Hôpitaux Universitaires Genève

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

Réponse immune humorale au SARS-CoV-2 chez les enfants ayant présenté un syndrome inflammatoire multisystémique post-COVID19 (MIS-C) par rapport aux enfants ayant eu une infection COVID19 non sévère

Colloque UIC du CRC du 7 février 2022

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Méd. Adjointe agrégée

Immunologie-Vaccinologie pédiatrique

May 2020: When everything started

Global report a new inflammatory syndrome affecting children

Same syndrome but different case definition and names

Table 1. Multisystem inflammatory syndrome in children case definitions

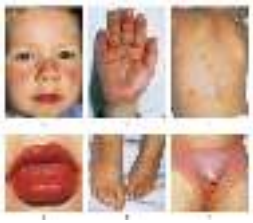
	Royal College of Paediatrics and Child Health[11]	Centers for Disease Control[12]	World Health Organization[13]
Fever	Persistent fever > 38.5°C	Fever > 38.0°C for ≥24 h, or report of subjective fever lasting ≥24 h	Fever > 3 days
Evidence of SARS-CoV2 infection or exposure	SARS-CoV-2 PCR testing may be positive or negative	Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms	Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with a person with COVID-19
Clinical features	Inflammation (neutrophilia, elevated CRP and lymphopenia) AND Evidence of single or multiorgan dysfunction (shock, cardiac, respiratory, renal, gastrointestinal, or neurological disorder) with additional features	Laboratory evidence of inflammation AND Multisystem (>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological)	Elevated markers of inflammation AND Two of the following: Rash/ mucocutaneous signs; Hypotension or shock; Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities; Coagulopathy; Acute gastrointestinal problems
Alternative diagnoses	Exclusion of any other microbial cause	No alternative plausible diagnoses	No other obvious microbial cause of inflammation
Level of care	Not specified	Hospitalization required	Not specified

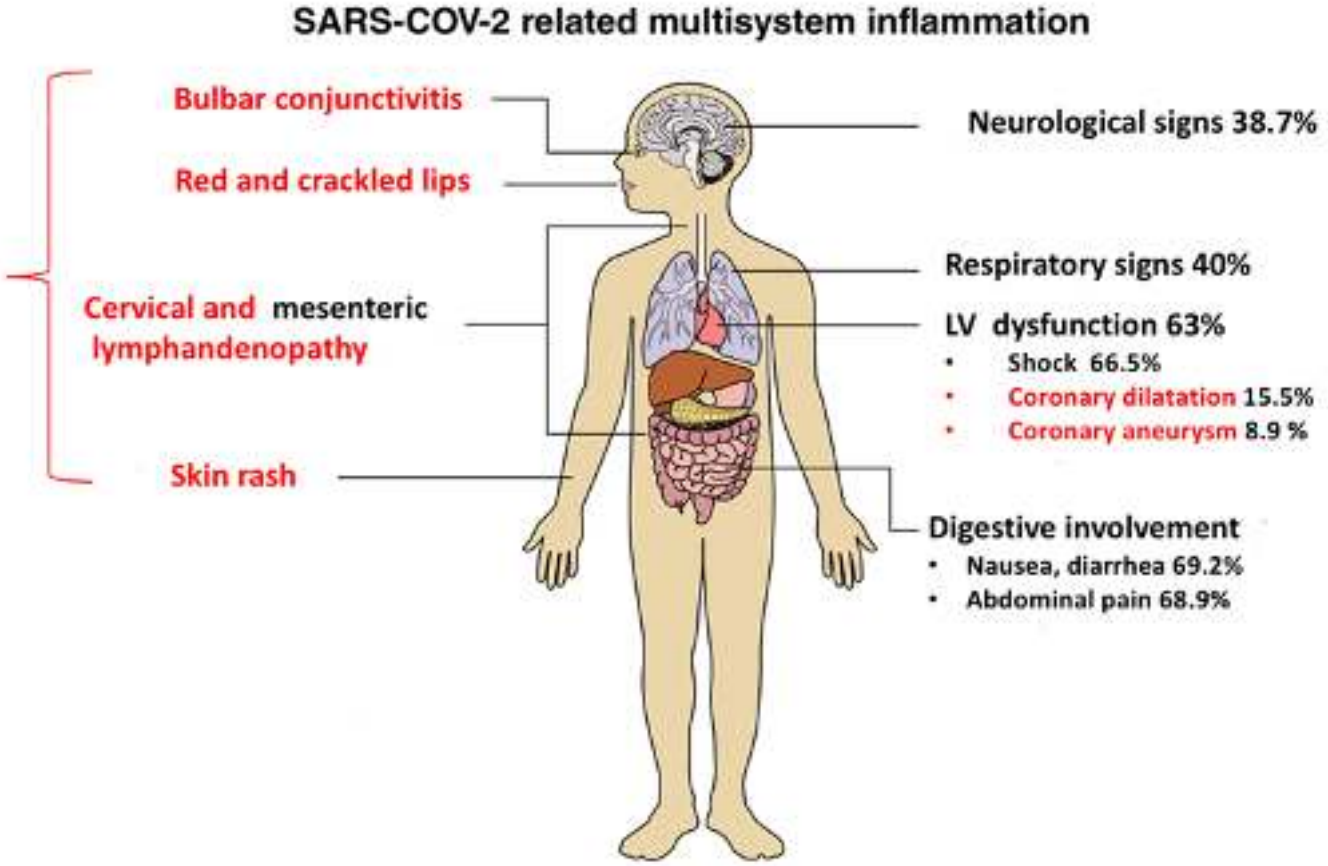
COVID-19, coronavirus disease 2019; CRP, C-reactive protein; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Roberts et al, Co-Rheumatology, 2021

MIS-C: clinical presentation

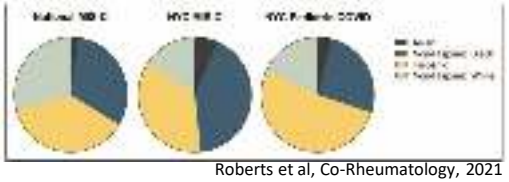
Complete Kawasaki disease





Picture modified from Belhadjer et al. *Circulation* 2020

- Male sex: 55.8 %
- Fever > 4 days and asthenia
- Ethnicity:



Roberts et al, Co-Rheumatology, 2021

- SARS-CoV-2 infection:
  - Positive RT-PCR: 34.7%
  - **Positive serological tests: 80.3%**

Timeline of MIS-C cases in Geneva

MIS-C: laboratory

- **Abnormal blood cell counts**:
  - Lymphocytopenia: 80 to 95%
  - Neutrophilia: 68-90 %
  - Mild anemia: 70%
- **Elevated inflammatory markers**:
  - CRP: 90-100%
  - ESR: 75-80%
  - D-dimer: 67-100%
  - Fibrinogen: 80-100%
  - Ferritin: 55-76%
  - Procalcitonin: 80-95 %
  - Interleukin-6: 80-100%
- **Elevated cardiac markers**:
  - Troponin: 50-90%
  - NT-pro-BNP: 73-90%
- **Hypoalbuminemia**: 48-95%
- **Mildly elevated liver enzymes**: 62-70%
- **Hypertriglyceridemia**: 70%

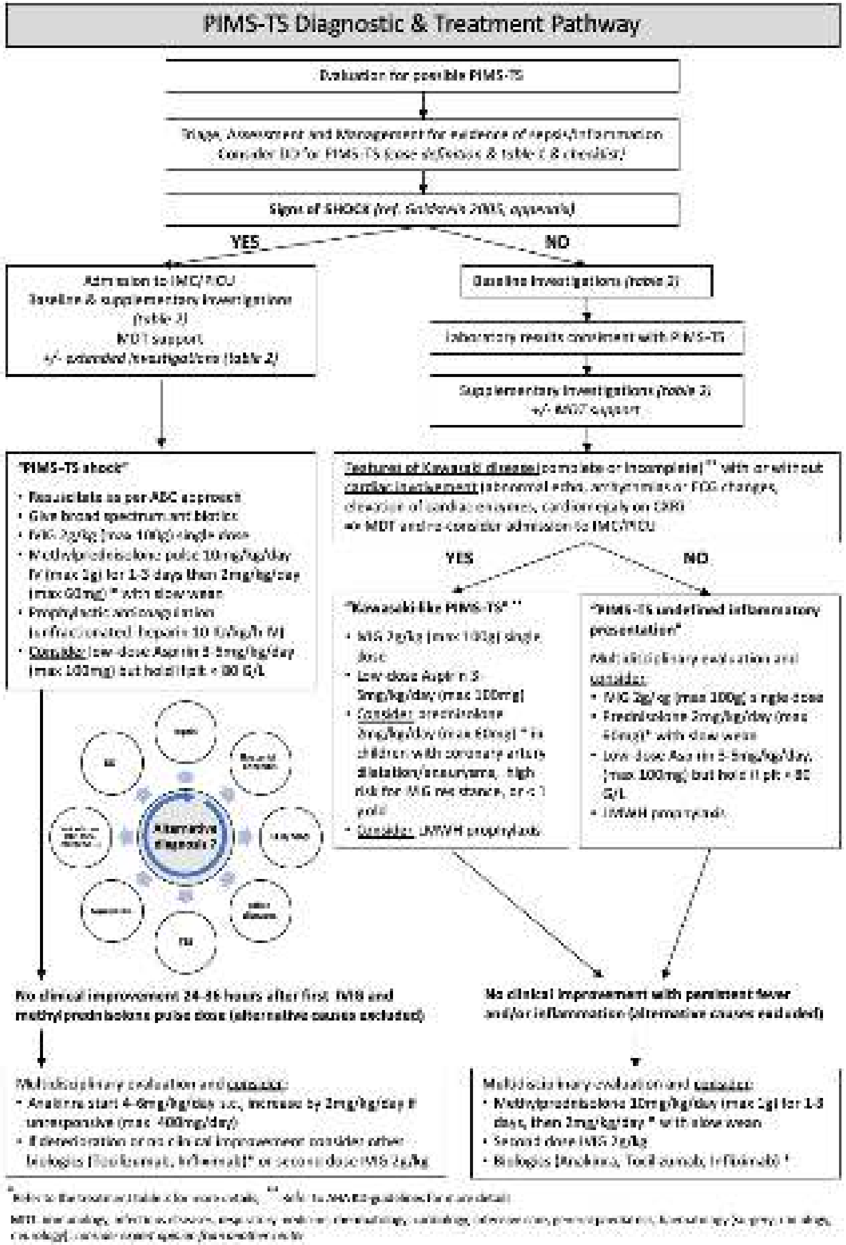
Feldstein *et al*. NEJM, 2020; Swann *et al*. BMJ 2020

frontiers in Pediatrics

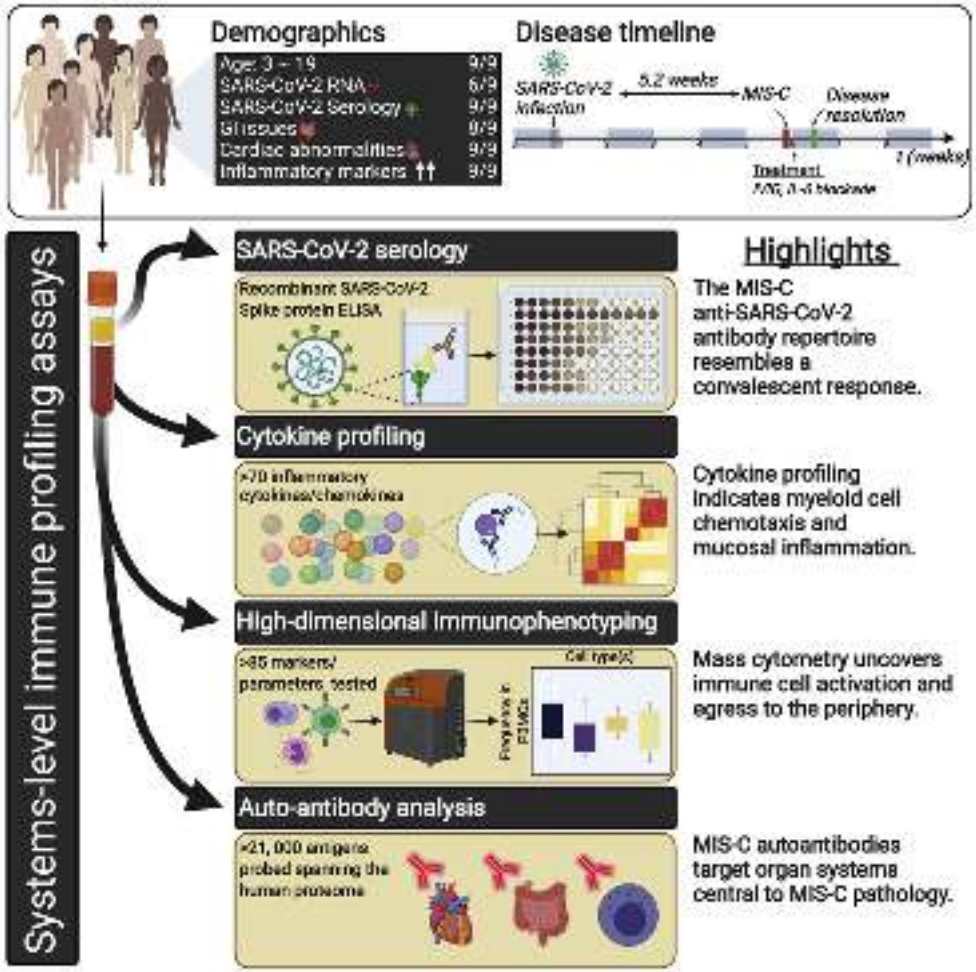
REVIEW
doi: 10.3389/fped.2021.667507

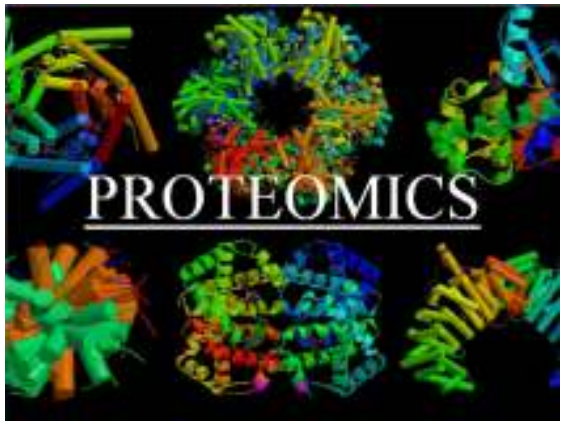
Best Practice Recommendations for the Diagnosis and Management of Children With Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2 (PIMS-TS; Multisystem Inflammatory Syndrome in Children, MIS-C) in Switzerland

Luregn J. Schlapbach, Maya C. Andre, Serge Grazioli, Nina Schöbi, Nicole Ritz, Christoph Aebi, Philipp Agyeman, Manuela Albisetti, Douggl G. N. Bailey, Christoph Berger, Géraldine Blanchard-Rohner, Sabrina Bressieux-Degueldre, Michael Hofer, Arnaud G. L'Huillier, Mark Marston, Patrick M. Meyer Sauteur, Jana Pachlopnik Schmid, Marie-Helene Perez, Bjarte Rogdo, Johannes Trück, Andreas Woerner, Daniela Wütz, Petra Zimmermann, Michael Levin, Elizabeth Whittaker, Peter C. Rimensberger and the PIMS-TS working group of the Interest Group for Pediatric Neonatal Intensive Care (IGPNI) of the Swiss Society of Intensive Care and the Pediatric Infectious Diseases Group Switzerland (PIGS)

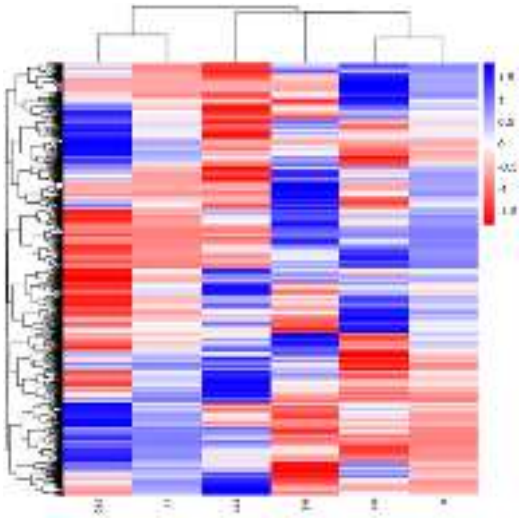


Exploring MIS-C immunopathogenesis





RNA seq



Gruber et al, Cell, 2020

MIS-C: Physiopathology

The effects that we see:

- Local vasculitis and inflammation of affected organs
- Endothelial cells damaged by auto-antibodies and complement (Consiglio et al. Cell 2020)
- Auto-antibodies of multiple specificities, incl. endothelial, gastrointestinal, cardiac and immune cells (Gruber et al. Cell 2020)

Physiopathology

Potential causes:

1. Direct effect of the virus as various autopsy reports have identified RNA in heart/brain endothelial cells, macrophages, neutrophils (Duarte-Neto et al. 2021; Dolhnikoff et al. 2020)
2. A part of SARS-CoV-2 S protein could act as a Super-Antigen → inducing polyclonal activation of T cells with important inflammatory response (Cheng et al. 2020)
3. Genetic susceptibility? Some ethnic groups over-represented, at least at the beginning: population-based genetic susceptibility, viral factors (variants)?

BUT No genetic predisposition found

MIS-C: What do we know?

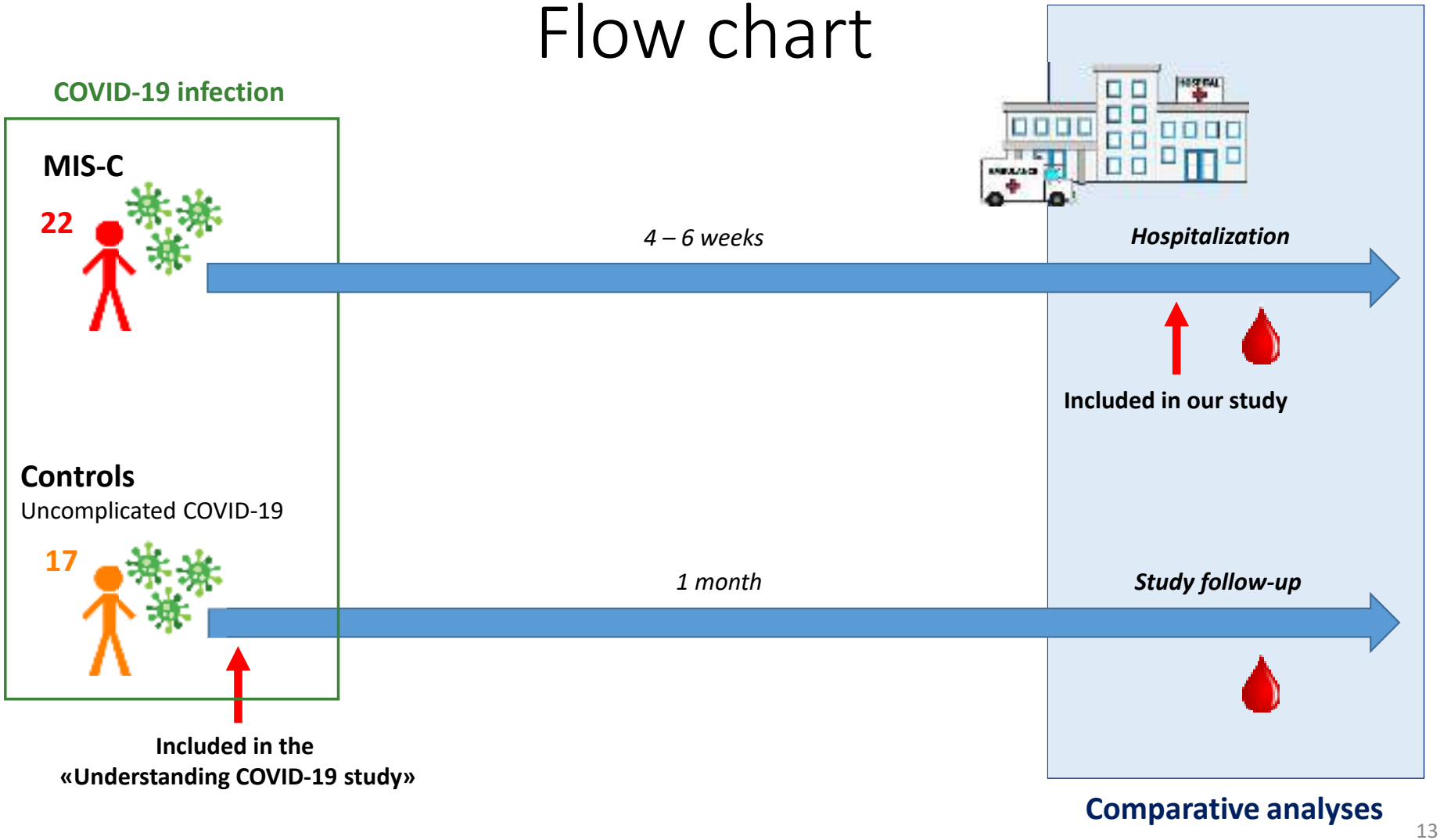
- MIS-C remains a “rare” condition
- Good response to aggressive immunomodulatory therapies
- Current evidence suggests a post-viral immunological reaction to SARS-CoV-2
- Immunopathogenesis is not completely understood but current data suggest autoimmune and superantigen-driven processes
- Multicenter international and national studies are in progress to determine the best management strategy and immunomodulatory treatment for patients diagnosed with MIS-C

Our study

- **Objectives:** To assess the humoral immune response of children with MIS-C in comparison to children who had an uncomplicated COVID-19 infection

- **Population:**
  - MIS-C patients < 18 years, hospitalized between March 2020 and March 2021 in Geneva (CCER 2020-00835)
  - Controls: Uncomplicated COVID-19 patients < 18 years recruited in the study « Understanding COVID-19 » (CCER 2020-00516) between March 2020 and January 2021

Flow chart


COVID-19 infection
MIS-C
22
Controls
Uncomplicated COVID-19
17
4 – 6 weeks
Hospitalization
Included in our study
1 month
Study follow-up
Included in the
«Understanding COVID-19 study»
Comparative analyses

Population

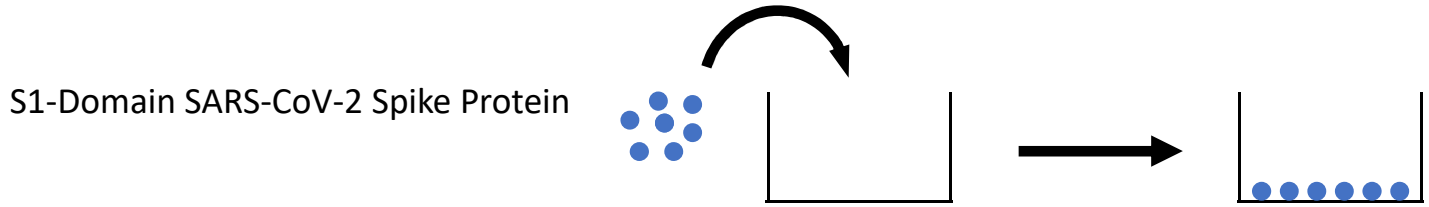
	MIS-C patients (n=22)	Control patients (n=17)
Age (years), mean (IQR)	9.9 (1-16)	8.1 (1-16)
Male (%)	17 (77)	12 (70)
Ethnicity, n (%) • **Caucasian** • **African** • **Hispanic** • **Asian** • **Mixed**	 • 7 (32) • 4 (18) • 2 (9) • 2 (9) • 7 (32)	 • 9 (53) • 3 (18) • 1 (6) • 0 (0) • 4 (24)

Methodes

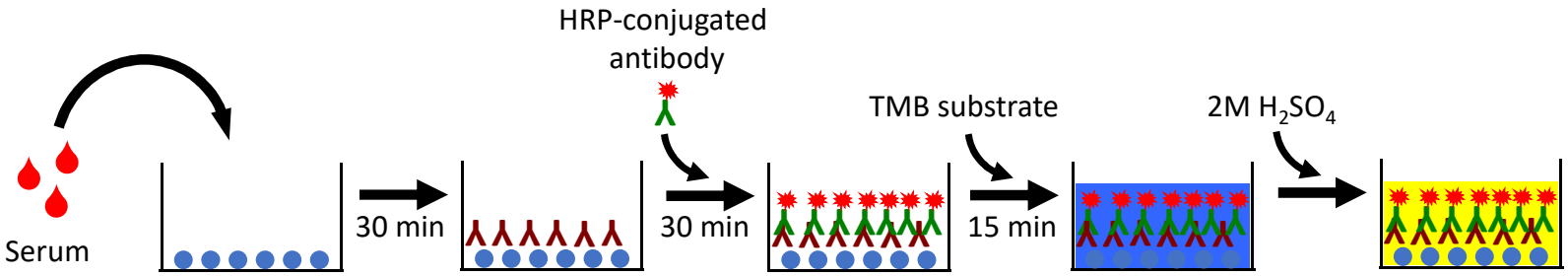
- ELISA : Enzyme-Linked Immunosorbant Assay
- LUMINEX : Multiplex immunoassay
- PRNT : Plaque Reduction Neutralization Test

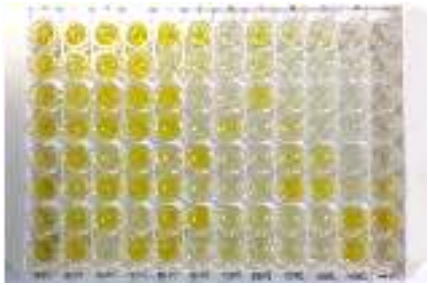
Enzyme-linked Immunosorbend Assay (ELISA)

1. Coating of 96-well plates with Antigen

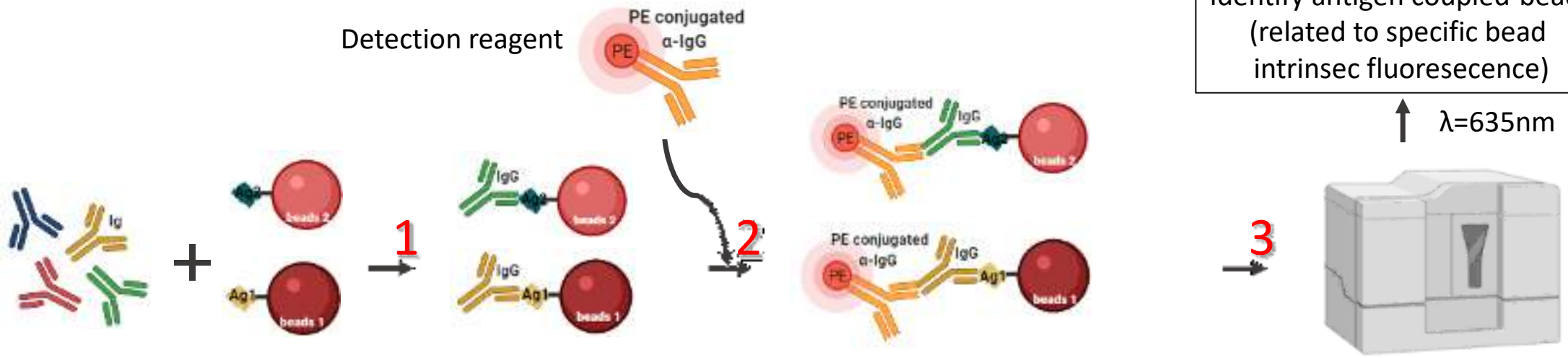


2. Antibody detection in patient serum





Luminex for isotype/subclass quantification: a multiplex immunoassay



Identify antigen coupled-beads (related to specific bead intrinsec fluoresecence)

λ=635nm

Serum

Antigen coupled-beads (fluorescent Magnetic)

Immune complex coupled-beads

Detection antibody α-IgG bound to immune complex

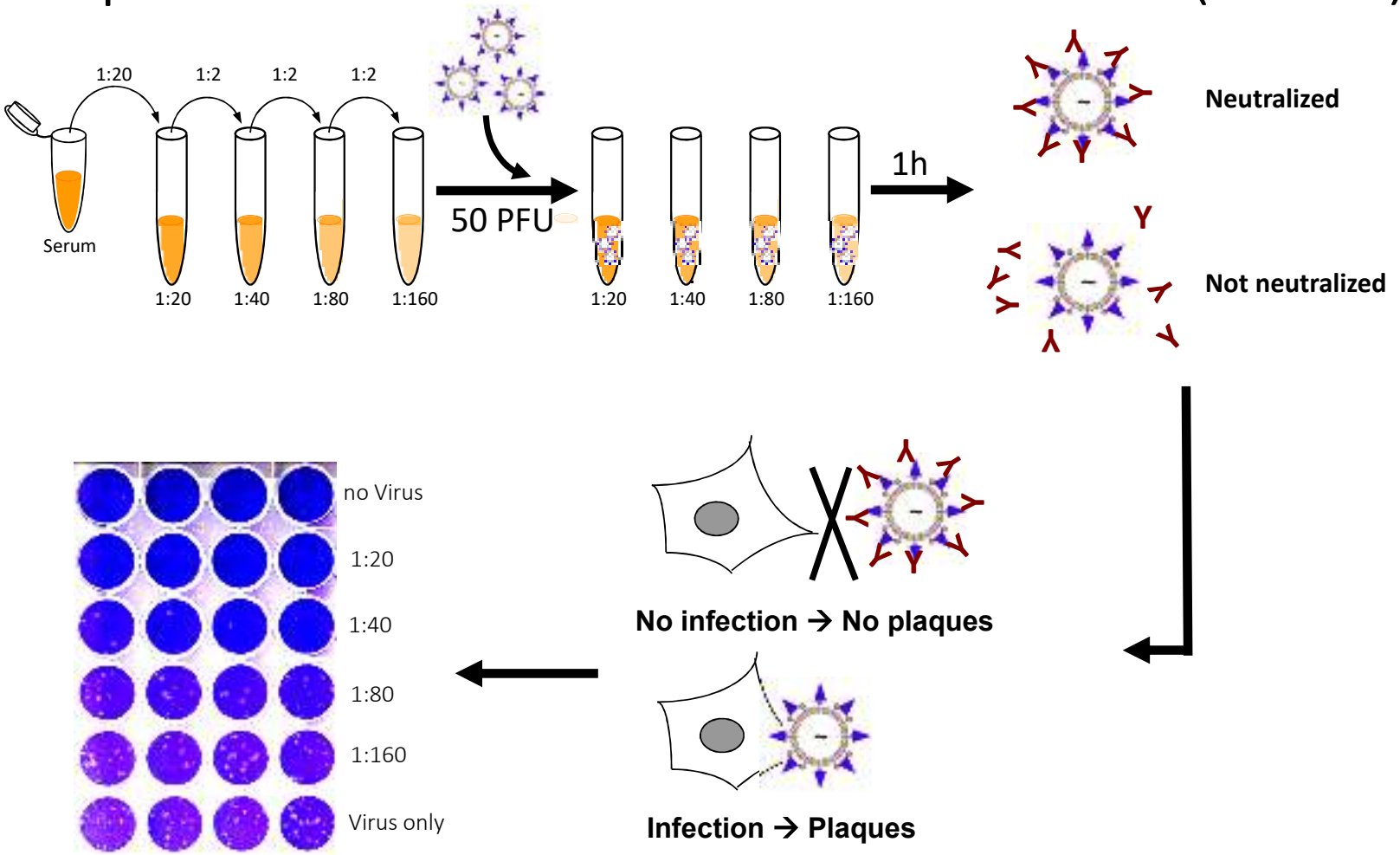
Bioplex200

λ=525nm

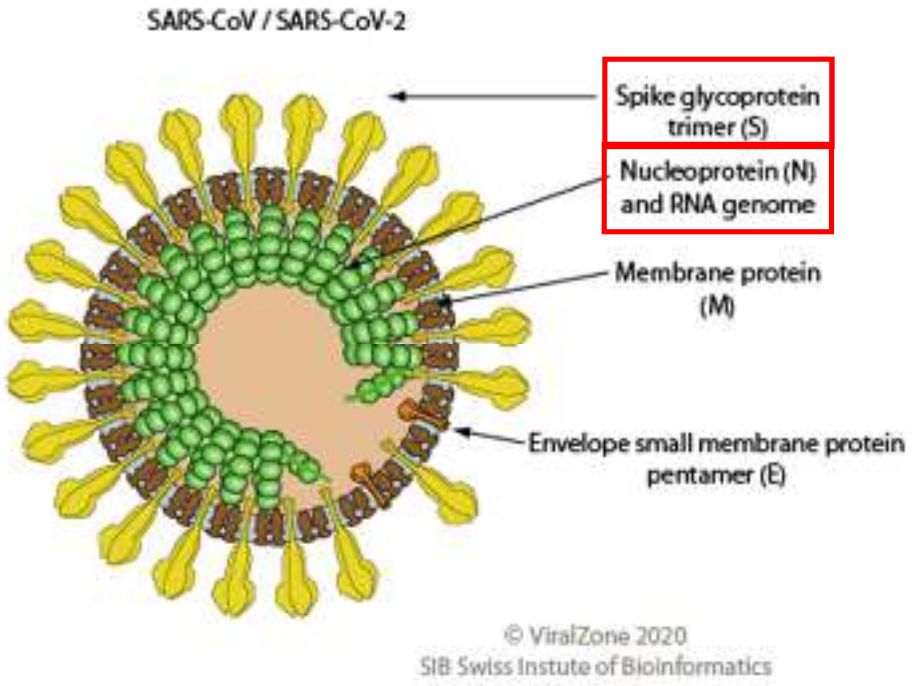
Quantify binding events (related to detection reagent, Result in MFI)

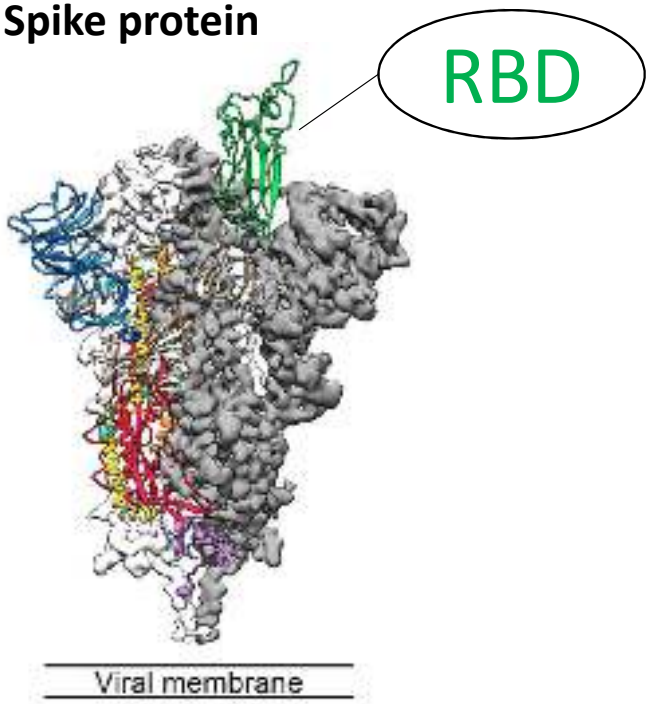
1 & **2** = incubation steps
3 = reading step

Plaque Reduction Neutralization Test (PRNT)



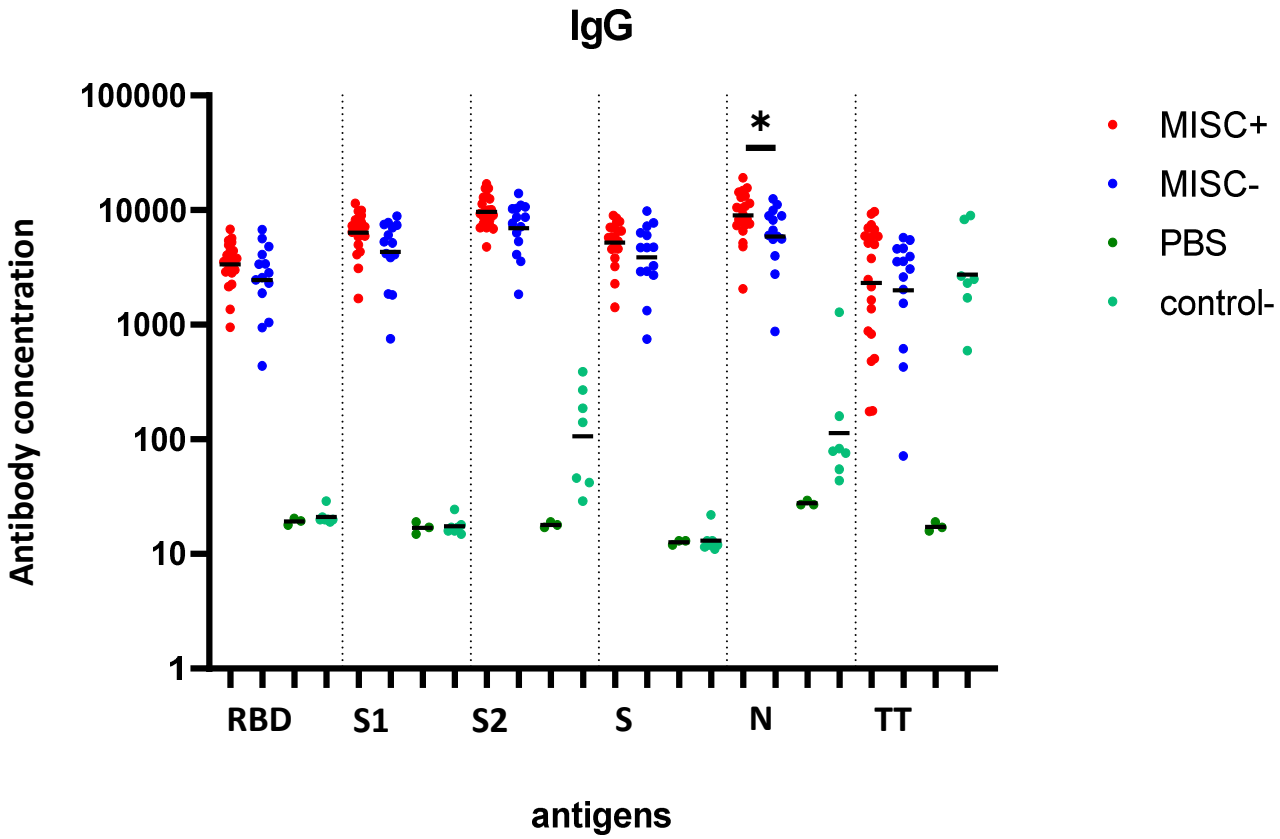
Viral proteins



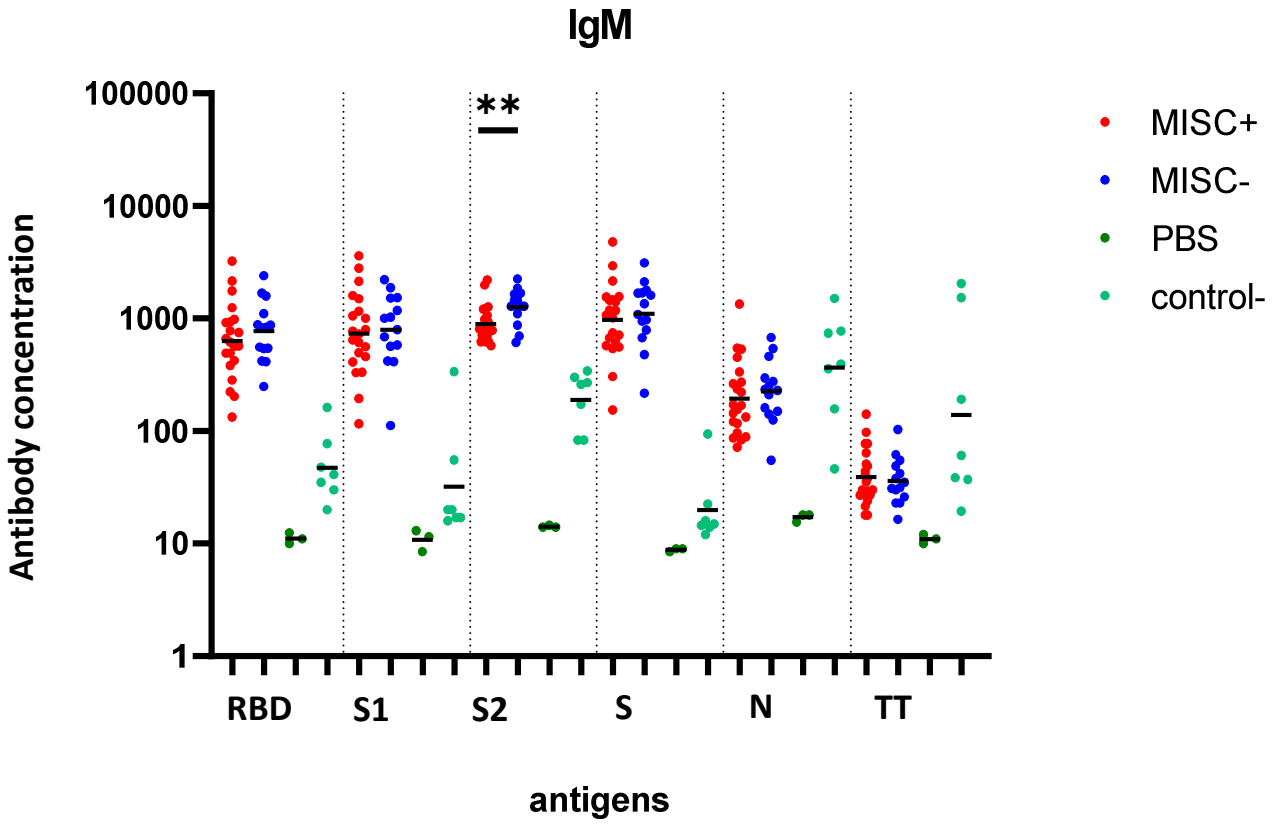


Source: D. Wrapp, et al., Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, eabb2507 (2020).

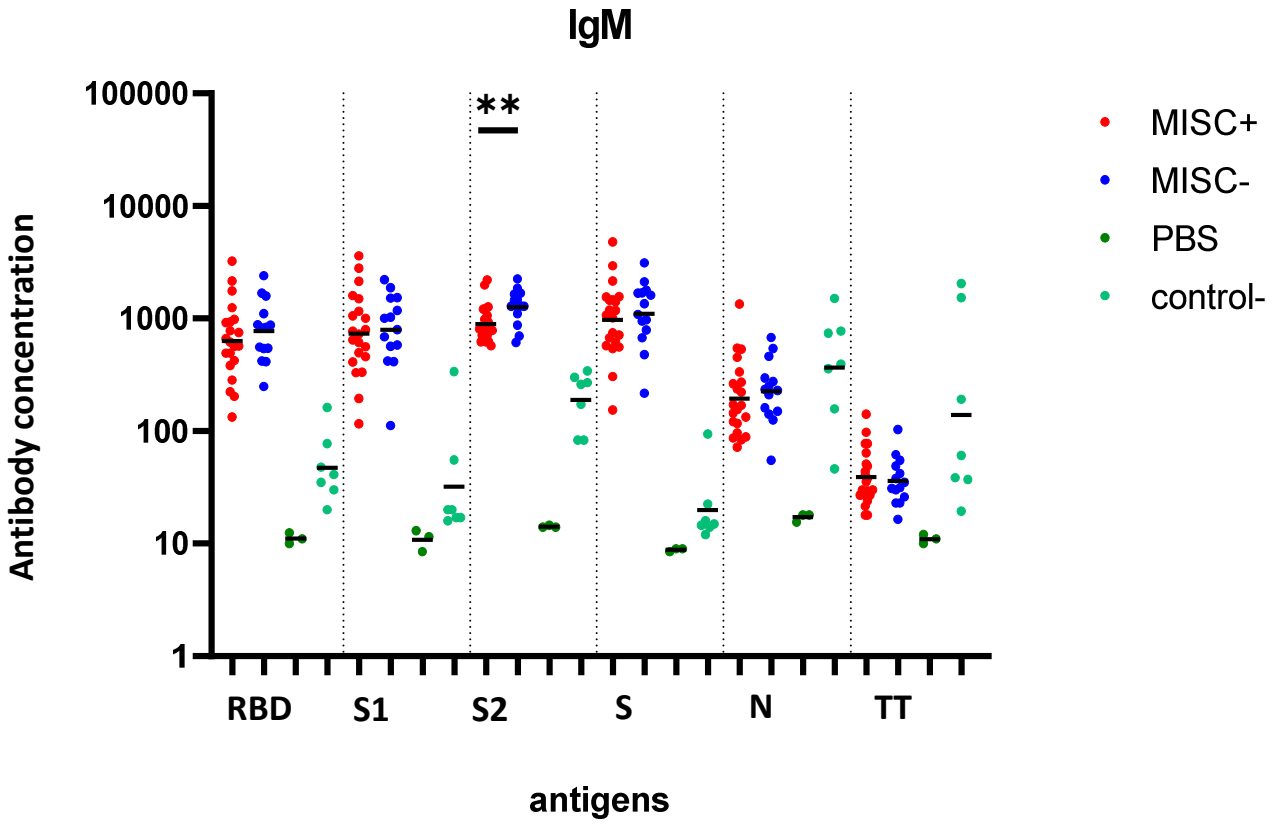
IgG responses against various viral proteins



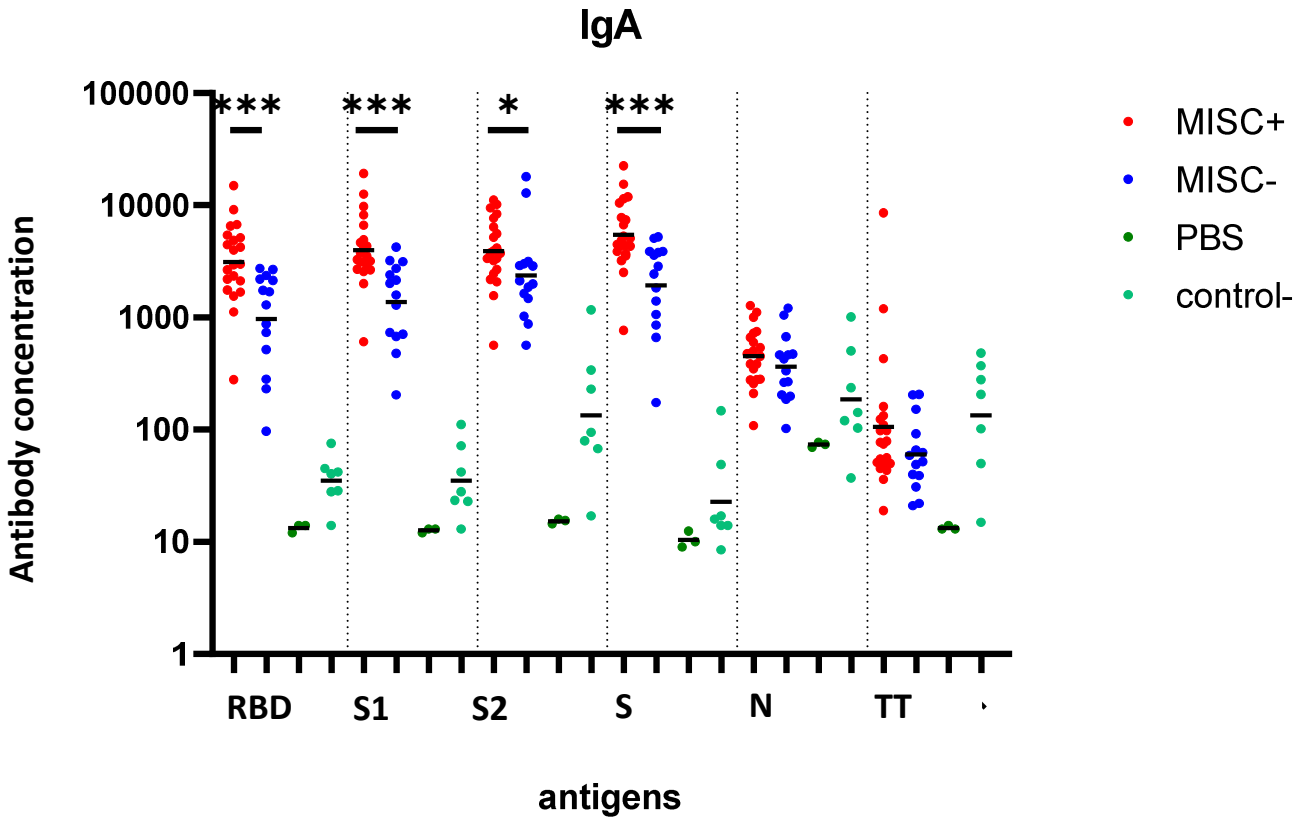
IgM responses for various viral proteins



IgM responses for various viral proteins

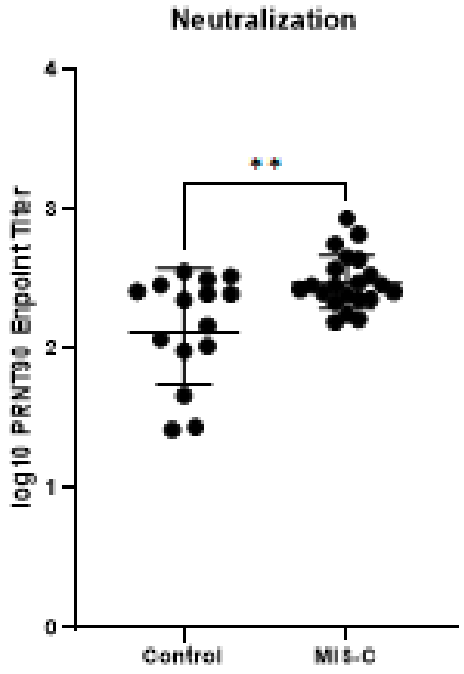


IgA responses for various viral proteins



Results

- Neutralising Abs significantly higher in MIS-C compared to controls



Conclusion

- MISC patients have a stronger IgA response and neutralizing antibodies than the control patients.
- Hypotheses:
  - Sustained inflammation in the gut due to persisting viremia in the intestine in some children?
  - Disturbed mucosal immune response ?
    - Different composition of intestinal microbiota
    - Previous GI tract infection with common cold coronaviruses

Next steps

- To assess IgG and IgA responses against
  - common cold coronaviruses
  - commensal respiratory /gut strains

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