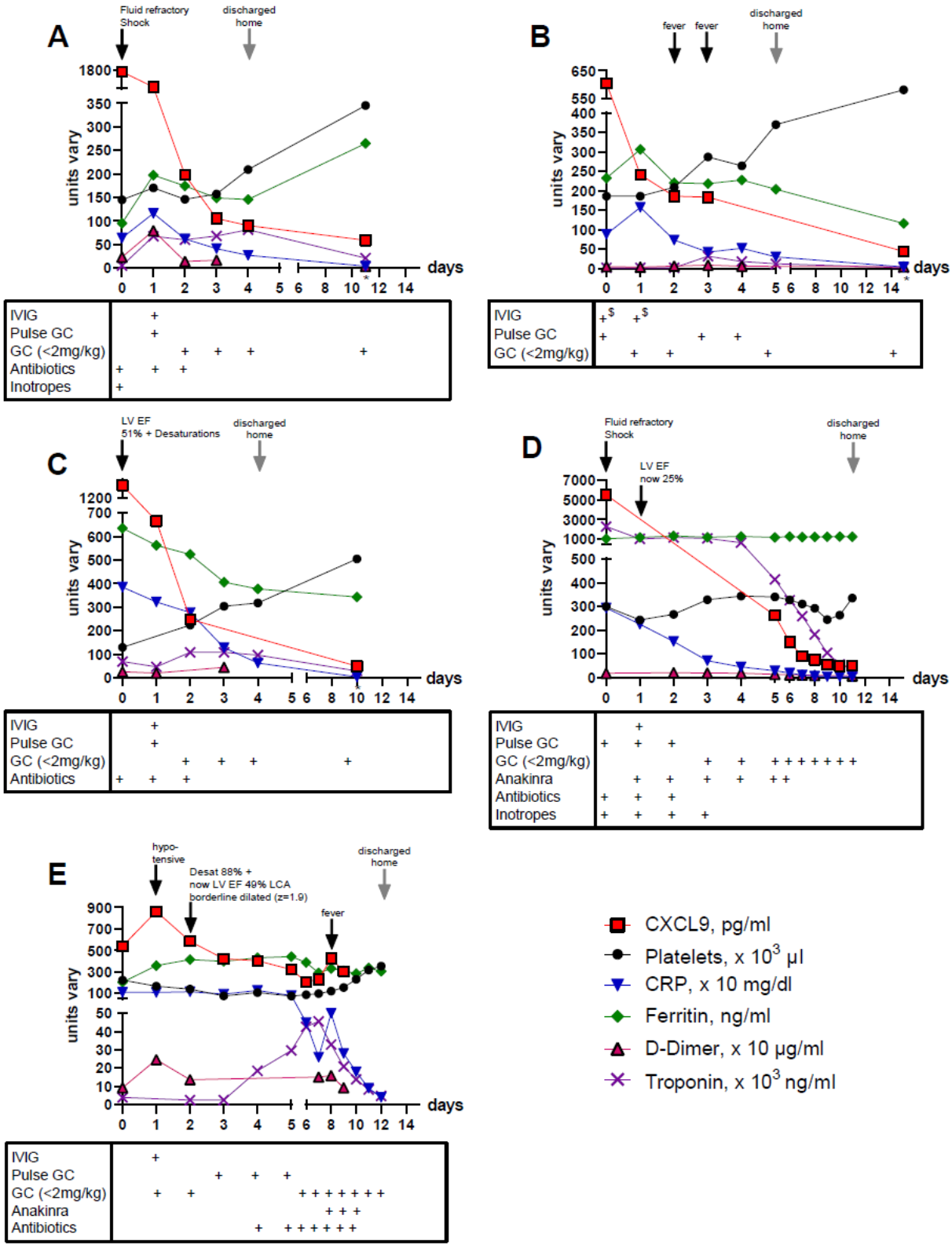
Unless noted otherwise, all patients were included in percentage calculations.
*Values reported for age and duration of symptoms are in median with 25% and 75% interquartile ranges (IQR)
§ biological sex
ǂ MAS criteria based on ferritin >684 ng/ml and two of the following: platelets ≤$181x10^9$ per L, AST >48, triglycerides ≥156 mg/dl and fibrinogen ≤360mg/dl



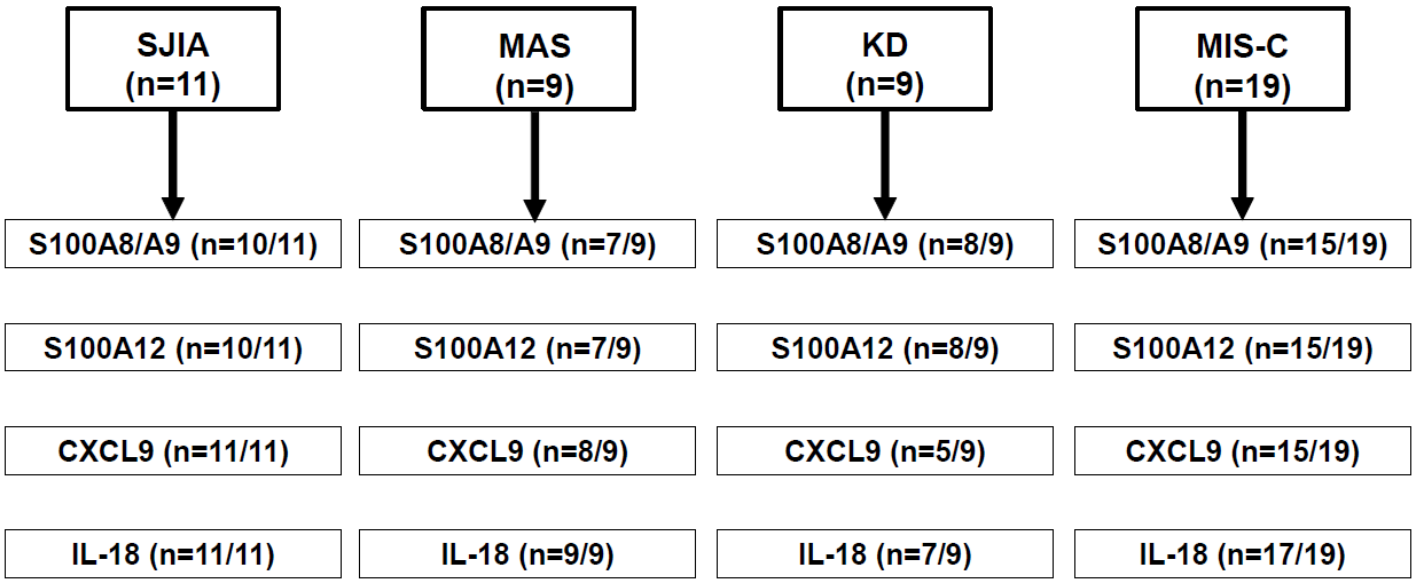
Appendix Figure 3: Cardiac markers and S100 proteins, cytokine and chemokines in MIS-C stratified by CXCL9 levels. All data are first sets of labs obtained during admission except for (A-C) which peak values are shown during admission. Red data points indicate outside the normal range for specific study. Data analyzed using unpaired t test if data was parametric or Mann-Whitney if nonparametric. . Reported are median with 25 and 75% interquartile ranges (IQR) * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$



Appendix figure 4: Hematology and laboratory results in MIS-C patients stratified by CXCL9 levels. All data are first sets of labs obtained during admission. n=5 low CXCL9 and n=10 high CXCL9 unless otherwise noted. Red data points indicate outside the normal range for specific study. Data was analyzed using unpaired t test if data was parametric or Mann-Whitney if nonparametric.Reported are median with 25 and 75% interquartile ranges (IQR). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.



Appendix Figure 5: Longitudinal trend of CXCL9 along with platelets, CRP, ferritin, D-Dimers, and high sensitivity troponin I during management of five MIS-C patients. For the purpose of graphing raw values for CRP and D-Dimers were multiplied by 10, and high sensitivity troponin I by 1000. *next to number of hospital day from admission refers to the first outpatient visit after discharge. Black arrows on top of graphs indicate clinical events that led to medical decision making. Gray arrow refers when patients were discharged. $ indicates that IVIG was stopped after 1 hour for presumed transfusion reaction and then resumed 12 hours later (day 1) without issues. GC= glucocorticoid steroids, pulse= 30mg/kg (max 1000mg).



Appendix Figure 6: Flow diagram showing patient group and overall sample number for specific biomarkers.