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Campylobacter jejuni infection and IgE sensitization in up to 2-year-old infants

Campylobacter jejuni infekcija i IgE senzibilizacija kod dece uzrasta do dve godine

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Abstract

Background/Aim. The "hygiene hypothesis" addresses the correlation between the occurrence of atopy and the frequency of infections in the earliest age, explaining an increase in the incidence of atopic diseases by living in good, infection-free, hygienic conditions. The aim of our study was to determine the conection between atopy and *Campylobacter* infection, and to analyze the association between serum concentrations of total IgE and *Campylobacter* infection in relation to atopy in children up to two years. **Methods.** A case control study was conducted with the sample of 98 infants of the average age of 8 months. Total serum IgE and Phadiatop infant multi-test were determined on Immunocap-100 (Phadia AB, Uppsala, Sweden). The presence of atopy was determined by detection of serum-specific IgE ≥ 0.35 kUA/L (Phadiatop infant positive) and serum IgM, IgA, IgG levels against *C. jejuni* were determined by a quantitative immuno-enzyme test - SERION ELISA classic. **Results.** Total IgE cut-off values ≥ 15 kU/L point to atopy in infants, and tIgE cut-off values ≥ 8.1 kU/L pointed to a *C. jejuni* infection in infants. Within the group of atopic children, tIgE levels ≥ 29.8 kU/L point to *C. jejuni* infection, and within the group of non-atopic children, tIgE levels ≥ 5.9 kU/L point to infection. Enteritis is not a predictor of *C. jejuni* infection, because of a high frequency of asymptomatic cases of infection. The risk factors for *C. jejuni* infection are age and tIgE, and the protective factors are breastfeeding and atopy. **Conclusion.** *C. jejuni* infection increases the total serum IgE level, which is predictive of infection, regardless of the presence of atopy. The presence of symptomatic *C. jejuni* infection reduces the risk of atopy in a child of the age of 5–24 months by the factor of 10.

Key words:
campylobacter jejuni; child; hypersensitivity, immediate; immunoglobulins; immunoglobulin e; immunologic tests.

Apstrakt

Uvod/Cilj. Povezanost nastanka atopije i učestalosti infekcija u najranijem uzrastu tema je kojom se bavi „hipoteza higijene", objašnjavajući porast incidencije atopijskih bolesti činjenicom da se živi u dobrim higijenskim uslovima, bez infekcija. Cilj naše studije bio je da utvrdimo povezanost atopije i infekcije kampilobakterijom, kao i da analiziramo povezanost serumske koncentracije ukupnih IgE i infekcije kamlobakterijom u odnosu na atopiju kod dece uzrasta do dve godine. **Metode.** Sprovedena je studija praćenja ukupno 98 dece, srednjeg uzrasta osam meseci. Postojanje atopije utvrđeno je detekcijom serumskih specifičnih IgE kvalitatitvnim multitestom *Phadiatop infant*, a serumske koncentracije IgM, IgA, IgG na C. *jejuni* određivane su kvantitativnim imunoenzimskim testom – *SERION ELISA classic.* **Rezultati.** Vrednost ukupnih IgE ≥ 15 kU/L ukazuje na to da dete ima atopiju, a tIgE ≥ 8,1 kU/L da dete ima *C. jejuni* infekciju. U grupi dece sa atopijom serumska koncentracija tIgE ≥ 29,8 kU/L ukazuje na to da dete atopičar ima infekciju, a u grupi dece bez atopije koncentracija ukupnih IgE ≥ 5,9 kU/L ukazuje na to da dete bez atopije ima *C. jejuni* infekciju. Enteritis nije prediktor *C. jejuni* infekcije, zbog velike učestalosti asimptomatskih slučajeva infekcije. Faktori rizika za *C. jejuni* infekciju su uzrast i količina tIgE, a protektivni faktori dojenje i atopija. **Zaključak.** *C. jejuni* infekcija povećava serumski nivo tIgE koji predstavlja prediktivni faktor infekcije bez obzira na postojanje atopije. Postojanje simptomatske *C. jejuni* infekcije 10 puta smanjuje rizik od atopije kod deca uzrasta 5–24 meseca.

Ključne reči:
campylobacter jejuni; deca; hipersenzibilnost, rana; imunoglobulini; IgE; imunološki testovi.

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Introduction

The "hygiene hypothesis" addresses the correlation between the occurrence of atopy and the frequency of infections in the earliest age, explaining an increase in the incidence of atopic diseases by living in good, infection-free hygienic conditions [1]. The concept of controlling the balance of Th-1/Th-2 cell response, which is protective for the occurrence of an allergic reaction, happens directly through early exposure of infants to microbes through the gastrointestinal tract, leading to stimulation and development of Th-1 in the environment of the dominant Th-2 response, as physiologically dominant in the breastfeeding period [2, 3].

Early childhood is considered as the most important period for "educating" of the immune system, when it is not yet mature, and when the immune tolerance to food and microbiotic antigens develops [4, 5]. Incapability of atopic children to develop oral tolerance to antigens in food in the first several years of life can be the consequence of belated maturation of the immune system [6]. Investigations of the effects of infection on allergic sensitization show that microorganisms can have a potentially modulatory role in the etiology and pathogenesis of atopic diseases [2]. The microbiotic hypothesis explains that the composition of enteral microorganisms in the earliest age is the main source of immune stimulation and an important factor in the development of oral tolerance [7]. A Danish study shows that a combined seropositivity to *Clostridium difficile*, *Campylobacter jejuni* and *Yersinia enterocolitica* results in an increased incidence of atopy (OR 1.7; 95% CI, 1.2 – 2.6), and that hepatitis A virus, *Helicobacter pylori* and *Toxoplasma gondii*, as poor hygiene pathogens, are related to a low prevalence of atopy [8].

C. jejuni is a species of gram-negative bacteria, considered as the most common cause of acute diarrhea in children aged 0–4 years [9]. *Campylobacter* is a classic extracellular bacteria eliminated dominantly by the humoral immune response that provides neutralization, opsonization and lysis of bacteria by activation of the complement system. van Spreeuwel et al. [10] also describe the intracellular presence of *C. jejuni* in epithelial cells of intestinal mucosa in patients suffering from colitis caused by these specific bacteria. *Campylobacter*, as a gram-negative microorganism, with antigens recognised by the receptors for molecular patterns of microorganisms, such as toll-like receptors 4 (TLR-4) for lipopolysaccharides (LPS), and TLR-5 for flagellin, and TLR-2 and 6 for lipopeptides, which induce signalling predominantly through (NF)kB [11], and the production of proinflammatory cytokines in epithelial and dendritic cells [12]. High levels of transforming growth factor (TGF)-β in the digestive tract in the presence of proinflammatory cytokines, such as IL-1 and IL-6, lead to the differentiation of naive T-cells into the Th-17 subgroup, the activation of which leads to the neutrophil infiltration and increased intestinal peristalsis manifested as an inflammatory diarrhea. The synthesis of IgA and IgG2 antibodies is promoted in the digestive tract, particularly as the response to T-independent antigens, such as polysaccharides from the capsule of *Campylobacter* or lipopolysaccharides from the bacterial cell wall (LPS and lipopoligosaccharides – LOS) in the presence of a proliferation including ligand, B/cell activating factor (APRIL, BAFF) or TGF-β cytokines [13, 14]. Over a 24-hour period, a dendritic cell starts to produce IL-12 necessary for the differentiation of naive T-cells into the Th-1 subgroup. Th-1 cells produce INF-γ that helps macrophages eliminate the bacteria by a higher level of phagocytosis and microbicidal activity, and creates conditions for the maturation of affinities and changes in the antibody heavy chain in the B-cell from IgM to IgG, along with the inhibition of Th-2 response [13, 14].

Studies methodologically involving children with diarrhea and isolation of *Campylobacter* from feces show that the frequency of *Campylobacter* infections is very variable. World Health Organization (WHO) studies conducted on a global level helped in determining that the rate of isolation of *Campylobacter* from feces in children with diarrhea up to 2 years of age is 4–38.8% [15, 16], but this percentage can reach up to 66% at the age of 5 in Mexico [17]. The isolation rate of *Campylobacter* from feces in children up to two years of age is correlated with the humoral response to surface antigens of *C. jejuni* [18]. It is known that the development of immunity to *C. jejuni* antigens plays a vital role in the decrease in the disease incidence at an older age, in the manifestation of the clinical form and severity of the disease, and in the duration of the microbe excretion phase in the period of recovery [19]. The infants continuously exposed to these bacteria develop antibodies in serum very early in life, and the symptoms subside [20]. Even in cases when cultivation could not prove the presence of *Campylobacter*, the seroconversion of all the three classes of immunoglobulin to *Campylobacter* antigens was detected, causing the opinion that serology is a more sensitive diagnostic method than cultivation [9]. A study carried out by Strid et al. [21] on 210 subjects with *Campylobacter* infection detected in feces involved serological testing for the presence of all the classes of serum antibodies to *Campylobacter*. The testing showed that an acute *Campylobacter* infection can be proved by individual analysis of antibodies, namely: IgM with the specificity of 60%, IgA with the specificity of 80%, and IgG with the specificity of 71%; however, the analysis of all the three antibodies detected infection with the sensitivity of 92% on the day 35 after the infection, and 90% in 3 months after the infection.

C. jejuni infection can have significant effects on the functionality of intestinal epithelium as a barrier and on the normal microflora composition, and therefore on the development of the immune system in the earliest age, which is also important for the phenotype expression of atopy [22, 23]. Studies focused only on the clinical phenotype of an atopic disease can underestimate the correlation between intestinal infection and an atopy, that is, the effects of intestinal microorganisms on the occurrence of atopic phenotypes [24, 25].

The aim of our study was to determine the connection between atopy and *Campylobacter* infection, and to analyze the association between serum concentrations of the total IgE and *Campylobacter* infection in relation to atopy in children up to two years.

Methods

The study was carried out in the town of Kragujevac in the period 2009 – 2011. The inclusion criteria were children (n = 167) aged 5 – 24 months, in good health, whose parents gave a written consent for their inclusion into the study. Data on symptoms of a gastrointestinal disease – vomiting and diarrhea – were obtained from a non-standard questionnaire, and were complemented by data from the database maintained in the primary health care centers by following the diagnoses according to the International Classification of Diseases (ICD) from the group A – intestinal infections of unspecified origin, and the group K – intestinal diseases manifested by vomiting and diarrhea. The exclusion criteria were children with the diagnoses A00–A04 and A06–A07 (*Salmonella* spp., *Shigella* spp., *E.coli*, protozoans, etc.), surgical intestinal diseases, and children with a positive test result for an antibody, specifically of the IgG class at the age of 5 and 6 months due to possible transplacental transmission (n = 5) or the IgM class against C. jejuni (n = 64).

The definitive sample consisted of 98 infants, while the average period from the presence of enterocolitic symptoms to the moment of testing was 3 months.

The presence of atopy was determined by detection of serum-specific IgE using the qualitative Phadiatop infant multi-test (cut-off value ≥ 0.35 kUA/L). Allergens against which the presence of specific IgE is determined by the Phadiatop infant multi-test, are proteins: egg white, cow milk, peanuts, shrimps, cat and dog hair, mites, silver birch pollen, timothy grass, ambrosia and nettle [26] . The Phadiatop infant test was carried out *in vitro* by immunofluorescence (Fluorescent Immunoassay) on Immunocap-100 (Phadia AB, Uppsala, Sweden). Total serum IgE levels were determined by the same technique. The group of atopic children included those in which the specific IgE serum levels ≥ 0.35 kUA/L were determined (Phadiatop infant positive). The group of non-atopic children included those in which specific serum IgE antibodies were not detected (Phadiatop infant negative).

IgM, IgA, and IgG serum levels to *C. jejuni* were determined by quantitative immuno-enzyme assay - SERION ELISA classic (Institute Virion/Serion GmbH, Würzburg, Germany). For IgM, the samples were treated with a rheumatoid factor absorbent - SERION Rheumatoid FactorAbsor-bent. The serum levels of specific antibodies to C. *jejuni* were calculated in SERION easy base 4PL-Softwarey evaluate. Since the manufacturer's cut-off values of specific antibody levels to C. *jejuni* were not defined for children, in our study we took the cut-off results as positive for the following values: IgM > 40 U/mL, IgA > 20 U/mL, and IgG > 20 U/mL. The cut-off results were repeated twice, and in case of a repeated cut-off value, the result would be included in the study as positive, and in case of a negative finding – as negative. *C. jejuni* infection was defined as the presence of at least two classes of antibodies (IgG+IgA; IgG+IgM; IgM+IgA, or IgM+IgA+IgG) in the levels higher than the listed cut-off values (n = 35); the infants free from a *C. jejuni* infection were those without a positive antibody result (n = 63). The frequencies of combinations of the classes of specific antibodies to *C. jejuni* in the group of infected children were: IgM+IgG 68.6% (n = 24/35), IgM+IgA 8.6% (n = 3/35), and IgM+IgA+IgG 22.9% (n = 8/35).

The infants with symptomatic *C. jejuni* infection were those having at least two positive antibodies to *C. jejuni*, plus diarrhea and/or vomiting reported in the medical history or database of the primary health care center (n = 15). The children with asymptomatic *C. jejuni* infection were those with at least two positive antibodies to *C. jejuni*, but without diarrhea and/or vomiting reported in the medical history or database of the primary health care center (n = 20).

The obtained results were processed statistically using the commercial software package SPSS 13.0 for Windows. The differences in the frequency of *C. jejuni* infections with respect to atopy were tested using the $\chi 2$ test and Fisher's exact test. The correlation between continuous variables was determined using the correlation test (Spearman). The continuous variables between atopic categories and *C. jejuni* infections were compared using non-parametric tests – Mann Vitney and Kruskal Wallis. ROC curve was used to determine the cut-off tIgE values as markers of atopy and *C. jejuni* infection. The correlation models between a dependent variable and independent variables were determined by logistic regression. The logistic regression result was presented by odds ratio (OR) values and the confidence interval for the accuracy of statement of 95% (CI 95%). The statistical significance of the model is based on the difference between the model in block 0 (the expected results of the analysis without any independent variables of which the model is consisted of) and block 1 (the results of analysis which include the examined characteristics), and is defined by the values of $\chi 2$ – C2 in the number of degrees of freedom and the number of cases included in the model, as well as *p* significance. The differences were considered to be significant when $p < 0.05$.

Ethical principles

The test was carried out in accordance with the ethical standards of the Declaration of Helsinki adopted in 1975 and revised in 1983. The study was approved by the Ethical Committee at the Public Health Institute in Kragujevac, and the Ethical Committee at the Faculty of Medical Sciences in Kragujevac, within the plan for prevention of allergic diseases in children. The biological material (serum) was collected from children in a health care institution under pediatric control, and with a consent from the parents previously informed of the procedures and objectives of the study.

Results

The descriptive statistical parameters of the studied group are shown in Table 1. The atopic children (n = 22), when compared to the non-atopic ones (n = 76), had a lower IgM serum levels to *C. jejuni* (Md = 23.2 *vs* 34.0 U/mL, z = 2.3; $p = 0.022$), similar IgA levels to *C. jejuni* (Md = 6.68 *vs* 6.00 U/mL, z = 0.99 $p = 0.321$) and lower IgG serum levels to *C. jejuni* (Md = 5.4 vs. 8.83 U/mL, z = -1.62, $p = 0.106$).

The comparison of tIgE serum levels in children with *C. jejuni* infection (Md = 10.2 kU/L, n = 35) and those in

Table 1

Data among children with and without *C. jejuni* infection

Parameters	All children (98, 100%)	Children with *C. jejuni* infection (35, 35.7%)	Children without *C. jejuni* infection (63, 64.3%)	*p* value
Gender, n (%)				
male	55 (56.1)	14 (40)	41 (65.1)	0.014*
female	43 (43.9)	21 (60)	22 (34.9)	
Age (months), n (%)				
5–6	50 (51)	10 (28.6)	40 (63.5)	
7–12	35 (35.7)	12 (34.3)	23 (36.5)	0.000***
13–24	13 (13.3)	13 (37.1)	0 (0)	
Breastfeeding at the time of testing, n (%)				
yes	52 (53.1)	10 (28.6)	42 (66.7)	0.000***
no	46 (46.9)	25 (71.4)	21 (33.3)	
Atopia, n (%)				
atopic	22 (22.4)	6 (17.1)	16 (25.4)	0.348
nonatopic	76 (77.6)	29 (82.9)	47 (74.6)	
Enteritis, n (%)				
yes	29 (29.6)	15 (42.9)	14 (22.2)	0.032*
no	69 (70.4)	20 (57.1)	49 (77.8)	
tIgE (kU/L), mean ± SD (median)	37.2 ± 133 (6.1)	85.5 ± 214 (10.2)	10.3 ± 14.4 (5.4)	0.020*
Abs to *C. jejuni* (U/mL), mean ± SD (median)				
IgM	85.9 ± 120 (33)	196 ±147 (128)	24 ± 8.3 (24.1)	
IgA	8.7 ± 7.5 (6.0)	15.2 ± 8.7 (12.6)	5.0 ± 3.1 (4.95)	
IgG	18.0 ± 19.7 (7.8)	39 ±18.8 (36.3)	6.3 ± 4.9 (4.9)	

Note: *p*-values were calculated using χ^2 test (for categorial variables) or Mann-Witney (for continuous variables); **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

children without C. *jejuni* infection (Md = 5.4 kU/L, n = 63) determined a statistically significant difference (Mann Witney U = 788, z = 2.33, $p = 0.020$) with the cut-off value of tIgE ≥ 8.1 kU/L showing that a child had *C. jejuni* infection (sensitivity 60%, specificity 70%). In the group of atopic children the cut-off value of tIgE serum level ≥ 29.8 kU/L showed that an atopic child had *C. jejuni* infection (area = 0.917, sensitivity 83.3%, specificity 87.5%), and in the group of non-atopic children the cut-off value of tIgE ≥ 5.9 kU/L showed that a non-atopic child had a *C. jejuni* infection (area = 0.635, sensitivity 62%, specificity 68%). The cut-off values for tIgE distinguished atopic from non-atopic children (Table 2).

In order to study a predictive significance of the studied features for the manifestation of C. *jejuni* infection in infants aged 5–24 months, the model of logistic regression included: age (0 = 5–6 months, 1 = 7–12 months, 2 = 13–24 months), gender (0 = male, 1 = female), breastfeeding at the time of study (0 = no, 1 = yes), enteritis (0 = no, 1 = yes), tIgE serum levels on the basis of determined cut-off values (0 = ≤ 5.9 kU/L; 1 = 5.91 – 29.79 kU/L, 2 = ≥ 29.8 kU/L) and atopy (0 = Phadiatop infant negative, 1 = Phadiatop infant positive) (Table 3). The predictive factors that were particularly statistically conclusive of the manifestation of *C. jejuni* infection were age and tIgE, and breastfeeding at the time of the study and atopy had a protective role. An increase in age in each category increased the risk of the presence of infection by the factor of 3.8 compared to the age of 5–6 months. An increase in tIgE serum levels in each category increased the risk of the presence of infection by the factor of 4.5 compared to the children with tIgE ≤ 5.9 kU/L. Breastfeeding reduced the risk of the presence of

Table 2

Biomarker for atopy tIgE - depending on the category of children according to the presence of symptoms of enteritis and *C. jejuni* infection

Groups	Atopia	tIgE median (kU/L)	*p*	Area	Cut-off (kU/L) ≥	Sensitivity (%)	Specificity (%)
Total	Atopic (n = 22)	13.1	0.000***	0.769	15.0	50	80
	Nonatopic (n = 76)	5.4					
Enteritis							
without C. *jejuni* infection	Atopic (n = 4)	19.4	0.065	0.825	11.9	75	80
	Nonatopic (n = 10)	2.9					
with C. *jejuni i*nfection	Atopic (n = 2)	688.5	0.027*	1.000	86.9	100	92
	Nonatopic (n = 13)	10.6					
Without enteritis							
without C. *jejuni* infection	Atopic (n = 12)	7.7	0.013*	0.740	5.9	75	70
	Nonatopic (n = 37)	4.6					
with C. *jejuni* infection	Atopic (n = 4)	203.8	0.011*	0.922	33.7	75	94
	Nonatopic (n =16)	7.4					

Note: *p* values were calculated using Mann-Witney; **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

Table 3

Logistic regression model that distinguishes children with *C. jejuni* infection

Phadiatop infant	Variable	B	S.E.	Wald	df	*p*	OR	CI 95%	
								lower	upper
Total	Age (kat)	1.33	0.42	10.22	1	0.001	3.78	1.67	8.55
	tIgE (kat)	1.50	0.48	9.71	1	0.002	4.48	1.74	11.51
Model	Breastfeeding	-1.24	0.58	4.65	1	0.031	0.29	0.09	0.89
c2 (6.98) = 46.4; $p = 0.000$	Atopy	-1.51	0.78	3.77	1	0.052	0.22	0.05	1.01
(37.7–51.8% variance)	Female	0.85	0.57	2.21	1	0.137	2.35	0.76	7.23
	Enteritis	0.56	0.63	0.81	1	0.367	1.76	0.52	6.00
	Constant	-2.24	0.68	10.88	1	0.001	0.11		
Non-atopic	Age (kat)	1.45	0.44	10.74	1	0.001	4.28	1.79	10.20
Model	tIgE (kat)	1.05	0.48	4.71	1	0.030	2.85	1.11	7.33
c2 (3.76) = 30.0. $p = 0.000$	Breastfeeding	-1.20	0.60	4.03	1	0.045	0.30	0.09	0.97
(32.6–44.4% variance)	Constant	-1.50	0.57	6.94	1	0.008	0.22		
Atopic	Age (kat)	2.11	1.32	2.54	1	0.111	8.24	0.62	109.9
Model	tIgE (kat)	4.10	1.92	4.57	1	0.033	60.7	1.40	2618.5
c2 (2.22) = 14.2. $p = 0.001$ (28.8–39.2% variance)	Constant	-8.36	3.78	4.88	1	0.027	0.000		

B – coeficient for usefulness of predictors; SE – standard error; W – Wald coeficient; OR – the ratio-change in the odds of the event of interest for a one-unit change in the predictor; CI 95% – confidience interval for 95%.

C. jejuni infection by the factor of 3.5, and atopy reduces the risk of the presence of infection by the factor of 4.5.

The model of logistic regression, differentiating the atopic children aged 5 – 24 months, revealed that an increase in tIgE serum level in each of the listed category raised the risk of the presence of atopy by the factor of 6.9, starting from tIgE serum levels ≤ 5.9 kU/L. The presence of *C. jejuni* infection gave a unique statistic predictor of the presence of atopy, reducing the risk by the factor of 5. The presence of symptomatic *C. jejuni* infection reduced the risk of the children aged 5 – 24 months being atopic by the factor of 10 (Table 4).

Table 4

Logistic regression model that distinguishes children with atopy

Phadiatop infant	Variable	B	S.E.	Wald	df	*p*	OR	CI 95%	
								lower	upper
Total	tIgE (kat)	1.93	0.50	14.80	1	0.000	6.89	2.58	18.40
	C. jejuni infection	-1.62	0.82	3.91	1	0.048	0.20	0.04	0.99
	Breastfeeding	0.36	0.60	0.35	1	0.554	1.43	0.44	4.66
Model	Enteritis	-0.23	0.65	0.13	1	0.719	0.79	0.22	2.81
c2 (6.98) = 22.0; $p = 0.001$	Age	0.05	0.49	0.01	1	0.919	1.05	0.40	2.75
(20.1–30.7% variance)	Female	-0.04	0.57	0.01	1	0.944	0.96	0.31	2.96
	Constant	-2.44	0.74	10.90	1	0.001	0.09		
Model	tIgE ≤ 5.9 kU/L			15.08	2	.001			
c2 (5.98) = 22.1. $p = 0.001$	tIgE 5.9–29.8 kU/L	1.73	0.66	6.89	1	0.009	5.63	1.55	20.45
(20.2–30.8% variance)	tIgE ≥ 29.8 kU/L	4.02	1.06	14.35	1	0.000	55.72	6.96	446.0
	No enteritis. No infection			5.53	3	0.137			
	Enteritis without infection	-0.00	0.77	0.00	1	0.997	0.98	0.22	4.50
	Asymptomatic infection	-1.63	.90	3.26	1	0.071	0.20	0.03	1.15
	Symptomatic infection	-2.32	1.13	4.22	1	0.040	.098	0.01	0.90
	Constant	-2.13	0.55	15.03	1	0.000	0.12		

B – coefficient for usefulness of predictors; SE – standard error; W – Wald coefficient; OR – the ratio-change in the odds of the event of interest for a one-unit change in the predictor; CI 95% – confidence interval for 95%.

Discussion

Humoral response in children in their first year of life shows the characteristics of its development that imply the following: the strongest is the IgM response and it reaches at the age of 12 months 75% of the levels of IgM response in adults; IgG synthesis exceeds the level of transplacentally transferred antibodies around the 4th month of age, reaching 60% of the serum levels in adults at the 12th month of age the IgA humoral response shows the slowest development [14, 21]. IgM production is dominant in the primary immune response to protein antigens, while the formation of other antibody isotypes at the larger extent is the characteristic of the secondary immune response [13]. In our study group, 68.6% of the children with *C. jejuni* infection had a combination of IgM+IgG classes, and in all combinations of antibodies the IgM class was always present. Lower IgM serum levels against *C. jejuni* detected in the atopic children can indicate a weaker immune response which does not correlate with the TH-1/TH-2 ratio.

Atopy is a subgroup of allergic hypersensitivities and is defined as a genetic predisposition for IgE production in response to exposure to allergens. Occurrence of specific IgE antibodies to allergens lies in the basis of the development of a clinical disorder (phenotype) of an atopic disease [27, 28]. In several studies the tIgE serum level is described as the marker of atopy, now widely used in the diagnosis of allergic diseases in children [29, 30]. Several studies determined increased levels of the total IgE in a group of sensitized children, but the cut-off values of the total IgE implying the presence of atopy in early childhood varied, ranging from 15.15 kU/L to 106 kU/L [29, 30]. In this study, tIgE was the marker of atopy with the cut-off value of tIgE ≥ 15 kU/L (sensitivity 50%, specificity 80.3%). Infection can have a role in a higher production of IgE during exposure to immunostimulants of bacterial origin [31]. Our study determined that a higher tIgE level can be a marker of the presence of *C. jejuni* infection in children aged 5–24 months, with the cut-off value ≥ 8.1 kU/L (60% sensitivity, 70% specificity). Th-2 response physiologically present in infancy also causes an increase in tIgE level during infection with pathogenic microorganisms, regardless of the presence of atopy. Reacting to antigenic stimulation, T-cells from the thymus (that reaches its highest cellularity at the age of 6-12 months) primarily differentiate into Th-2 producing IL-4 and IL-13. Primarily lower Th-1 cell response in infants is interpreted by lower IL-12 production from dendritic cells in response to LPS, lower expression of CD40-ligand on CD4+ cells after activation (which additionally reduces IL-12 expression in dendritic cells), lower expression of STAT4 transcription factor and higher gene methylation for INF-γ [32]. The possibility for Th-1 response during bacterial infections to be responsible for the growth of tIgE antibodies is excluded, and only TH-2 helper cells participate in this process [33]. According to our results, serum levels of tIgE < 5.9 kU/L show that a child has neither an infection nor atopy, whereas tIgE ≥ 29.8 kU/L shows that a child has both atopy and infection. Proper interpretation of tIgE serum levels in this age is additionally complicated by the fact that a *C. jejuni* infection can also be asymptomatic by itself. Namely, if a child has no enteritis symptoms, it can be atopic even with the levels of tIgE ≥ 5.9 kU/L in the case of absence of infection, and if a child has a asymptomatic *C. jejuni* infection, the serum level of tIgE ≥ 33.7 kU/L can imply that a child is atopic. If an atopic child aged 5–24 months has enteritis, the symptoms can be the consequence of food allergy only (cut-off value of tIgE ≥ 11.9 kU/L) or can be an intestinal infection on the ground of an Th-2 inflammation already present in the intestines (cut-off value of tIgE ≥ 86.9 kU/L), implying that *C. jejuni* infection in an atopic child strongly stimulates Th-2 response. Therefore, the tIgE serum level range 5.9 -33.7 kU/L in children without gastrointestinal symptoms, and the range 11.9 – 86.9 kU/L in children who had enteritis , are the "grey zones" where it is always necessary to test both for atopy and an infection. If an infant up to two years of age has extremely high levels of tIgE, there is a danger for a diagnostician to focus his attention on atopy, leaving the infection unnoticed. On the other hand, tIgE reference levels in atopic children without an infection imply the danger of not noticing atopy. tIgE serum level cannot be a reliable marker of atopy in infants up to two years of age, unless this is considered in the context of infection.

In our study, enteritis symptoms did not have a predictive significance for *C. jejuni* infection, because of a large number of asymptomatic cases. Tenkate and Stafford [34] indicate a decreasing rate of symptomatic infections during the first two years of life. New conclusions show that *C. jejuni* colonization is most often asymptomatic, but clinical manifestations also depend on the host-specific factors, such as: age, immune competence and health condition [35].

In our territory, the gender-specific differences in illness caused by *C. jejuni* infection in children are related to a higher frequency of breastfeeding of male children. Diarrhea caused by *C. jejuni* is less frequently and less intensively manifested in breastfed children [34.] Heresi et al. [35] stated that breastfeeding reduces the frequency of symptomatic campylobacteriosis, but does not reduce the colonization with these bacteria. In addition to the lysis of bacteria, lactoferrin in mother's milk has a role in the reduction of inflammatory response by immature enterocytes, and the interraction between glycans from mother's milk, microflora and glycans in mucin from the intestinal epithelium assist the development of mucosal immunity and protect an infant from infection and inflammatory intestinal diseases. A mother's exposure to *Campylobacter* will activate specific clones of T- and B-cells to these bacteria, and they will reach the breast tissue causing mother's milk to contain immunoglobulins IgA, but also IgG and IgM. Titres of secretory antibodies to surface *C. jejuni* antigens are the highest in colostrum, but persist through the whole period of lactation [34]. Significant quantities of immunoregulatory cytokines, such as TGF-β, IL-10, erythropoietin and lactoferrin, reduce excessive inflammatory response in the intestines of an infant [13, 14].

Even though the socioepidemiologic indicators of infections suggest an inverse correlation with atopy [36], immunopathogenic studies on the correlation between bacterial infections and allergies are less consistent [37]. It is known that infection cannot generate an atopic condition, and some microbes manifest antagonistic activity to the development of atopy, as well as the normal flora in the gastrointestinal tract necessary for the development of oral tolerance to antigens/allergens [38, 39]. Our study shows that atopy points to a lower probability of the presence of *C. jejuni* infection, which is in line with the opinion that atopy protects a child from developing gastrointestinal infections at the earliest age [6, 40]. In our study, however, most children with infection had a positive result for the IgM+IgG combination of antibodies to C. *jejuni*, whose antibodies are predominantly produced in Th-1 immune response. Furthermore, the result showing that symptomatic *C. jejuni* infection reduces the risk of atopy in a child is interpreted by a lower probability that an atopic child will develop a significant neutrophil inflammation dependent on Th-17 response that is in synergy with Th-1 immune response. Atopic children have a lower immune response regulation function (TGF- beta and IL-10), along with genetically conditioned mechanisms for the de-

velopment of Th-2 response. Weaker immune response regulation, with lower TGF-β at the beginning of infection, leads to a reduced joint effect of this cytokine with IL-1 and IL-6 in the production of Th-17 cells, and a lower development of neutrophil inflammation in atopic children [14]. However, lower TGF-β can result in the lack of the immunosupressive role both to Th-1 and Th-2 response in infection in inplants. *Campylobacter* with its antigens (primarily lipopolysaccharides) causes primarily Th-1/Th-17 immune response, but in atopic children the immune response to these bacteria is directed to Th-2 response, which is also indicated by a high tIgE level, as an indicator of a strong Th-2 immune response correlated with the severity of infection. Namely, an increase of tIgE level in atopic patients within the defined categories raises the risk of an infection by the factor of 60, and an increase of tIgE in non-atopic children can point to a risk of infection higher only by the factor of 2.8. Prescott et al. [41] showed that the development of allergy is correlated with the continuation of Th-2 response (Il-4, Il-13) and a reduced capacity for Th-1 response (INF-γ) to allergens, while non-atopic patients showed a strong Th-2 response only at birth, after which it decreased and the Th-1 response increased over the first six months of life.

The Th-1/Th-2 cell response balance is undoubtedly significant for the development of allergy, but the establishment of this balance is increasingly under consideration from the aspect of variability of the inborn immunity function, and the effector functions of T-cell immunity and regulatory T-lymphocytes, which may be compromised in the earliest age [2]. Nowadays efforts in overcoming the problem of non-compliant results direct to more sophisticated methods that could, in the future, enable studies of genomes of the normal intestinal flora and its influence on the development of immune response correlated with the individual genetic predisposition [2].

Conclusion

C. jejuni infection increases the total serum IgE level, which is predictive of infection, regardless of the presence of atopy. The presence of symptomatic *C. jejuni* infection reduces the risk of atopy in a child of the age of 5–24 months by the factor of 10.

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