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ORIGINAL RESEARCH

Pro-Inflammatory and Anti-Inflammatory Cytokines Levels are Significantly Altered in Cerebrospinal Fluid of Unruptured Intracranial Aneurysm (UIA) Patients

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Introduction: Identifying all the relevant "players" in the formation and development of brain aneurysms may help understand the mechanisms responsible for the formation of an aneurysm, as well as in the search for non-invasive targets for aneurysm pharmacotherapy.

Aim: The evaluation of the concentration of pro-inflammatory and anti-inflammatory cytokines in cerebrospinal fluid (CSF) and serum of patients with unruptured intracranial aneurysms (UIA) in comparison to individuals without vascular lesions in the brain.

Methods: The concentration of 27 proteins in the CSF and serum of UIA patients (N = 40) and individuals without vascular lesions in the brain (N = 15) was evaluated using a multiplex ELISA kit (Bio-Plex Pro Human Cytokine 27-Plex Panel).

Results: In the CSF 13 out of 27 proteins evaluated presented a concentration 1.36-fold or greater in UIA patients in comparison to the control group. Significantly higher were IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-7, IL-8, IL-12, IL-13, TNF-α, INF-γ, MCP-1, and VEGF. In the serum none of the proteins evaluated significantly differ between UIA patients and the control group. The correlation coefficient analysis showed that CSF IL-1β, IL-8, and TNF-α positively, while IL-13 negatively correlated with the size of aneurysms. CSF IL-6 and MCP-1 concentrations positively correlated with the number of aneurysms.

Conclusion: In patients with UIA, pro-inflammatory and anti-inflammatory mechanisms are activated simultaneously, because the concentration of promoting and suppressing inflammatory response proteins was significantly higher in CSF of UIA patients compared to the control group. The preventive therapy of brain aneurysm development should be focused on IL-1β, IL-6, IL-8, MCP-1, and TNF-α, the concentration of which in CSF positively correlated with the size and number of aneurysms.

Keywords: cerebrospinal fluid, cytokines, inflammation, interleukins, intracranial aneurysm, unruptured intracranial aneurysms

Introduction

Inflammation within the arterial vessel due to hemodynamic changes is a critical element in the formation of an intracranial aneurysm (IA).[1–8] Understanding the mechanisms leading to the formation of brain aneurysms and identifying all the relevant "players" in this process is essential for the development of an effective pharmacotherapy that could prevent life-threatening subarachnoid hemorrhage from rupture of IA.

Both the literature data and our previous research indicated, that the process of formation and development of brain aneurysms is multifactorial and dependent, among other things, on interleukins, cytokines, chemokines, growth factors, or matrix metalloproteinases.[4,5,9–16] Researchers also highlighted the importance of immune cells in this process (monocytes/macrophages, neutrophils, T lymphocytes, natural killer cells (NK), mast cells) that infiltrate the vessel wall and promote its pathogenic remodeling.[17–20] The most critical importance in the formation of brain aneurysms is attributed to macrophages secreting inflammatory cytokines,[19,21] proteolytic enzymes, or reactive oxygen species, thus inducing apoptosis and fibrosis of smooth muscle cells within the IA wall.[22–26] Persistent

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chronic inflammation has a modulating effect on the arterial wall, promoting its degradation, but also supports repair mechanisms.[27] However, the advantage of degenerative over repair mechanisms contributes to the critical thinning of the vessel wall and eventual rupture, leading to aneurysmal subarachnoid hemorrage.[28]

A possible mechanism for the development of aneurysm-related inflammation has been attributed to the imbalance between the pro- and anti-inflammatory systemic response.[10] Typical pro-inflammatory cytokines are interleukins (ILs) such as IL-1, −6, −8, −15, −17, −18, −23, tumor necrosis factor-alpha (TNF-α) and chemokines like monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and macrophage inflammatory protein-1β (MIP-1β).[29,30] Also, IL-2, −5, −7, −12, interferon-γ (IFN- γ), granulocyte colony-stimulating factor (G-CSF), granulocyte monocyte colony-stimulating factor (GM-CSF), regulated on activation, normal T cell expressed and secreted (RANTES), interferon gamma-induced protein 10 (IP-10) are recognized as pleiotropic regulators of inflammation.[31–36] Major anti-inflammatory cytokines encompass IL-1 receptor antagonist (IL-1ra), IL-4, −9, −10, −11, and IL-13.[30,37]

Studies that can "visualize" the body's response to the formation of an aneurysm in the brain may help understand the mechanisms responsible for their formation as well as in the search for non-invasive targets for aneurysm pharmacotherapy. Therefore, the current study aimed to assess the concentrations of pro- and anti-inflammatory cytokines in cerebrospinal fluid (CSF) and serum of patients with unruptured intracranial aneurysms (UIA) in comparison to individuals without vascular changes in the brain. It should be highlighted that most of the conducted studies to date have been focused on animal models or performed in patients with ruptured aneurysms.[16,18,38–41] The commercially available panel selected by us included 27 cytokines such as IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1ra, TNF-α, IFN-γ, GM-CSF, G-CSF, Eotaxin, IP-10, MCP-1, MIP-1-α, MIP-1β, RANTES, VEGF, PDGF-BB, and bFGF. We also attempted to check if these proteins are related to the size and number of aneurysms.

Materials and Methods

Subjects

The study group consisted of 40 patients with unruptured intracranial aneurysms (UIA). The patients were operated on at the Department of Neurosurgery at the Clinical Hospital of the Medical University of Bialystok. All patients underwent craniotomy and direct surgical clipping of an unruptured intracranial aneurysm. Detailed information about the procedure can be found in our previous publications.[4,5] The control group consisted of 15 patients suffering from trigeminal neuralgia because of an anatomical conflict between the trigeminal nerve and the cerebellar artery. All patients within this group were revealed to be unresponsive to conservative treatment and were qualified for posterior fossa craniotomy and microvascular decompression. Table 1 presents clinical characteristics and basic laboratory parameters in UIA patients and the control group. Figure 1A–D illustrates the localization and geometry of aneurysms in UIA patients. Table 2 presents modifiable risk factors of intracranial aneurysm development in the group of UIA patients and the control individuals without vascular lesions in the brain.

Exclusion criteria for both groups were comprised of neurodegenerative conditions like multiple sclerosis, neuroinfection, and a brain tumor in medical history, surgery or major trauma, antibiotics, anti-inflammatory, or corticosteroid administration in the preceding months.

The study was conducted according to the guidelines of the Declaration of Helsinki and the protocol was approved by the Bioethics Human Research Committee of the Medical University of Bialystok (Permission No. R-I-002/383/2015 and APK.002.9.2021). All subjects gave their informed consent for inclusion before they participated in the study.

Cerebrospinal Fluid (CSF) and Serum Collection and Storage

Neurosurgical procedures to obtain CSF were conducted in the standard manner. For details please refer to the publications of Kamińska et al[4,5] Particular care was taken to prevent any contamination of the CSF with blood or warm saline solution used as irrigation. All the aforementioned steps were taken at the start of each procedure, before any bleeding might have occurred. Blood samples were collected into 5 mL tubes without anticoagulant (S-Monovette,

Table 1 Clinical Characteristics and Basic Laboratory Parameters in UIA Patients and the Control Group

Clinical Characteristics					
Variable	**Control Group**		**UIA Group**		**p-value**
	Mean ± SD	**Median (interquartiles)**	**Mean ± SD**	**Median (interquartiles)**	
Age [years]	62 ± 9	61 (56–68)	57 ± 12	59 (53–66)	0.1552^{NP}
Sex: Female/Male	8/7	-	33/7	-	0.0623$^{\&}$
Weight [kg]	81 ± 14	82 (70–90)	73 ± 16	69 (63–83)	0.0804^{NP}
Growth [m]	1.67 ± 0.08	1.68 (1.60–1.72)	1.64 ± 0.05	1.64 (1.62–1.68)	0.2424^{NP}
BMI [kg/m^2]	28.84 ± 4.30	29.41 (25.69–32.87)	26.99 ± 5.40	25.78 (23.18–30.09)	0.1608^{NP}
Systolic BP [mmHg]	142 ± 15	143 (127–153)	138 ± 20	137 (123–150)	0.4440^{PT}
Diastolic BP [mmHg]	84 ± 12	85 (70–95)	81 ± 9	80 (76–90)	0.5939^{NP}
Heart rate [minute]	74 ± 13	70 (63–83)	71 ± 15	74 (65–78)	0.7864^{NP}
BASIC LABORATORY PARAMETERS					
	Mean ± SD	**Median (interquartiles)**	**Mean ± SD**	**Median (interquartiles)**	**p-value**
WBC [x10^3/μL]	6.18 ± 1.71	5.97 (4.71–7.52)	7.51 ± 2.18	7.28 (6.30–8.60)	**0.0378**^{PT}
RBC [x10^6/μL]	4.56 ± 0.36	4.51 (4.45–4.73)	4.47 ± 0.48	4.45 (4.18–4.77)	0.5416^{PT}
HGB [g/dl]	13.55 ± 0.91	13.50 (12.90–14.00)	13.47 ± 1.26	13.65 (12.80–14.25)	0.8202^{PT}
HCT [%]	40.19 ± 2.71	40.20 (37.50–41.40)	41.07 ± 9.37	39.60 (37.50–42.50)	0.8444^{NP}
PLT [x10^3/μL]	263 ± 53	274 (238–300)	229 ± 66	233 (184–280)	**0.0497**^{NP}
MPV [fl]	10.3 ± 0.6	10.3 (9.8–10.7)	11.0 ± 1.0	11.0 (10.3–11.6)	**0.0155**^{PT}
P-LCR[%]	33.8 ± 25.3	28.2 (23.2–30.5)	32.9 ± 8.0	33.3 (26.8–37.7)	0.0679^{NP}
PT [s]	12.8 ± 0.6	12.9 (12.2–13.1)	12.3 ± 0.5	12.2 (11.9 12.6)	**0.0012**^{PT}
INR	0.97 ± 0.05	0.99 (0.91–1.02)	0.92 ± 0.05	0.91 (0.88–0.94)	**0.0006**^{PT}
APTT [s]	28.1 ± 3.4	27.1 (25.9–29.9)	28.5 ± 2.2	28.5 (27.1–29.9)	0.5544^{PT}
Fibrinogen [mg/dl]	319 ± 73	300 (266–383)	350 ± 64	337 (298–388)	0.1198^{NP}
Na^+ [mmol/l]	138 ± 4	139 (136–140)	138 ± 3	138 (137–140)	0.6070^{NP}
K^+ [mmol/l]	4.7 ± 0.5	4.7 (4.3–5.1)	4.4 ± 0.4	4.4 (4.1–4.7)	**0.0335**^{PT}
Glucose [mg/dl]	99 ± 18	99 (88–103)	101 ± 19	95 (88–111)	0.8298^{NP}
Urea [mg/dl]	35 ± 17	29 (22–49)	32 ± 10	31 (26–41)	0.4114^{PT}
Creatinine [mg/dl]	0.80 ± 0.14	0.81 (0.70 −0.87)	0.78 ± 0.12	0.79 (0.69–0.85)	0.6235^{PT}
eGFR [mL/min/1.73 m^2]	94 ± 24	98 (77–105)	87 ± 16	89 (76–98)	0.2807^{PT}

Notes: The bold font of p-value means statistical significance (p<0.05). Conversion factors to SI units are as follows: for WBC – 1.0, for RBC – 1.0, for HGB – 10.0, for PLT – 1.0, for glucose – 0.0555, for creatinine – 88.4. $^{\&}$Yates' chi-square test, ^{PT}parametric Student's *t*-test, ^{NP}non-parametric U Mann–Whitney test.

Abbreviations: APTT, activated partial thromboplastin; BMI, body mass index; eGFR, estimated glomerular filtration rate; HCT, hematocrit; HGB, hemoglobin; INR, International Normalized Ratio Time; K^+, potassium ion; MPV, mean platelet volume; Na^+, sodium ion; P-LCR, platelet-large cell ratio; PLT, platelet count; PT, prothrombin time; RBC, red blood cell count; SD, standard deviation; UIA, unruptured intracranial aneurysm; WBC, white blood cell count.

SARSTEDT). Both the CSF and serum samples were centrifuged for 20 minutes at 1000 × g and in the next step aliquoted and placed in storage at −75°C until further analysis.

Methods

To detect the presence of 27 cytokines in CSF and serum in UIA patients and the control group the multiplex ELISA method was applied (Bio-Plex Pro Human Cytokine 27-Plex Panel, catalog no: #M500KCAF0Y, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Concentrations of the following molecules were determined: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1ra, TNF-α, IFN-γ, GM-CSF, G-CSF, Eotaxin, IP-10, MCP-1, MIP-1-α, MIP-1β, RANTES, VEGF, PDGF-BB, and bFGF.

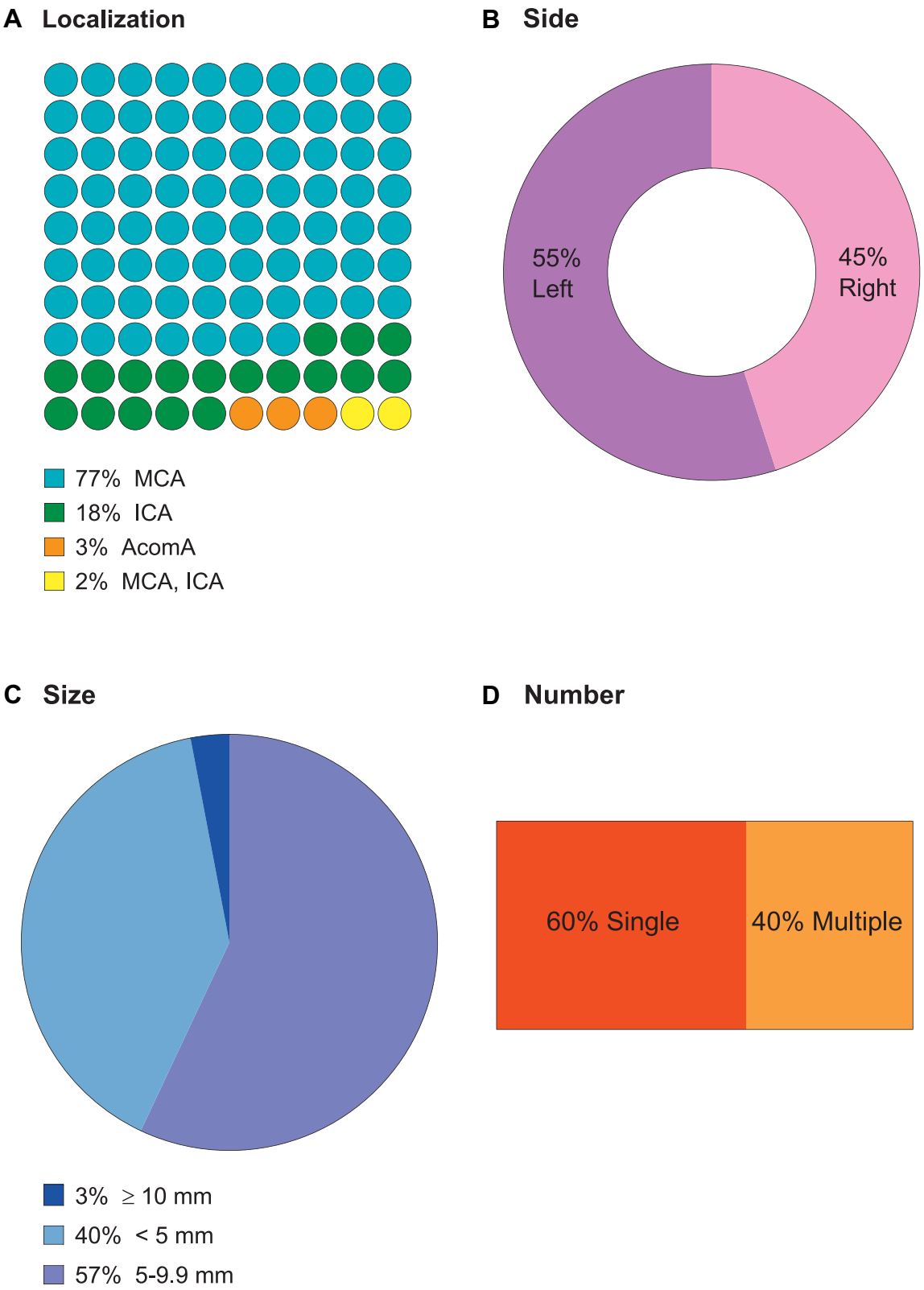

Figure 1 (**A**–**D**). Localization (**A**) and geometry (**B**–**D**) of aneurysms of UIA patients (n=40).
Abbreviations: AcomA, anterior communicating artery; ICA, internal carotid artery; MCA, middle cerebral artery; mm, millimeter; UIA, unruptured intracranial aneurysm.

Table 2 Risk Factors of Intracranial Aneurysm Development in the Group of UIA Patients and the Control Group

Risk Factors	Control Group N (%)	UIA Group N (%)	p-value
Smoking tobacco[#]	4 (27)	21 (53)	0.0866
Drinking alcohol[@]	3 (20)	5 (13)	0.7842[&]
Diabetes	4 (27)	3 (8)	0.1484[&]
Obesity[$]	7 (47)	11 (28)	0.3046[&]
Hypertension	7 (47)	28 (70)	0.1091
Cardiovascular diseases	0 (0)	7 (18)	0.2005[&]
Head injury	0 (0)	3 (8)	- [%]

Notes: [&]Yates' chi-square, [#]More than 20 cigarettes a day for over 12 months and more, [@]More often than occasionally, [$]body mass index (BMI) > 30.0 (kg/m^2), [%]Too few cases, to perform the statistic test.
Abbreviations: N, number; UIA, unruptured intracranial aneurysm.

Statistical Analysis

The obtained results were analyzed with the use of the STATISTICA 12.0 PL software (StatSoft Inc., Tulsa, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, USA). In the first step, the normality of the distribution of the examined parameters was assessed using the Shapiro–Wilk test. When comparing measurable variables in two independent samples in the absence of a normal distribution, the non-parametric Mann–Whitney *U*-test was used. The Student's *t*-test was used when normal distribution was present in both tested groups. The ANOVA rank Kruskal–Wallis test was used for the comparison of three samples. If significant differences were detected, the post-hoc test was conducted to assess which groups were different. Correlation coefficients were obtained by applying Spearman's rank correlation and presented as a heat map. Differences were considered significant for $p < 0.05$.

Results

Interleukins, Cytokines, Chemokines, and Growth Factor Concentrations in UIA Patients in Comparison to the Control Group

Analysis of interleukins, cytokines, chemokines, and growth factor concentrations in the CSF showed that 13 out of 27 proteins evaluated presented a concentration 1.36-fold or greater in UIA patients when compared to the control group (Table 3). Significantly higher were: IL-1β, IL-1ra, IL-2, IL-4, IL- 5, IL-7, IL-8, IL-12, IL-13 (Figure 2A–I), TNF-α, INF-γ, MCP-1, and VEGF (Figure 3A–D). Analysis of serum interleukins, cytokines, chemokines, and growth factor concentrations showed that none of the 27 proteins evaluated significantly differ between UIA patients and the control group (Table 4).

Interleukins, Cytokines, Chemokines, and Growth Factor Concentrations in CSF When Compared to the Serum

In the group of UIA patients CSF IL-2, IL-6, IL-8, MIP-1α, and MCP-1 concentrations were significantly higher when compared to values obtained in the serum. In the control group, only MCP-1 concentration was significantly higher in the CSF when compared to the serum (Figure 4A–E).

Interleukins, Cytokines, Chemokines, and Growth Factor Concentrations Depending on the Aneurysm's Location

Proteins analysis depending on an aneurysm's location showed that only CSF IL-1β significantly differ between UIA patients with MCA location compared to those with ICA location (Figure 5). Neither in serum, nor in the CSF did we find any other differences regarding the aneurysm's location.

Table 3 CSF Concentrations of Interleukins, Cytokines, Chemokines, and Growth Factors in UIA Patients and the Control Group

Variable	Control Group	UIA Group	
CSF [pg/mL]	**Median (Interquartiles)**	**Median (Interquartiles)**	**2-Tailed *p*-value**
IL-1β	0.15 (0.04–0.20)	0.30 (0.18–0.49)	**0.0006***
IL-1ra	32.19 (32.19–84.77)	114.96 (39.65–163.43)	**0.0165***
IL-2	1.22 (0.85–1.40)	2.04 (1.36–2.49)	**0.0004***
IL-4	0.21 (0.13–0.35)	0.54 (0.29–0.84)	**0.0014***
IL-5	12.73 (6.53–20.27)	74.51 (29.93–96.85)	**<0.0001***
IL-6	2.15 (0.90–3.06)	2.05 (1.61–2.86)	0.5556
IL-7	1.55 (0.90–1.61)	2.13 (1.55–3.62)	**0.0037***
IL-8	13.73 (12.11–16.58)	18.79 (14.49–26.75)	**0.0133***
IL-9	23.34 (13.53–56.99)	17.21 (11.54–43.08)	0.6070
IL-10	1.14 (0.31–1.94)	1.68 (0.17–2.47)	0.3066
IL-12	0.17 (0.17–1.09)	1.31 (0.17–2.37)	**0.0266***
IL-13	0.40 (0.07–1.40)	0.97 (0.30–1.16)	**0.0065***
IL-15	#	155.36 (105.34–229.84)[&]	-
IL-17	1.54 (0.54–2.62)	1.96 (1.41–3.61)	0.1392
TNF-α	1.94 (1.44–4.04)	4.53 (3.06–8.67)	**0.0002***
IFN-γ	4.93 (3.78–5.49)	7.41 (5.96–9.63)	**0.0002***
Eotaxin	4.19 (3.40–4.69)	4.88 (3.90–5.65)	0.1977
IP-10	3439.31 (3009.68–4052.98)	3865.06 (2405.82–5007.00)	0.5705
MCP-1	123.93 (108.53–160.50)	169.10 (122.70–213.30)	**0.0205***
MIP-1α	2.89 (1.56–4.35)	4.22 (2.16–11.01)	0.0545
MIP-1β	24.68 (11.64–34.93)	34.18 (14.34–71.73)	0.2329
RANTES	72.44 (42.95–186.74)	44.52 (20.80–132.77)	0.3156
GM-CSF	#	0.66 (0.21–0.94)	-
G-CSF	117.70 (62.67–189.48)	156.00 (93.72–389.28)	0.0771
VEGF	10.86 (10.86–51.99)	63.80 (16.70–115.83)	**0.0497***
PDGF-BB	24.97 (18.27–28.25)	26.05 (11.29–39.99)	0.4710
bFGF	9.21 (7.23–13.20)	14.06 (8.74–20.65)	0.1109

Notes: [#]Concentration not detected, [&]Concentration detected only in five cases,*And bold of a 2-tailed p-value – the level of significance < 0.05.

Abbreviations: CSF, cerebrospinal fluid; IL, interleukin; TNF-α, tumor necrosis factor-α; INF-γ; interferon-γ; GM-CSF, granulocyte monocyte colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP-1-α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation, normal T cell expressed and secreted; VEGF, vascular endothelial growth factor; PDGF-BB, platelet-derived growth factor-BB; Bfgf, basic fibroblast growth factor; UIA, unruptured intracranial aneurysm.

Correlation Coefficient Results in the CSF

The complex relationship between interleukins, cytokines, chemokines, and growth factors in the CSF of UIA patients is presented on the heat map (Figure 6). We also analyzed the correlations between protein concentrations and the size and number of aneurysms. The correlation coefficient analysis showed that CSF IL-1β, IL-8, and TNF-α positively, while IL-13 negatively correlated with the size of aneurysms. Only CSF IL-6 and MCP-1 concentrations positively correlated with the number of aneurysms (Figure 7A–F).

CSF Interleukins, Cytokines, Chemokines, and Growth Factor Concentrations Depending on Confounding Factors

We also analyzed whether CSF concentrations of analyzed proteins may differ depending on confounding factors such as sex, age, smoking, cardiovascular disease, drinking, obesity, or hypertension. We demonstrated that in the UIA group CSF IFN-γ concentration was significantly higher in females compared to males (Supplementary Table 1). CSF TNF-α

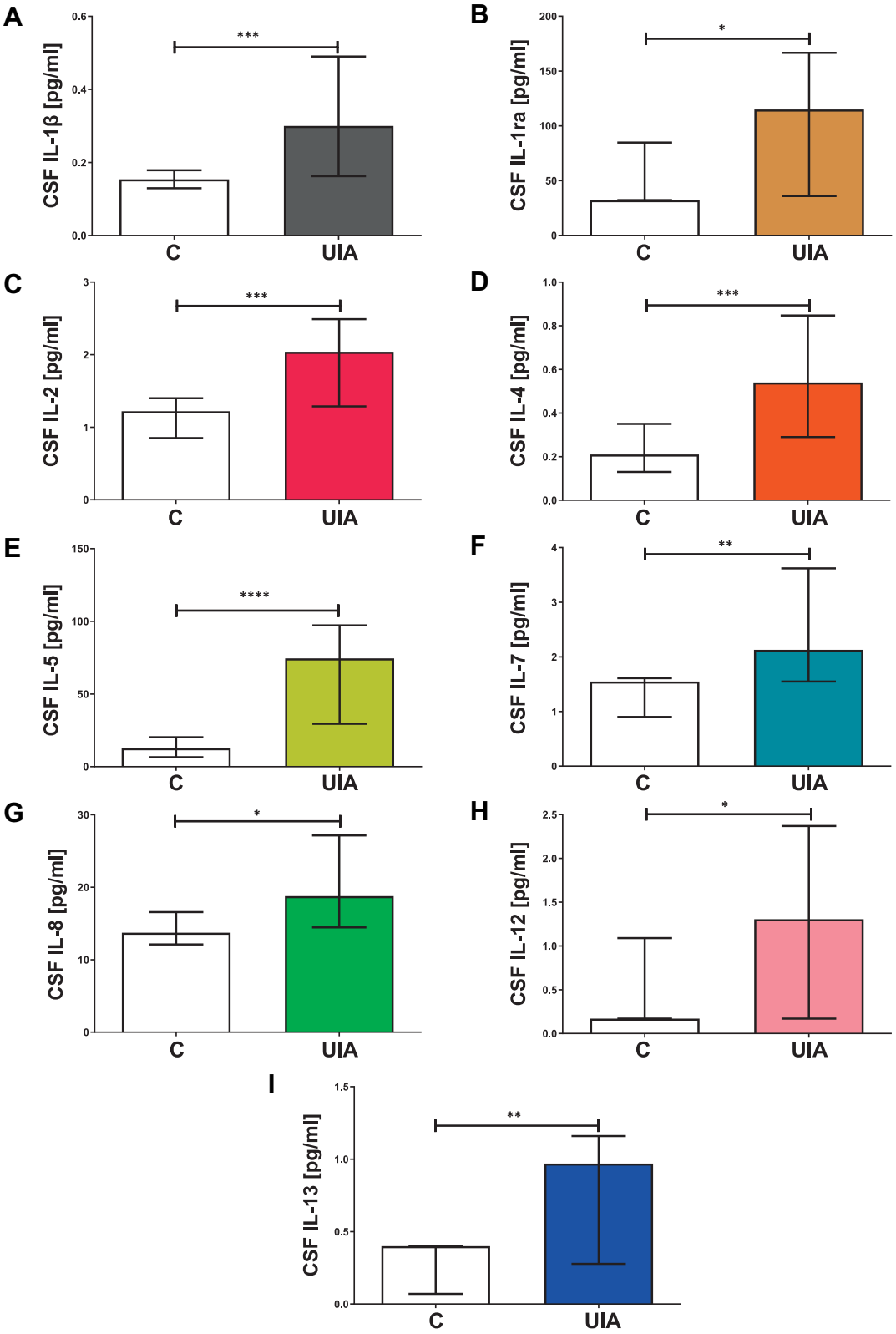

Figure 2 (**A**–**I**). CSF IL-1β (**A**), IL-1ra (**B**), IL-2 (**C**), IL-4 (**D**), IL-5 (**E**), IL-7 (**F**), IL-8 (**G**), IL-12 (**H**), and IL-13 (**I**) concentrations in UIA patients (n=40) and the control group (n=15).

Note: *p < 0.05, ** p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

Abbreviations: C, control group; CSF, cerebrospinal fluid; IL, interleukin; IL-1ra, IL-1 receptor antagonist; UIA, unruptured intracranial aneurysm.

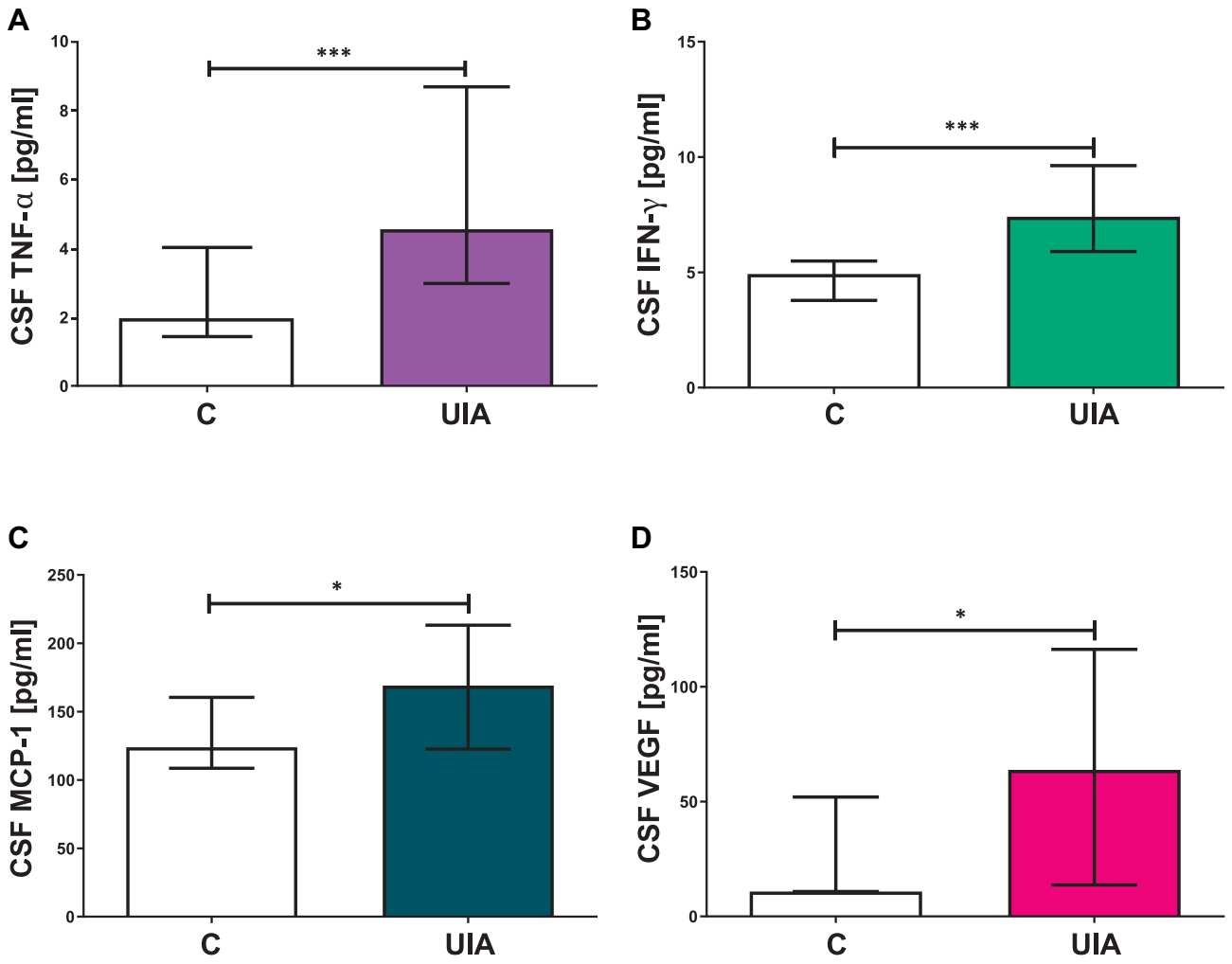

Figure 3 (**A**–**D**). CSF TNF-α (**A**), IFN-γ (**B**), MCP-1 (**C**), and VEGF (**D**) concentrations in UIA patients (n=40) and the control group (n=15).
Note: *p < 0.05, ***p ≤ 0.001.
Abbreviations: C, control group; CSF, cerebrospinal fluid; IFN-γ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; UIA, unruptured intracranial aneurysm; VEGF, vascular endothelial growth factor.

concentration was significantly higher in UIA patients >60 years old compared to patients ≤60 years old (Supplementary Table 2). CSF IL-8 concentration was significantly lower in UIA patients who smoke compared to non-smokers (Supplementary Table 3). CSF IL-1ra and IL-13 concentrations were significantly lower in UIA patients with cardiovascular disease compared to those without cardiovascular disease (Supplementary Table 4). Drinking, obesity, and hypertension had no influence on the CSF protein concentrations (Supplementary Tables 5–7).

Discussion

The formation and development of intracranial aneurysms is a complex process and depends on the interaction of many molecules, including interleukins, cytokines, chemokines, growth factors, or matrix metalloproteinases.[42,43] Alteration of the biomechanical properties of the arterial wall, apparently associated with inflammation and repair mechanisms, leads to formation and rupture of intracranial aneurysms.[44] Therefore, studies evaluating the protein panel in unruptured intracranial aneurysm (UIA) patients seem to be extremely valuable. To the best of our knowledge, our study is the first to assess 27 proteins in CSF and serum that can positively and negatively modulate the inflammatory response in UIA patients.

Our study showed that in the CSF of UIA patients, the concentration of 13 out of 27 tested proteins was significantly higher compared to individuals without vascular lesions in the brain. High CSF concentrations of these proteins indicated

Table 4 Serum Concentrations of Interleukins, Cytokines, Chemokines, and Growth Factors in UIA Patients and the Control Group

Variable	Control Group	UIA Group	
Serum [pg/mL]	**Median (Interquartiles)**	**Median (Interquartiles)**	**2-Tailed *p*-value**
IL-1β	1.44 (1.14–1.70)	1.39 (1.19–1.39)	0.3213
IL-1ra	128.76 (128.76–457.24)	128.76 (128.76–394.74)	0.7430
IL-2	0.76 (0.56–1.52)	0.38 (0.38–1.14)	0.7430
IL-4	4.76 (3.64–6.48)	5.49 (4.41–6.67)	0.7430
IL-5	50.92 (50.92–50.92)	#	-
IL-6	0.98 (0.56–6.42)	0.70 (0.70–0.98)	0.9626
IL-7	14.48 (14.48–24.32)	14.48 (10.61–14.48)	0.2766
IL-8	7.30 (4.46–31.40)	6.93 (4.82–8.36)	0.7430
IL-9	631.96 (582.24–684.16)	620.91 (618.92–677.46)	0.5414
IL-10	1.24 (1.24–7.24)	2.37[$]	-
IL-12	4.36 (4.36–4.36)	#	-
IL-13	0.66 (0.28–3.92)	#	-
IL-15	#	#	-
IL-17	18.26 (15.96–24.04)	20.28 (17.24–24.29)	0.9026
TNF-α	22.48 (19.08–25.84)	22.95 (21.02–24.40)	0.9626
IFN-γ	12.30 (10.66–14.32)	11.89 (10.23–11.89)	0.2359
Eotaxin	127.44 (65.86–170.30)	123.73 (96.20–166.22)	0.7866
IP-10	1541.98 (1126.02–2028.18)	1451.39 (1069.70–1587.32)	0.7133
MCP-1	26.00 (19.20–41.34)	21.34 (19.65–25.27)	0.3079
MIP-1α	2.22 (1.84–3.22)	2.28 (1.57–2.81)	0.5414
MIP-1β	260.60 (245.34–299.62)	268.80 (263.47–274.52)	0.4807
RANTES	10631.40 (7868.50–13850.88)	12801.02 (11396.66–13736.82)	0.1426
GM-CSF	0.83[&]	22.52[$]	-
G-CSF	122.76 (104.80–171.74)	133.87 (100.02–162.97)	0.7880
VEGF	#	#	-
PDGF-BB	3127.40 (2583.08–3986.14)	2401.26 (2191.16–3165.48)	0.6441
bFGF	34.94 (33.04–42.06)	33.05 (33.05–36.85)	0.8148

Notes: [#]Concentration not detected, [$]Concentration detected only in two cases, [&]Concentration detected only in one case.
Abbreviations: IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; GM-CSF, granulocyte monocyte colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP-1-α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation, normal T cell expressed and secreted; VEGF, vascular endothelial growth factor; PDGF-BB, platelet-derived growth factor-BB; bFGF, basic fibroblast growth factor; UIA, unruptured intracranial aneurysm.

their local synthesis within the central nervous system (CNS), and can be alternatively regarded as evidence of inflammatory response at the site of an aneurysm.[45,46] Thus, the increased CSF concentration of IL-1β, IL-1ra, IL-2, IL-4, IL- 5, IL-7, IL-8, IL-12, IL-13, TNF-α, INF-γ, MCP-1, and VEGF in UIA patients as compared to individuals without vascular lesions in the brain may indicate that these molecules can modulate UIA development. The results of our research also draw attention to the cytokines IL-1ra, IL-2, IL-4, IL-5, IL-7, and IL-13, whose role in the formation and development of brain aneurysms has so far, not been widely studied. In this context, our study also provides new insight into understanding the process of intracranial aneurysm formation. Additionally, in UIA patients, contrary to subjects without vascular lesions in the brain, IL-2, IL-6, IL-8, and MIP-1α concentrations were significantly higher in CSF when compared to the serum values, which indicates that these molecules are synthesized locally within the CNS,[4,5,47] possibly in response to the development of a brain aneurysm. Current research also strengthens our previous findings indicating the significant role of IL-6, IL-8, and MCP-1 in the formation and development of brain aneurysms in humans.[4,5]

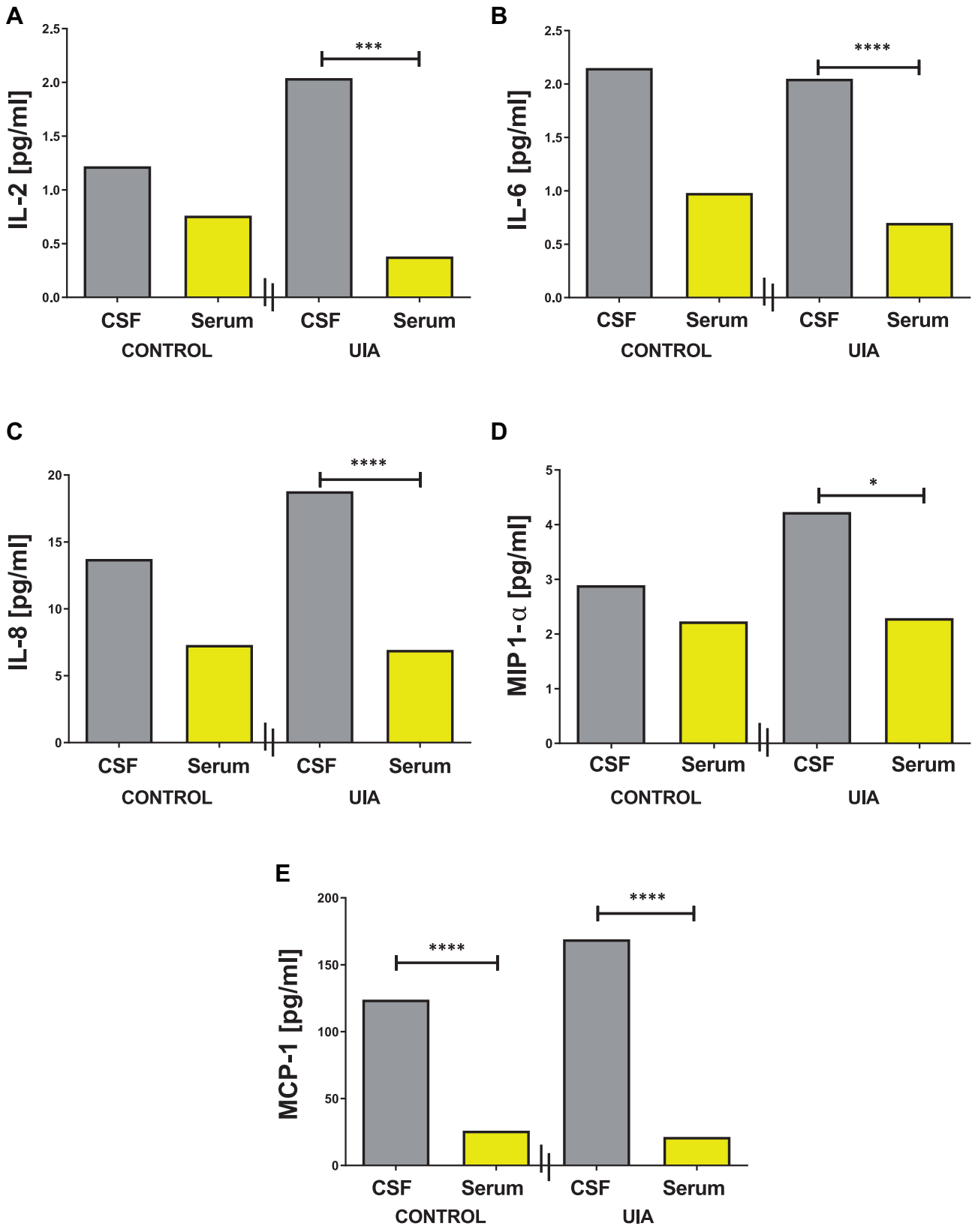

Figure 4 (**A**–**E**). CSF IL-2 (**A**), IL-6 (**B**), IL-8 (**C**), MIP-1α (**D**), and MCP-1 (**E**) concentrations as compared to the serum values in UIA patients (n=40) and the control group (n=15).

Note: *p < 0.05, ***p ≤ 0.001, ****p ≤ 0.0001.

Abbreviations: CSF, cerebrospinal fluid; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory proteins-1α; UIA, unruptured intracranial aneurysm.

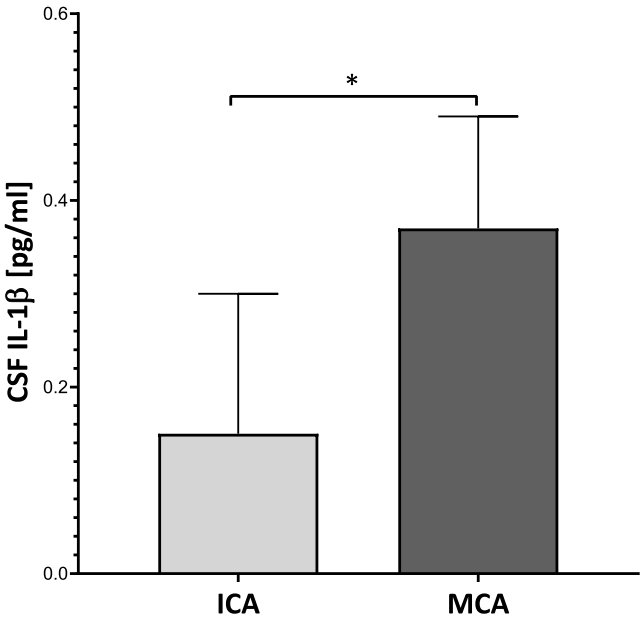

Figure 5 CSF IL-1β concentration in UIA patients depending on aneurysm's location.
Note: *p < 0.05.
Abbreviations: CSF, cerebrospinal fluid; ICA, internal carotid artery; IL-1β, interleukin 1 beta; MCA, middle cerebral artery; UIA, unruptured intracranial aneurysm.

Studies in animal models showed increased mRNA and protein expression of IL-1β, IL-8, TNF-α, and MCP-1 in endothelial cells in intracranial aneurysms (IA).[16,18,40,41,48] Chalouhi et al[12] showed significantly higher concentrations of IL-8, IL-17, MCP-1, RANTES, MIG, IP-10, in UIA patients and Eotaxin in plasma obtained from the lumen of the cerebral aneurysm compared to the plasma obtained from the femoral artery. These studies suggest the involvement of the above-mentioned cytokines in the pathomechanism of aneurysm formation. Although it is difficult to indicate beyond doubt, whether these cytokines are the cause or the result of aneurysm development, it is certain that, along with immunocompetent cells, they play a part in the pathological remodeling of the cerebral vessel. Pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, and IFN-γ are mainly secreted by macrophages, having the greatest contribution to the formation of brain aneurysms.[23,49] Additionally, studies conducted in our population of UIA patients confirmed the involvement of these cytokines in the formation of brain aneurysms. However, apart from interleukins and TNF-α, our study showed a significantly higher concentration of VEGF in CSF in patients with UIA compared to those without vascular lesions in the brain. VEGF is one of the most potent angiogenic factors involved in physiological and pathological angiogenesis by inducing a proliferation of endothelial cells. Our research, therefore, may suggest a possible role for VEGF in the pathogenesis of brain aneurysms.

Moriwaki et al[40] in an experimental mouse model of IA showed that IL-1β expression is induced in the aneurysmal wall in the early stages of its development. Moreover, they also found increased expression of IL-1β in smooth muscle cells (SMC), which promoted their apoptosis and thus the process of brain aneurysm formation. In addition, the progression of the aneurysm was slower in the mutant mice (IL-1β -/-) compared to the wild-type mice.[40] The relationship between IL-1β and aneurysm development was also confirmed in our study, which showed a positive correlation between the concentration of IL-1β in CSF and the size of the aneurysm. Zhang et al[10] showed a significantly higher concentration of MCP-1 and TNF-α in the plasma of patients with ruptured brain aneurysms compared to patients with unruptured brain aneurysms and patients with multiple brain aneurysms, thus indicating the participation of these cytokines primarily in the progression of the vascular lesion. This finding provides a link between ultrastructural alterations and modifications of the biomechanical properties at the rupture zone. The above-mentioned hypothesis is confirmed by the results of our study, which showed that the concentration of MCP-1 in CSF positively correlated with the number of aneurysms, and the concentration of TNF-α in CSF with the size of the aneurysm. It should be emphasized that IL-1β promotes the synthesis of the chemokine MCP-1.[49] Another protein, that upregulates the expression of MCP-

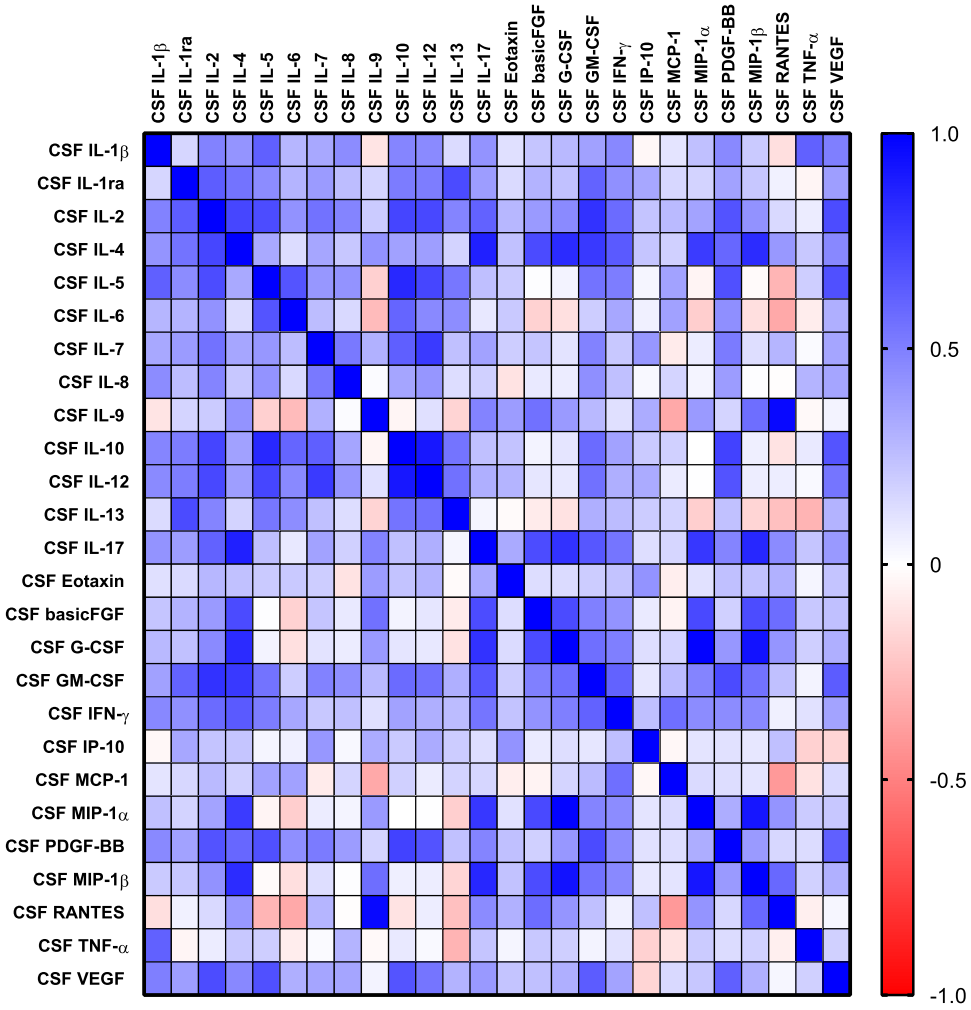

Figure 6 The heat map of the relationship between interleukins, cytokines, chemokines, and growth factors in the CSF of UIA patients (n=40).
Abbreviations: CSF, cerebrospinal fluid, IL, interleukin, TNF-α, tumor necrosis factor-α, INF-γ, Interferon-γ, GM-CSF, granulocyte monocyte colony-stimulating factor, G-CSF, granulocyte colony-stimulating factor, IP-10, Interferon gamma-induced protein 10, MCP-1, monocyte chemoattractant protein-1, MIP-1-α, macrophage inflammatory protein-1α, MIP-1β, macrophage inflammatory protein-1β, RANTES, regulated on activation, normal T cell expressed and secreted, VEGF, vascular endothelial growth factor, PDGF-BB, platelet-derived growth factor-BB, bFGF basic fibroblast growth factor, UIA, unruptured intracranial aneurysm.

1, is tissue-type plasminogen activator (tPA), which stimulates the infiltration of macrophages within the aneurysmal wall. Thus, tPA indirectly contributes to the IA formation and rupture through a pro-inflammatory pathway.[50] All this data taken together suggests the presence of a feedback loop between cytokines in patients with UIA, which was also confirmed by our current research showing many significant correlations between the concentrations of the analyzed cytokines in the CSF.

It should also be noted that the positive correlation of CSF IL-1β, IL-8, and TNF-α levels with aneurysms' size and CSF IL-6 and MCP-1 levels with aneurysms' number might be due to the volume of the lesions (the total number of cells producing cytokines). Undoubtedly, the issue of whether increased protein concentrations are associated with the presence of smaller or larger lesions, requires further analysis.

Brain aneurysm development, like atherosclerosis, is the result of chronic inflammation involving Th1 lymphocytes,[49,51] the differentiation of which is dependent on IL-12 and IFN-γ. Th1 lymphocytes mainly produce IFN-γ and TNF-α, thus stimulating NK cells to secrete IFN-γ, IL-4, and IL-13, and activate macrophages to the pro-inflammatory phenotype M1.[52] IFN-γ produced by Th1 lymphocytes and activated M1 macrophages inhibits both SMC proliferation and collagen production, contributing to the apoptosis of smooth muscle cells.[49,51,53–55] Moreover, IFN-γ, acting synergistically with IL-1β and TNF-α, induces the expression of leukocyte adhesion molecules, which

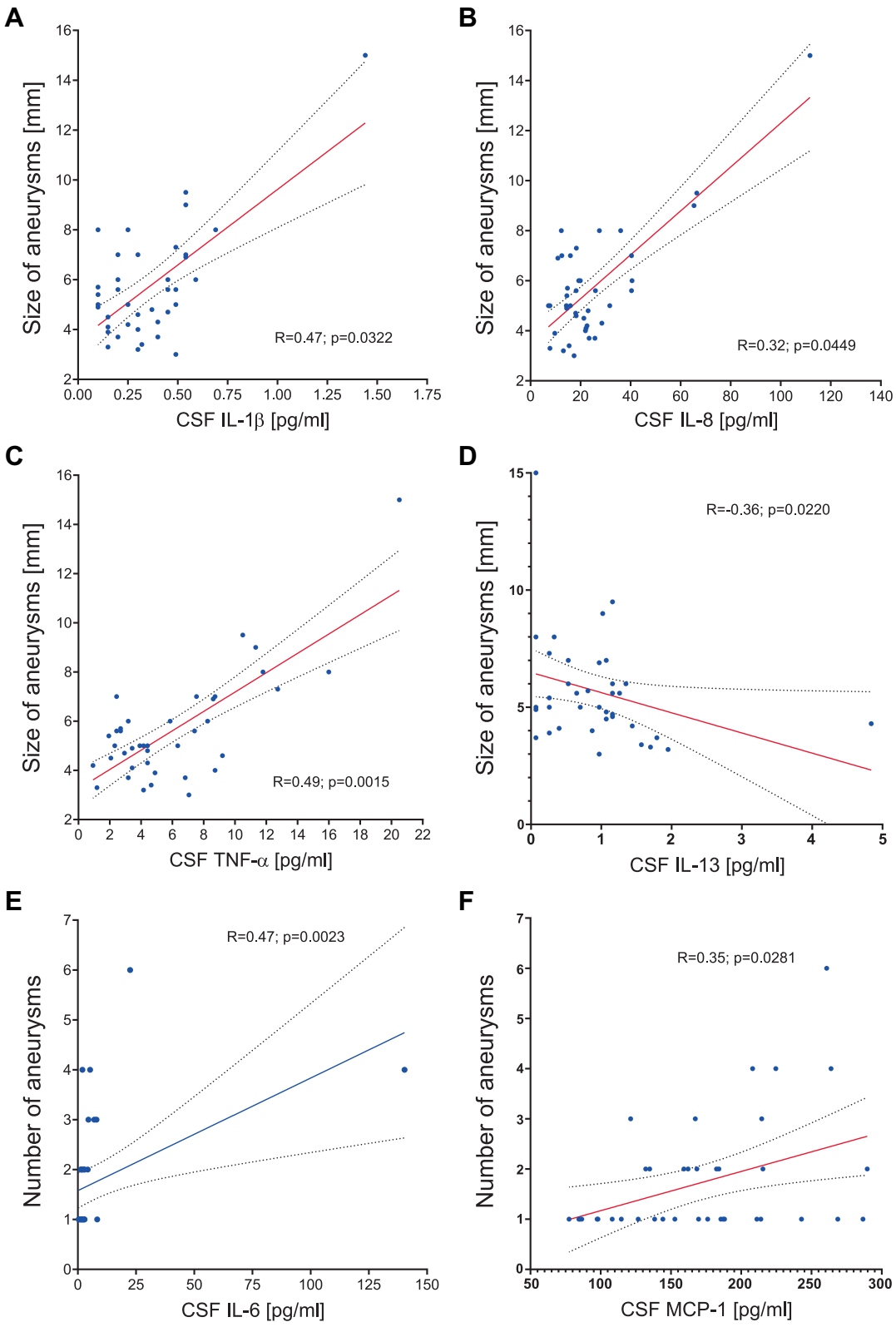

Figure 7 (**A**–**F**). Correlation coefficient analysis of CSF IL-1β (**A**), IL-8 (**B**), TNF-α (**C**), and IL-13 (**D**) concentrations with the size of aneurysms and CSF IL-6 (**E**) and MCP-1 (**F**) concentrations with the number of aneurysms.

Abbreviations: CSF, cerebrospinal fluid; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α.

also promotes inflammatory remodeling of the arterial vessel.[56] In our study, the concentration of IL-12, IFN-γ, IL-4, and IL-13 in CSF was significantly higher in UIA patients compared to those without vascular lesions in the brain, which indirectly indicates the involvement of Th1-dependent cellular response in the pathogenesis and development of brain aneurysms.

The results of our studies also revealed that in UIA patients there is a significant increase in the secretion of anti-inflammatory cytokines. We showed for the first time that in UIA patients, the concentration of IL-1ra, which inhibits the binding of both IL-1α and IL-1β to cell surface receptors, was significantly higher compared to individuals without vascular lesions in the brain. Arend et al[57] indicated that inhibition of a biological IL-1 response requires at least a 100-fold excess of IL-1ra over IL-1. In our study, we demonstrated an over 380-fold excess of IL-1ra to IL-1β. We also discovered that the concentrations of IL-4, influencing the differentiation of T helper cells into anti-inflammatory Th2 cells,[58] and IL-5, inhibiting the differentiation of pro-inflammatory Th1 and Th17 lymphocytes,[59] were significantly higher in UIA patients compared to individuals without vascular lesions in the brain. This allows us to hypothesize that in UIA patients mechanisms aimed to inhibit the development of the aneurysm are also activated. However, the anti-inflammatory effects of cytokines may be annulled by the pleiotropism of the pro-inflammatory cytokines and the predominance of cells with an inflammatory over an anti-inflammatory potential. Especially since Jayaraman et al[23] suggested that although anti-inflammatory Th2 lymphocytes are present in the wall of the brain aneurysm, this is not where they are activated, and thus they do not achieve their function.

IL-2 is a cytokine, whose role in the formation and progression of brain aneurysms has not been fully discovered.[60] In our study, the concentration of IL-2 was significantly higher in CSF of UIA patients compared to individuals without vascular lesions in the brain. Zhang et al in animal IA model studies showed that administration of IL-2 significantly increased the expression of the checkpoint molecule Tim-3 in Treg cells, thereby boosting their proliferation.[60] IL-2 not only ensures the homeostasis of Treg lymphocytes but also influences the differentiation of T helper lymphocytes into Th1 and Th2 subpopulations. Indirectly, it may promote pro and anti-inflammatory mechanisms, and thus the evaluation of IL-2 importance in the formation of brain aneurysms requires further study.[61,62]

Study Limitation

We would like to stress, that CSF collection via lumbar puncture is not routine in patients with UIA. Thus, the Local Bioethics Committee gave permission for CSF collection only within neurosurgery. In order to reliably assess the concentration of the analyzed molecules in the CSF, the comparative group needed to have the fluid taken from the same interspace in order to make the samples relatively homogenous. For this reason, the control group was not composed of entirely healthy people, but patients suffering from trigeminal neuralgia. Nevertheless, we were still able to demonstrate statistically significant differences.

Another study limitation could be the number of cases included to the study. However, we would like to clarify that only CSF samples with a red blood cell count that equaled 0/μL were used for further analysis, which significantly limited the number of patients included to the study. Moreover, only trigeminal neuralgia patients, who did not respond to conservative treatment, were qualified for posterior fossa craniotomy and microvascular decompression.

Conclusions

The current study showed, that in the CSF 13 out of 27 proteins evaluated were significantly higher in UIA patients in comparison to individuals without vascular lesions in the brain. On the contrary, in the serum none of the proteins tested significantly differ between UIA patients and the comparison group. These results indicated, that CSF is a better material for searching molecules implicated in IA formation and development. High CSF concentrations of these proteins indicated their local synthesis within the CNS, and can be alternatively regarded as evidence of inflammatory response at the site of an aneurysm. Our results also suggest that in patients with UIA, pro-inflammatory and anti-inflammatory mechanisms are activated simultaneously, because the concentration of both promoting and suppressing the inflammatory response proteins was significantly higher in UIA patients compared to individuals without vascular lesions in the brain. We suggest that the molecules responsible for the formation of IA are primarily IL-2, IL-6, IL-8, MIP-1α, and MCP-1, the concentration of which in UIA patients was significantly

higher in CSF compared to serum, indicating their local synthesis within the central nervous system. On the other hand, the preventive therapy of brain aneurysm development should be focused on IL-1β, IL-6, IL-8, MCP-1, and TNF-α, whose concentration in CSF positively correlated with the size and number of aneurysms.

Abbreviations

APTT, activated partial thromboplastin; bFGF, basic fibroblast growth factor; BMI, body mass index; CNS, central nervous system; CSF, cerebrospinal fluid; eGFR, estimated glomerular filtration rate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte monocyte colony-stimulating factor; HCT, hematocrit; HGB, hemoglobin; IA, intracranial aneurysm; IFN-γ, interferon-γ; IL, interleukin; IL-1ra, IL-1 receptor antagonist; INR, International Normalized Ratio Time; IP-10, interferon gamma-induced protein 10; K+, potassium ion; M1, macrophages 1; MCP-1, monocyte chemoattractant protein-1; MIG, chemokine monokine induced by interferon (IFN)-γ; MIP-1-α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; MPV, mean platelet volume; mRNA, messenger ribonucleic acid; Na+, sodium ion; NK, natural killer; PDGF-BB, platelet-derived growth factor-BB; P-LCR, platelet-large cell ratio; PLT, platelet count; PT, prothrombin time; RANTES, regulated on activation, normal T cell expressed and secreted; RBC, red blood cell count; SD, standard deviation; SMC, smooth muscle cells; Th, T helper cells; Tim-3, T-cell immunoglobulin and mucin domain-3; TNF-α, tumor necrosis factor-α; UIA, unruptured intracranial aneurysms; VEGF, vascular endothelial growth factor; WBC, white blood cells count.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author (J.K.) on reasonable request.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki and the protocol was approved by the Bioethics Human Research Committee of the Medical University of Bialystok (Permission No. APK.002.9.2021). All subjects gave their informed consent for inclusion before they participated in the study.

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Disclosure

The authors declare that they have no competing interests in this work.

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