COVID

MDPI

Article

The Role of Intestinal Epithelial Permeability in Multisystem Inflammatory Syndrome in Children: A Case–Control Study

Cathal Roarty, Clare Mills, Claire Tonry, Helen E. Groves, Chris Watson and Thomas Waterfield *

Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast BT97BL, UK; croarty01@qub.ac.uk (C.R.); clare.mills@qub.ac.uk (C.M.); chris.watson@qub.ac.uk (C.W.)
* Correspondence: t.waterfield@qub.ac.uk

Abstract: Background: Multisystem inflammatory syndrome in children (MIS-C) occurs after SARS-CoV-2 infection, with gastrointestinal symptoms a prominent feature. This syndrome has been proposed to be triggered by persistent SARS-CoV-2 antigenemia due to increased intestinal epithelial permeability. We obtained evidence for this in this study. Methods: In a single-centre study, we recruited 83 children and analysed blood samples to quantify the circulating markers of increased intestinal permeability following SARS-CoV-2 infection. Publicly available proteomics MIS-C datasets were also accessed to assess the evidence for increased intestinal permeability. We further quantified SARS-CoV-2 antigenemia and the humoral response to SARS-CoV-2 spike protein. Results: Following SARS-CoV-2 infection, healthy children demonstrated no dysregulation of the intestinal epithelial barrier. In MIS-C, considerable increases in markers of epithelial dysfunction were observed, with similar increases noted in febrile controls. Furthermore, we found little evidence of persistent SARS-CoV-2 antigenemia in MIS-C. Conclusions: Our results suggest that although increased intestinal epithelial permeability is a feature of MIS-C, it is not unique to the condition, and persistent SARS-CoV-2 antigenemia does not occur.

Keywords: COVID; SARS-CoV-2; PIMS-TS; MIS-C

Citation: Roarty, C.; Mills, C.; Tonry, C.; Groves, H.E.; Watson, C.; Waterfield, T. The Role of Intestinal Epithelial Permeability in Multisystem Inflammatory Syndrome in Children: A Case–Control Study. *COVID* **2024**, *4*, 1355–1367. https://doi.org/10.3390/covid4090096

Academic Editor: Bruno Megarbane

Received: 10 July 2024
Revised: 10 August 2024
Accepted: 14 August 2024
Published: 24 August 2024

1. Introduction

Multisystem inflammatory syndrome in children (MIS-C) is an immune-mediated hyperinflammatory condition estimated to affect between 0.026 to 0.045% of children infected with SARS-CoV-2 [1–4]. MIS-C typically occurs four to six weeks after acute SARS-CoV-2 infection and clinically overlaps with a childhood vasculitis known as Kawasaki disease (KD) and the superantigen-mediated toxic shock syndrome (TSS). As with KD, there is often evidence of vasculitis, coronary artery inflammation, dermatological involvement, and myocarditis. Unlike KD, many children with MIS have significantly impaired cardiac function during the acute phase, requiring inotropic support, along with multiorgan failure like that observed in acute TSS [5,6].

The underlying immune mechanisms remain unclear, although MIS-C does appear to be a postinfective phenomenon, with a sizeable proportion of cases not having evidence of concurrent SARS-CoV-2 infection whilst having a serological antibody response consistent with recent SARS-CoV-2 infection [5,7,8]. The delay between initial infection with SARS-CoV-2 and subsequent MIS-C makes studying the immune response challenging. Several studies have reported immune cell population changes, with skewing of B- and T-cell repertoires [9–11].

Due to the overlapping clinical and immune phenotypes between TSS and MIS-C, it has been suggested that MIS-C may be driven by a response to a persistent superantigen [12,13]. In support of this, computational modelling of SARS-CoV-2 spike (S) protein identified several epitopes with potential superantigenic activity [14]. These included a base sequence prior to the S1/S2 cleavage region, which is uniquely present in SARS-CoV-2 compared to

the other viruses in the Betacoronavirus genus. Unlike TSS however, the immune response to a potential superantigen occurs weeks after the initial infection. One of the leading hypotheses proposed to explain this delayed response is that the persistence of SARS-CoV-2 within the gut causes localised inflammation and increased gut permeability, with an associated persistent SARS-CoV-2 antigenemia leading to MIS-C [15]. Clinically, this hypothesis fits with the frequently observed abdominal symptoms reported in children with SARS-CoV-2 infection and the commonly reported severe abdominal symptoms observed with MIS-C [5,16–18]. In addition, markers of gut permeability such as zonulin, a regulator of epithelial tight junctions, and lipopolysaccharide binding protein (LPB) were shown to be elevated in MIS-C compared with healthy controls [15]. No comparison has been made, however, between the circulating levels of these markers in MIS-C and a hospitalised paediatric cohort until now.

An antagonist to zonulin, larazotide, was suggested as a potential therapeutic for MIS-C, and has been used in combination with immunomodulators to treat a series of MIS-C cases [19]. It is not currently recommended for use in MIS-C [20,21].

The objectives of this study were to quantify the markers of intestinal epithelial permeability following SARS-CoV-2 infection in children with and without MIS and to measure these markers in children admitted to hospital with febrile illnesses not related to SARS-CoV-2.

2. Materials and Methods

2.1. Study Design

The protocol for the COVID Warriors study was previously published and adhered to the STROBE statement for observational cohort studies. Initially designed as a seroprevalence study, the COVID Warriors study was amended to include children hospitalised with either COVID-19 or MIS-C [22]. The analysis planned for the additional samples recruited through this amendment was made available prior to analysis [23]. The case–control study of hospitalised children was conducted at the only tertiary children's hospital in Northern Ireland between May 2020 and January 2023.

2.2. Participants

Children under 16 years of age admitted to hospital with MIS-C or serious infection were eligible for inclusion [18,20]. MIS-C was defined as per the Centers for Disease Control and Prevention (CDC). The diagnosis of MIS-C was made by a paediatric infectious diseases specialist independent. Febrile controls were selected from hospitalised children diagnosed with a suspected severe infection and no evidence of concurrent SARS-CoV-2 infection. Severe infection was defined as a bacterial or viral infection resulting in hospital admission. Healthy children with samples available before and after SARS-CoV-2 infection were selected from the COVID Warriors study, matched to the MIS-C cohort by age and sex.

2.3. Clinical Variables and Data Sources

Participants were screened for eligibility by clinical staff at the time of admission, and study data were recorded using an electronic CRF. All participants in the study received clinical care as per local guidance without delay. Study data included demographic details, clinical features, laboratory results, treatments received, final diagnosis, levels of care, and paediatric sequential organ failure assessment (SOFA) scores.

2.4. Blood Sample Collection

Venous blood samples were collected into a BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) EDTA and serum-separating tubes by clinical staff. Where possible, blood sampling was timed with routine phlebotomy events to minimise the number of phlebotomy events performed for each child. The venous blood sample was centrifuged at 1500× g for 15 min within one hour of sample collection. Aliquoted samples were stored at −80 °C until required.

2.5. Additional Laboratory Analysis

LC-MS/MS Measurement of Protein Markers

Depleted plasma samples were digested and analysed on an LC-MS/MS system. Zonulin, ICAM-1, LBP, and CD14 were identified by their respective Uniprot identifier [24]. Protein abundance values were log2-normalised, and missing values were imputed with the median minus two standard deviations of each protein.

2.6. Circulating Spike Protein Quantification

Serum samples were analysed using the commercially available S-Protein (S1RBD) ELISA (ab284402, ABCAM), as previously described [25]. Serum samples were diluted 1:4 with provided sample diluent and analysed in duplicate following the manufacturer's instructions.

2.7. Quantification of Anti-RBD IgG, IgM, and IgA Antibodies

We used an enzyme-linked immunoassay as previously described [26]. Serum samples were 3-fold serially diluted from 1:100 to 1:2700, incubated for 1 h, and then washed. The optical density at 450 nm (OD450) was plotted for each dilution, and the area under the curve (AUC) was calculated, with a baseline set as the mean + 3 standard deviations of a pool of seronegative samples for each antibody subtype.

2.8. Statistical Methods

The study population was described in terms of demographic characteristics, clinical features, levels of care, admission to intensive care units, and survival using descriptive statistics. Statistical analysis and graph generation were performed using GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, CA, USA). Comparative analysis between before and after SARS-CoV-2 infection protein abundances was performed using the paired sample *t* test. Multiple comparative analyses were made using a one-way ANOVA with Tukey's post hoc test. Correlation and regression analyses between age and protein abundances were performed using Pearson's correlation. Categorical comparisons were made using Fishers exact test. $p < 0.05$ was taken to be significant unless otherwise stated.

2.9. Patient and Public Involvement

On commencing the original COVID Warriors study, we convened groups of parents and young people to assist with the design and conduct of this study. They have informed the design of this study, providing feedback on participant information, the conduct of clinics, and on how to make the procedures more comfortable. The COVID Warriors PPI group were also involved with the dissemination of the results and the promotion of this study. This included television interviews. For this additional work to investigate MIS-C, additional PPI members with experience with MIS-C were included. They helped to secure funding for this study and influenced the design and conduct of the case–control component.

3. Results

3.1. Cohort Description

The median age of the entire cohort was 103 months (IQR 54 to 152 months). There were 41 boys and 42 girls included. A total of 25 MIS-C cases were included, with 25 SARS-CoV-2 seropositive controls and 33 febrile controls. The sex makeup of the groups was similar, while the febrile controls had a significantly lower median age (58 months, IQR 30 to 125) than the MIS-C group (119 months, IQR 85 to 163 months, $p = 0.011$). GI symptoms were more common in the MIS-C group, with 21 (84%) presenting with GI symptoms, than in the febrile control group, with 14 (42%) reporting GI symptoms during their admission ($p < 0.0001$).

Further details can be found in Tables 1 and 2.

One-way ANOVA with Tukey's multiple comparison was used for age, and the chi squared test was used for sex and GI symptoms to assess for significant differences.

Table 1. Cohort summary.

	MIS-C (*N* = 25)	Febrile Controls (*N* = 33)	Seropositive Healthy Controls (*N* = 25)	*p*-Value
Male, *n* (%)	13 (52%)	14 (42.4%)	14 (56%)	0.564
Age, months, Median (IQR)	119 (85, 163)	58 (30, 125)	112(85 to 158)	0.065
GI symptoms reported, *n* (%)	21 (84%)	NA	7 (38.9%)	0.0001

Table 2. Clinical features.

	MIS-C (*N* = 25)	Febrile Controls (*N* = 33)	*p*-Value
Pre-existing health condition, *n* (%)	6(14%)	18(56%)	0.014
Steroid administered, *n* (%)	18 (72%)	11 (33%)	0.004
Antibiotic administered, *n* (%)	9 (41%)	28 (85%)	<0.001
Inotrope administered, *n* (%)	10 (40%)	5 (15%)	0.032
Respiratory support, *n* (%)	6 (27%)	21 (64%)	0.008
ICU admission, *n* (%)	11 (44%)	16 (50%)	0.65
Length of hospital stay, median (IQR)	8.0 (6.0, 9.0)	7.0 (5.0, 13.5)	0.88
Deceased, *n* (%)	0(0%)	3(9.1%)	0.25

3.2. The Effects of SARS-CoV-2 Infection on the Intestinal Epithelial Permeability of Healthy Children

Given the existing evidence of GI symptoms and a change in the circulating markers of intestinal epithelial permeability in MIS-C, we first assessed the levels of these markers in a cohort of healthy children with longitudinal samples both before and after SARS-CoV-2 infection as indicated by positive serology [22]. The levels of zonulin, ICAM-1, and LBP, as measured in depleted plasma by liquid chromatography tandem mass spectrometry (LC-MS/MS), were not significantly different in these longitudinal samples with $p = 0.464$, $p = 0.844$, and $p = 0.087$, respectively. CD14 was lower in the postinfection seropositive samples ($p = 0.0082$) (Figure 1A). We performed subgroup analysis of the healthy controls based on the presence of gastrointestinal symptoms. There was no significant difference in the abundance of circulating zonulin, ICAM-1, LBP, or CD14 between the healthy controls who experienced GI symptoms and those who did not ($p = 0.467$, $p = 0.679$, $p = 0.504$, $p = 0.576$, respectively) (Figure 1B).

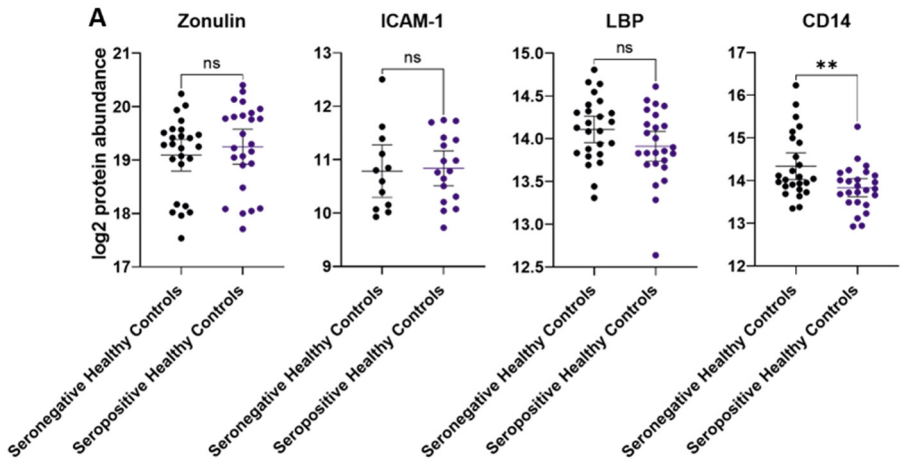

Figure 1. *Cont.*

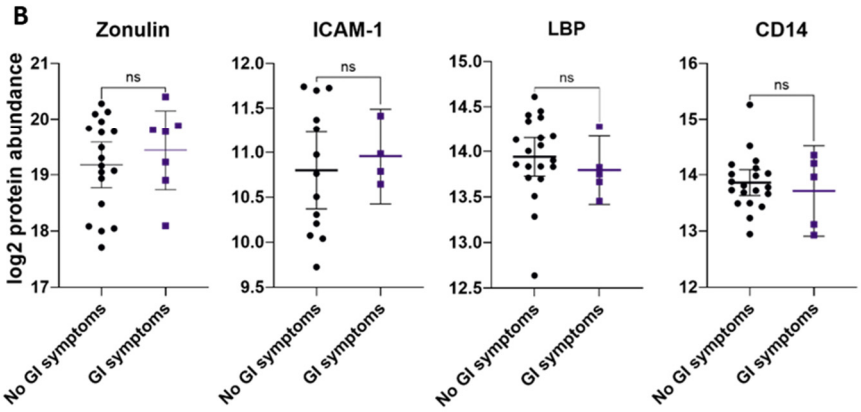

Figure 1. (**A**) Plasma abundances of zonulin, ICAM-1, LBP, and CD14 were measured by LC-MS in healthy children (n = 25) before (seronegative) and after (seropositive) SARS-CoV-2 infection, compared using a paired samples *t* test. (**B**). Plasma abundances of zonulin, ICAM-1, LBP, and CD14,measured by LC-MS in healthy children (n = 25) reporting GI symptoms or no GI symptoms following SARS-CoV-2 infection, compared using unpaired *t* test. ** $p < 0.01$, ns =not significant. Mean values and 95% confidence intervals are shown.

3.3. Intestinal Epithelial Permeability in Children with MIS-C Compared with That of Control Groups

We next compared the levels of circulating zonulin, ICAM-1, LBP, and CD14 between MIS-C cases, febrile controls, and seropositive healthy controls. The levels of zonulin, ICAM-1, LBP, and CD14 in the MIS-C cases were significantly higher than those in the seropositive healthy controls ($p < 0.0001$, $p < 0.0001$, $p = 0.0153$, and $p = 0.0004$, respectively) (Figure 2A). The levels of circulating zonulin, ICAM-1, LBP, and CD14 were however, not significantly different compared with those of the matched febrile controls ($p = 0.171$, $p = 0.052$, $p = 0.199$, and $p = 0.422$, respectively).

To further investigate the increased intestinal epithelial permeability in children with MIS-C, we assessed the translocation of mucosal immunoglobulin A (IgA) into the circulation by measuring the blood levels of the heavy chain constant region of both IgA isoforms (immunoglobulin heavy constant alpha 1 (IGHA1) and immunoglobulin heavy constant alpha 2 (IGHA2)). Both IgA isoform levels were higher in MIS-C than in seropositive healthy controls (IGHA1, $p = 0.0415$; IGHA2, $p = 0.0291$). In contrast, there were no significant differences observed between MIS-C and febrile controls in either IGHA1 or IGHA2 levels ($p = 0.083$ and $p = 0.996$, respectively) (Figure 2B).

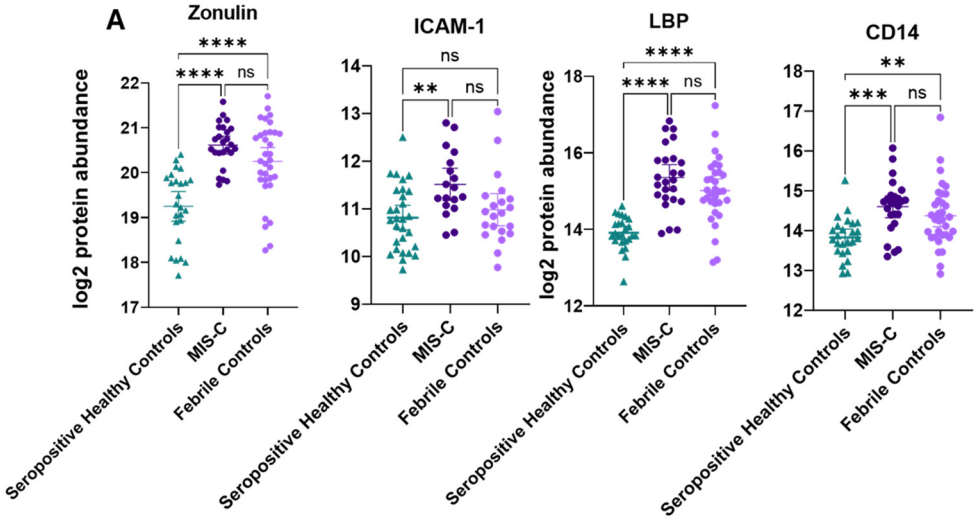

Figure 2. *Cont.*

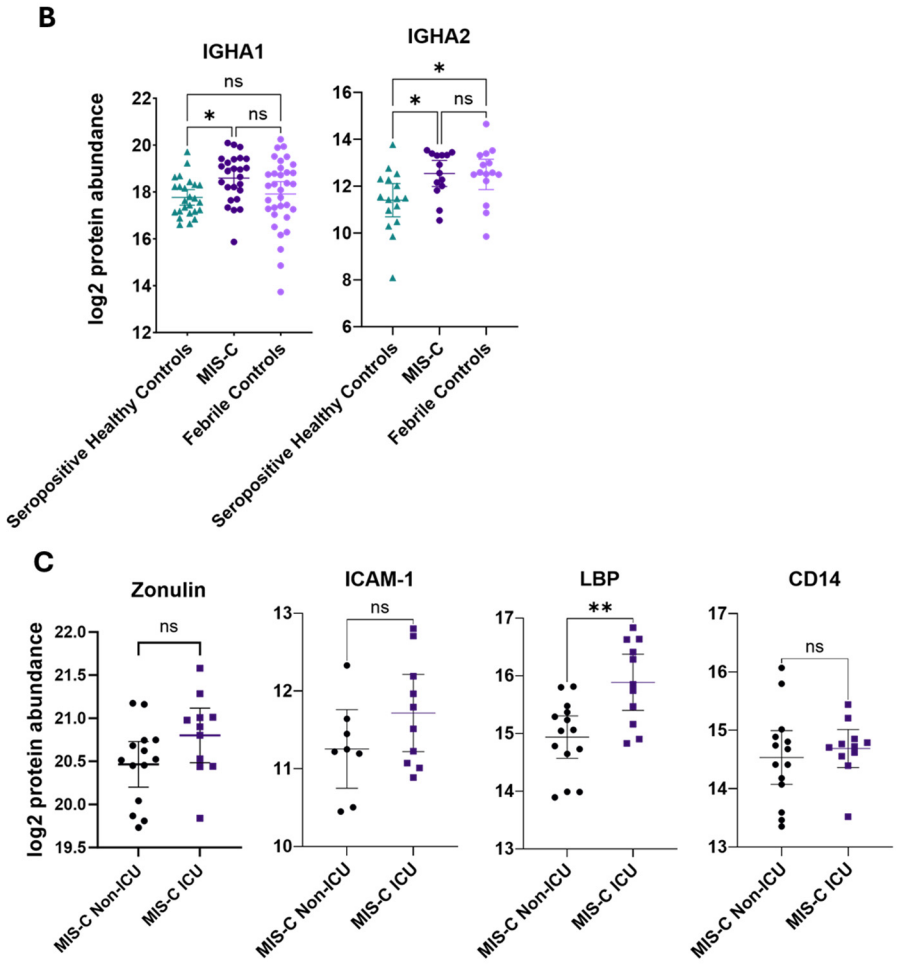

Figure 2. (**A**)**.** Plasma abundances of zonulin, ICAM-1,LBP, and CD14 measured by LC-MS in healthy children (n = 25) following SARS-CoV-2 infection, MIS-C (n = 25), and febrile controls (n = 33) compared by one-way ANOVA with multiple comparisons. (**B**). Plasma abundances of IGHA1 and IGHA2 measured by LC-MS in healthy children (n = 25) following SARS-CoV-2 infection, MIS-C (n = 25), and febrile controls (n = 33) compared by one-way ANOVA with multiple comparisons. (**C**). Plasma abundances of zonulin, ICAM-1, LBP, and CD14 measured by LC-MS in MIS-C cases admitted to the ICU (n = 11) and MIS-C not admitted to the ICU (n = 14) compared by unpaired *t* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Mean values and 95% confidence intervals are shown.

There were no significant differences in the levels of circulating zonulin, ICAM-1, and CD14 between MIS-C cases admitted to the ICU and MIS-C cases not admitted to the ICU (Figure 2C). The circulating levels of LBP were significantly higher in the MIS-C cases admitted to the ICU than in cases not admitted to the ICU.

3.4. Circulating Levels of SARS-CoV-2 Spike Protein Following SARS-CoV-2 Infection in Children with and without MIS-C

To assess persistent viral protein antigenemia in MIS-C, we performed an ELISA measuring the receptor binding domain (RBD) of the spike protein, as previously reported [25]. For a large minority of both MIS-C (n = 9, 45%) and seropositive healthy controls (n = 4, 26.67%), no circulating RBD was detected. The levels of the RBD portion of the spike protein were not significantly different between MIS-C and seropositive healthy controls ($p = 0.6992$) or seronegative healthy controls ($p > 0.999$) (Figure 3A). To further investigate persistent viral antigenemia driving the pathogenesis of MIS-C, we quantified antibody subclasses to the spike RBD. In concordance with the results for the circulating RBD protein,

there were no significant differences in the anti-spike RBD IgG, IgM, or IgA between the MIS-C and the seropositive healthy controls ($p = 0.196$, $p = 0.648$, $p = 0.287$) (Figure 3B).

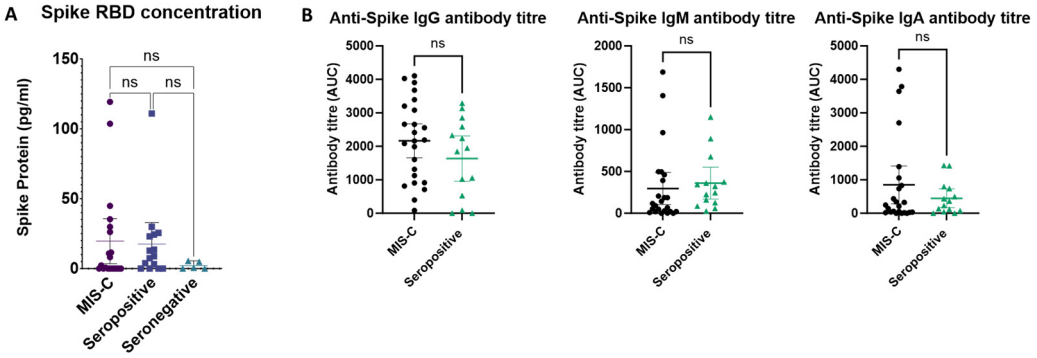

Figure 3. (**A**). Circulating levels of spike protein measured by ELISA in MIS-C (n = 25), seropositive healthy controls (n = 16), and seronegative healthy controls (n = 5) compared by one-way ANOVA. Mean values and 95% confidence intervals are shown. (**B**) Circulating titres of antibodies to spike protein measured by ELISA in MIS-C (n = 25) and seropositive healthy controls (n = 25) compared with unpaired *t* test. ns = not significant. Mean values and 95% confidence intervals are shown.

3.5. In Silico Investigation of Markers of Intestinal Epithelial Permeability

To investigate the markers of gut permeability that are increased in MIS-C, we assessed their levels in publicly available datasets of MIS-C proteomes. Three datasets meeting our criteria were identified [25,27,28]. Zonulin was present in dataset A [27]. Although the individual peptide-level data were not available, the mean levels were higher in MIS-C than in the healthy controls ($p < 0.0001$). Similarly, in this dataset, the levels of ICAM-1 ($p < 0.0001$), LBP ($p < 0.0001$), and CD14 ($p < 0.0001$) were all higher in both disease groups than in the healthy controls (Figure 4A). In dataset B, zonulin was not present [28]. The levels of ICAM-1 were significantly higher in patients with MIS-C than in the healthy controls ($p < 0.0001$) but not significantly different from cases of severe acute SARS-CoV-2 infection (Figure 4B). The levels of LBP ($p < 0.0001$) and CD14 ($p < 0.0001$) were likewise higher in those with MIS-C than in healthy controls but not significantly raised compared to in those with severe acute SARS-CoV-2 infection. In dataset C, ICAM-1 and LBP data were available [25]. The levels of both were not significantly different between patients with MIS-C and children with acute SARS-CoV-2 infection or healthy controls (Figure 4C).

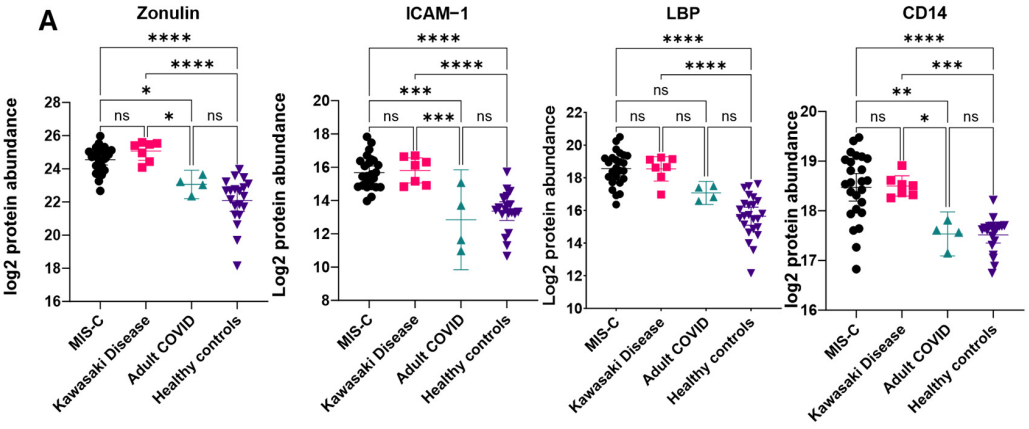

Figure 4. *Cont.*

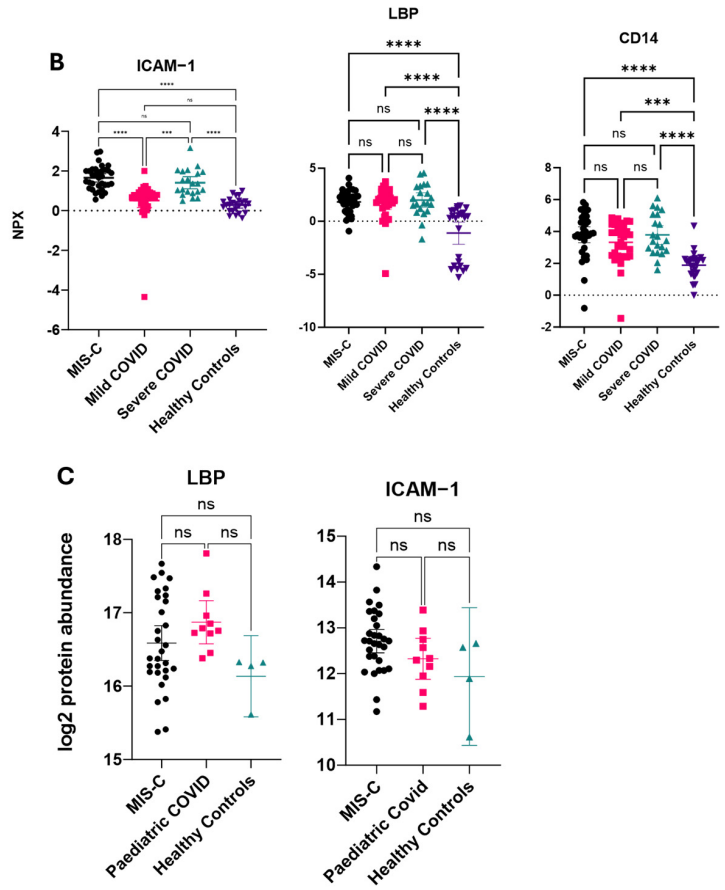

Figure 4. (**A**) Circulating titres of zonulin, ICAM-1, LBP, and CD14 in dataset A. (**B**) Circulating titres of ICAM-1, LBP, and CD14 in dataset B. (**C**) Circulating titres of ICAM-1 and LBP in dataset C. All compared by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant. Mean values and 95% confidence intervals are shown.

4. Discussion

The aim of this study was to test the hypothesis that the persistence of SARS-CoV-2 within the gut causes localised inflammation and increased gut permeability, with the associated persistent SARS-CoV-2 antigenemia leading to MIS-C [13,15] (Figure 5). In adults, the severity of SARS-CoV-2 infection has been shown to correlate with the abundance of the circulating markers of intestinal epithelial permeability such as zonulin, LBP, and CD14 [29–31]. The translocation of the contents of the gut lumen due to the dysfunction of epithelial tight junctions in severe disease may provide a mechanism for the development of MIS-C through the persistence of circulating antigen. Acute SARS-CoV-2 infections in children are usually mild, with less than 1% of cases requiring hospital admission [32]. There has been no correlation demonstrated between the severity of initial SARS-CoV-2 infection and the risk of developing MIS-C [33,34]. There was no difference observed in the levels of zonulin ($p = 0.464$), ICAM-1 ($p = 0.844$), and LBP ($p = 0.087$) amongst healthy controls before and after SARS-CoV-2 infection, which does not support an increase in intestinal permeability following SARS-CoV-2 infection in healthy children.

It has been suggested that MIS-C occurs secondarily to persistent antigenemia, specifically the spike protein translocating from the gut to the bloodstream. This has been proposed to be due to the persistence of viral infection in the intestinal epithelium, and SARS-CoV-2 viral proteins have been found in the stool of individuals numerous days after symptoms develop [15,35–37]. In contrast, the results of this study do not support a humoral response in keeping with persistent viral infection, with no significant difference in the antibody response to the RBD of S protein in healthy children with SARS-CoV-2 or

MIS-C. This is in keeping with findings in other MIS-C cohorts, with the antibody response being shown to be of a similar proportion to that of normal infection [11,38,39]. The titres of anti-spike IgM and anti-spike IgA, which, in a situation of recent stimulation by an antigen, were not significantly different between MIS-C and the seropositive group (p = 0.648, p = 0.287). Additionally, we found no evidence of increased spike protein in the circulation of MIS-C in comparison to seropositive healthy controls (p= 0.6992) [38,39].

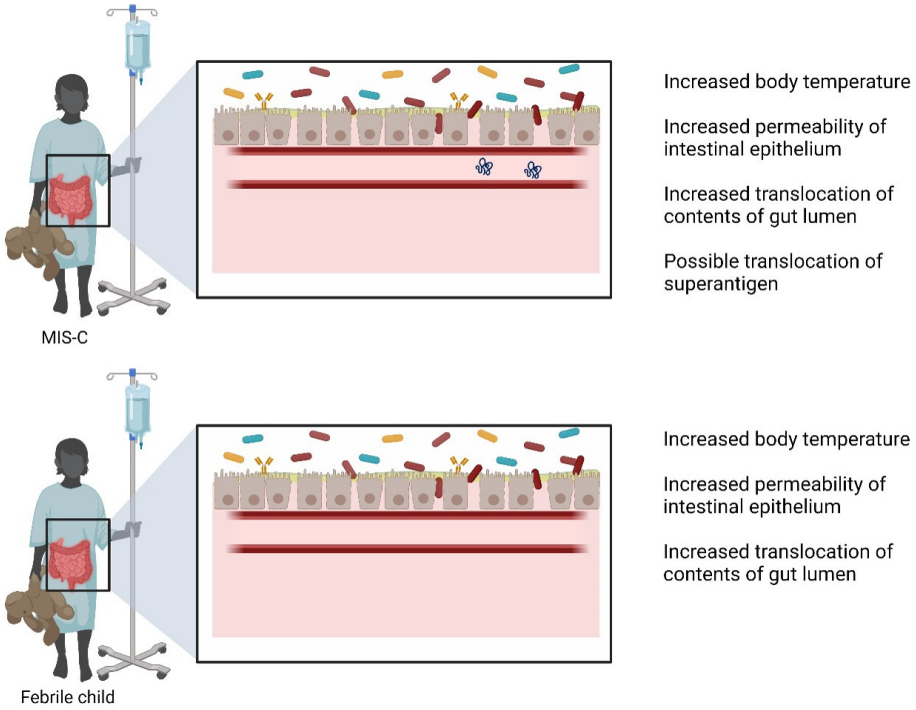

Figure 5. Diagram showing proposed increase in intestinal epithelial permeability in MIS-C and febrile children.

Although an increase in intestinal epithelial permeability markers was not observed following SARS-CoV-2 infection in the healthy control cohort, it was observed in MIS-C with elevated levels of zonulin, ICAM-1, LBP, or CD14 compared to healthy controls (p = 0.465, p = 0.844, p = 0.087, p = 0.0082). These results are similar to the findings from the in silico validation, demonstrating a similar trend with regard to zonulin, ICAM-1, and LBP having higher levels in MIS-C than in healthy controls [25,27,28].

Unlike previous research, our study is the first to compare MIS-C with matched febrile controls experiencing a similar disease severity. We found no significant difference in the circulating levels of zonulin, ICAM-1, LBP, or CD14 between MIS-C and febrile controls (p = 0.171, p = 0.052, p = 0.199, p = 0.422). This indicates that increased gut permeability is not unique to MIS-C and occurs to a similar extent in febrile children with a variety of clinical conditions [40–46].

Fever is common in response to infection or inflammation and is associated with increased intestinal barrier permeability [40–43]. Similarly intestinal dysfunction can occur secondary to an initial insult to a distant organ, and this has been described in a number of cases such as liver cirrhosis, stroke, and burns [44,45]. The presence of proinflammatory mediators leads to the increased permeability of the intestinal epithelial barrier, local intestinal inflammation, and to GI tissue damage, culminating in an acute failure of the intestine [46–48]. It is plausible that the increased body temperature present in MIS-C, coupled with the increased levels of circulating cytokines, leads to an increase in intestinal epithelial barrier permeability through a mechanism like that of any severe febrile childhood illness. Our findings of increased markers of intestinal epithelial permeability in febrile controls suggests that an increase in intestinal epithelial permeability may occur

more frequently than currently considered in febrile children hospitalised with an infectious cause, as previously suggested [49].

Our study has a few limitations. Due to the rarity of MIS-C, only a moderate number of cases were recruited. It is possible that the differences in the levels of circulating S1 protein in our samples and in other datasets [25,28] may be due to the use of dithiothreitol to release antibody-bound spike [50]. Several of the comparisons *p*-values are close to the significance threshold, which should be considered when interpreting the findings. A particular strength of this study is the matched febrile and seropositive controls, which allowed for a direct comparison between MIS-C and these cohorts. In addition, our sample collection occurred early during the admission course for the hospitalised cohorts, and samples were all processed within an hour of collection. As this was an observational study with no changes to routine clinical care, the MIS-C and febrile groups received different medications, which may have played a role in the functioning of the intestinal epithelium. In particular, the use of antibiotics in the febrile control group may have contributed to a change in the gut flora, which in turn affected intestinal permeability.

5. Conclusions

In our study, we found no evidence of the persistent elevation of the markers of intestinal epithelial permeability following SARS-CoV-2 infection in healthy children or evidence of persistent spike protein antigenemia. We were able to demonstrate that MIS-C is temporally associated with an increase in intestinal epithelial permeability, but this was not unique to MIS-C and occurred to a similar extent in children with fever due to non-SARS-CoV-2-related causes. As such, increased markers of intestinal permeability may not be used as markers specific to MIS-C. Our findings do not support the hypothesis that increased gut permeability following SARS-CoV-2 infection and the associated persistent spike protein antigenemia drives MIS-C.

Author Contributions: Conceptualisation, T.W., C.W. and C.R.; methodology, C.T., C.M., T.W. and C.W.; formal analysis, C.R., C.W., T.W., C.T., C.M. and H.E.G.; investigation, C.R., C.T. and C.M.; resources, T.W. and C.W.; data curation, C.R. and C.T.; writing—original draft preparation, C.R., T.W. and C.T.; writing—review and editing, C.R., C.M., C.T., H.E.G., C.W. and T.W.; supervision, T.W., C.W. and H.E.G.; project administration, C.M. and C.R.; funding acquisition, T.W. and C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Public Health Agency of Northern Ireland, grant number COM/5712/22, and Northern Ireland Chest Heart and Stroke, 2021_H03.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the London-Chelsea Research Ethics Committee (REC Reference—20/HRA/1731). The Belfast Health & Social Care Trust Research Governance (Reference 19147TW-SW) provided the local research governance.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data are available on request.

Acknowledgments: We wish to acknowledge the children and their families that participated in this research.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Shingleton, J.; Williams, H.; Oligbu, G.; Powell, A.; Cohen, J.; Arditi, M.; Watson-Koszel, T.; Kenny, S.; Gent, N.; Ladhani, S.N. The changing epidemiology of PIMS-TS across COVID-19 waves: Prospective national surveillance, January 2021 to July 2022, England. *J. Infect.* **2022**, *85*, 702–769. [CrossRef]

2. Shingleton, J.; Burton, L.; Williams, H.E.; Finnie, T.J.R.; Bennett, E.; Birrell, P.; Kenny, S.; Watson-Koszel, T.; Viner, R.; Arditi, M.; et al. Risk of paediatric multisystem inflammatory syndrome (PIMS-TS) during the SARS-CoV-2 alpha and delta variant waves: National observational and modelling study, 2020–2021, England. *Front. Pediatr.* **2022**, *10*, 2166. [CrossRef] [PubMed]
3. Payne, A.B.; Gilani, Z.; Godfred-Cato, S.; Belay, E.D.; Feldstein, L.R.; Patel, M.M.; Randolph, A.G.; Newhams, M.; Thomas, D.; Magleby, R.; et al. Incidence of Multisystem Inflammatory Syndrome in Children Among US Persons Infected With SARS-CoV-2. *JAMA Netw. Open* **2021**, *4*, e2116420. [CrossRef] [PubMed]
4. Roarty, C.; Waterfield, T. Review and future directions for PIMS-TS (MIS-C). *Arch. Dis. Child.* **2022**, *108*, e2. [CrossRef]
5. Feldstein, L.R.; Rose, E.B.; Horwitz, S.M.; Collins, J.P.; Newhams, M.M.; Son, M.B.F.; Newburger, J.W.; Kleinman, L.C.; Heidemann, S.M.; Martin, A.A.; et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. *N. Engl. J. Med.* **2020**, *383*, 334–346. [CrossRef] [PubMed]
6. Feldstein, L.R.; Tenforde, M.W.; Friedman, K.G.; Newhams, M.; Rose, E.B.; Dapul, H.; Soma, V.L.; Maddux, A.B.; Mourani, P.M.; Bowens, C.; et al. Characteristics and Outcomes of US Children and Adolescents with Multisystem Inflammatory Syndrome in Children (MIS-C) Compared with Severe Acute COVID-19. *JAMA J. Am. Med. Assoc.* **2021**, *325*, 1074–1087. [CrossRef]
7. Flood, J.; Shingleton, J.; Bennett, E.; Walker, B.; Amin-Chowdhury, Z.; Oligbu, G.; Avis, J.; Lynn, R.M.; Davis, P.; Bharucha, T.; et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS): Prospective, national surveillance, United Kingdom and Ireland, 2020. *Lancet Reg. Health Eur.* **2021**, *3*, 100075. [CrossRef]
8. Hoste, L.; Van Paemel, R.; Haerynck, F. Multisystem inflammatory syndrome in children related to COVID-19: A systematic review. *Eur. J. Pediatr.* **2021**, *180*, 2019–2034. [CrossRef]
9. Porritt, R.A.; Paschold, L.; Rivas, M.N.; Cheng, M.H.; Yonker, L.M.; Chandnani, H.; Lopez, M.; Simnica, D.; Schultheiß, C.; Santiskulvong, C.; et al. HLA class I–associated expansion of TRBV11-2 T cells in multisystem inflammatory syndrome in children. *J. Clin. Investig.* **2021**, *131*, e146614. [CrossRef]
10. Vella, L.A.; Giles, J.R.; Baxter, A.E.; Oldridge, D.A.; Diorio, C.; Kuri-Cervantes, L.; Alanio, C.; Pampena, M.B.; Wu, J.E.; Chen, Z.; et al. Deep immune profiling of MIS-C demonstrates marked but transient immune activation compared to adult and pediatric COVID-19. *Sci. Immunol.* **2021**, *6*, eabf7570. [CrossRef] [PubMed]
11. Ramaswamy, A.; Brodsky, N.N.; Sumida, T.S.; Comi, M.; Asashima, H.; Hoehn, K.B.; Li, N.; Liu, Y.; Shah, A.; Ravindra, N.G.; et al. Immune dysregulation and autoreactivity correlate with disease severity in SARS-CoV-2-associated multisystem inflammatory syndrome in children. *Immunity* **2021**, *54*, 1083–1095.e7. [CrossRef] [PubMed]
12. Kouo, T.; Chaisawangwong, W. SARS-CoV-2 as a superantigen in multisystem inflammatory syndrome in children. *J. Clin. Investig.* **2021**, *131*, e149327. [CrossRef] [PubMed]
13. Noval Rivas, M.; Porritt, R.A.; Cheng, M.H.; Bahar, I.; Arditi, M. Multisystem Inflammatory Syndrome in Children and Long COVID: The SARS-CoV-2 Viral Superantigen Hypothesis. *Front. Immunol.* **2022**, *13*, 3480. [CrossRef] [PubMed]
14. Hongying Cheng, M.; Zhang, S.; Porritt, R.A.; Noval Rivas, M.; Paschold, L.; Willscher, E.; Binder, M.; Arditi, M.; Bahar, I. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 25254–25262. [CrossRef] [PubMed]
15. Yonker, L.M.; Gilboa, T.; Ogata, A.F.; Senussi, Y.; Lazarovits, R.; Boribong, B.P.; Bartsch, Y.C.; Loiselle, M.; Noval Rivas, M.; Porritt, R.A.; et al. Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier. *J. Clin. Investig.* **2021**, *131*, e149633. [CrossRef]
16. Swann, O.V.; Holden, K.A.; Turtle, L.; Pollock, L.; Fairfield, C.J.; Drake, T.M.; Seth, S.; Egan, C.; Hardwick, H.E.; Halpin, S.; et al. Clinical characteristics of children and young people admitted to hospital with covid-19 in United Kingdom: Prospective multicentre observational cohort study. *BMJ* **2020**, *370*, 5. [CrossRef]
17. Whittaker, E.; Bamford, A.; Kenny, J.; Kaforou, M.; Jones, C.E.; Shah, P.; Ramnarayan, P.; Fraisse, A.; Miller, O.; Davies, P.; et al. Clinical Characteristics of 58 Children with a Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2. *JAMA J. Am. Med. Assoc.* **2020**, *324*, 259–269. [CrossRef]
18. Waterfield, T.; Watson, C.; Moore, R.; Ferris, K.; Tonry, C.; Watt, A.; McGinn, C.; Foster, S.; Evans, J.; Lyttle, M.D.; et al. Seroprevalence of SARS-CoV-2 antibodies in children: A prospective multicentre cohort study. *Arch. Dis. Child.* **2020**, *106*, 680–686. [CrossRef]
19. Yonker, L.M.; Swank, Z.; Gilboa, T.; Senussi, Y.; Kenyon, V.; Papadakis, L.; Boribong, B.P.; Carroll, R.W.; Walt, D.R.; Fasano, A. Zonulin Antagonist, Larazotide (AT1001), As an Adjuvant Treatment for Multisystem Inflammatory Syndrome in Children: A Case Series. *Crit. Care Explor.* **2022**, *10*, e0641. [CrossRef]
20. Henderson, L.A.; Canna, S.W.; Friedman, K.G.; Gorelik, M.; Lapidus, S.K.; Bassiri, H.; Behrens, E.M.; Kernan, K.F.; Schulert, G.S.; Seo, P.; et al. American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS–CoV-2 and Hyperinflammation in Pediatric COVID-19: Version 3. *Arthritis Rheumatol.* **2022**, *74*, e1–e20. [CrossRef]
21. Harwood, R.; Allin, B.; Jones, C.E.; Whittaker, E.; Ramnarayan, P.; Ramanan, A.V.; Kaleem, M.; Tulloh, R.; Peters, M.J.; Almond, S.; et al. A national consensus management pathway for paediatric inflammatory multisystem syndrome temporally associated with COVID-19 (PIMS-TS): Results of a national Delphi process. *Lancet Child. Adolesc. Health* **2020**, *5*, 133–141. [CrossRef]
22. Corr, M.; Christie, S.; Watson, C.; Maney, J.; Fairley, D.; Ladhani, S.N.; Lyttle, M.D.; McFetridge, L.; Mitchell, H.; Shields, M.D.; et al. Seroprevalence of SARS-CoV-2 antibodies in children of United Kingdom healthcare workers: A prospective multicentre cohort study protocol. *BMJ Open* **2020**, *10*, e041661. [CrossRef] [PubMed]

23. Roarty, C.; Mills, C.; Tonry, C.; Cosgrove, P.; Norman-Bruce, H.; Groves, H.; Watson, C.; Waterfield, T. Study protocol: Medium throughput, deep proteomic characterization of children with PIMS-TS, and identification of candidate diagnostic biomarkers. *medRxiv* **2022**. [CrossRef]
24. Consortium, T.U.; Bateman, A.; Martin, M.J.; Orchard, S.; Magrane, M.; Ahmad, S.; Alpi, E.; Bowler-Barnett, E.H.; Britto, R.; Bye-A-Jee, H.; et al. UniProt: The Universal Protein Knowledgebase in 2023. *Nucleic Acids Res.* **2022**, *51*, D523–D531. [CrossRef] [PubMed]
25. Sacco, K.; Castagnoli, R.; Vakkilainen, S.; Liu, C.; Delmonte, O.M.; Oguz, C.; Kaplan, I.M.; Alehashemi, S.; Burbelo, P.D.; Bhuyan, F.; et al. Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19. *Nat. Med.* **2022**, *28*, 1050–1062. [CrossRef]
26. Amanat, F.; Stadlbauer, D.; Strohmeier, S.; Nguyen, T.H.O.; Chromikova, V.; McMahon, M.; Jiang, K.; Arunkumar, G.A.; Jurczyszak, D.; Polanco, J.; et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat. Med.* **2020**, *26*, 1033–1036. [CrossRef]
27. Porritt, R.A.; Binek, A.; Paschold, L.; Rivas, M.N.; McArdle, A.; Yonker, L.M.; Alter, G.; Chandnani, H.K.; Lopez, M.; Fasano, A.; et al. The autoimmune signature of hyperinflammatory multisystem inflammatory syndrome in children. *J. Clin. Investig.* **2021**, *131*, e151520. [CrossRef]
28. Diorio, C.; Shraim, R.; Vella, L.A.; Giles, J.R.; Baxter, A.E.; Oldridge, D.A.; Canna, S.W.; Henrickson, S.E.; McNerney, K.O.; Balamuth, F.; et al. Proteomic profiling of MIS-C patients indicates heterogeneity relating to interferon gamma dysregulation and vascular endothelial dysfunction. *Nat. Commun.* **2021**, *12*, 7222. [CrossRef]
29. Giron, L.B.; Dweep, H.; Yin, X.; Wang, H.; Damra, M.; Goldman, A.R.; Gorman, N.; Palmer, C.S.; Tang, H.Y.; Shaikh, M.W.; et al. Plasma Markers of Disrupted Gut Permeability in Severe COVID-19 Patients. *Front. Immunol.* **2021**, *12*, 686240.
30. Hoel, H.; Heggelund, L.; Reikvam, D.H.; Stiksrud, B.; Ueland, T.; Michelsen, A.E.; Otterdal, K.; Muller, K.E.; Lind, A.; Muller, F.; et al. Elevated markers of gut leakage and inflammasome activation in COVID-19 patients with cardiac involvement. *J. Intern. Med.* **2021**, *289*, 523–531. [CrossRef]
31. Messner, C.B.; Demichev, V.; Wendisch, D.; Michalick, L.; White, M.; Freiwald, A.; Textoris-Taube, K.; Vernardis, S.I.; Egger, A.S.; Kreidl, M.; et al. Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. *Cell Syst.* **2020**, *11*, 11–24.e4. [CrossRef] [PubMed]
32. Viner, R.M.; Mytton, O.T.; Bonell, C.; Melendez-Torres, G.J.; Ward, J.; Hudson, L.; Waddington, C.; Thomas, J.; Russell, S.; Van Der Klis, F.; et al. Susceptibility to SARS-CoV-2 Infection Among Children and Adolescents Compared With Adults: A Systematic Review and Meta-analysis. *JAMA Pediatr.* **2021**, *175*, 143–156. [CrossRef] [PubMed]
33. Rhedin, S.; Lundholm, C.; Horne, A.C.; Smew, A.I.; Osvald, E.C.; Haddadi, A.; Alfvén, T.; Kahn, R.; Król, P.; Brew, B.H.; et al. Risk factors for multisystem inflammatory syndrome in children—A population-based cohort study of over 2 million children. *Lancet Reg. Health Eur.* **2022**, *19*, 100443. [CrossRef] [PubMed]
34. Abrams, J.Y.; Oster, M.E.; Godfred-Cato, S.E.; Bryant, B.; Datta, S.D.; Campbell, A.P.; Leung, J.W.; Tsang, C.A.; Pierce, T.J.; Kennedy, J.L.; et al. Factors linked to severe outcomes in mul-tisystem inflammatory syndrome in children (MIS-C) in the USA: A retrospective surveillance study. *Lancet Child. Adolesc. Health* **2021**, *5*, 323–331. [CrossRef]
35. Mayordomo-Colunga, J.; Vivanco-Allende, A.; López-Alonso, I.; López-Martínez, C.; Fernández-Vega, I.; Gil-Peña, H.; Rey, C. SARS-CoV-2 Spike Protein in Intestinal Cells of a Patient with Coronavirus Disease 2019 Multisystem Inflammatory Syn-drome. *J. Pediatr.* **2022**, *243*, 214–218.e5. [CrossRef] [PubMed]
36. Lehmann, M.; Allers, K.; Heldt, C.; Meinhardt, J.; Schmidt, F.; Rodriguez-Sillke, Y.; Kunkel, D.; Schumann, M.; Böttcher, C.; Stahl-Hennig, C.; et al. Human small intestinal infection by SARS-CoV-2 is characterized by a mucosal infiltration with activated CD8+ T cells. *Mucosal Immunol.* **2021**, *14*, 1381–1392. [CrossRef] [PubMed]
37. Lamers, M.M.; Beumer, J.; Vaart, J.; Van Der Knoops, K.; Puschhof, J.; Breugem, T.I.; Ravelli, R.B.G.; Schayck, J.P.; Van Mykytyn, A.Z.; Duimel, H.Q.; et al. SARS-CoV-2 productively infects human gut enterocytes. *Science* **2020**, *369*, 50–54. [CrossRef]
38. Gruber, C.N.; Patel, R.S.; Trachtman, R.; Lepow, L.; Amanat, F.; Krammer, F.; Wilson, K.M.; Onel, K.; Geanon, D.; Tuballes, K.; et al. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). *Cell* **2020**, *183*, 982–995.e14. [CrossRef]
39. Bartsch, Y.C.; Wang, C.; Zohar, T.; Fischinger, S.; Atyeo, C.; Burke, J.S.; Kang, J.; Edlow, A.G.; Fasano, A.; Baden, L.R.; et al. Humoral signatures of protective and pathological SARS-CoV-2 infection in children. *Nat. Med.* **2021**, *27*, 454–462. [CrossRef]
40. Leroy, O.; Gangneux, J.P.; Montravers, P.; Mira, J.P.; Gouin, F.; Sollet, J.P.; Carlet, J.; Reynes, J.; Rosenheim, M.; Regnier, B.; et al. Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: A multicenter, prospective, observational study in France (2005–2006). *Crit. Care Med.* **2009**, *37*, 1612–1618. [CrossRef]
41. Young, P.J.; Bellomo, R.; Bernard, G.R.; Niven, D.J.; Schortgen, F.; Saxena, M.; Beasley, R.; Weatherall, M. Fever control in critically ill adults. An individual patient data meta-analysis of randomised controlled trials. *Intensiv. Care Med.* **2019**, *45*, 468–476. [CrossRef]
42. Pires, W.; Veneroso, C.E.; Wanner, S.P.; Pacheco, D.A.S.; Vaz, G.C.; Amorim, F.T.; Tonoli, C.; Soares, D.D.; Coimbra, C.C. Association Between Exercise-Induced Hyperthermia and Intestinal Permeability: A Systematic Review. *Sports Med.* **2016**, *47*, 1389–1403. [CrossRef]
43. Dokladny, K.; Moseley, P.L.; Ma, T.Y. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *Am. J. Physiol. Liver Physiol.* **2006**, *290*, G204–G212. [CrossRef]

44. Stanley, D.; Mason, L.J.; MacKin, K.E.; Srikhanta, Y.N.; Lyras, D.; Prakash, M.D.; Nurgali, K.; Venegas, A.; Hill, M.D.; Moore, R.J.; et al. Translocation and dissemination of commensal bacteria in post-stroke infection. *Nat. Med.* **2016**, *22*, 1277–1284. [CrossRef]
45. Wang, J.; Li, F.; Wei, H.; Lian, Z.X.; Sun, R.; Tian, Z. Respiratory influenza virus infection induces intestinal immune injury via mi-crobiotamediated Th17 cell-dependent inflammation. *J. Exp. Med.* **2014**, *211*, 2397–2410. [CrossRef] [PubMed]
46. Bruewer, M.; Luegering, A.; Kucharzik, T.; Parkos, C.A.; Madara, J.L.; Hopkins, A.M.; Nusrat, A. Proinflammatory Cytokines Disrupt Epithelial Barrier Function by Apoptosis-Independent Mechanisms 1 [Internet]. *J. Immunol.* **2003**, *171*, 6164–6172. [CrossRef]
47. Heller, F.; Florian, P.; Bojarski, C.; Richter, J.; Christ, M.; Hillenbrand, B.; Mankertz, J.; Gitter, A.H.; Bürgel, N.; Fromm, M.; et al. Interleukin-13 Is the Key Effector Th2 Cytokine in Ulcerative Colitis That Affects Epithelial Tight Junctions, Apoptosis, and Cell Restitution. *Gastroenterology* **2005**, *129*, 550–564. [CrossRef] [PubMed]
48. Capaldo, C.T.; Nusrat, A. Cytokine regulation of tight junctions. *Biochim. Biophys. Acta BBA Biomembr.* **2008**, *1788*, 864–871. [CrossRef]
49. Sturgeon, J.P.; Bourke, C.D.; Prendergast, A.J. Children with Noncritical Infections Have Increased Intestinal Permeability, Endotoxemia and Altered Innate Immune Responses. *Pediatr. Infect. Dis. J.* **2019**, *38*, 741–748. [CrossRef] [PubMed]
50. Yonker, L.M.; Swank, Z.; Bartsch, Y.C.; Burns, M.D.; Kane, A.; Boribong, B.P.; Davis, J.P.; Loiselle, M.; Novak, T.; Senussi, Y.; et al. Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis. *Circulation* **2023**, *147*, 867–876. [CrossRef]