

## Supplemental Online Content

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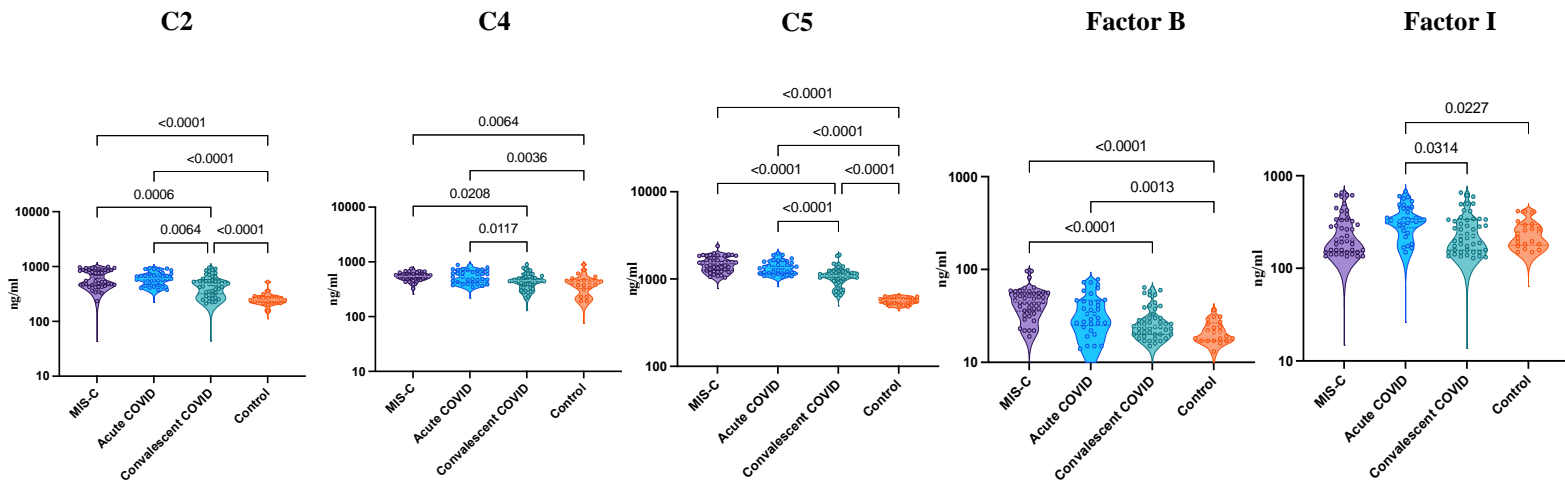
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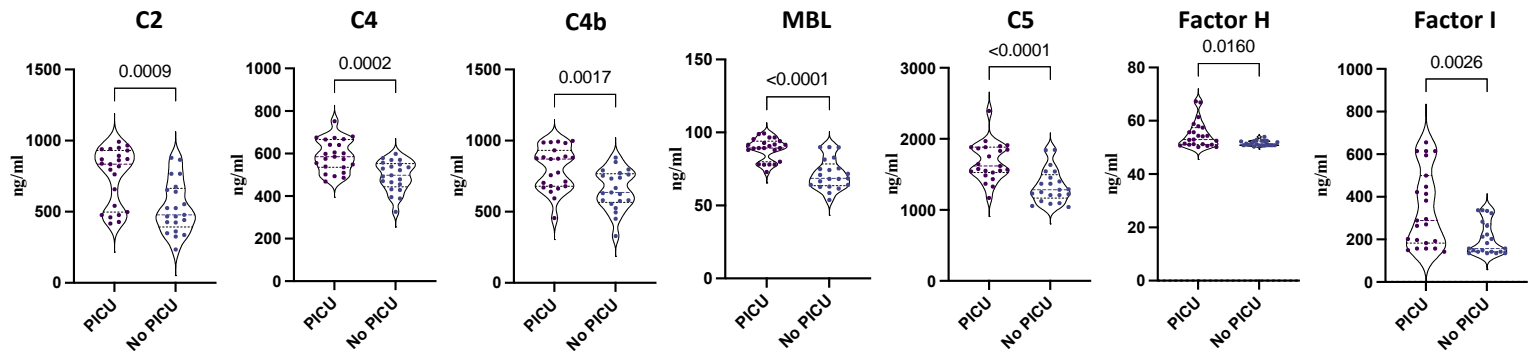
This supplemental material has been provided by the authors to give readers additional information about their work.

**eFigure 1. MIS-C and acute COVID-19 children are associated with elevated levels of complement activation**



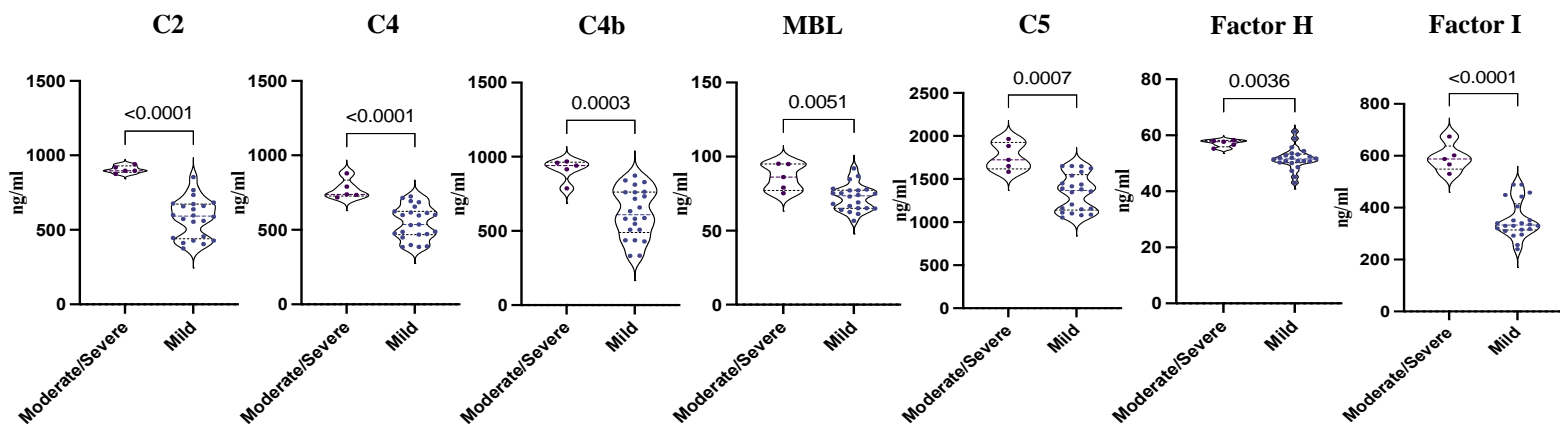
**eFigure.1.** Levels of the specified complement components, complement regulators and complement activation products C2, C4, C4b and MBL, C5, and iC3b, factor B, factor D, and factor I in plasma samples from MIS-C (n=44), Acute COVID-19 (n=33), convalescent COVID-19 (n=47) and control (n=21) children. The data are represented as scatter plots with each circle representing a single individual. P values were calculated using the Kruskal-Wallis test with Dunn’s post-hoc for multiple comparisons.

**eFigure 2. Enhanced complement activation is associated with disease severity in MIS-C children**



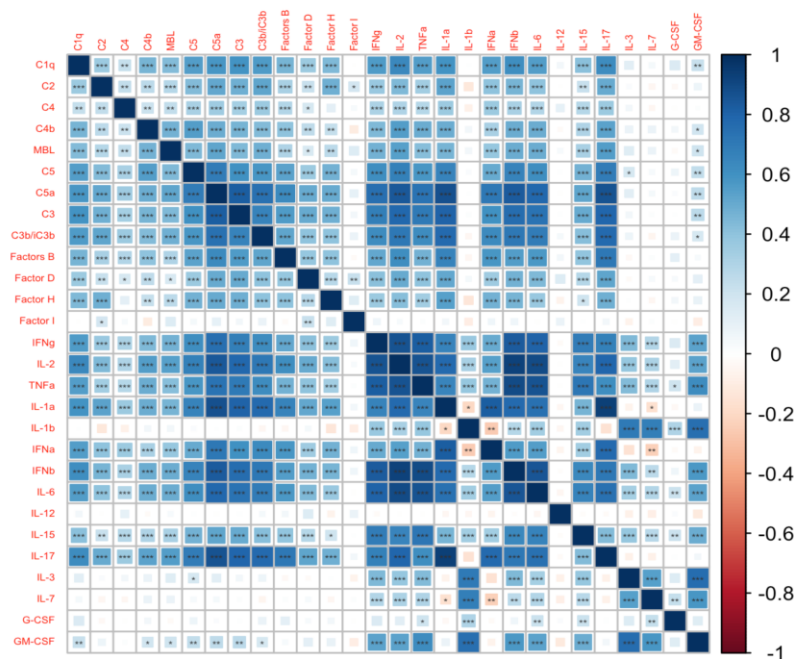
**eFigure.2.** Levels of the specified complement components, complement regulators and complement activation products C2, C4, C4b, MBL, C5, factor H and factor I were measured using plasma samples in children with MIS-C requiring PICU care (n = 23) and children in wards [who did not require PICU care] (n = 21). The data are represented as scatter plots with each circle representing a single individual. p values were calculated using the Mann-Whitney test with Holm's correction for multiple comparisons.

### eFigure 3. Enhanced complement activation is associated with disease severity in acute COVID-19



**eFigure 3.** Levels of the specified complement components, complement regulators and complement activation products C2, C4, C4b, MBL, C5, factor H and factor I were measured using plasma samples in children with moderate to severe (n = 5) COVID-19, and children with mild (n = 22) and asymptomatic (n = 5) COVID-19. The data are represented as scatter plots with each circle representing a single individual. P values were calculated using the Kruskal—Wallis test with Dunn’s post—hoc for multiple comparisons.

**eFigure 4. Correlation of plasma complement components, complement regulators and complement activation products with laboratory parameters and pro-inflammatory cytokines**



**eFigure.4.** Correlation between plasma levels of complement components (C1q, C2, C3, C4, C4b, MBL, C5), regulators (factor B, factor D, factor H and factor I) and activation products (iC3b, C5b) and pro-inflammatory cytokines (IFN $\gamma$ , IL-2, TNF $\alpha$ , IL1 $\alpha$ , IL1 $\beta$ , IFN $\alpha$ , IFN $\beta$ , IL-6, IL-12, IL-15, IL-17, IL-3, IL-7, G-CSF and GM-CSF) levels in all children with MIS-C, acute COVID-19, convalescent COVID-19 and control. Spearman's correlation method was used for the analysis. \* indicates p=0.05, \*\* indicates p= 0.01 and \*\*\* indicates p=0.001 the blue colour denotes the positive correlation and the red colour denotes the negative correlation.

**eTable 1. Demographics and haematology parameters of the study population**

	<b>MIS-C n = 44</b>	<b>Acute COVID-19 n = 29</b>	<b>Convalescent COVID-19 n = 47</b>	<b>Controls n=21</b>
<b>Age median (years, IQR)</b>	7 y (1 – 14y)	5 (1 – 17 yr)	4.4 y (1 – 17 y)	6 y, (1 – 15 y)
<b>Male n (%)</b>	19 (43%)	20 (60%)	35 (74%)	11 (52%)
<b>Female n (%)</b>	25 (57%)	9 (40%)	12 (26%)	10 (48%)
<b>Serology IgG positive n (%)</b>	44 (100%)	0	47 (100%)	0
<b>CRP mean (&lt; 5 mg/L)</b>	169 (39 – 473)	5 (<5 – 181)	5 (<5 – 181)	NA
<b>Lymphocyte(/mm<sup>3</sup>) (1500 – 4000) median (IQR)</b>	1386 (330 - 2200)	2945 (650 – 12000)	3890 (650 – 12000)	3200 (2600 – 4000)
<b>Neutrophils (/mm<sup>3</sup>) (1500 – 7000) median (IQR)</b>	11658 (9918 - 14878)	3681 (120 – 13160)	6300 (120 – 13160)	4500 (3500 – 6500)
<b>Platelets (200 – 450)x10<sup>9</sup>/L median (IQR)</b>	110 (62 – 210)	278 (180 – 400)	327 (100 – 540)	350 (350 -450)
<b>Sodium (135 – 145mmol/l) median (IQR)</b>	134 (127 -138)	136 (135 – 145)	138 (135 – 148)	139 (135 – 145)
<b>D-Dimer(100–500ng/ml FEU) median (IQR)</b>	4890 (2446–10 000)	NA	NA	NA
<b>Ferritin (7 to 140ng/mL) median (IQR)</b>	605 (38–2571)	NA	NA	NA
<b>LDH (125–243U/L) median (IQR)</b>	494 (200–905)	NA	NA	NA

**eTable 2. Geo Mean and Fold change of complement components of the study groups**

<b>Parameters</b>	<b>MIS-C Geo Mean ng/ml</b>	<b>Acute COVID-19 Geo Mean ng/ml</b>	<b>Convalescent COVID-19 Geo Mean ng/ml</b>	<b>Control Geo Mean ng/ml</b>	<b>Acute COVID- 19 Fold change</b>	<b>Convalescent COVID-19 Fold change</b>	<b>Control Fold change</b>
<b>C1q</b>	61.48	56.92	41.57	24.14	1.1	1.5	2.5
<b>C2</b>	605.8	606.4	448.1	255.9	1.0	1.4	2.4
<b>C4</b>	537.4	539.1	441.8	396.9	1.0	1.2	1.4
<b>C4b</b>	712.4	640.7	562.3	351.5	1.1	1.3	2.0
<b>MBL</b>	79.42	69.56	65.02	49.32	1.1	1.2	1.6
<b>C5</b>	1487	1364	1076	561.9	1.1	1.4	2.6
<b>C5a</b>	2614	1826	886	462.5	1.4	3.0	5.7
<b>C3</b>	318.2	237.7	161.1	123.4	1.3	2.0	2.6
<b>C3b/iC3b</b>	3971	3702	2646	2039	1.1	1.5	1.9
<b>Factor B</b>	47.62	44.58	36.64	27.53	1.1	1.3	1.7
<b>Factor D</b>	43.95	33.84	26.89	21.28	1.3	1.6	2.1
<b>Factor H</b>	53.11	50.82	47.23	43.62	1.0	1.1	1.2
<b>Factor I</b>	242.4	332.7	242	233.9	0.7	1.0	1.0

**eTable 3. Geo Mean and Fold Change of Complement Components Between PICU vs No PICU**

<b>Parameters</b>	<b>PICU Geo Mean ng/ml</b>	<b>No PICU Geo Mean ng/ml</b>	<b>Fold change</b>
<b>C1q</b>	72.31	51.47	1.4
<b>C2</b>	718.9	502.2	1.4
<b>C4</b>	585.7	489	1.2
<b>C4b</b>	788.6	637.4	1.2
<b>MBL</b>	88.26	70.75	1.2
<b>C5</b>	1659	1320	1.3
<b>C5a</b>	2806	2418	1.2
<b>C3</b>	341.1	294.9	1.2
<b>C3b/iC3b</b>	4247	3690	1.2
<b>Factor B</b>	52.02	43.22	1.2
<b>Factor D</b>	54.44	34.77	1.6
<b>Factor H</b>	54.58	51.54	1.1
<b>Factor I</b>	300.2	191.7	1.6



**eTable 4. Geo Mean and Fold change of complement components between Moderate/Severe Vs Mild**

<b>Fig.3</b>	<b>Moderate/Severe</b>	<b>Mild</b>	<b>Fold change</b>
<b>C1q</b>	89.44	54.71	1.6
<b>C2</b>	905.8	565.8	1.6
<b>C4</b>	770	536.4	1.4
<b>C4b</b>	911.5	597.9	1.5
<b>MBL</b>	85.75	71.78	1.2
<b>C5</b>	1756	1329	1.3
<b>C5a</b>	2608	1845	1.4
<b>C3</b>	311.8	229.3	1.4
<b>C3b/iC3b</b>	4450	3597	1.2
<b>Factor B</b>	52.71	44	1.2
<b>Factor D</b>	71.01	33.55	2.1
<b>Factor H</b>	57.16	51.54	1.1
<b>Factor I</b>	590.5	347.7	1.7

**eTable 5. Geo Mean and Standard deviation of complement activation is associated with disease severity in acute COVID-19.**

<b>eFig.3</b>	<b>Moderate/Severe GM and SD</b>	<b>Mild</b>	<b>p value</b>
<b>C2</b>	905.8 [131.0]	565.8 [24.6]	<0.0001
<b>C4</b>	770 [110.5]	536.4 [65.8]	<0.0001
<b>C4b</b>	911.5 [166.3]	597.9 [74.0]	0.0003
<b>MBL</b>	85.8 [8.8]	71.8 [9.0]	0.0051
<b>C5</b>	1756.0 [159.4]	1329.0 [212.1]	0.0007
<b>Factor H</b>	57.2 [4.0]	51.5 [1.3]	0.0036
<b>Factor I</b>	590.5 [70.7]	347.7 [53.0]	<0.0001

**eTable.6. Geo Mean and Standard deviation of complement activation is associated with disease severity in acute COVID-19.**

<b>Fig.4</b>	<b>Moderate/Severe GM and SD</b>	<b>Mild</b>	<b>p value</b>
<b>C1q</b>	89.4 [14.0]	54.7 [8.0]	<0.0001
<b>C3</b>	311.8 [65.3]	229.3 [51.8]	0.0121
<b>C3b/iC3b</b>	4450.0 [692.5]	3597.0 [327.1]	0.0152
<b>C5a</b>	2608.0 [420.8]	1845.0 [128.8]	0.0002
<b>Factor B</b>	52.7 [5.3]	44.0 [4.0]	0.0034
<b>Factor D</b>	71.0 [11.5]	33.6 [5.7]	<0.0001