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Assessment of selected inflammatory markers in bacterial and viral neuroinfections in children

Ocena wybranych markerów zapalnych w bakteryjnych i wirusowych zakażeniach układu nerwowego u dzieci

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Abstract

Aim: The aim of the study was to assess the suggested biomarkers' usefulness in diagnosing central nervous system infections in order to optimise treatment and minimise adverse outcomes. The study included a comprehensive comparison of the known parameters and a search for correlations with proposed biomarkers. **Materials and methods:** The data of 73 hospitalised children were reviewed. According to their final diagnoses, 42 participants were assigned to the control group, 13 to the cohort with bacterial and 18 to the cohort with viral neuroinfections. The children underwent clinically indicated blood and cerebrospinal fluid tests. The serum interleukin (IL)-1β, IL-6 and neopterin concentrations, and S100B protein and matrix metalloproteinase (MMP)-9 levels in the cerebrospinal fluid were determined. The results were compared between the groups and correlations were sought. **Results:** Serum IL-6 levels were found to have increased in viral ($p = 0.0412$) and bacterial ($p < 0.0001$) infections, with a predominance of the latter ($p = 0.0403$). In terms of serum neopterin and IL-1β, the neuroinfection cohort did not differ from the control group. The level of S100B in the cerebrospinal fluid in bacterial disease was higher compared with the viral aetiology ($p = 0.0325$). The cerebrospinal fluid S100B correlated positively with serum IL-6 ($p = 0.0138$, $R = 0.6396$) and reversely with IgA and IgG levels ($p = 0.0499$, $R = -0.5325$; $p = 0.0022$, $R = -0.7451$, respectively) in the neuroinfection cohort. The cerebrospinal fluid MMP-9 was linked with cerebrospinal fluid cytosis in patients with viral ($p = 0.0018$, $R = 0.7547$) and bacterial ($p = 0.0124$, $R = 0.6935$) disease. The serum IL-6 levels correlated with IgA in the viral aetiology ($p = 0.0374$, $R = 0.9$). **Conclusions:** The MMP-9 level correlated with blood–brain barrier permeability, expressed by cerebrospinal fluid proteins and cytosis, which may indicate the possibility of sequelae. The higher serum concentrations of IL-6 and S100B in bacterial neuroinfections may reflect a more intense immune reaction associated with this aetiology.

Keywords: CNS infections, interleukins, neopterin, matrix metalloproteinase 9, inflammation mediators

Streszczenie

Cel: Zaplanowano ocenę użyteczności diagnostycznej wybranych biomarkerów w zakażeniach ośrodkowego układu nerwowego, w celu zoptymalizowania leczenia i zminimalizowania ryzyka powikłań, a także kompleksowe porównanie wykorzystywanych obecnie parametrów oraz poszukiwanie ich korelacji z badanymi markerami. **Materiał i metody:** Przeanalizowano dane 73 hospitalizowanych dzieci. Na podstawie ostatecznego rozpoznania 42 uczestników przyporządkowano do grupy kontrolnej, 13 – do podgrupy z zakażeniami bakteryjnymi, a 18 – z neuroinfekcją wirusową. Dzieci zostały poddane badaniom krwi obwodowej oraz płynu mózgowo-rdzeniowego. Przeanalizowano stężenie interleukiny (IL)-1β, IL-6 oraz neopteryny w surowicy oraz poziomy białka S100B i metaloproteinazy macierzy (MMP)-9 w płynie

mózgowo-rdzeniowym. Wyniki zostały porównane w grupach oraz poszukiwano wzajemnych zależności. **Wyniki:** Stężenie IL-6 w surowicy wzrastało w zakażeniach wirusowych ($p = 0{,}0412$) oraz bakteryjnych ($p < 0{,}0001$), istotnie w drugiej grupiej ($p = 0{,}0403$). Stężenie neopteryny w surowicy pacjentów z neuroinfekcją nie różniło się od wartości w grupie kontrolnej. Stężenie S100B w płynie mózgowo-rdzeniowym pacjentów z infekcją bakteryjną było wyższe niż w zakażeniu wirusowym ($p = 0{,}0325$). Stężenie S100B w płynie mózgowo-rdzeniowym wprost korelowało ze stężeniem IL-6 w surowicy ($p = 0{,}0138$, $R = 0{,}6396$) oraz odwrotnie z poziomem IgA i IgG w surowicy (odpowiednio $p = 0{,}0499$, $R = -0{,}5325$; $p = 0{,}0022$, $R = -0{,}7451$) wśród pacjentów z neuroinfekcją. Stężenie MMP-9 w płynie mózgowo-rdzeniowym odpowiadało cytozie płynu mózgowo-rdzeniowego pacjentów z zakażeniem wirusowym ($p = 0{,}0018$, $R = 0{,}7547$) i bakteryjnym ($p = 0{,}0124$, $R = 0{,}6935$). Stężenia IL-6 i IgA w surowicy korelowały w neuroinfekcji wirusowej ($p = 0{,}0374$, $R = 0{,}9$). **Wnioski:** MMP-9 odzwierciedla przepuszczalność bariery krew–mózg, wyrażoną poprzez cytozę i stężenie białka w płynie mózgowo-rdzeniowym, co może odpowiadać prawdopodobieństwu wystąpienia powikłań. Wyższe stężenia IL-6 i S100B w zakażeniach bakteryjnych mogą odpowiadać silniej wyrażonej reakcji immunologicznej we wspomnianej etiologii.

Słowa kluczowe: infekcje OUN, interleukiny, neopteryna, metaloproteinaza macierzy 9, markery zapalenia

INTRODUCTION

Neuroinfections in childhood are recognised as a relevant clinical issue. The most common aetiological factors remain bacterial and viral agents, which were responsible for 41% and 59% of central nervous system (CNS) infections in Poland in 2019 (Ochocka et al., 2021). The epidemiological status varies year to year due to regional and global circumstances, such as the COVID19 pandemic (Mrozowska-Nyckowska et al., 2022). *Borrelia burgdorferi* (*B. burgdorferi*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *Neisseria meningitidis* (*N. meningitidis*) are known to be the most common causative agents of bacterial neuroinfections, whereas enterovirus and tick-borne encephalitis virus (TBEV) dominate among the viral causes of meningitis and encephalitis, respectively (Ochocka et al., 2021).

In view of the dynamic epidemiological situation, there is a need for biomarkers enabling fast and accurate differentiation to introduce adequate treatment and minimise adverse outcomes. The immune responses to viral and bacterial infections differ (Verhoef et al., 2019), which seems to be the key to the detection of new diagnostic parameters.

The selected markers from various immunological pathways appear in different inflammation phases. Interleukin (IL)-1 and IL-6 belong to proinflammatory particles responsible for the initial immune reaction, triggering generalised reactions, e.g. fever, malaise, and weakness. Moreover, IL-1 maintains chronic inflammation in neurodegenerative diseases (Ye et al., 2013), and IL-6 converts from the acute to prolonged reaction (Ye et al., 2016). Neopterin reflects Th1mediated immunity, including macrophage activities, indirectly illustrating free radical production (Berdowska and Zwirska-Korczala, 2001; Dale et al., 2009). In cerebrospinal fluid (CSF), matrix metalloprotease (MMP)-9, and S-100B were chosen. The former belongs to the family of proteins decomposing collagen, resulting in increased

		Neuroinfection cohort			
Control group	***n***	**Bacterial**	***n***	**Viral**	***n***
Seizures:		Meningitis:		Meningitis:	
• first episode	7	• *B. burgdorferi* spp.	4	• enterovirus	3
• epilepsy	6	• *N. meningitidis*	3	• VZV	3
Excluded CNS infection suspicion	11	• unidentified agent	1	• EBV	1
Involuntary movements	3	Septic SIRS	2	• HHV-6	1
Sight disturbances	3	*S. pneumoniae*-associated encephalomeningitis	1	• mixed EBV and enterovirus	3
Gait/balance impairment	2	*M. pneumoniae*-triggered acute tic disorder	1	• mixed VZV and enterovirus	1
Psychophysical retardation	2	*S. agalactiae* sepsis with neuroinfection	1	• unidentified agent	4
Prolonged fatigue	2			Mixed HSV-1 + EBV encephalomeningitis	1
Consciousness loss	2			Congenital cytomegaly	1
Head trauma	1				
CNS tumour	1				
Abductor nerve neuropathy	1				
Toxocariasis follow-up	1				

B. burgdorferi* spp.** – *Borrelia burgdorferi* species; **CNS** – central nervous system; **EBV** – Epstein–Barr virus; **HHV** – human herpes virus; **HSV-1** – herpes simplex virus type 1; ***M. pneumoniae – *Mycoplasma pneumoniae*; ***N. meningitidis*** – *Neisseria meningitidis*; **SIRS** – systemic inflammatory response syndrome; ***S. agalactiae*** – *Streptococcus agalactiae*; ***S. pneumoniae*** – *Streptococcus pneumoniae*; **VZV** – varicella-zoster virus.

Tab. 1. The study group in subdivisions based on the diagnosis

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blood–brain barrier (BBB) permeability (Yong, 2005). The latter's concentration is linked to glial tissue damage due to its increased level in astrocytic cells (Di Stefano et al., 2020).

The aim was to assess the usefulness of selected biomarkers in distinguishing and differentiating viral and bacterial neuroinfections to optimise treatment and minimise adverse outcomes. The study included a comparison of the known parameters between diseases of other origins and a search for correlations with proposed new biomarkers.

MATERIALS AND METHODS

The study was conducted in a tertiary centre specialising in infectious diseases and child neurology between August 2018 and January 2020, and involved patients with suspected neuroinflammation. The children whose CSF or blood parameters could not be assessed, and children with autoimmune disease, were excluded. The final diagnosis determined assignment to the control group or one of the study's two neuroinfection groups. The control cohort consisted of 42 patients with excluded autoimmune and infectious pathogenesis, suffering, e.g. from head trauma, epileptic seizures or chronic diseases. The heterogeneity of diagnoses in the control group reduces the risk of bias associated with the patterns of results which are characteristic for the specific aetiologies.

Moreover, the participants of the control group and neuroinfection cohort had a comparable availability of medical procedures' outcomes, increasing the credibility of analyses. The neuroinfection group included 31 patients: 13 with bacterial and 18 with either confirmed or highly probable viral aetiology. In four participants, mixed aetiology was found; in eight children, we failed to identify the triggering factor, but the clinical course and response to treatment univocally suggested a bacterial or viral aetiology. The characteristics of the study population are shown in Tab. 1.

CSF samples and serum from centrifuged venous blood were collected and stored at −80°C before the laboratory procedures. The parameters, including general CSF examination, were evaluated with standard laboratory analysers. The reference values (RV) for selected parameters are listed in Tab. 2. The serum IL-1β (Elabscience, 2019, Houston, TX, USA), IL-6 (Elabscience, 2019, Houston, TX, USA) and neopterin (MyBioSource, Inc., 2019, San Diego, CA, USA), as well as CSF MMP-9 (Abcam, 2019, Cambridge, UK) and S100B (Abcam, 2019, Cambridge, UK) concentrations were assessed with commercial kits.

The statistical analyses were carried out with PQStat v.1.8.4.152 (PQStat Software, 2022, Poznań, Poland). For the groups' quantity and standard distribution, data suitably underwent the Mann–Whitney *U* test, *t*-test for independent groups, Kruskal–Wallis ANOVA (KW) or one-way ANOVA for independent groups test (ANOVA). A *p*-value below 0.05 was considered statistically significant. Parameters assessed with KW or ANOVA achieving $p < 0.05$ underwent

Parameter	Age range	Reference value
WBC [10^3/μL]	1–12 m/a	4.0–20.0
	1–6 y/a	4.5–13.0
	7–12 y/a	4.0–12.0
	>12 y/a	4.0–10.0
HGB [g/dL]	1–2 m/a	10.0–13.5
	2–3 m/a	9.5–13.0
	3–6 m/a	10.0–13.0
	6–9 m/a	10.5–13.0
	9–24 m/a	11.0–14.0
	2–6 y/a	10.9–14.2
	7–12 y/a	12.0–15.5
	>12 y/a	12.0–16.0 (female) 14.0–18.0 (male)
PLT [10^3/μL]	Any age	150–400
IgG [mg/dL]	1–3 m/a	270–780
	4–6 m/a	190–860
	7–12 m/a	350–1180
	1–3 y/a	520–1360
	4–5 y/a	540–1420
	6–7 y/a	570–1410
	8–9 y/a	730–1410
	10–11 y/a	730–1350
	12–13 y/a	770–1510
	>13 y/a	700–1600
IgA [mg/dL]	1–3 m/a	6–58
	4–6 m/a	10–96
	7–12 m/a	36–165
	1–3 y/a	45–135
	4–5 y/a	52–220
	6–7 y/a	65–240
	8–9 y/a	108–200
	10–11 y/a	91–255
	12–13 y/a	108–325
	>13 y/a	70–400
IgM [mg/dL]	1–3 m/a	12–87
	4–6 m/a	25–120
	7–12 m/a	30–104
	1–3 y/a	46–190
	4–5 y/a	40–200
	6–7 y/a	55–210
	8–9 y/a	55–175
	10–11 y/a	66–155
	12–13 y/a	70–150
	>13 y/a	40–230
CRP [mg/dL]	Any age	<0.5
PCT [ng/mL)	Any age	<0.5
CSF-P [mg/dL]	Any age	15–45
CSF-L [μL^{-1}]	Any age	1–5

CRP – C-reactive protein; **CSF-L** – cerebrospinal fluid cytosis; **CSF-P** – cerebrospinal fluid protein concentration; **HGB** – haemoglobin concentration; **IgA** – serum immunoglobulin A concentration; **IgG** – serum immunoglobulin G concentration; **IgM** – serum immunoglobulin M concentration; **m/a** – months of age; **PCT** – procalcitonin concentration; **PLT** – platelet count; **WBC** – white blood cells; **y/a** – years of age.

Tab. 2. Reference values of selected parameters

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	All participants		Control group		Neuroinfection cohort			
					Bacterial		Viral	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age [years]	4.23	2.18–9.97	4.35	2.18–10.2	3.73	0.84–4.28	8.09	3.62–13.98
CSF – S100B [pg/mL]	266.4	181.4–387.3	270	212.5–314.1	379[c]	210.4–649.5	201.6[c]	128.4–322.3
CSF – MMP-9 [ng/mL]	82.2	55.7–245.6	66.6	45.1–75.3	124.3	55.7–383.9	154.6	76.9–279.9
s – IL-1 [pg/mL]	3.195	2.817–3.658	2.906	2.789–3.239	3.22	3.153–3.574	3.364	3.173–5.161
s – IL-6 [pg/mL]	4.954	4.209–7.203	4.496	4.044–5.622	8.636[ac]	7.192–120.818	5.155[c]	4.33–6.649
s – neopterin [ng/mL]	8.674	7.226–17.421	8.404	7.408–12.902	15.644	12.795–20.381	7.634	6.993–22.733
WBC [10^3/µL]	8.94	6.96–13.16	7.85	6.4–11.45	13.1	7.79–15.15	10.47	8.09–13.41
HGB [g/dL]	12.15	11.2–13.4	12.1	11.3–13.2	11.2	10.2–13.1	12.8	11.5–13.7
PLT [10^3/µL]	295	219.75–369.5	287	219–374	291	235–349	318	234.75–367.5
CRP [mg/dL]	0.2	0.2–1.1175	0.2	0.2–0.43	2.13[a]	0.2–17.52	0.585	0.205–1.435
PCT [µg/L]	0.155	0.0825–1.9175	0.13	0.1–0.48	30.79[ac]	1.98–89.2	0.075[c]	0.0575–0.175
Glucose [mg/dL]	94	86–104	93	83–96.5	90[d]	87–98	106.5[ad]	101.5–117.5
CSF protein [mg/dL]	24	18–38	19	14–26	34.5[a]	21–75.5	38[a]	24–60.5
CSF cytosis [μL^{-1}]	2	2–39	2	1–2	46[a]	13–192	66.5[a]	16–250
IgG [mg/dL]	720	581–898	754.5	491–1017	617	582.5–681	758	739–894
IgA [mg/dL]	54	36–114.25	86	37–123	38	24–52.5	62	55–107
IgM [mg/dL]	95	66–137	107	70.25–135	66.5[bd]	49.5–68	160[bd]	151–178
APTT [s]	31	29.1–32.9	30.4	29.5–32.6	32.4	30.2–33.8	31	28.6–32.4
PT [s]	12.9	11.625–13.95	12.6	11.55–13.15	13.8[a]	12.8–16	13.25	12.425–14.1
Fibrinogen [mg/dL]	306.5	241.3–390.3	276.5	216.5–335.5	364.5[a]	313–749	389.5[a]	278–435
D-dimer [mg/dL]	0.31	0.2–0.66	0.3	0.2–0.49	0.95	0.27–5.14	0.3	0.25–0.94

APTT – activated partial thromboplastin time; **CRP** – C-reactive protein; **CSF** – cerebrospinal fluid; **HGB** – haemoglobin; **Ig** – immunoglobulin; **IL** – interleukin; **IQR** – inter-quartile range; **MMP-9** – metalloproteinase-9; **PCT** – procalcitonin; **PLT** – platelet count; **PT** – prothrombin time; **s** – prefix for serum parameters; **WBC** – white blood cell count.
[a] Comparison with the control group, $p < 0.05$ (Kruskal–Wallis ANOVA with Dunn–Bonferroni post-hoc test).
[b] Comparison with the control group, $p < 0.05$ [one-way ANOVA for independent groups with Fisher (LSD) post-hoc test].
[c] Comparison of bacterial and viral groups, $p < 0.05$ (Mann–Whitney *U* test).
[d] Comparison of bacterial and viral groups, $p < 0.05$ (*t*-test for independent groups).

Tab. 3. Median values and inter-quartile ranges of analysed parameters

	All participants		Control group		Neuroinfection cohort					
					Whole cohort		Bacterial		Viral	
	p	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*
s – IL-6 vs. s – IL-1β	0.0696	0.3418	0.2942	0.2796	0.6286	–0.1484	0.6	–0.4	0.8647	0.0667
s – IL-6 vs. CSF – MMP-9	0.5689	0.1831	-	-	0.3403	0.3374	0.6	0.4	0.6998	0.2029
s – IL-6 vs. s – neo.	0.3766	0.1982	0.9828	–0.007	0.2763	0.3818	0.6667	–0.5	0.1482	0.6071
s – IL-6 vs. CSF – S100B	0.2839	0.2022	0.4053	–0.2236	0.0138*	0.6396*	0.2	0.8	0.2145	0.4303
s – IL-1β vs. CSF – MMP-9	0.7109	–0.1197	-	-	0.9866	0.0061	0.6	–0.4	0.9131	–0.058
s – IL-1β vs. s – neo.	0.534	–0.1401	0.5193	–0.2067	0.1869	–0.4545	0.6667	–0.5	0.4821	–0.3214
s – IL-1β vs. CSF – S100B	0.7023	0.0728	0.3678	–0.2414	0.3919	0.2484	0.8	–0.2	0.1076	0.5394
CSF – MMP-9 vs. s – neo.	0.699	–0.1802	-	-	0.6990	–0.1802	0.6667	0.5	0.6	–0.4
CSF – MMP-9 vs. CSF – S100B	0.8912	0.0223	0.8168	0.0682	0.7436	0.0674	0.7456	0.1049	0.3701	0.2596
s – neo. vs. CSF – S100B	0.1375	0.3269	0.2551	0.3566	0.7261	0.1273	0.6667	–0.5	0.879	0.0714

* $p < 0.05$ (Spearman's rank test).
s – prefix for serum parameters; **CSF** – prefix for cerebrospinal fluid parameters.
IL – interleukin; **MMP-9** – metalloproteinase-9; **neo.** – neopterin; ***R*** – correlation coefficient.

Tab. 4. Correlation between analysed parameters

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		All participants		Control group		Neuroinflammation cohort					
						Whole cohort		Bacterial		Viral	
		p	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*
CSF S100B	**CSF-P**	0.9494	−0.0086	0.41	−0.1621	0.5709	0.1098	0.8729	0.0548	0.3915	0.1081
	CSF-L	0.8748	−0.0211	0.2169	0.2409	0.6416	−0.0886	0.5412	−0.1961	0.1311	0.604
	s – CRP	0.368	0.1204	0.7056	0.0747	0.2435	0.2196	0.3358	0.3045	−0.09281	0.7142
	s – IgA	0.6798	−0.0816	0.0165*	0.6264	0.0499*	−0.5325	0.1875	−0.48335	−0.4	0.50465
	s – IgM	0.6257	−0.0964	0.0534	0.5259	0.06765	−0.5017	0.4444	−0.29249	-	-
	s – IgG	0.1067	−0.31147	0.46437	0.2132	0.0022*	−0.7451	0.0096*	−0.8	−0.6	0.2848
	PT	0.398	0.1163	0.2392	0.2443	0.426	0.1509	0.3306	0.3077	−0.061	0.81
	fibr	0.7226	0.0499	0.8101	−0.0518	0.3296	0.1877	0.2466	0.3818	−0.0609	0.8103
CSF MMP-9	**CSF-P**	0.0054*	0.4375	1	0	0.0504	0.3954	0.1232	0.4932	0.3674	0.261
	CSF-L	<0.0001*	0.6681	0.95	0.0184	<0.0001*	0.7358	0.0124*	0.6935	0.0018*	0.7547
	s – CRP	0.0203*	0.3656	1	0	0.0893	0.3399	0.5729	0.1813	0.0018*	0.7542
	s – IgA	0.1554	−0.3299	0.9554	−0.0238	0.368	−0.2857	0.5755	−0.2167	0.3333	0.866
	s – IgM	0.0924	−0.3864	0.4198	0.3333	0.0455*	−0.5855	0.0525	−0.6611	0.3333	−0.866
	s – IgG	0.0084*	−0.5723	0.3518	−0.381	0.0208*	−0.6549	0.244	−0.4333	0.3333	−0.866
	PT	0.0036*	0.4611	0.4604	−0.2359	0.0033*	0.5544	0.0625	0.5524	0.027*	0.5881
	fibr	0.0027*	0.4857	0.5712	−0.1922	0.0027*	0.5735	0.1173	0.5	0.0109*	0.6557
Serum IL-1β	**CSF-P**	0.6488	0.0867	0.0427*	−0.5118	0.5156	0.1898	0.4	−0.6	0.2114	0.4329
	CSF-L	0.8485	0.0364	0.005*	−0.6639	0.4685	−0.2112	0.6	0.4	0.2202	−0.4255
	s – CRP	0.9687	−0.0075	0.5819	−0.149	0.0968	−0.4614	1	0	0.1557	−0.4847
	s – IgA	0.2478	−0.3181	0.3374	−0.4286	0.6703	−0.1796	-	-	0.6238	−0.3
	s – IgM	0.8544	0.0518	0.8175	−0.1081	1	0	0.6667	−0.5	0.7471	−0.2
	s – IgG	0.8894	−0.0393	-	-	0.5702	−0.2381	0.6667	−0.5	0.1881	−0.7
	PT	0.1065	0.306	0.6801	0.1162	0.9821	−0.0066	0.6	0.4	0.6992	0.1402
	fibr	0.4724	0.1389	0.8247	−0.0626	0.714	−0.1077	0.2	−0.8	0.8548	0.0667
Serum IL-6	**CSF-P**	0.4006	0.1592	0.5968	−0.1432	0.6682	0.1258	0.6	−0.4	0.6372	0.1707
	CSF-L	0.5283	0.1198	0.0837	−0.4456	0.6365	0.1386	0.4	−0.6	0.8675	0.0608
	s – CRP	0.0147*	0.4409	0.5548	0.1596	0.2787	0.3113	0.2	−0.8	0.8266	0.0798
	s – IgA	0.9849	0.0054	0.7017	−0.1786	0.6081	−0.2156	-	-	0.0374*	0.9
	s – IgM	0.5538	−0.1662	0.1925	−0.5586	0.3199	−0.4048	0.6667	0.5	0.391	0.5
	s – IgG	0.6571	0.125	0.4821	0.3214	0.3199	−0.4048	0.6667	0.5	0.8729	0.1
	PT	0.0003*	0.6195	0.1014	0.4393	0.0023*	0.7431	0.6	0.4	0.048*	0.6361
	fibr	0.0819	0.3284	0.9698	0.0107	0.2668	0.3187	0.8	0.2	0.9867	0.0061
Serum neopterin	**CSF-P**	0.9881	0.0034	0.6482	−0.1471	0.7001	−0.1398	-	-	0.2103	−0.5406
	CSF-L	0.264	0.2489	0.6888	0.1293	0.3001	0.3647	-	-	0.504	0.3063
	s – CRP	0.5011	0.1514	0.3541	−0.2937	0.2276	0.4195	-	-	0.3325	0.4325
	s – IgA	0.7186	0.123	0.6238	−0.3	0.5774	0.2899	-	-	1	0
	s – IgM	0.0549	0.5923	0.0048*	0.9747	0.5441	0.3143	-	-	0.8	−0.2
	s – IgG	0.65	0.1545	0.5046	−0.4	0.8717	0.0857	-	-	0.8	−0.2
	PT	0.3243	0.2204	0.8799	0.049	0.6628	0.1581	0.6667	−0.5	0.6701	0.1982
	fibr	0.4757	0.1605	0.8799	0.049	0.777	0.103	0.6667	0.5	1	0

* $p < 0.05$ (Spearman's rank test).
CRP – C-reactive protein; **CSF** – cerebrospinal fluid; **CSF-L** – cerebrospinal fluid leukocyte count; **CSF-P** – cerebrospinal fluid protein concentration; **fibr** – fibrinogen; **Ig** – immunoglobulin; **IL** – interleukin; **MMP-9** – metalloproteinase-9; **PT** – prothrombin time; ***R*** – correlation coefficient; **s** – prefix for serum parameters.

Tab. 5. Correlation between analysed parameters and PT, fibr, CRP, immunoglobulin, CSF protein concentration, and CSF leukocyte count

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Dunn–Bonferroni or Fisher (LSD) posthoc tests, respectively. The Bioethics Committee at the Poznan University of Medical Sciences approved the study design (No. 1283/18). The legal guardians or/and patients expressed their written consent to participate in the study, as required by law.

RESULTS

The analysis included data collected for a total of 73 hospitalised children; 48 (65.8%) were male and 25 (34.2%) female. The median age equalled 4.23 years (interquartile range, IQR 2.18–9.97).

Serum IL-6 concentrations differed ($p = 0.048$) in the control group compared to the viral ($p = 0.0412$) and bacterial ($p < 0.0001$) aetiology. A direct comparison between the two infection types showed a predominance in the latter ($p = 0.0403$). The IL-1β and neopterin levels in the study population revealed no statistical difference. The CSF S100B level in patients with bacterial neuroinfection exceeded its concentration in the viral aetiology ($p = 0.0325$). The data distribution in the study groups is summarised in Tab. 3.

In the complete neuroinfection cohort, a weak association between serum IL-6 and CSF S100B ($p = 0.0138$, $R = 0.6396$) was observed. Also, a correlation between the latter and immunoglobulin concentration emerged – positive with immunoglobulin (Ig)-A in the control group ($p = 0.0165$, $R = 0.6264$), reverse with IgA and IgG in patients with neuroinfection ($p = 0.0499$, $R = -0.5325$; $p = 0.0022$, $R = -0.7451$, respectively), and separately with IgG in bacterial disease ($p = 0.0096$, $R = -0.8$).

In the study population, CSF MMP-9 was associated with higher CSF-P levels ($p = 0.0054$, $R = 0.4375$). The mentioned parameter increased the CSF-L level in the entire study population ($p < 0.0001$, $R = 0.6681$) and in patients with bacterial ($p = 0.0124$, $R = 0.6935$) or viral ($p = 0.0018$, $R = 0.7547$) neuroinfections as well as considered in a merged group ($p < 0.0001$, $R = 0.7358$). The CSF MMP-9 c oncentration rises with the serum C-reactive protein (CRP) level ($p = 0.0203$, $R = 0.365$), especially in viral neuroinfection ($p = 0.0018$, $R = 0.7542$). In the group of children with neuroinfection, the parameter decreases with IgM ($p = 0.0455$, $R = -0.5855$) and IgG ($p = 0.0208$, $R = -0.6549$) concentrations. CSF MMP-9 positively correlated with prothrombin time (PT) and fibrinogen (fibr) in all participants ($p = 0.0036$, $R = 0.4611$; $p = 0.0027$, $R = 0.4857$, respectively) and all children with neuroinfection ($p = 0.0033$, $R = 0.5544$; $p = 0.0027$, $R = 0.5735$, respectively) – especially with virus-triggered ($p = 0.027$, $R = 0.5881$; $p = 0.0109$, $R = 0.6557$, respectively). Serum IL-1β concentration reversely correlates with CSF-P ($p = 0.0427$, $R = -0.5118$) and CSF-L ($p = 0.005$, $R = -0.6639$) in the control group. Serum IL-6 positively affects CRP in the whole population ($p = 0.0147$, $R = 0.4409$), IgA in children with viral disease ($p = 0.0374$, $R = 0.9$), and PT in the whole population ($p = 0.0003$, $R = 0.6195$), patients with neuroinfections ($p = 0.0023$, $R = 0.7431$) – especially of viral origin ($p = 0.048$, $R = 0.6361$). In the control group, a strong correlation between neopterin and IgM serum concentrations ($p = 0.0048$, $R = 0.9747$) was demonstrated.

The detailed analyses are shown in Tabs. 4 and 5.

DISCUSSION

S100B

S100B belongs to calcium-binding proteins, stored predominantly in astrocytes (Di Stefano et al., 2020). S100B is released non-specifically in cell stress – such as damage and chronic and acute inflammation. Lower concentrations cause leukocyte chemotaxis and impact cell proliferation and macrophage activity (Donato, 1999); higher levels lead to toxicity and apoptosis (Hajduková et al., 2015). The concentration correlates with the extent of damage, reaching a high level in the CSF, especially in patients developing severe adverse outcomes. However, the affected tissue volume does not directly reflect the sequelae (Watson and Scott, 1995).

The study revealed a mutual S100B correlation with IL-6. The association finds confirmation in Alzheimer's disease and bacterial meningitis in which S100B induction by IL-6, IL-1β and tumour necrosis factor (TNF)-α was previously proven. Interestingly, the extensive synergy between proinflammatory cytokines leads to decreased BBB selectivity preceding the nucleated cell inflow to the intrathecal compartment (Hamed et al., 2009; de Souza et al., 2013).

The analysis revealed significantly higher S100B concentrations in bacterial than viral infections. The finding may be associated with a lower prevalence of cytokine storm and milder course of the aseptic disease (Wu et al., 2020), leading to a minor injury to the CNS tissues. However, S100B with ferritin, neopterin and IL-6 may differentiate HSV encephalitis, probably due to the extensive necrotic process (Di Stefano et al., 2020; Sindic et al., 1985).

The analysis revealed a negative correlation between S100B and IgA, and IgG in the neuroinfection cohort. Although similar dependencies are not reported in the literature, hypothetically, S100B appears in the first inflammatory phase in which specific immunoglobulins are not produced. In the later stages, S100B decreases, while the IgA/IgG production increases.

MMP-9

MMP-9 belongs to extracellular proteolytic enzymes responsible for embryogenesis, early CNS development, regeneration, and recovery. However, unregulated high production of MMP-9 increases BBB permeability, indirectly leading to immune overactivation, leukocyte infiltration, and neuron apoptosis. In neuroinfections, it may forecast a poor outcome (Kang et al., 2013; Yong, 2005).

When comparing the aetiologies of neuroinfections, MMP-9 was hypothesised to predominate in the bacterial cohort.

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Although the preceding article showed higher CSF MMP-9 concentrations in patients with neuroinfection (Lubarski et al., 2022), no difference between the viral and bacterial subgroups was shown in our study.
The MMP-9 correlation with CSF-P and CSF-L was seen in the entire study population. Other reports also confirmed a CSF MMP-9 association with CSF-P (Baunbæk Egelund et al., 2017). The findings remain closely linked to proteolytic function, decreasing BBB selectivity. Furthermore, the protein stimulates the production of other proinflammatory cytokines and the migration of inflammatory cells to the intrathecal compartment (Williams et al., 2002).
The MMP-9 was found to be associated with fibrinogen and PT in the whole population and in the neuroinfection cohort. A similar finding was noted in patients with acute coronary syndrome. In the article, serum MMP-9 correlated not only with fibrinogen but also with IL-18 and CRP levels. Moreover, the authors observed a progressive increase in serum MMP-9 concentration with disease severity (Yang et al., 2022).

Interleukin-1

IL-1β co-initiates the immune pathway, signalling other cytokines, e.g. IL-6, which is described below. However, it also triggers generalised reactions via prostaglandin induction, thus increasing the production of adhesion molecules that enable the immunocompetent cells to penetrate diseased tissues. IL-1β is known to affect the bone marrow, increasing myeloid cell differentiation (Dinarello, 2009).
This article revealed a reverse correlation of IL-1β with CSF-P and CSF-L, but significance was observed only in the control group. The literature describes a correlation between CSF cytosis and IL-1β regardless of aetiology. A frequent explanation is that it promotes both active and passive leukocyte migration to tissues (Cape et al., 2014; Rigor et al., 2012) and increased adhesive molecule production (Dinarello, 2009). The induced inflammatory process is postulated to result in higher cytotoxic activity and increased inflammatory protein production, elevating the total CSF-P level. Furthermore, the IL-1β concentration may correlate with sequelae, probably due to the underlying inflammation (Azuma et al., 1997).

Interleukin-6

IL-6 fluctuations are observed in neuroinfections. The cytokine belongs to proinflammatory agents, non-specifically indicating the intensity of inflammation (Kang et al., 2013). Included data suggests a correlation between IL-6 and CRP in patients with neuroinflammation, as initially hypothesised. The results find confirmation in other types of injuries. Kalabalikis et al. reported that peak IL-6, measured during the first hours after head trauma, correlated with peak CRP. Interestingly, the authors failed to demonstrate any links between CRP and IL-6 and patient outcomes (Kalabalikis et al., 1999).
Our analysis revealed a significant increase in bacterial aetiology compared to the control group and viral disease. Previous works confirmed the prevalence of IL-6 in bacterial infections (Dalal et al., 2003; Watson and Scott, 1995). However, Dalal et al. also observed a significant difference in CSF IL-6 between the control and viral cohorts. Moreover, the authors assessed the IL-6 positive and negative predictive values at 100% and 73%, respectively (Dalal et al., 2003). Our study found no evidence to confirm this relationship in serum.

Neopterin

Neopterin is known to rise non-specifically in neuroinfections, autoimmune diseases, and post-transplant complications due to macrophage activation as a marker of cellular response. The protein induces free radicals, enhancing cytotoxicity (Huber et al., 1984). In some studies, neopterin was found to correlate with IL-6 and CRP (Yadav et al., 2012); in others, e.g. focused on rheumatic arthritis, no association with CRP was identified (El-Lebedy et al., 2017). Likewise, our study showed no correlation with any of the analysed inflammatory parameters. The analysis, however, revealed neopterin's positive correlation with total IgM concentration. Even though the published manuscript does not address this association, there are reports that in viral infections high serum neopterin accompanies specific IgM seroconversion (Schennach et al., 1994).

CONCLUSIONS

Our study shows that the incorporation of novel markers into routine practice would enrich physicians' knowledge about individual disease courses. The analysis detected several potentially critical relationships between viral and bacterial neuroinfections. Regarding the most significant findings, a correlation between MMP-9 and BBB permeability, assessed with increased CSF-P and CSF-L, was confirmed, potentially reflecting patients' adverse outcomes (Kang et al., 2013). The proposed novel parameters appear suitable for prospective clinical usage, though further analyses are needed, especially in paediatrics, which is a discipline in which data is limited. A broader population enrolled in future studies would minimise statistical bias and decrease the effect of specific aetiologies that show an atypical biochemical profile, such as the S100B protein in HSV infection (Di Stefano et al., 2020).

Conflict of interest
The authors declare no significant conflict of interest according to this article.

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