**Anti-SARS-CoV2 antibody responses in serum and cerebrospinal fluid of COVID-19 patients with neurological symptoms**

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**Summary:** COVID-19 patients with neurological symptoms had IgG against the SARS-CoV-2 S/S1 protein both in serum (81%) and CSF (56%). All patients with elevated markers of CNS damage also had IgG against SARS-CoV-2 in CSF.

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**ABSTRACT**

Antibody responses to SARS-CoV-2 in serum and CSF from 16 COVID-19 patients with neurological symptoms were assessed using two independent methods. IgG specific for the virus spike protein was found in 81% of cases in serum and in 56% in CSF. SARS-CoV-2 IgG in CSF was observed in two cases with negative serology. Levels of IgG in both serum and CSF were associated with disease severity ($p<0.05$). All patients with elevated markers of CNS damage in CSF also had CSF antibodies ($p=0.002$), and CSF antibodies had the highest predictive value for neuronal damage markers of all tested clinical variables.

**Keywords**

COVID-19, SARS-CoV-2, serology, CSF, IgG, neurological symptoms

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## BACKGROUND

Coronaviruses (CoVs), including SARS-CoV-2, are associated with neurological manifestations including cerebrovascular disorder, encephalopathy, autoimmune and neuropsychiatric complications[1].

PCR for SARS-CoV-2 in CSF is typically negative[2] but a small number of positive cases have been described[3-6]. CSF work-up is often normal, although markers of CNS injury can be elevated[5-8]. Benamour et al. investigated and found immunoglobulin G antibodies (IgG) against subunit 1 (S1) of the SARS-CoV-2 spike protein (S) in CSF from three COVID-19 patients who developed encephalopathy and encephalitis[9]. High-titers of anti-SARS-CoV-2 IgG were additionally detected in CSF of eight patients with neurological symptoms. One of these patients also showed signs of intrathecal production of anti-SARS-CoV-2 IgG indicated by high IgG-index[10]. These reports provide important initial findings but the data are scarce and immune responses in COVID-19 patients with neurological symptoms require further characterization. Here, we describe IgG responses against the SARS-CoV-2 S and S1 in serum and CSF of patients with mild to severe CNS symptoms.

## METHODS

### Patients and study design

The National Ethical Review Authority approved this study (2020-01883 and 2014/148). Informed consent was obtained from each patient, or next-of-kin if a patient was unable give consent. Patients with confirmed COVID-19 and at least one new-onset neurological symptom including altered mental status, cranial nerve symptoms and/or paresis, extrapyramidal, cerebellar and sensory symptoms were prospectively included from April until September 2020. Patients had either a positive PCR test result for SARS-CoV-2 in nasopharynx samples (*n*=15) or SARS-CoV-2-specific IgG against nucleoprotein-based antigen in serum (*n*=1). Matched CSF and serum samples were analyzed



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serologically alongside their routine work-up. LP was carried out in conjunction with or after the period of most severe symptoms. Five patients were sampled >2 months after COVID-19 debut due to residual cognitive and/or neurological symptoms. Four of these cases had previously been treated in intensive care at some stage of their disease. The patients were examined by a neurologist. The NIH criteria for COVID-19 severity were used to classify patient clinical status as mild, moderate, severe or critical[11].

**Detection of IgG in serum and CSF**

The levels of IgG in serum and in CSF against SARS-CoV-2 S or S1 were assessed in two independent laboratories using an enzyme-linked immunosorbent assay (ELISA) and a suspension immunoassay (SIA), respectively.

*SARS-CoV-2 spike timer-based ELISA*

A SARS-CoV-2 ELISA assay based on native-like spike trimers was used as previously described[12]. Briefly, 96-well ELISA plates (Nunc MaxiSorp) were coated with spike trimers (100 µl of 1 ng/µl) in PBS overnight at 4°C. Plates were washed six times with 300 ml PBS-Tween-20 (0.05%) and blocked using PBS-5% no-fat milk (Sigma). Thawed serum was diluted 1:100 and CSF diluted 3,33:100 blocking buffer and samples incubated overnight at 4°C, before washing as before. Wells were then incubated for 1 hour at room temperature with secondary HRP-conjugated anti-human goat anti-human IgG (Southern Biotech, 2014-05) diluted 1:10,000 in blocking washed with with TMB Stabilized Chromogen (Invitrogen) and the reaction was stopped using 1M sulphuric acid. Results are expressed as optical density (OD) values measured at 450 nm using an Asys Expert 96 ELISA reader (Biochrom Ltd.). Six standard deviations from the mean of negative control samples ran on the same day (blood donors from the spring of 2019) was used to set the cut-off for the assay.



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*SARS-CoV-2 S1-based suspension immunoassay (SIA)*

Assays were performed using the MagPix system (Luminex Corp., Austin, TX, USA) as described earlier[13]. Ten µg SARS-CoV-2 S1 protein (40591-V08H, Sino Biological, Inc., Wayne, PA, USA) was covalently coupled to 2.5 x 106 carboxylated paramagnetic beads (MagPlex microspheres, Luminex) using sulfo-N-hydroxysulfosuccinimide and 1-ethyl-3-[3 dimethylaminopropyl]carbodiimide hydrochloride (Pierce Biotechnology-ThermoFisher, Waltham, MA USA). Aliquots of 50 µl bead mix (25 beads/µl PBST (0.05% Tween 20)) and 50 µl diluted serum (1:25 in PBST) or 50 µl diluted CSF (1:10 in PBST) were mixed and incubated in a 96-well plate for 60 min in the dark at room temperature on a plate shaker (at 600 rpm). Beads were then washed with 100 µl of PBST using a magnet plate separator (Life technologies-ThermoFisher, Carlsbad, CA, USA). Next, 100 µl of biotinylated protein G (Pierce) was added (2 µg/ml PBST). The beads were incubated for 30 min as described above, washed once, followed by 15 min incubation with 100 µl of phycoerythrin-conjugated streptavidin (Invitrogen-ThermoFisher, Waltham, MA, USA) (2 µg/ml PBST). Following re-suspension in 100 µl PBST, the beads were analyzed by measuring the fluorescence of 50 beads/sample at default settings, using xPONENT software (Luminex). The results were expressed as median fluorescence intensity (MFI). Six standard deviations from the mean of negative controls plus 10% (blood donors from 2018) was used to set the cut-off for the assay.

**CSF biomarkers for CNS injury, blood-brain barrier (BBB) injury and intrathecal IgG production**

Markers for neurological damage were measured in CSF samples. Total-tau protein (T-tau) was measured using Lumipulse technology according to the manufacturer's instructions (Fujirebio, Ghent, Belgium). Neurofilament light protein (NfL) and glial fibrillary acidic protein (GFAp) were

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measured using in-house ELISAs, as previously described [14]. Albumin and IgG levels in serum and CSF were measured on a Roche Cobas Analyzer (Roche Diagnostics, Bromma, Sweden).

### Statistics

Correlation analysis was performed using Spearman's rank correlation for variables with skewed distribution. Antibody levels above cut-off in either or both methods were classified as positive, while those negative in both methods were classified as negative. These binary variables were compared to categories for clinical variables using a likelihood ratio. Student's T-tests were used for groupwise comparisons of age. To assess the strength of possible predictors in relation to each other, a forward binary logistic regression model was used. The statistical analysis was performed using SPSS version 27 (IBM Corp, Armonk, NY).

## RESULTS

### Patients demographics and characteristics

Clinical characteristics and demographics are presented in Table 1. Routine CSF work up showed that only two had increased number of cells/pleocytosis. All patients were negative PCR for SARS-CoV-2 in CSF at the time point measured in this study (Table 1). The median ± IQR CSF/serum albumin ratio was 8.9 ± 3.7 and IgG index of 0.45 ± 0.08.

### Comparison of KI and UU methods for IgG serology

The correlation analysis of IgG against the SARS-CoV-2 S and S1 using ELISA based on S-trimers or a SIA based on the S1 subunit of S was high both in serum (rho=0.659 p=0.006) and CSF (rho=0.867 p<0.001) (Figure 1A). In total, thirteen patients (81%) were classified as seropositive in serum and nine (56%) in CSF by either or both methods (Figure 1B). Both methods identified twelve patients as



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positive for anti-S/S1 IgG in serum and eight for anti-S/S1 IgG in CSF. The individual result with either method is presented in Figure 1C.

**SARS CoV-2 S and S1 antibodies in CSF**

There was no correlation between anti-S/S1 IgG in serum and CSF using either method (ELISA: rho=0.271, p=0.310; SIA: rho=0.235, p=0.380). Two cases were found to have anti-S/S1 IgG in CSF but not in serum, although one case, sampled 12 days after symptom debut, was only detected by the SIA method. Both cases presented with altered mental state and fever and had elevated levels of NfL in CSF. One of the patients recovered within days, while the other required intensive care. Neuroimaging was negative in both cases.

CSF/serum albumin ratio correlated strongly with anti-S/S1 IgG in CSF by both methods (ELISA rho=0.694, p=0.004, SIA rho=0.798, p<0.001) but no correlation could be observed with IgG index. Neither did the IgG index correlate with anti-S/S1 IgG in CSF detected by any of the methods. Patients with positive findings of SARS CoV-2 S and S1 IgG in CSF had significantly higher CSF/albumin ratio (8.9± 3.6) compared to those with negative findings (4.5± 0.9; p=0.016), no significant differences could be seen in IgG index between these groups.

**SARS CoV-2 S/S1 antibodies in CSF in relation to clinical variables**

SARS CoV-2 S/ S1 IgG in CSF were explored for possible correlation to clinical presentation and/or outcome. The distribution of symptoms in patients with and without IgG in CSF is shown in Table 1 and in Figure 1D. There was a positive correlation between the NIH criteria for COVID-19 severity and the levels of anti-S/S1 IgG in serum (p<0.05) and CSF (p<0.05). COVID-19 severity did not correlate with CSF/serum albumin ratio. Seven patients with anti-S/S1 IgG in CSF required intensive care and



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six required invasive ventilation and six had a moderate to severe GCS (≤ 12) at some point before LP. Importantly, all patients with at least one elevated biomarker for neurological damage (n=7) had anti-S/S1 IgG in CSF (p=0.002).

Using a forward binary logistic regression model, the presence of anti-S/S1 IgG in CSF was the only factor that predicted elevated markers of CNS damage with an odds ratio of 40 (95% CI=2–794, p=0.016). The other factors tested in the model were not significant, including COVID-19 severity, age group, invasive ventilation, gender, intensive care, days since symptoms onset. Restricting the IgG-positive group to only the eight patients identified using both methods in CSF did not change the results.

**DISCUSSION**

COVID-19 patients with neurological symptoms had IgG against the SARS-CoV-2 S/S1 protein both in serum (81%) and CSF (56%). CSF antibody-positive cases were over-represented among patients requiring invasive ventilation. Furthermore, all patients with elevated markers of CNS damage also had IgG against SARS-CoV-2 in CSF. The results support previous findings of CSF IgG in individuals with severe disease and encephalopathy[10].

It remains uncertain if SARS-CoV-2-specific antibodies in CSF indicates intrathecal production or a passive diffusion due to BBB impairment that could be linked to COVID-19 severity. We found a strong significant correlation between detection of SARS-CoV-2-specific IgG in CSF and COVID-19 severity, level of consciousness and respiratory symptoms. Increased CSF/serum albumin ratio indicates disturbance of the BBB and values above reference range were found in three patients, all with SARS-CoV-2-specific antibodies in CSF. Those three patients were treated in the ICU and had critical COVID-19. No correlation was seen between IgG index and the SARS-CoV-2 antibodies in CSF. There was no correlation between serum and CSF levels of IgG against SARS-CoV-2. We also found SARS-CoV-2 IgG in CSF for two cases with negative serology. The choroid plexus is lacking tight junctions and might serve for an easier breach of barrier.



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Our findings support and extend the results in a previous report of three patients with encephalopathy or encephalitis, elevated levels of cytokines and laboratory-confirmed coronavirus disease with increased CSF levels of anti-S1 IgM but negative PCR[9] and with a study of two patients with COVID-19 encephalopathy who had elevated antibody titers against SARS-CoV-2 S1, S2 and nucleocapsid in CSF[15]. A strength is the use of two different methods for IgG analysis (solid phase and suspension), which gave mainly concordant results. Differences in the detected IgG response between the two methods may be due to the use of different S antigen preparations and/or different detection methods. Further analysis of antibody responses in the CSF in patients suffering from neurological symptoms during or following COVID-19 may help explain disease sequelae following SARS CoV-2 infection.

This population although larger than previous publications, represents few patients with heterogeneous clinical presentations and a wide span in sampling dates which may have affected the IgG levels. The measurement of IgG was semi-quantitative and binary categorization of the data was used in the main analysis for this reason. In two patients who were anti-S/S1 IgG negative, the lumbar puncture was performed only four days after symptom onset which was probably too early for formation of IgG. This study identified patients with severe COVID-19 who displayed SARS- CoV-2-specific IgG in CSF and were at high risk of developing CNS damage detected by CSF biomarkers. These patients may benefit from early detection and early treatment interventions. Negative serology did not exclude CSF positivity for SARS-CoV-2 IgG as this was observed in two cases. Further studies are needed to investigate if detection of anti-SARS-CoV-2 IgG in CSF is a result of intrathecal production. Future work should establish the effects of the virus on the CNS and examine the relationship between CSF positivity and treatment response and prognosis.

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**Conflicts of interest/Competing interests**

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. The other authors report no disclosures relevant to the manuscript.

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**Figure 1.**

**A)** Anti- SARS-CoV-2 spike protein IgG (anti-S/S1 IgG) in serum and CSF using the enzyme-linked immunosorbent assay (ELISA) and suspension immunoassay (SIA). Cut-offs at 0.25 for optical density (OD450) and 300 median fluorescence intensity (MFI) are indicated. Twelve cases with anti-S/S1 IgG values over cut-off in serum (orange) and eight in CSF (red) were identified with both methods, one case over cut-off with only one method was noted for serum (yellow) and one for CSF (pink). **B)** Presenting the relationship between serum and CSF levels of anti-S/S1 IgG in individual cases for the SIA and ELISA. Both methods detected a case with higher levels in CSF compared to serum and an additional case was identified with the SIA. **C)** An UpSet plot to provide an overview of the distribution of patients with different combinations of variables in the cohort. The Red rectangle indicates the overlap in Anti-S/S1 IgG in CSF Anti-S/S1 IgG over cut-off using both method (red) or one method (pink) in CSF with Damage markers (high levels of CNS injury markers (NfL, GFAp, T-tau) in 8 cases. The other clinical variables (sets), based on the time-period from debut to sample collection, include the severity of COVID-19 graded mild, moderate, severe or critical, Glasgow Coma Scale (GCS) when under 12 and requiring intensive care unit (ICU). Anti-S/S1 IgG over cut-off using both method (orange) or one method (yellow) in serum are indicated. One patient with mild disease did not have IgG above cutoff.

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**Table 1.** Characteristics and CSF findings of patients positive and negative for anti-S/S1 IgG in CSF. Neurological symptoms and respiratory support at some stage in the disease course before lumbar puncture.

| | CSF anti-S/S1 IgG | |
|---|---|---|
| | Positive (n=9) | Negative (n=7) |
| Days since symptoms onset (Median, IQR) | 30 (21–74) | 43 (4–124) |
| Age, years (Median, IQR) | 64 (48–73) | 43 (36–54) |
| Gender, male (%) | 5 (56) | 3 (43) |
| Mild COVID-19, n (%) | 0 (0) | 3 (43) |
| Moderate COVID-19, n (%) | 3 (33) | 1 (14) |
| Severe COVID-19, n (%) | 1 (11) | 2 (29) |
| Critical COVID-19, n (%) | 5 (56) | 1 (14) |
| ICU, n (%) | 7 (78) | 3 (33) |
| ICU, median days (IQR) | 10 (2–27) | 0 (0–2) |
| GCS ≤12, n (%) | 6 (67) | 1 (14) |

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| | | |
|---|---|---|
| High flow oxygen, n (%) | 2 (22) | 3 (43) |
| Invasive ventilation, n (%) | 6 (67) | 1 (14) |
| Altered mental status, n (%) | 8 (89) | 5 (71) |
| Cranial nerve affection, n (%) | 2 (22) | 0 (0) |
| Anosmia/ ageusia, n (%) | 3 (33) | 3 (43) |
| Vertigo, n (%) | 3 (33) | 4 (57) |
| Headache, n (%) | 3 (33) | 7 (100) |
| Peripheral paralysis, n (%) | 2 (22) | 1 (14) |
| Central paralysis, n (%) | 3 (33) | 3 (43) |
| Sensory symptoms, n (%) | 1 (11) | 3 (43) |
| Pleocytosis, n (%) | 0 (0) | 2 (29) |
| CSF/serum albumin x $10^3$ (median, IQR) | 7.4 (6.5–12.5)) | 4.5 (3.8–5.3) |
| IgG Index (median, IQR) | 0.44 (0.42–0.49) | 0.42 (0.38–0.44) |
| OCB, n (%) | 1 (11) | 1 (14)[4] |
| Elevated IgG[1], in CSF, n (%) | 3 (33) | 0 (0) |
| Elevated T-tau, n (%) | 5 (56) | 1 (14)[2] |
| Elevated NfL, n (%) | 7 (78) | 0 (0)[3] |
| Elevated GFAp, n (%) | 2 (22) | 0 (0)[3] |

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Increase of the T-tau, NfL and GFAp biomarkers was determined in relation to age-related normal reference limits.ICU = intensive care unit; GCS = Glasgow Coma Scale; OCB = oligoclonal bands; T-tau = total tau; NfL = neurofilament light chain; GFAp = glial fibrillary acidic protein.

Reference ranges for these assays were: T-tau: age < 50 years, < 360 ng/L; age > 50 years, < 479 ng/L; NfL: age 30–40 years, < 560 ng/L; age 40–60 years, < 890 ng/L; age > 60 years, < 1,850 ng/L; GFAp: age 20–60 years, < 750 ng/L, age > 60 years, < 1,250 ng/L. The CSF/serum albumin ratio was calculated as [CSF albumin/serum albumin] a measure of the BBB function (15 years–45 years = <6.8, >45 years = <10.2), while the IgG index was calculated as [(CSF-IgG/serum-IgG)/(CSF-albumin/serum-albumin)] as a measure for intrathecal IgG production (>15 years = <0.63).

note. [1] non-specific increase of CSF IgG; [2] Missing data for three individuals; [3] Missing data for one individual; [4] unique for CSF.

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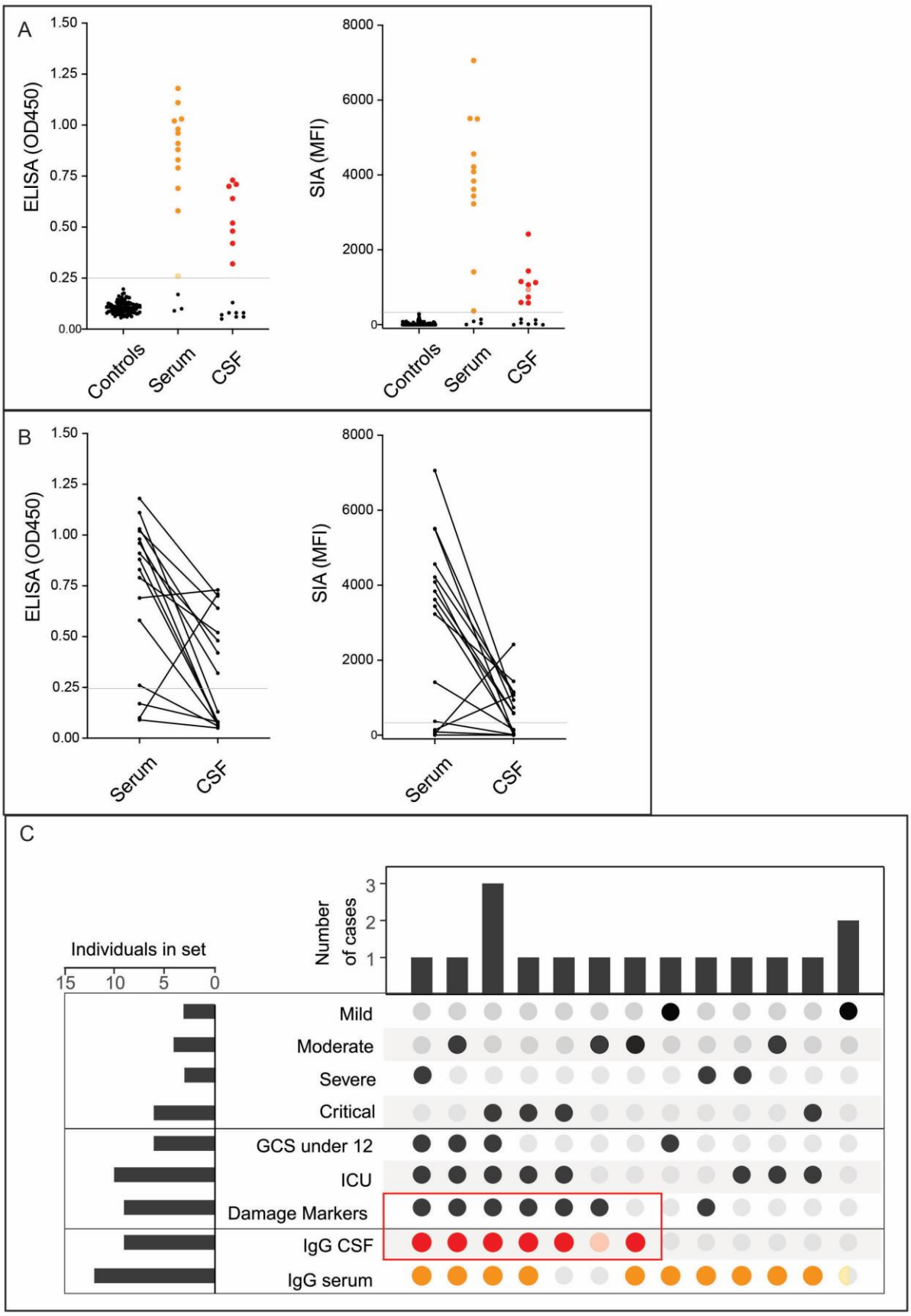
Figure 1

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