SUPPLEMENTARY INFORMATION

Multiplex Metal-Detection based Assay (MMDA) for COVID-19 diagnosis and identification of disease severity biomarkers

Materials and methods

Reagents

The recombinant N and S protein were purchased from GeneMedi Biotechnology. The mouse anti-human IgM secondary antibody, rabbit anti-human IgG Fc gamma secondary antibody, goat anti-human IgA secondary antibody, Pierce™ NHS-activated magnetic beads, magnetic stand and borate buffer (pH 8.5) were purchased from Life Technologies, the MaxPAR antibody conjugation kits were bought from Fluidigm. The deionized water (18.2 MΩ.cm) was used throughout the experiments.

Facilities and Apparatus

A quadrupole-based inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Agilent Technologies, USA), equipped with a glass concentric nebulizer and an impact spray chamber, was used throughout the experiments.

Magnetic beads functionalization with antigens.

To conjugate antigen on magnetic beads, N-hydroxysuccinimide (NHS) activated magnetic beads were used to covalently immobilize proteins through reaction with primary amines on proteins. After removing the preservation buffer using a magnetic stand, the NHS activated magnetic beads were activated in ice-cold hydrochloric acid (1 mM, pH 3.0). The antibodies (25 μg) that remained to be labeled were added into magnetic beads (1mg) immediately after removing hydrochloric acid. The mixture of antibodies and magnetic beads were incubated for two hours at room temperature on a rotator. The glycine (0.1 M, pH 2.0) were added after incubation and the antibody labeled magnetic beads were washed with ultrapure water twice followed by quenching the reaction in ethanolamine (3 M, pH 9.0). The magnetic beads were then washed with ultrapure water and PBS twice and then resuspended in PBS contained 0.05% sodium azide at a final concentration of 10 mg/mL for the protein-conjugated magnetic beads.

Antibody conjugation with metal-polymer tags

The anti-human secondary IgM, IgG and IgA antibodies were labelled with ^{167}Er, ^{175}Lu and ^{149}Sm individually using the MaxPAR antibody conjugation kits according to the company's recommended protocol. After determining the concentration of metal labelled antibodies using BCA calibration, the metal-labeled antibodies were preserved in PBS for long-term storage at 4 °C.

Enzyme linked immunosorbent assay (ELISA) for NP and spike RBD[1]

Briefly, 96-well immunoplates (Nunc Immuno modules; Nunc, Denmark) were coated with 100 μl/well (0.1 μg/well) of SARS-CoV-2 NP or spike RBD in 0.05 M $NaHCO_3$ (pH 9.6) overnight at 4°C and then followed by incubation with a blocking reagent. After blocking, 100 μL heat-inactivated serum samples at 1:100 dilution was added to the wells and incubated at room temperature for 1 h. For the determination of IgM, the serum was first mixed with an IgG inactivation agent (Gullsorb) to inactivate serum IgG. The attached antibodies were detected using horseradish-peroxidase-conjugated goat anti-human IgG or IgM antibody (Invitrogen, Thermo Fisher Scientific, Waltham,

MA, USA). The reaction was developed by adding diluted 3,3',5,5'-tetramethylbenzidine single solution and stopped with 0.3M H_2SO_4. The optical density (OD) was read at 450 nm. A single positive sample was included in each run as positive control. An archived anonymous sample from 2018 used in our previous study was used as negative control.[2]

Serum samples for antibody assay

Under the approval by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13-372 and UW 14-249), archived serum samples of COVID-19 patients and control subjects (healthy blood donors prior to or during the COVID-19 outbreak) were obtained from Queen Mary Hospital in Hong Kong.[3] The serum samples were heat-inactivated by incubation at 56°C for 30 min before stored at -80°C until further use.

SARS-CoV-2 antibody profiling in serum by ICP-MS

The antigen functionalized magnetic beads were blocked in 2.5% BSA for 30 min at room temperature. The serum samples were diluted (1:50) using assay buffer (PBS contained 2.5% BSA) and 100 μL of diluted samples were incubated with antigen functionalized magnetic beads for 30 min at room temperature. The magnetic beads were then washed with PBS three times followed by incubation with metal tag labeled secondary antibodies (^{175}Lu-anti-human IgG, ^{149}Sm-anti-human IgA and ^{167}Er-IgM antibodies) for 30 min at room temperature. The magnetic beads were then washed with PBS four times and 50 μL 1% HNO_3 was added to release the captured antibodies. The released solution was diluted in 500 μL 1% HNO_3 with 0.5 ppb indium (In) as internal standard for metal quantification by ICP-MS. ICP-MS was tuned using a standard tuning solution before each measurement.

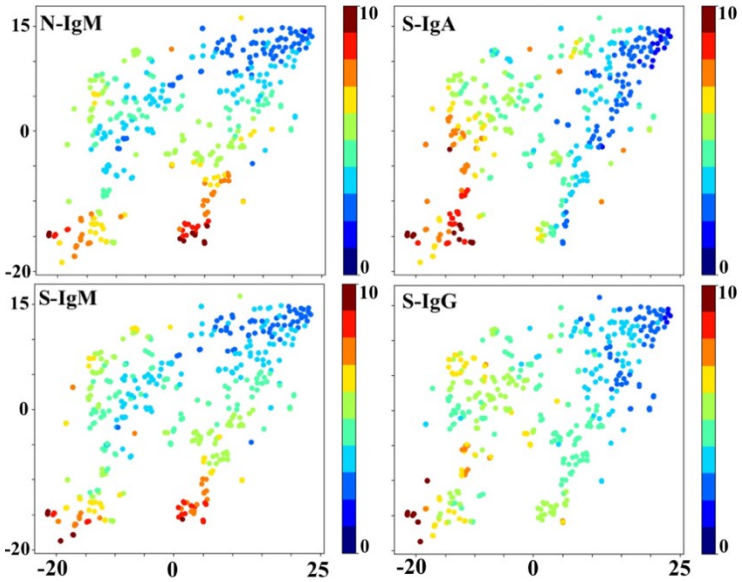

Figure S1. tSNE map shows the intensity distribution of four antibodies (N protein specific IgM and S protein specific IgA, IgM and IgG) across all the individuals included in this study (217 cases). The alteration of antibody response intensity among individuals was demonstrated by a colour change from blue (low intensity) to red (high intensity).

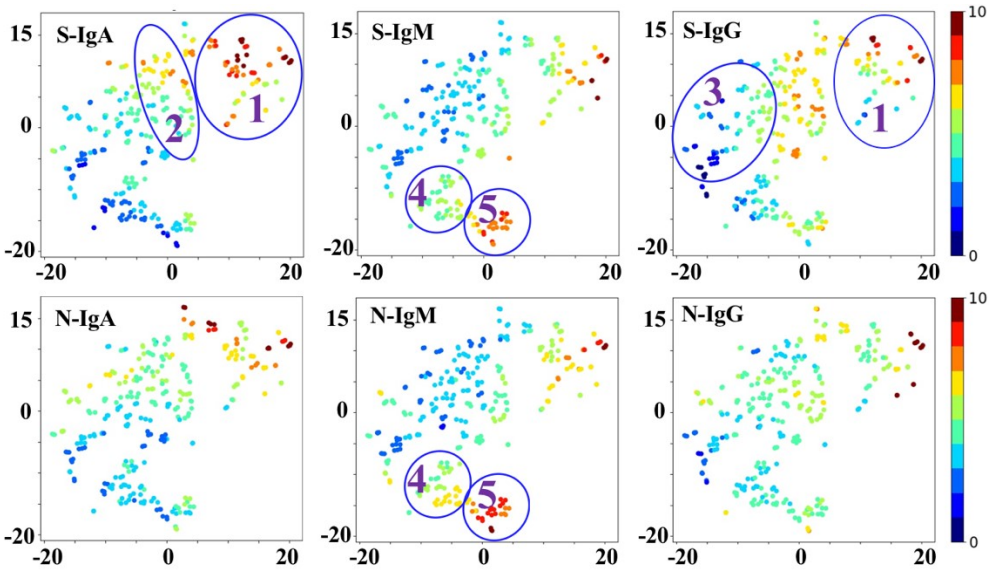

Figure S2. tSNE map shows the intensity distribution of six antibodies (N and S protein specific IgA, IgM and IgA) across all the COVID-19 patients (107 cases) for subpopulation classification. The alteration antibody intensity among COVID-19 patients was demonstrated by a colour change from blue (low intensity) to red (high intensity).

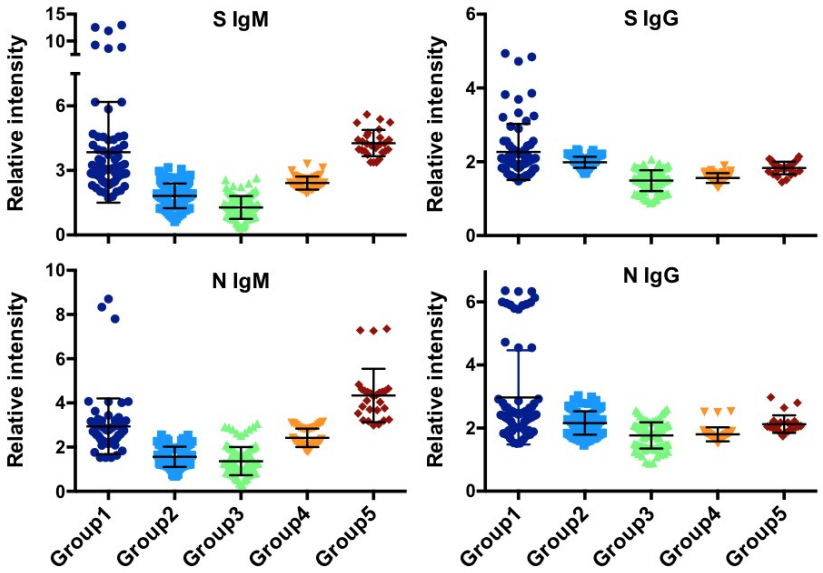

Figure S3. Comparison of S and N protein specific IgM and IgG responses between different subgroups classified by tSNE.

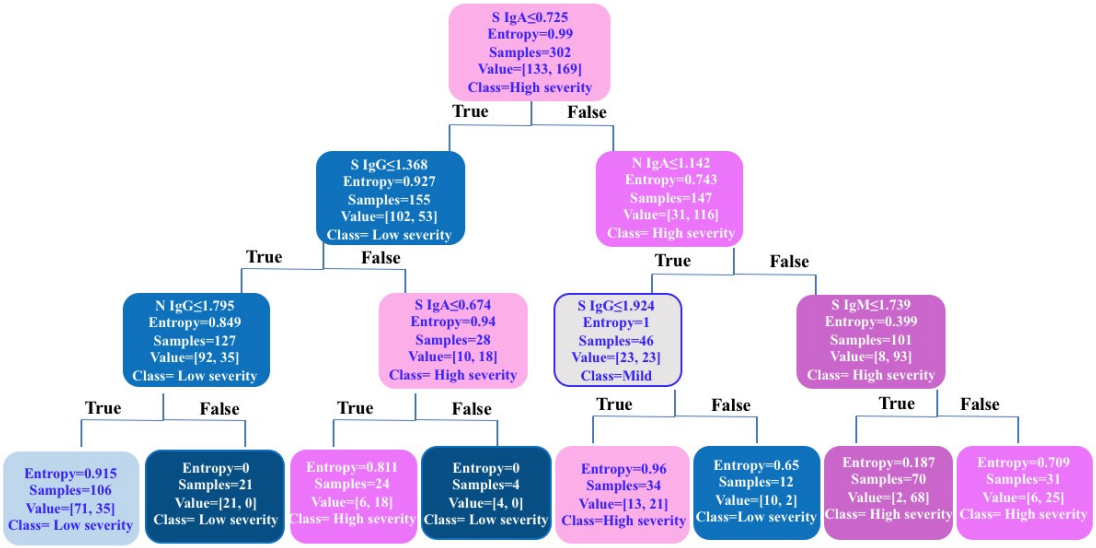

Figure S4. Graphical representation of the decision tree model based on antibody features for disease severity classification. The colour of nodes indicates the severity level, i.e. blue: mild; pink: non-mild (moderate/severe/death). 235 cases (302 cases in total) are correctly classified (accuracy= 78%). The darker colour indicates a higher proportion of patients included in the indicated class. Entropy indicated in the decision tree stands for homogeneity in each classified subgroup.

Table S1 The clinical characteristics (gender, age, collection date, infection status) of cases included in this study.

Donor number	Sex (M: male; F: female)	Age	Day of symptom onset	Severity	Asymptomatic
6	M	61	17	Mild	
7	M	62	12	Mild	
8	F	64	10	Moderate	
9	M	81		Mild	Asymptomatic
10	M	53		Moderate	Asymptomatic
16	M	71	10	Mild	
26	F	51	12	Mild	
27	F	29	14	Mild	
28	M	60	13	Mild	
29	M	66	13	Mild	
30	M	78	13	Death	
31	F	64	12	Mild	
32	M	13	13	Mild	
33	F	75		Mild	Asymptomatic
34	F	54	10	Mild	
35	F	40		Mild	Asymptomatic
41	F	40	9	Mild	
42	F	32		Mild	Asymptomatic
44	M	39	10	Mild	
45	M	74	3	Mild	
46	M	59	14	Moderate	
47	F	60	14	Mild	
48	F	48	11	Mild	
49	M	31		Mild	Asymptomatic
50	F	32		Mild	Asymptomatic
61	F	93	13	Mild	
62	M	55		Moderate	Asymptomatic
64	M	25	11	Mild	
65	M	48	14	Moderate	
71	F	54	10	Mild	
72	M	82	22	Mild	
73	M	54	10	Mild	
74	M	31	10	Moderate	
75	M	67	5	Mild	
76	F	62		Mild	Asymptomatic
77	F	44	13	Mild	
78	M	65		Mild	Asymptomatic
79	M	59	14	Moderate	

80	M	62	10	Mild	
91	F	20	37	Mild	
92	F	50	11	Mild	
93	M	31		Mild	Asymptomatic
94	F	37		Mild	Asymptomatic
95	M	37		Mild	Asymptomatic
106	F	58	10	Mild	
107	M	43	8	Moderate	
108	M	60	11	Moderate	
109	M	60	17	Severe	
110	M	43	22	Mild	
111	M	45	10	Mild	
112	M	36	12	Severe	
113	F	58	12	Mild	
114	M	59	8	Mild	
115	F	30	7	Mild	
116	F	22		Mild	Asymptomatic
123	M	69		Mild	Asymptomatic
125	F	80	15	Moderate	
126	F	42	10	Mild	Asymptomatic
127	M	55	14	Moderate	
129	M	21	8	Mild	
130	M	46	12	Moderate	
151	F	52	13	Moderate	
152	F	73		Mild	Asymptomatic
153	F	48	13	Moderate	
154	F	91	16	Mild	
155	M	27	9	Mild	
156	F	11	17	Mild	
157	M	1	27	Mild	
158	F	46		Mild	Asymptomatic
160	F	46	3	Mild	
172	M	49		Mild	Asymptomatic
173	M	53	13	Mild	
174	M	45	17	Severe	
175	F	66		Mild	Asymptomatic
181	F	80	17	Mild	
182	F	44	7	Mild	
183	F	8	23	Mild	
184	F	60	8	Mild	
185	M	63	3	Mild	
186	F	40		Mild	Asymptomatic
187	F	55	6	Mild	
188	F	32		Mild	Asymptomatic
189	F	41		Mild	Asymptomatic

190	F	17		Mild	
191	F	29		Mild	Asymptomatic
192	M	30		Mild	Asymptomatic
193	F	5		Mild	Asymptomatic
194	M	30		Mild	Asymptomatic
195	F	49		Mild	Asymptomatic
196	M	25		Mild	Asymptomatic
197	F	63		Mild	Asymptomatic
198	M	59		Mild	Asymptomatic
199	F	49		Mild	Asymptomatic
200	F	42		Mild	Asymptomatic
201	M	78	4	Severe	
202	F	67	2	Severe	
203	F	76	2	Severe	
204	M	79	3	Severe	
205	M	68	2	Severe	
206	F	70	7	Severe	
207	F	66	3	Severe	
208	M	71	2	Severe	
209	M	70	3	Severe	
210	M	72	5	Severe	
211	F	68	4	Severe	
212	M	36	6	Severe	
213	M	78	4	Severe	

Table S2 Comparison of the accuracy for the quantification of IgA, IgM, IgG antibodies against viral S and N proteins in serum samples. Evident decrease of the false negative and false positive results was obtained for our developed method compared with ELISA.

	ICP-MS Based method						ELISA					
Antibody type	N IgA	S IgA	N IgM	S IgM	N IgG	S IgG	N IgA	S IgA	N IgM	S IgM	N IgG	S IgG
False negative rate (%)	42.9	57.1	0	14.3	0	0	85.7	100	14.3	28.6	14.3	71.4
False positive rate (%)	60	60	0	0	0	0	40	60	0	40	0	80

References

1. Chen, Y.; Li, L., *Lancet Infect. Dis.* **2020,** *20* (5), 515-516.
2. Chen, L. L.; Wu, W. L.; Chan, W. M.; Fong, C. H. Y.; Ng, A. C. K.; Ip, J. D.; Lu, L.; Dissanayake, T. K.; Ding, X.; Cai, J. P.; Zhang, A. J. X.; Tam, S.; Hung, I. F. N.; Chan, K. H.; Yuen, K. Y.; To, K. K. W., *Epidemiol. Infect.* **2019,** *147*, e279.
3. To, K. K.-W.; Tsang, O. T.-Y.; Leung, W.-S.; Tam, A. R.; Wu, T.-C.; Lung, D. C.; Yip, C. C.-Y.; Cai, J.-P.; Chan, J. M.-C.; Chik, T. S.-H., *Lancet Infect. Dis.* **2020,** *20* (5), 565-574.