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Cytokine profiles in children with acute intussusception in South Africa

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Abstract

Serum specimens of children hospitalized with acute intussusception (IS; n = 407) were analyzed for various pro- and anti-inflammatory cytokines to identify host markers specifically for IS compared to other surgical conditions (n = 235) or acute gastroenteritis (AGE; n = 68) in a cross-sectional study design. We showed that children with IS had elevated levels of pro-inflammatory cytokines IFN-γ, TNF-α, MIP-1β, IL-1β, IL-2, IL-6, IL-7, IL-8, and IL-17 as well as anti-inflammatory cytokines IL-1RA, IL-4, IL-5, and IL-13 compared to those admitted with surgical conditions or AGE symptoms, indicating these cytokines as markers for IS. In addition, we showed an increase in C-reactive protein (CRP) levels in children with IS. This study is the first to show a broad cytokine profile and identify cytokine markers in children with IS.

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CRediT authorship contribution statement

Theresa K. Bessey: Investigation, Writing - original draft, Methodology, Validation, Formal analysis, Visualization. **Umesh D. Parashar:** Supervision, Writing - review & editing, Conceptualization, Resources, Funding acquisition. **Jacqueline E. Tate:** Writing - review & editing, Formal analysis. **Shabir A. Madhi:** Conceptualization, Methodology, Data curation. **Baoming Jiang:** Conceptualization, Supervision, Writing - review & editing, Methodology, Resources. **Michelle J. Groome:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision.:.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2021.155639.

Keywords

Intussusception; Surveillance; C-reactive protein; Pro-inflammatory cytokines; Rotavirus vaccination; Acute Gastroenteritis; IFN-γ

1. Introduction

Intussusception (IS), the invagination of one bowel segment into the adjacent segment, is one of the leading causes of childhood bowel obstruction. The incidence is greatest in infants and usually peaks between 4 and 10 months of age [1,2]. If untreated, blood circulation of the affected part can be compromised, resulting in bowel obstruction, intestinal ischemia, perforation or fatality. IS rates vary by population due to differences in ethnicity, environmental factors, and access to health care among others [1,3,4]. The etiology of IS remains unclear but various pathogens, especially adenovirus, have been reported to be associated with the onset of acute IS in children [5]. Viral and bacterial infectious diseases can lead to the increase of Interleukin (IL)- 6 and Tumor-Necrosis-Factor (TNF)-α in serum of patients [6,7]. Both of these cytokines are known to affect gastrointestinal motility which could affect or promote IS development [8]. However, no broad cytokine profiling of patients with natural intussusception has been reported to date.

Following the licensing of the oral rotavirus (RV) vaccine RotaShield in 1999, an association between IS and this vaccine was found leading ultimately to its withdrawal from the market in the United States [9,10]. New generation oral, live-attenuated RV vaccines, Rotarix and RotaTeq, were evaluated for an association between vaccination and IS in large clinical trials [11,12]. No association was found, leading to the licensure of those vaccines for the global market in 2006 [9,13]. However, post-licensure surveillance in several high- and middle-income countries has shown a low risk of intussusception associated with both RotaTeq and Rotarix [14-16]. Interestingly, no risk of intussusception was found with Rotarix vaccination in a large post-licensure evaluation of this vaccine in seven low-income African countries and with Rotavac, an Indian-made rotavirus vaccine licensed in 2014, in a post-licensure study in India [3,17]. These data suggest that intussusception risk associated with rotavirus vaccination is likely related to intestinal replication and inflammation caused by the live oral vaccine strain. Other factors such as interference with other co-infecting pathogens, malnutrition, or level of maternal antibody that might affect vaccine virus replication in the gut can also result in IS development [18].

The current study presents data generated from post-licensure monitoring of IS in South Africa after the introduction of Rotarix vaccine into the national immunization program in 2009 [19]. The primary study did not find an association of intussusception with rotavirus vaccination in South African infants [19]. Cytokine profiles in children hospitalized with acute IS were analyzed to identify markers for natural occurring IS compared to other surgical conditions or acute gastroenteritis (AGE).

2. Materials and Methods

2.1. Patients and sample collection

Patients were enrolled from 8 hospital complexes in South Africa during an active intussusception surveillance program from 2013 to 2018 [19]. The enrollment hospitals included Chris Hani Baragwanath Academic Hospital (CHBAH) and Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg; Red Cross War Memorial Children's and Tygerberg Hospitals in Cape Town; Steve Biko Academic/Kalafong Hospital in Pretoria; East London Hospital Complex in East London; Universitas Hospital in Bloemfontein and Inkosi Albert Luthuli Hospital in Durban. Cases were patients <3 years of age hospitalized with evidence of intussusception according to Level 1 Brighton criteria [20]. Control patients hospitalized with non-intussusception surgical conditions were enrolled from all hospitals and control patients hospitalized with acute gastroenteritis symptoms were only enrolled from CHBAH hospital. Control patients were matched with case patients on age (born±90 days), date of hospitalization (within 90 days of the case) and hospital. Vaccination status and basic demographics were collected upon enrollment. Informed consent was obtained from all caregivers before participating in this study.

Approvals were obtained from the ethics committees of the University of the Witwatersrand (M120934), University of KwaZulu-Natal (BE263/14), University of Cape Town (587/2013), University of Stellenbosch (N12/12/085), University of the Free State (ETOVS 84/2014), University of Pretoria (345/2013) and Walter Sisulu University (051/012).

Blood was collected from patients when first presented to the hospital site and serum was prepared by centrifugation, subsequently aliquoted and stored at −80 °C.

2.2. C-reactive protein (CRP) assay

Serum CRP levels were measured using the Quantikine ELISA Human C-reactive Protein Immunoassay (R&D Systems, Minneapolis, MN USA) according to manufacturer's instructions. For initial screening, samples were diluted 200-fold and tested in duplicate. Samples with an optical density (OD) above the OD of the standard curve were retested at a 400- and 800-fold dilution each in duplicate for accurate calculation of CRP concentration. The normal serum CRP concentration in children is below 5 mg/L.

2.3. Multi-plex cytokine assay

Serum was analyzed by using the Bio-plex Pro Human Cytokine 27-plex Assay (BioRad, Hercules, CA USA) for IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Eotaxin, basic FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF. Serum was diluted 4-fold and assay was performed according to manufacturer's instructions. Samples were measured using MAGPIX (Luminex, Austin, TX USA) reader. Data were analyzed by using xTAG Data Analysis Software (Luminex, Austin, TX USA) and BioPlex Manager Software (BioRad, Hercules, CA, USA).

2.4. Statistical analysis

Statistical analysis between Case, Surgical and AGE Control groups was done using the one-way ANOVA with Turkey's multiple comparison test and a 95% confidence interval or unpaired, two-tailed *t* test for 2 variables. Statistical significance was accepted when $p < 0.05$ with *: $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$; ns: $p \geq 0.05$ and performed with GraphPad Prism for Windows (Version 5.02, GraphPad Software, San Diego, CA USA).

3. Results

The active intussusception (IS) surveillance program in South Africa recruited IS patients and controls from September 2013 to January 2018. Serum specimens were obtained from 407 IS case patients, 235 surgical control patients, and 68 AGE control patients and used for cytokine testing (Table 1). Of the 407 children admitted with IS, pneumatic reduction was used for around 68% and surgical reduction was used for around 30% of cases for the initial treatment (Supplementary Table 1). Gender distribution of IS cases and AGE controls were comparable; however, we observed a significantly higher number of male patients in the surgical control group (73.2%) compared to IS cases (55%; $p < 0.001$) and AGE control group (58.8%; $p < 0.001$). The majority of children in all groups were vaccinated against RV with at least one dose (88% in IS case patients; 90.2% in surgical control patients; 94.1% in AGE control patients; $p = 0.37$). In the surgical control group, children were admitted with a variety of elective surgery conditions (Supplementary Table 2). The largest proportion of children were admitted with inguinal hernia (37%) and anorectal malformation (18.7%).

Serum CRP concentrations, a liver enzyme upregulated in serum during various inflammatory conditions, were significantly higher in IS case patients (mean 25.3 mg/ml) compared to surgical control patients (mean 9.1 mg/ml) and AGE control patients (mean 11.8 mg/ml) (Fig. 1, **** $p < 0.0001$). CRP levels in AGE control patients were also elevated compared to surgical controls however the difference was not statistically significant ($p = 0.43$).

Serum analysis showed a significant increase in a variety of pro-inflammatory and anti-inflammatory cytokines in IS cases compared to surgical control and AGE control patients (Table 2). The largest increase in pro-inflammatory cytokines was seen for IL-1β, IL-6, and IL-8 compared to surgical control and AGE control patients. In addition, pro-inflammatory cytokines IFN-γ, TNF-α, TNF-1β, PDGF-BB, IL-2, IL-7, and IL-17 were significantly upregulated in IS cases as well. No difference could be detected between cytokine levels in surgical control and AGE control patients. Interestingly, we observed significantly elevated IL-6, and CRP expression as well as a trend for elevated TNF-α expression in only IS patients that underwent surgical reduction for IS compared to pneumatic reduction (Supplementary Fig. 1). Anti-inflammatory cytokines IL-1RA, IL-4, IL-5, IL-13, and basic FGF were significantly elevated in IS cases compared to surgical control patients. Also, we observed elevated level of IL-1RA in AGE control patients, but the increase was not statistically significant compared to surgical control patients.

Besides cytokines exclusively upregulated in IS case patients, we observed elevated levels of pro-inflammatory cytokines IL-12p70, IL-15, GM-CSF, MCP-1, and MIP-α in serum of

IS case and surgical control patients compared to AGE control patients (Table 3). Mean cytokine levels of IL-12p70, IL-15, and GM-CSF in IS cases were significantly elevated compared to levels in AGE control patients, but only IL-15 is significantly upregulated comparing surgical to AGE controls. However, the mean cytokine levels in surgical control patients were elevated compared to AGE control patients though not statistically significant. Additionally, we measured an elevated expression of the anti-inflammatory cytokine G-CSF in IS cases and surgical control patients compared to AGE control patients although not statistically significant. No specific cytokine upregulation for the different subgroups of surgical control patients could be detected (data not shown).

In addition, we detected two cytokines, the pro-inflammatory cytokine eotaxin and the anti-inflammatory cytokine IL-10, significantly upregulated in IS cases and AGE control patients compared to surgical control patients (Table 4). The pro-inflammatory cytokine IP-10 was the only cytokine significantly upregulated exclusively in AGE control patients compared to IS cases and surgical controls (Table 4) ($p < 0.001$). We could not detect any differences in cytokine levels when comparing viral AGE with bacterial AGE cases in this control group (data not shown).

No changes in sera of IS case, surgical control and AGE control patients were observed for the pro-inflammatory cytokines IL-9, RANTES, and VEGF (Supplementary Table 3).

4. Discussion & Conclusion

During this study, we identified various pro- and anti-inflammatory cytokines in sera of children with acute IS compared to surgical control and AGE control patients. Specifically, we showed the increase of pro-inflammatory IFN-γ, TNF-α, MIP-1β, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-17, and PDGF-BB as well as anti-inflammatory IL-1RA, IL-4, IL-5, IL-13, and basic FGF in children with acute IS compared to surgical control patients. In addition, we showed a significant increase of CRP in sera of children with IS compared to the two control groups. An increase in IFN-γ, IL-6, TNF-α and CRP has been previously associated with IS severity in children and experimental animal models [8,21-23].

IS can be treated by pneumatic reduction or surgical reduction, both with potential resection of the affected bowel if reduction is not successful. A previous study compared different IS reduction methods and found that children with surgical IS reduction showed an increase of IL-6 and TNF-α, and a significant increase of CRP levels compared to children with non-operative IS conditions (pneumatic reduction), positively correlating CRP levels and disease severity [21]. In our study, we also detected an increase of IL-6, TNF-α, and CRP in IS cases that needed surgical reduction compared to pneumatic reduction (Supplementary Fig. 1). However, the difference between pneumatic and surgical reduction was not statistically significant for TNF-α due to reduced sample size.

Previous studies identified a lack of association of upregulated pro-inflammatory cytokines MIP-1β, PDGF-BB, IL-1β, IL-2, IL-7, and IL-8, and anti-inflammatory cytokines IL-1RA, IL-4, IL-5, IL-13, and basic FGF with IS. However, these cytokines have been associated with various inflammatory conditions, infection, autoimmune diseases, and chronic

inflammation in patients [24-27]. By contrast, the presented study is the first of its kind to show a broad cytokine upregulation in children with acute IS. Interestingly, IL-5 and eotaxin were linked to eosinophilic enteritis, a condition that can cause intussusception and is characterized by increased eosinophil recruitment in the intestine [28-30]. Here, we also showed the upregulation of IL-5 and Eotaxin in IS patients. A direct link of these two cytokines and the manifestation of natural occurring IS needs further investigation.

Vaccinations are known to alter cytokine profiles upon administration. Here we showed that only the expression of IL-7 ($p = 0.0352$) and IL-12p70 ($p = 0.0201$) was significantly elevated in non-vaccinated children compared to vaccinated children with acute IS (Supplementary Fig. 2). Due to the low number of non-vaccinated children in this study setting, no significant increase or decrease in cytokine expression could be found for any of the other cytokines analyzed. In addition, rotavirus vaccination did not seem to alter the cytokine profile in children in the present study. Only 18 children were admitted with acute IS within 10 days of receiving any rotavirus vaccination dose. When analyzing the cytokine profiles of children admitted <10 days compared to children admitted ≥10 days after receiving a rotavirus vaccine, only IL-1RA was significantly elevated in children <10 days (data not shown). Rotavirus infection and vaccination is known to induce Th1 cytokines like IFN-γ, IL-2, and IL12 as well as Th2 cytokines like IL4, IL-6, and IL-10 [31,32]. However, here we showed that IL-2, IL-6, IL-4, and IFN-γ were associated with IS rather than rotavirus infection or vaccination. An effect of other vaccinations on the cytokine profile could not fully be excluded since no records of vaccinations beside rotavirus were noted in this study.

Increase in the pro-inflammatory cytokines/chemokines IL-12, IL-15, GM-CSF, MCP-1, and MIP-1α were seen in IS cases as well as in surgical control patients. While those cytokines have been associated with various inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, celiac disease, and autoimmune diseases [33-36], the relation of these cytokines has not been previously investigated with IS. GM-CSF and G-CSF are known to be induced by IL-1β, TNF-α, or IL-12 [37,38]. We showed that these three cytokines were upregulated in IS patients which could explain the increased production of GM-CSF and G-CSF in IS patients. We could not detect cytokines exclusively upregulated in surgical control patients compared to the other groups as well as no specific cytokine upregulation for the different surgical conditions most likely due to the diversity of this group. The surgical control group in this study was presented as a heterogenous group and cytokine profiles and levels were dependent on the type of (inflammatory) condition as well as the time the children were admitted to the hospital. Rapid increase and decrease of cytokines in serum of children could have influenced the detection of cytokine levels in the presented study.

Of note, detection of cytokines in AGE control patients was limited in this study to an increase in eotaxin, IL-10, and IP-10 as well as an intermediate increase in CRP levels when compared to IS cases and surgical controls. During bacterial and viral infections, various cytokines are up or down regulated, depending on the nature of the pathogen. However, the cytokines detected here are known to have broad reactivity to various inflammatory and infectious conditions. No differences were seen when analyzing the cytokines in bacterial

versus viral AGE (data not shown). For CRP, it has been shown that bacterial infection leads to a stronger increase in serum CRP than viral infections [39,40]. However, we were not able to draw this conclusion from this study. As seen for surgical control patients, in AGE control patients the nature of infection as well as severity and duration of hospitalization may play a crucial role in the detection of serum cytokines. In addition, the smaller sample size of the AGE control patients limited the statistical analysis for most cytokines.

The present study has limitations. Incidence rate of RV vaccine associated IS is highest up to 21 days after vaccination [41]. In this study, the average time of IS onset after vaccination was >100 days indicating no correlation of RV vaccination in the development of IS [19]. Only 30 cases were admitted with IS that occurred up to 21 days after RV vaccination and because of small sample size, those cases were not excluded in the analysis. Further studies are needed to examine specific biomarkers in response to IS from RV vaccination among children in middle- or high-income settings where RV vaccines are associated with rare but severe IS. Nevertheless, this study is the first to identify a wide range of cytokines upregulated exclusively in IS patients compared to control patients. Our data may help increase our understanding of host responses to IS and identify potential biomarkers to better manage and improve the outcome of this disease in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

IS	Intussusception
AGE	Acute gastroenteritis
RV	Rotavirus

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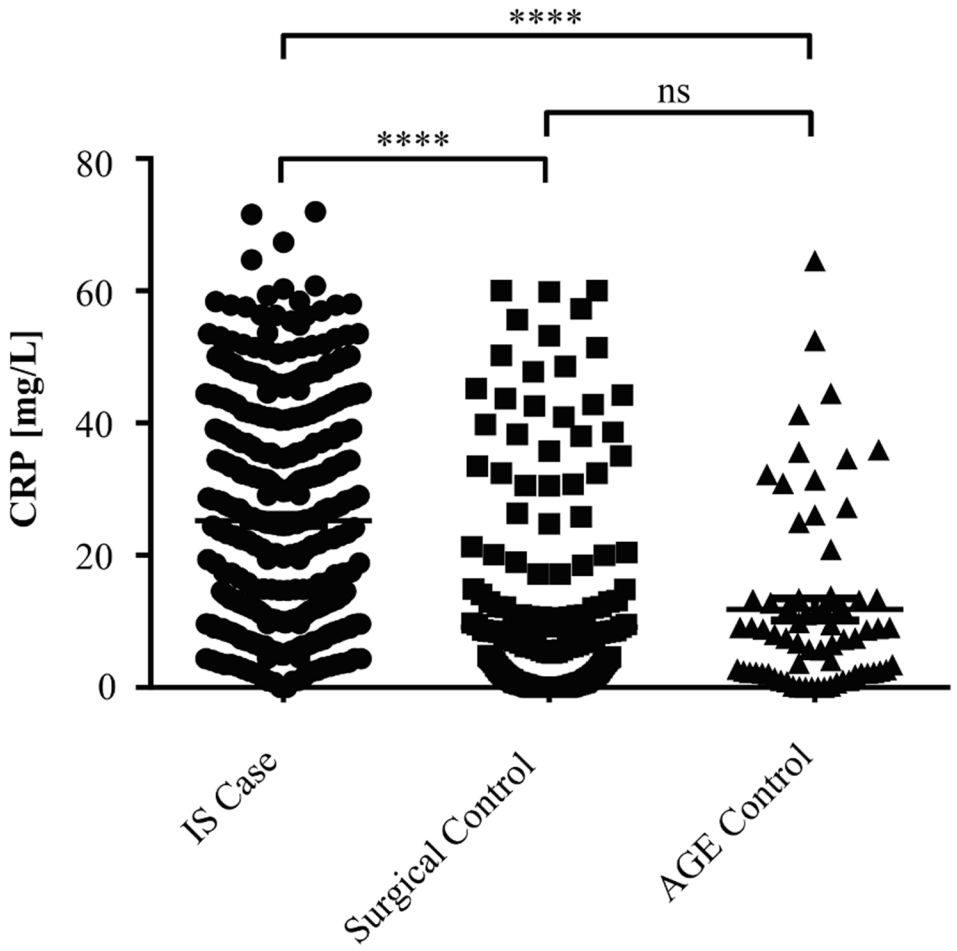

Fig. 1.

Increased detection of CRP in the serum of children with acute Intussusception. Serum was collected from children admitted for acute intussusception (IS Case), routine surgical procedures (surgical Control) or acute gastroenteritis symptoms (AGE Control). IS Case n = 407; Surgical Control n = 235; AGE Control n = 68. Statistical analysis was performed to compare mean CRP levels with SEM by using one-way ANOVA with Tukey's multiple comparison test. ns: not significant $p \geq 0.05$; **** $p < 0.0001$. CRP: C-reactive protein; IS: Intussusception; AGE: Acute Gastroenteritis.

Table 1

Characteristics of the study participants enrolled from September 2013 to January 2018.

	IS Case Patients	Surgical Control Patients	AGE Control Patients
Total number	407	235	68
Gender	N *(%)*	N *(%)*	N *(%)*
Female	182 *(44.7%)*	59 *(25.1%)*	28 *(41.2%)*
Male	224 *(55.0%)*	172 *(73.2%)***	40 *(58.8%)*
Unknown	1 *(0.3%)*	4 *(1.7%)*	0 *(0%)*
RV vaccination status	N *(%)*	N *(%)*	N *(%)*
RV vaccinated (at least one dose)	358 *(88.0%)*	212 *(90.2%)*	64 *(94.1%)*
Non-RV vaccinated	44 *(10.8%)*	17 *(7.2%)*	3 *(4.4%)*
Unknown	5 *(1.2%)*	6 *(2.6%)*	1 *(1.5%)*
Age	N *(%)*	N *(%)*	N *(%)*
<1 month	0 *(0%)*	1 *(0.4%)*	0 *(0%)*
1–6 months	235 *(57.7%)*	117 *(50.2%)*	34 *(50.0%)*
7–12 months	138 *(33.9%)*	91 *(38.7%)*	25 *(36.8%)*
>12 months	32 *(7.9%)*	22 *(9.4%)*	9 *(13.2%)*
Unknown	2 *(0.5%)*	4 *(1.7%)*	0 *(0%)*

IS: Intussusception; AGE: Acute Gastroenteritis; RV: Rotavirus. ** $p < 0.01$ with Chi-square test.

Table 2

Pro- and anti-inflammatory cytokines upregulated in the serum of IS case patients compared to surgical and AGE control patients.

Cytokines	IS Case [pg/ml]	Surgical Control [pg/ml]	AGE Control [pg/ml]
Pro-inflammatory Cytokines			
IFN-γ	41.2 (0.03–518.1) ***/*	18.2 (0.3–334.5)	21.9 (0.4–214.4)
TNF-α	138.8 (3.0–2,036) */ns	97.9 (3.0–1,436)	89.7 (17.4–366.0)
MIP-1β	297.4 (0.4–9,971) */ns	180.5 (28.0–1,974)	165.3 (85.9–531.2)
IL-1β	79.5 (0.06–5,634) */ns	3.9 (0.06–135.2)	2.6 (0.3–27.4)
IL-2	23.2 (0.07–426.6) */*	14.9 (0.2–288.1)	9.4 (1.0–60.8)
IL-6	348.5 (0.48–15,326) **/ns	61.3 (0.2–4,909)	11.0 (1.6–117.4)
IL-7	39.5 (0.63–161.6) ***/ns	28.6 (1.6–106.4)	34.1 (7.4–73.2)
IL-8	2,538 (1.13–113,307) **/ns	318.8 (1.3–18,615)	79.7 (12.6–1,243)
IL-17	63.7 (1.64–724.5) */ns	50.2 (1.64–738.0)	35.4 (7.3–175.1)
PDGF-BB	10,755 (333–35,761) ***/***	7,763 (781–22,374)	7,894 (50.4–17,986)
Anti-inflammatory Cytokines			
IL-1RA	5,337 (150.5–71,576) ***/**	1,193 (46.1–19,085)	2,386 (161.3–15,024)
IL-4	9.3 (0.06–64.8) ***/ns	6.5 (0.01–54.5)	6.9 (1.8–16.7)
IL-5	58.0 (0.5–564.4) ***/ns	37.5 (1.1–502.4)	38.6 (3.4–126.6)
IL-13	3.5 (0.1–33.6) */ns	2.8 (0.02–23.1)	3.1 (0.8–8.6)
Basic FGF	55.1 (1.7–433.3) **/ns	43.5 (3.9–339.5)	41.07 (17.1–119.4)

Values are mean (min-max). IL: Interleukin; IFN: Interferon; TNF: Tumor-Necrosis-Factor; MIP: Macrophage inflammatory protein; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; IS: Intussusception; AGE: Acute Gastroenteritis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns not significant $p \geq 0.05$, comparing IS case patients to surgical (first Asterix indicator) and AGE control (second Asterix indicator) patients using one-way ANOVA with Tukey's multiple comparison test. Difference between surgical control group and AGE control group were not statistically significant.

Table 3

Pro- and anti-inflammatory cytokines upregulated in the serum of IS case and surgical control patients compared to and AGE control patients.

Cytokines	IS Case [pg/ml]	Surgical Control [pg/ml]	AGE Control [pg/ml]
Pro-inflammatory Cytokines			
IL-12p70	7.0 (0.2–52.0) *	6.5 (0.1–85.4) ns	4.3 (0.7–15.9)
IL-15	155.9 (1.2–1,270) **	137.9 (1.2–1,790) *	65.4 (7.2–343.7)
GM-CSF	5.3 (0.04–89.4) **	4.2 (0.2–54.4) ns	2.3 (0.03–11.8)
MCP-1	252 (0.4–7,191) ns	197.0 (0.5–9,913) ns	64.1 (7.2–869.4)
MIP-1α	203.0 (0.2–6,039) ns	166.6 (0.3–4,483) ns	16.5 (0.6–252.5)
Anti-inflammatory Cytokine			
G-CSF	2,428 (6.6–42,904) ns	1,978 (17.4–24,705) ns	1,092 (260.2–12,644)

Values are mean (min – max). IL: Interleukin; GM-CSF: Granulocyte-macrophage colony-stimulating factor; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; G-CSF: Granulocyte colony-stimulating factor; IS: Intussusception; AGE: Acute Gastroenteritis. * $p < 0.05$; ** $p < 0.01$; ns: not significant $p \geq 0.05$, comparing IS case patients or surgical control patients to AGE control patients using one-way ANOVA with Tukey's multiple comparison test. Difference between IS case patients and surgical control patients are not significant for all cytokines shown here.

Table 4

Pro- and anti-inflammatory cytokines upregulated in the serum of IS case or AGE control patients compared to surgical control patients.

Cytokines	IS Case	Surgical Control	AGE Control
Pro-inflammatory Cytokine			
Eotaxin	109.7 (0.1–650.0) ***	67.9 (5.9–381.2)	116.9 (27.5–338.8) ***
Anti-inflammatory Cytokine			
IL-10	35.5 (0.4–755.0) ***	13.9 (0.9–140.8)	25.2 (1.7–145.7) ns
Pro-inflammatory Cytokine			
IP-10	1,759 (3.1–18,874) ns	1,769 (66.9–16,574)	3,027 (490.7–11,669) ***

Values are mean (min – max). IL: Interleukin; IP: Interferon-γ induced protein; IS: Intussusception; AGE: Acute Gastroenteritis. *** $p < 0.001$; ns: not significant $p \geq 0.05$, comparing IS case or AGE control patients to surgical control patients using one-way ANOVA with Tukey's multiple comparison test.