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ORIGINAL PAPER

The concentration of IL-6, TNF-α, s-ICAM-1, and EBV DNA load – predictive factors of hepatological complications in children with infectious mononucleosis. A pilot study

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ABSTRACT

Introduction: The aim of the study was to assess the relationship between the concentration of pro-inflammatory cytokines such as interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), soluble intercellular adhesion molecule 1 (s-ICAM-1), and Epstein-Barr virus (EBV) DNA viraemia in children with infectious mononucleosis and hepatitis.

Material and methods: The Epstein-Barr virus DNA load in the plasma of 36 immunocompetent patients aged 2.5–18 years with a symptomatic (sore throat, cervical lymph node enlargement, fatigue, and fever), antibody-confirmed EBV primary infection was assessed using a quantitative real-time polymerase chain reaction assay. The concentration of IL-6, s-ICAM-1, and TNF-α in serum was determined using enzyme-linked immunosorbent assay tests.

Results: In a group of patients with infectious mononucleosis caused by EBV and viraemia > 3.5 log10 copies/ml, shorter duration of symptoms, and higher serum levels of s-ICAM-1 and C-reactive protein (CRP) were confirmed. In group of children with EBV hepatitis and CRP > 5 mg/l, levels of s-ICAM-1, γ-glutamyl transpeptidase activity, count of total white blood cells and lymphocytes, and EBV DNA load were significantly higher than in patients with CRP < 5 mg/l. Among patients with EBV hepatitis the increase of s-ICAM-1 concentration correlated with the increase of IL-6 and TNF-α serum levels.

Conclusions: The results suggest a higher risk of cholestatic complications in children with elevated CRP level. They indicate that EBV DNA viraemia in conjunction with an s-ICAM-1, IL-6, and TNF-α assessment may be helpful in selecting patients requiring hospitalization.

KEY WORDS:

EBV infection, EBV DNA load, cytokines, s-ICAM-1.

INTRODUCTION

The pathomechanism of hepatocyte damage induced by Epstein-Barr virus (EBV) infection is still not clearly defined. The virus does not act directly on liver cells or the biliary tract epithelium [1]. Research shows that pro-inflammatory cytokines such as interferon γ or tumour necrosis factor α (TNF-α) are responsible for damage to

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liver cells [1, 2]. Epstein-Barr virus hepatitis may contribute to the development of acute acalculous cholecystitis, which in the paediatric population accounts for 50–70% of all cases of acute cholecystitis [3]. The excretion of bile acids depends on an efficiently functioning hepatobiliary system. Microbial products, such as lipopolysaccharide (LPS) or endotoxin, directly stimulate hepatocytes and Kupffer cells to secrete cytokines that inhibit the expression of bile duct transporters, especially Na^+-taurocholate co-transporting polypeptide and bile salt export pump. The group of cytokines involved in the above-mentioned processes includes, for example, TNF-α and interleukin 6 (IL-6) [4–6]. Viral infections, including the Epstein-Barr virus, may be among the factors contributing to endothelium damage and may increase in leukocyte adhesion. The adhesion process of both leukocytes and monocytes is related to the presence of adhesive particles, i.e. ICAM-1 (intercellular adhesion molecule-1) and vascular target adhesion molecule-1 [7]. The induction of ICAM-1 secretion and an increase in the concentration of its soluble form in the serum (s-ICAM-1) is the result of the mediators of inflammation, e.g. TNF-α, IL-1, interferon γ, and bacterial LPS [8, 9].

The importance of EBV DNA viral load assessment in the diagnosis and monitoring of cancers and lymphoproliferative diseases associated with EBV infection in immunocompromised patients has already been well documented [10, 11]. In immunocompetent patients, the assessment of EBV DNA viral load, especially in the case of primary infection, is usually not performed. In this group of patients, a relationship between the amount of viraemia and the need for hospitalization, the duration of symptoms, or age has been reported [12–14].

The aim of the study was to assess the concentrations of IL-6, soluble intercellular adhesion molecule-1 (s-ICAM-1), and TNF-α in patients with confirmed EBV infection, and to demonstrate the correlation between the concentrations of the tested proinflammatory cytokines in the group of patients with accompanying hepatological complications of EBV infection. The study also attempted to demonstrate the relationship between the levels of pro-inflammatory cytokines, i.e. TNF-α, IL-6, and s-ICAM-1, with EBV DNA viral load.

MATERIAL AND METHODS

Thirty-six immunocompetent patients, aged 2.5–18 years, with a clinically suspected, primary EBV infection were included in the study. Clinical signs observed in children were as follows: sore throat, cervical lymph node enlargement, fatigue, and fever. In each of the subjects an EBV infection was confirmed using serological and molecular methods. In all the patients, the concentrations of IL-6, TNF-α, s- ICAM-1, C-reactive protein (CRP), alanine aminotransferase (ALT), and white blood cell (WBC) count were determined. Gamma-glutamyl transpeptidase (GGTP) activity was assessed in 31 patients. Blood for laboratory tests was collected during the first 48 hours of hospitalization.

This study was approved by the Bioethics Committee of Nicolaus Copernicus University in Torun and Ludwik Rydygier Collegium Medicum in Bydgoszcz.

SERUM ALT, GGTP, AND CRP SERUM LEVELS

The serum samples underwent an analysis by a standard enzymatic-colorimetric assay using an ALT, GGTP assay kit in accordance with the manufacturer's protocols (COBAS INTEGRA 400/400 Plus, Roche). The reference value (IU/l) for ALT and GGTP was established on the basis of "reference ranges for adult and children preanalytical considerations" [15].

The C-reactive protein concentration was determined by the immunoturbidimetric method using a commercially available kit according to the manufacturers protocol (analyser-COBAS INTEGRA 400/400 Plus analyser, Roche).

WBC, MONOCYTE, AND LYMPHOCYTE COUNTS

The samples underwent an analysis by a standard fluorescence flow cytometry assay using an XS-1000i analyser (Sysmex, Poland).

ENZYME-LINKED IMMUNOSORBENT ASSAY

The concentrations of IL-6 and TNF-α (Cusabio Biotech, USA) in the serum were determined using a commercially available kit according to the manufacturer's protocol (analyser-Etimax 3000).

s-ICAM-1 (Wuhan Fine Biotech, China) in serum was determined using a commercially available kit according to the manufacturer's protocol (analyser-Etimax 3000).

EPSTEIN-BARR VIRUS DETECTION

Epstein-Barr virus antibodies were detected by a LIAISON® EBV IgM test (DiaSorin) using a chemiluminescence immunoassay technology. The test has to be performed on a LIAISON® XL analyser. The DNA isolation was carried out using a Sherlock AX (A&A Biotechnology) kit. The TaqMan™ Master Mix II with no UNG (Applied Biosystems) and the DNA quantitative standard for EBV (Vircell) were used to carry out the reaction.

STATISTICAL ANALYSIS

The summary statistics for normally distributed continuous variables are presented as mean and standard deviation and as median and interquartile range (Q1, Q3) for non-normally distributed variables. Differences

between continuous normally distributed variables were analysed by the t-test for independent samples. In this case the data were not normally distributed, and the differences were tested by the Mann-Whitney *U* test. Spearman's correlation coefficient ρ was used to examine the dependencies between selected continuous variables. The results were considered as statistically significant when the *p*-value was less than 0.05. The statistical analysis was performed with the use of the R-software version 3.0.3.

RESULTS

The study included 36 patients aged 2.5–18 years, with median age 10.6 (6.2–16.2) years. Epstein-Barr virus DNA viraemia was confirmed in all the children. The median EBV DNA was 2831 (1358–8751) copies/ml.

An analysis of EBV DNA concentrations among the analysed children (n = 36) showed values of < 3.5 $\log_{10}$ copies/ml in 19 patients and > 3.5 $\log_{10}$ copies/ml in 17 patients (Tab. 1).

As noted in Table 1, there were statistically significant differences in the concentration of s-ICAM-1, CRP, and the duration of symptoms preceding hospitalization between the group with viraemia > and < 3.5 $\log_{10}$ copies/ml (p = 0.034, p = 0.003, p = 0.036, respectively). Median concentrations of TNF-α and IL-6 were higher in the group with viraemia > 3.5 $\log_{10}$ copies/ml; however, the differences were not statistically significant (211.0 vs. 64.0 pg/ml, p = 0.071; 5.54 vs. 4.2 pg/ml, p = 0.059, respectively). In the group of patients with viraemia > 3.5 $\log_{10}$ copies/ml, higher median ALT and GGTP activities were found, but they were not statistically significant (82.0 vs. 56.0 U/l, p = 0.447; 61.0 vs. 20.0 U/l, p = 0.056, respectively).

In the group of all children (n = 36), the analysis showed statistically significant positive correlations between the EBV DNA viraemia level and the s-ICAM-1 concentration (Spearman's correlation coefficient ρ = 0.42, p = 0.01) (Figure 1), CRP concentration (ρ = 0.62, p < 0.001) (Figure 1), and the number of leukocytes (ρ = 0.36, p = 0.03) and monocytes (ρ = 0.36, p = 0.03) (data not shown). On the other hand, a negative statistically significant correlation was found between the EBV DNA viral load and the duration of symptoms (ρ = –0.41, p = 0.013) (Figure 1). A positive correlation was shown between the concentrations of s-ICAM-1 and the number of leukocytes (ρ = 0.39, p = 0.02) and TNF-α (ρ = 0.78, p < 0.001) (Figure 2). Moreover, a statistically significant correlation was demonstrated between the concentration of IL-6 and the concentration of TNF-α and s-ICAM-1 (ρ = 0.51, p = 0.002; ρ = 0.47, p = 0.004, respectively) (Figure 2). A correlation between the EBV DNA load and the activity of ALT, GGTP as well as IL-6 concentration (data not shown) was not confirmed.

Hepatitis (defined as an increase ALT activity) was diagnosed in 26/36 (72%) children. In these patients, as in the entire study group, statistically significant correlations were found between EBV DNA load and the concentration of s-ICAM-1 (ρ = 0.4, p = 0.041), TNF-α (ρ = 0.38, p = 0.053), the concentration of CRP (ρ = 0.57, p = 0.002) (Figure 3), and the count of leukocytes (ρ = 0.43, p = 0.03) and monocytes (ρ = 0.53, p = 0.005) (data not

TABLE 1. Values of the tested parameters for Epstein-Barr virus DNA viral load < and > 3.5 $\log_{10}$ copies/ml

Parameters	Overall (N = 36)	EBV DNA $\log_{10}$ [copies/ml]		*p*-value
		< 3.5 (n = 19)	> 3.5 (n = 17)	
Age (years)	10.6 (6.2–16.2)	10.7 (6.2–16.2)	10.5 (6.5–16.0)	0.975
EBV DNA load [copies/ml]	2831 (1358–8751)	1363 (380–1804)	9832 (5594–26636)	–
Hospitalisation (days)	5.0 (4.0–6.0)	5.0 (3.0–6.0)	5.0 (4.0–7.0)	0.185
Duration of symptoms (days)	5.5 (3–8.5)	7.0 (4.0–10.0)	4.0 (2.0–6.0)	0.036
Total WBC count [10^3/µl]	12.87 (5.72)	11.37 (5.32)	14.54 (5.84)	0.099
Lymphocyte count [10^3/µl]	7.62 (4.1)	6.95 (4.33)	8.37 (3.82)	0.305
Monocyte count [10^3 /µl]	1.10 (0.45)	1.03 (0.33)	1.18 (0.55)	0.328
ALT [IU/L]	57.5 (26.5–112.5)	56.0 (32.0–86.0)	82.0 (26.0–207.0)	0.447
GGTP [IU/l]	25.0* (16.0–80.5)	20.0** (15–40)	61.0*** (21–105)	0.056
CRP [mg/l]	7.7 (2.8–18.7)	4.7 (1.2–8.5)	11.8 (7.1–19.4)	0.003
IL-6 [pg/ml]	5.5 (3.2–9.5)	4.2 (3.2–6.4)	9.5 (4.7–11.2)	0.059
TNF-α [pg/ml]	89.0 (43.0–202.5)	64.0 (43.0–117.5)	211.0 (530.0–855.0)	0.071
s-ICAM-1 [ng/ml]	13.3 (9.1)	10.3 (7.8)	16.7 (9.4)	0.034

ALT – alanine aminotransferase, CRP – C-reactive protein, EBV – Epstein-Barr virus, GGTP – γ-glutamyltranspeptidase, IL-6 – interleukin 6, TNF-α – tumour necrosis factor α, s-ICAM-1 – soluble intercellular adhesion molecule-1, WBC – white blood cell

Quantitative data are expressed as mean ±SD or median and interquartile range.

** n = 31*

*** n = 17*

**** n = 14*

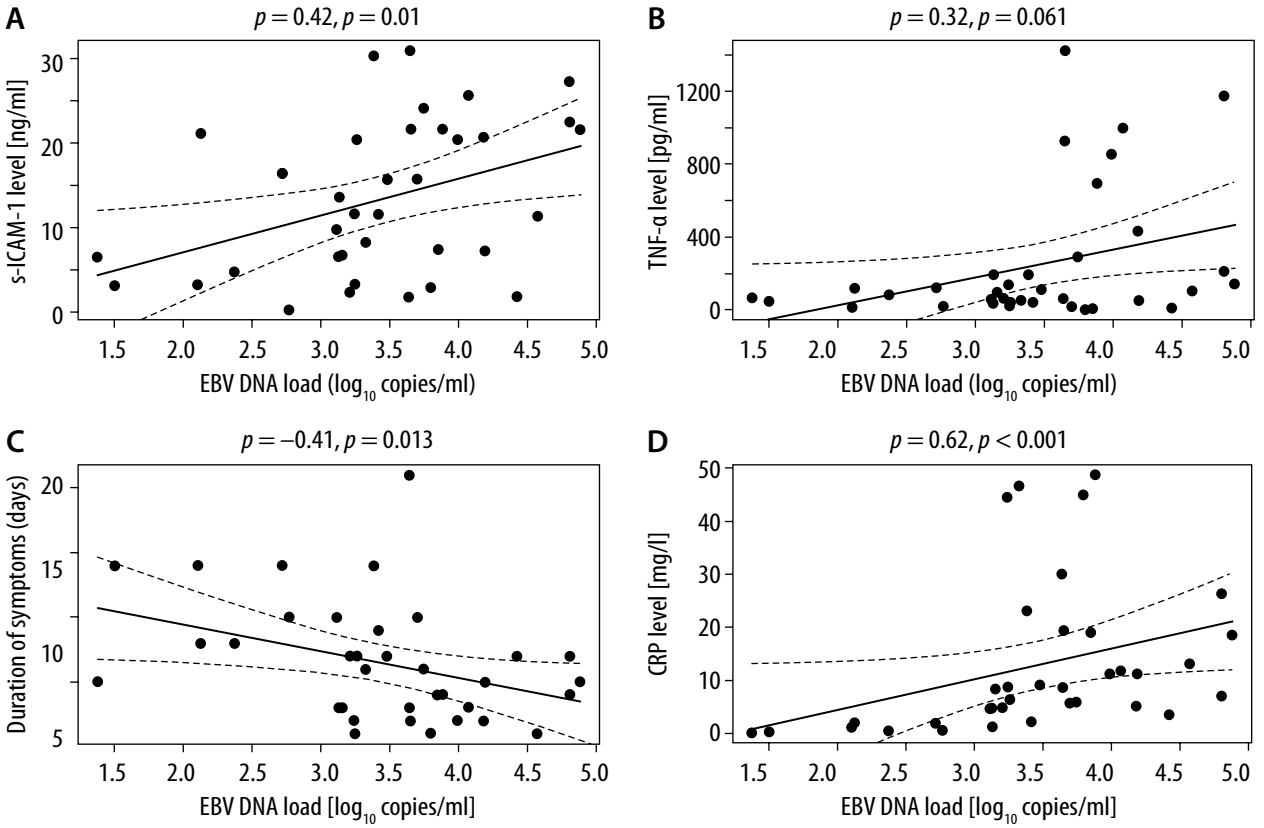

FIGURE 1. The association between Epstein-Barr virus DNA Load, serum levels of s-ICAM-1, TNF-α, C-reactive protein and duration of symptoms with linear regression curves with 95% confidence interval (overall group, $N = 36$)

CRP – C-reactive protein, EBV – Epstein-Barr virus, s-ICAM-1 – soluble intercellular adhesion molecule-1, TNF-α – tumour necrosis factor α 17,705 children hospitalized in the Department of Infectious Diseases and Child Neurology ICD-A6.

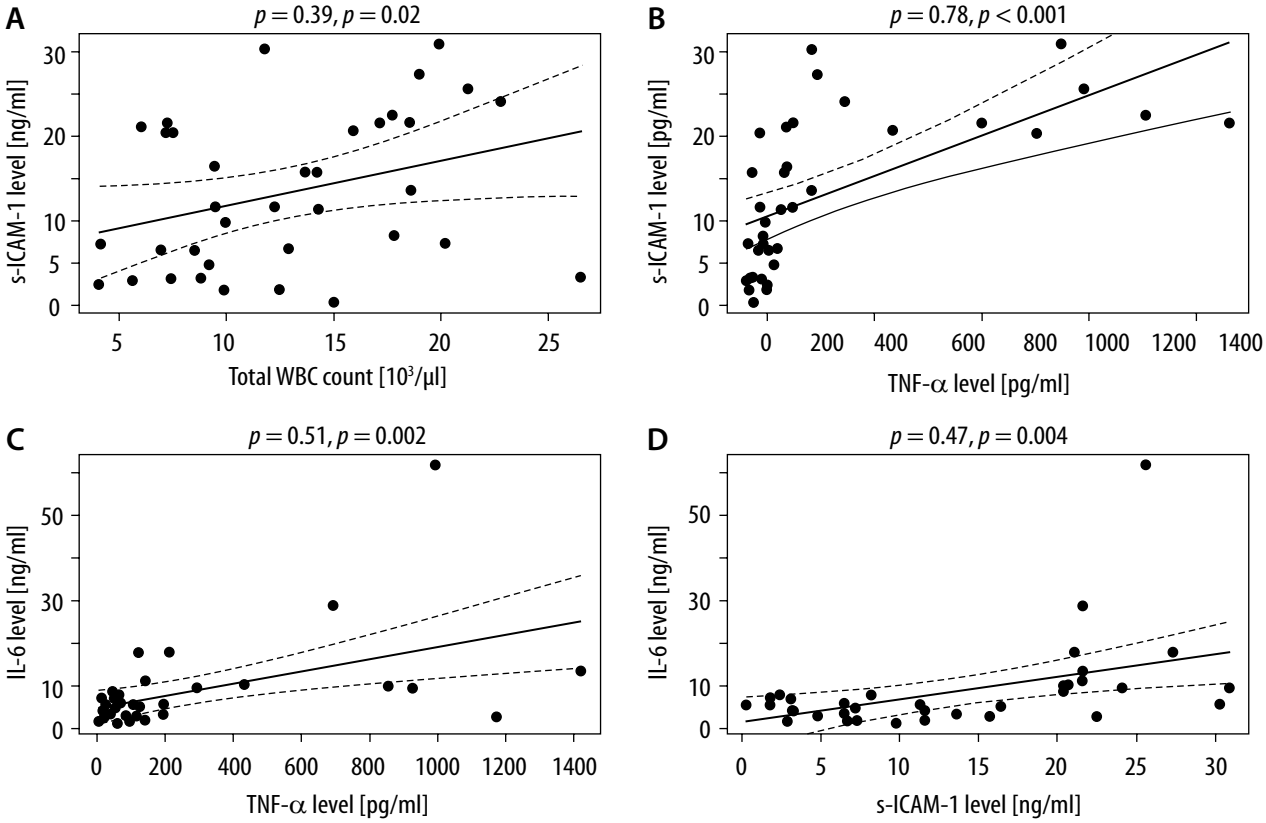

FIGURE 2. The association between serum levels of soluble intercellular adhesion molecule-1, tumour necrosis factor α, IL-6, and total white blood cell count with linear regression curves with 95% confidence interval (overall group, $N = 36$)

IL-6 – interleukin 6, s-ICAM-1 – soluble intercellular adhesion molecule-1, TNF-α – tumour necrosis factor α, WBC – white blood cell

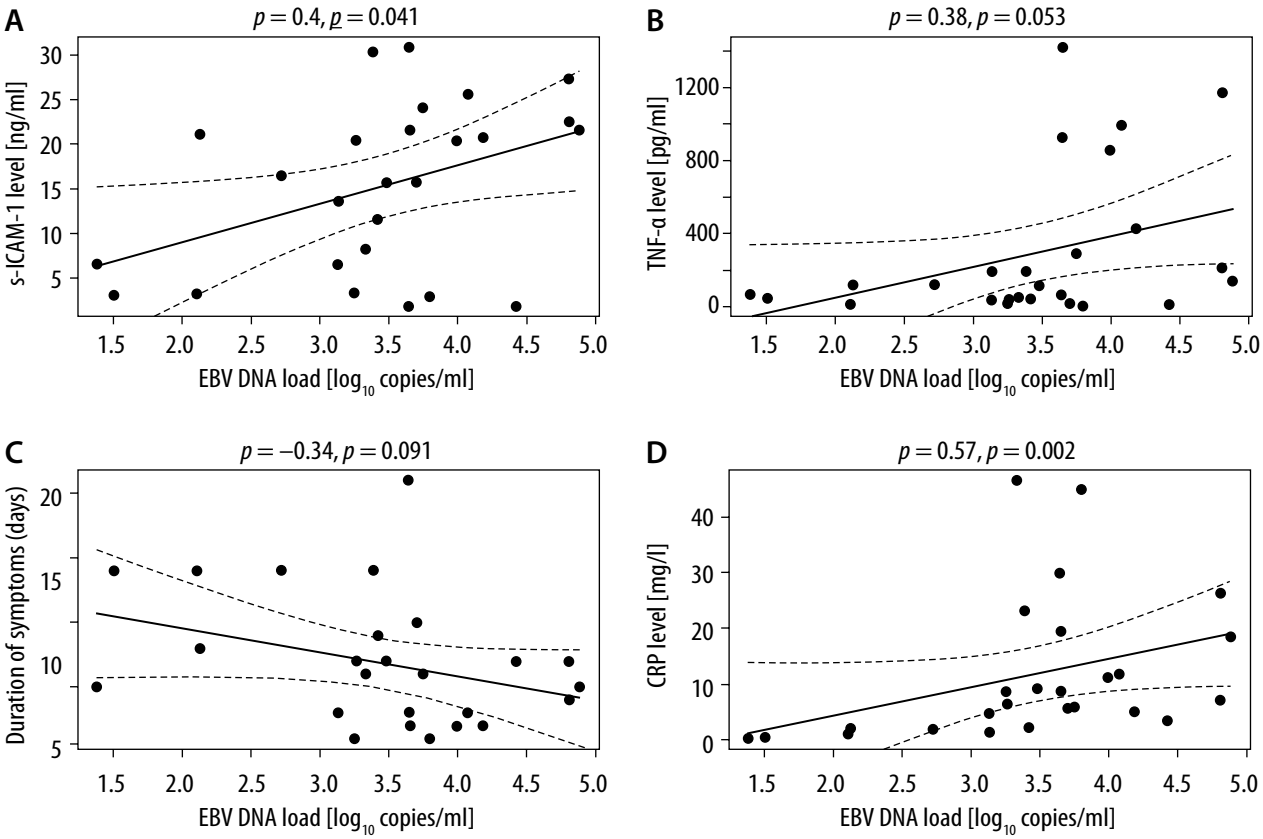

FIGURE 3. The association between Epstein-Barr virus DNA load, serum levels of soluble intercellular adhesion molecule-1, tumour necrosis factor α, C-reactive protein, and duration of symptoms with linear regression curves with 95% confidence interval (hepatitis group, $n = 26$)

CRP – C-reactive protein, EBV – Epstein-Barr virus, s-ICAM-1 – soluble intercellular adhesion molecule 1, TNF-α – tumour necrosis factor α

shown). In this group of patients, a correlation between the concentration of s-ICAM-1 and TNF-α and the count of leukocytes ($\rho = 0.8$, $p < 0.001$; $\rho = 0.46$, $p = 0.018$) was also confirmed (Figure 4). A positive correlation was also demonstrated between the concentration of IL-6 and the concentration of TNF-α and s-ICAM-1 ($\rho = 0.53$, $p = 0.005$; $\rho = 0.53$, $p = 0.006$, respectively) (Figure 4).

Patients with hepatitis were divided into 2 groups: with normal and with increased GGTP activity (Tab. 2). The median age for the group of children with hepatitis ($n = 26$) was 11.2 (6.0–16.7) years, while the median EBV DNA viral load was 3711 (1363–9832) copies/ml. As noted in Table 2, the median age among patients with hepatitis was higher in the group with normal GGTP activity, while the median EBV DNA viral load was higher in the group with increased GGTP activity (14.9 vs. 7.7 years, $p = 0.439$; 4737 vs. 2283 copies/ml, $p = 0.297$, respectively); ALT activity was significantly higher in the group of patients with increased vs. normal GGTP activity; median 180.5 (97.0–286.0) vs. 53.5 (48.5–61.5) U/l, $p < 0.001$, respectively. A shorter duration of clinical symptoms prior to hospitalization was also demonstrated in the group of patients with increased GGTP activity, but the differences were not statistically significant (7.0 vs. 4.5 days, $p = 0.187$, Tab. 2).

As shown in Table 2, there were no statistically significant differences between the groups in terms of the length of hospitalization ($p = 1$), count of leukocytes ($p = 0.207$), lymphocytes ($p = 0.282$), and monocytes ($p = 0.412$), CRP concentration ($p = 0.368$), IL-6 ($p = 0.189$), and s-ICAM-1 ($p = 0.983$). In children with hepatitis, the median concentration of TNF-α in the group with increased GGTP activity was higher compared to the group with normal enzyme activity (118.5 vs. 86.0 ng/ml, respectively), but the difference was not statistically significant ($p = 0.738$) (Tab. 2). In patients with increased GGTP activity and hepatitis ($n = 14$), the positive correlation between the concentration of s-ICAM-1, TNF-α, IL-6, and the count of leukocytes ($\rho = 0.86$, $p < 0.001$; $\rho = 0.54$, $p = 0.046$; $\rho = 0.61$, $p = 0.021$) was confirmed. A positive correlation was also demonstrated between EBV DNA virus load and CRP concentration and monocyte count ($\rho = 0.59$, $p = 0.026$; $\rho = 0.56$, $p = 0.037$). Moreover, positive correlations between the concentration of TNF-α and the count of leukocytes and lymphocytes ($\rho = 0.56$, $p = 0.039$; $\rho = 0.57$, $p = 0.032$) were also confirmed (data not shown).

The group of patients with hepatitis ($n = 26$) was analysed for normal and increased markers of inflammation (Table 2). Elevated CRP concentration > 5 mg/l was found in 17 patients (65%), and in 9 (35%) the CRP concentration was within a normal range (< 5 mg/l). The median age in children with CRP > 5 mg/l was lower than in children with a normal result (9.0 vs. 14.9 years), and the median duration of clinical

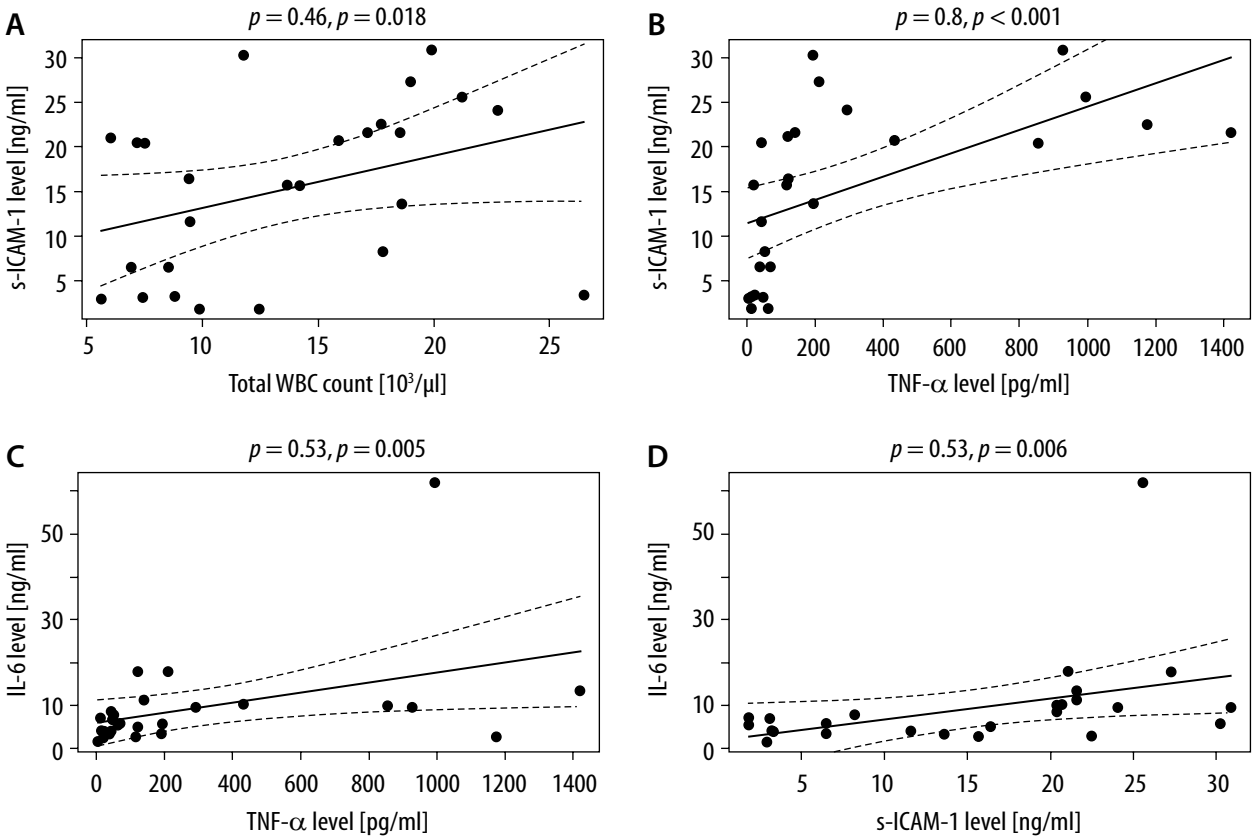

FIGURE 4. The association between serum levels of soluble intercellular adhesion molecule-1, tumour necrosis factor α, IL-6, and total white blood cell count with linear regression curves with 95% confidence interval (hepatitis group, $n = 26$)

IL-6 – interleukin 6, s-ICAM-1 – soluble intercellular adhesion molecule-1, TNF-α – tumour necrosis factor α, WBC – white blood cell

symptoms prior to the hospitalization in the first group was shorter than in children with normal CRP concentration (5.0 vs. 8.0 days, respectively), but these differences were not statistically significant. In the group of children with elevated CRP, the median CRP concentration was 11.2 (7.1–23.1) mg/l. As shown in Table 2, these patients also demonstrated statistically significantly higher amounts of leukocytes and lymphocytes, GGTP activity, and s-ICAM-1 concentration than in the group with normal CRP ($p = 0.005$, $p = 0.045$, $p = 0.043$, $p = 0.009$, respectively). The median viral load in the group with elevated CRP was 5025 (3033–11867) copies/ml, and in children with a normal CRP it was 524 (127–1363) copies/ml. The difference was statistically significant ($p = 0.001$).

DISCUSSION

The specific cellular response in the course of EBV infection consists of increased activity of T CD8+ lymphocytes, which secrete large amounts of cytokines, i.e. TNF-α, IL-1β, and IL-6. It has been proven that the number of these lymphocytes positively correlates with the severity of symptoms of infectious mononucleosis [12, 16]. Also, Horneff and others demonstrated a statistically significant higher concentration of TNF-α and IL-6 in patients with infectious mononucleosis under the influence of LPS stimulation compared to a control group [17].

Excessive and uncontrolled macrophage proliferation induced by EBV infection, leading to the development of macrophage activation syndrome, is an example of a potentially fatal disease in which TNF-α is attributed to play a major role [18]. Tumour necrosis factor is considered a prognostic factor in patients with hemophagocytic syndrome, the concentration of which correlates with the severity of clinical symptoms [19].

The importance of s-ICAM-1 in patients with extra- and intrahepatic cholestasis has been described in many studies, which confirmed statistically significant correlations between the concentration of s-ICAM-1 and the activity of GGTP, ALP, AST or the bilirubin concentration [9, 20]. Takahara *et al.*, in their study, confirmed the increase in s-ICAM-1 secretion in patients with EBV-induced nasal NK/T cell lymphoma, induced by EBV infection, expressing latent membrane protein-1. A decrease in the concentration of s-ICAM-1 molecules after treatment may suggest the usefulness of s-ICAM-1 in the assessment of tumour progression [21]. Tomasiewicz *et al.* confirmed a statistically significant increase in s-ICAM-1 concentration in patients with infectious mononucleosis as compared to the healthy control group [22].

In the course of hepatitis, damage to hepatocytes results, inter alia, from blood circulation disorders, contributed to by damage to the endothelium. Endothelial damage, which

TABLE 2. Values of the studied parameters depending on γ-glutamyltranspeptidase activity and C-reactive protein concentration in patients with hepatitis

Parameters	Overall ($N = 26$)	↑GGTP activity		*p*-value	CRP serum concentration		*p*-value
		No ($n = 12$)	Yes ($n = 14$)		< 5.0 ($n = 9$)	> 5.0 ($n = 17$)	
Age (years)	11.2 (6.0–16.7)	14.9 (9.6–16.6)	7.7 (5.9 – 11.8)	0.371	14.9 (10.7–15.9)	9.0 (5.5– 16.7)	0.608
EBV DNA load [copies/ml]	3711 (1363–9832)	2283 (748–7170)	4737 (1778– 11867)	0.186	524 (27–1363)	5025 (3033–11867)	0.001
Hospitalisation (days)	5.0 (4.0–7.0)	5.0 (3.5–6.5)	5.0 (4.0–7.0)	1.0	5.0 (5.0–6.0)	5.0 (3.0–7.0)	0.583
Duration of symptoms (days)	6.0 (3.0–9.0)	7.0 (4.5–11.5)	4.5 (3.0–7.0)	0.187	8.0 (5.0–14.0)	5.0 (2.0–7.0)	0.098
Total WBC count [10^3/µl]	13.62 (5.88)	12.05 (5.10)	14.96 (6.34)	0.207	9.74 (3.80)	15.67 (5.81)	0.005
Lymphocyte count [10^3/µl]	8.11 (4.23)	7.13 (3.94)	8.94 (4.44)	0.282	6.04 (3.11)	9.2 (4.41)	0.005
Monocyte count [10^3/µl]	1.11 (0.47)	1.03 (0.32)	1.18 (0.57)	0.412	0.94 (0.23)	1.20 (0.54)	0.107
ALT [IU/l]	86.0 (56.0–207.0)	53.5 (48.5–61.5)	180.5 (97.0–286.0)	< 0.001	8.0 (49.0–154.0)	87.0 (57.0–207.0)	0.767
GGTP [IU/l]	35.5 (19.0–92.0)	–	–	–	17.0 (15.0–40.0)	53.0 (21.0–93.0)	0.043
CRP [mg/l]	6.7 (2.2–18.5)	4.9 (1.7–15.3)	8.7 (5.1–18.5)	0.368	–	–	–
IL-6 [pg/ml]	6.4 (4.1–10.0)	7.5 (4.9–11.7)	5.3 (2.8–9.5)	0.189	5.1 (4.2–6.9)	8.6 (4.1–10.2)	0.374
TNF-α [pg/ml]	117.5 (43.0–292.0)	86.0 (43.0–202.5)	118.5 (38.0–432.0)	0.738	48.0 (38.0–120.0)	194.0 (52.0–855.0)	0.059
s-ICAM-1 [ng/ml]	15.2 (9.4)	15.2 (9.7)	15.3 (9.5)	0.983	9.3 (6.7)	18.4 (9.3)	0.009

ALT – alanine aminotransferase, CRP – C-reactive protein, EBV – Epstein-Barr virus, GGTP – γ-glutamyltranspeptidase, IL-6 – interleukin 6, TNF-α – tumour necrosis factor α, s-ICAM-1 – soluble intercellular adhesion molecule-1, WBC – white blood cell
Quantitative data are expressed as mean ±standard deviation or median and interquartile range.
CRP and GGTP data were not analysed statistically if they constitute a criterion for the division of particular groups.

is the result of, inter alia, the secretion of TNF-α [23], leads to an increase in leukocyte adhesion and an increase in s-ICAM-1 secretion. In our study positive correlations between s-ICAM-1, IL-6, and TNF-α in children with EBV hepatitis were confirmed. Hepatitis in the course of infectious mononucleosis is a common complication of self-limiting nature. Kimura *et al.* [24] and Banko *et al.* [12] showed no significant correlation between ALT activity and the amount of EBV DNA viraemia. An analysis conducted by Banko *et al.* in 33 patients with molecularly confirmed infectious mononucleosis showed a statistically significant positive correlation between viraemia and the necessity of hospitalization due to the clinical condition. Bauer *et al.* showed significantly higher values of EBV DNA viral load in hospitalized patients compared to outpatient patients [13]. There are many reports on the positive correlation between the EBV DNA viral load and the severity of infectious mononucleosis symptoms as well as the need for hospitalization [13, 24, 25], which may suggest the importance of viral load in predicting the clinical course of patients with infectious mononucleosis. In their study, Kimura *et al.* assessed the relationship between the amount of EBV DNA viraemia and the severity of the clinical course of infectious mononucleosis in 33 children [24]. The value to which the viral load was compared was the duration of fever (< and > 10 days). In the group of patients with a fever lasting longer than 10 days, a higher viral load was found than in the group with a fever lasting for a shorter period than 10 days; however, this result was not statistically significant.

In our study, the CRP level was considered as an important criterion determining the severity of the clinical course. In a group of 26 children with hepatitis and elevated CRP level, the mean of viral load, count of leukocytes, lymphocytes, and GGTP activity were statistically significantly higher than in children with CRP < 5 mg/l. The mean age of patients with CRP > 5 mg/l was lower than in the group with CRP within normal range. These data suggest that the possibility of developing cholestatic complications increases with increasing CRP and concerns younger children. Moreover, they confirm that the determination of EBV DNA viral load may be helpful in the differential diagnosis of hepatological disorders, especially in the youngest group of patients, in which the percentage of false-positive results in the determination of heterophile or specific antibodies directed against the capsid antigen (VCA IgM) is the highest [26–28]. Balfour *et al.*, while analysing the relationship between EBV DNA viraemia and IL-6 concentration, confirmed statistically significantly higher IL-6 concentration in patients with EBV viremia > 3.5 $\log_{10}$ copies/ml compared to patients whose viral load did not exceed this level or was undetectable [25]. In our own study, we did not obtain statistical significance between IL-6 serum level and EBV DNA load.

In our study there were statistically significant differences in serum levels of s-ICAM-1, CRP, and the duration of symptoms prior to hospitalization between the group

with viraemia > and < 3.5 $\log_{10}$ copies/ml. In the group of patients with viraemia > 3.5 $\log_{10}$ copies/ml, mean levels of s-ICAM-1 and CRP were higher and the mean duration of symptoms prior to hospitalization was shorter compared to the group with viraemia < 3.5 $\log_{10}$ copies/ml. The presence of a higher EBV DNA load in patients with a shorter duration of symptoms was confirmed in a study by Pitetti *et al.* [14].

The clinical forms of EBV infection can be very diverse, from primary syndromes to lymphoproliferative syndromes. Infectious mononucleosis is the most common form of acute EBV infection. Shi *et al.* confirmed that the group of most often hospitalized patients due to infectious mononucleosis syndrome are children aged 1–3 years [29]. In their study, they emphasized the essence of EBV DNA viral load determination, especially in patients under 7 years of age, who manifest symptoms that may suggest a disease induced by EBV infection, due to the fact that in the youngest group of children we may not obtain a positive result for the presence of heterophile or specific antibodies against EBV IgM capsid antigens [26–28]. Our study concerned a group of immunocompetent patients with infectious mononucleosis, in whom, in clinical practice, EBV DNA viral load is not routinely measured to predict the course of primary infection [12, 29]. However, the analysis by Banko and Shi *et al.* indicates that EBV DNA viraemia should become an important element in the diagnosis of EBV infection. It seems that the inclusion of a routine EBV DNA viral load determination in the group of the youngest immunocompetent children may significantly accelerate the diagnosis and treatment of EBV-induced cholestatic hepatitis.

A limitation of the study is the small size of the test group. The confirmation of the results of this publication will require further research on a larger number of patients.

CONCLUSIONS

The data obtained in the present study allow us to confirm the hypothesis about the possibility of using the EBV DNA viral load, IL-6, TNF-α, and s-ICAM-1 concentrations as risk factors for the occurrence of hepatological complications in patients with infectious mononucleosis. A quick diagnosis will shorten the hospitalization period and reduce costs resulting from extensive diagnostics.

DISCLOSURE

The authors declare no conflict of interest.

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