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BDNF, sHLA-G, and sTREM-1 are useful blood biomarkers for identifying grade IV glioma patients

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Inflammation and immunity belong to the main factors influencing tumor growth. In this study, we attempted to identify a profile of biomarkers associated with gliomas. We found decreased serum levels of sTREM-1 (soluble triggering receptor expressed on myelocytes) and increased levels of IL-10 in all grades of glioma patients in comparison with healthy controls (sTREM-1: grade II: p=0.0051, grade III: p=0.02, grade IV: p=0.01; IL-10: grade II: p=0.0017, grade III: p=0.03, grade IV: p=0.007). However, we did not find any combination of tested markers with good sensitivity and specificity in grades II and III of glioma patients to discriminate them from healthy controls. In grade IV glioma patients, two sets of markers showed promising results in distinguishing patients from healthy people. For the first set consisting of four selected markers, sTREM-1, sHLA-G, BDNF, and IL-13, the ROC curves indicate a good discriminatory capability for glioblastoma patients (AUC=0.9510). The best discriminatory capability for glioblastoma patients (AUC=0.9534) was found for the second set consisting of three selected markers sTREM-1, sHLA-G, and BDNF with 79.2% sensitivity and 94.1% specificity.

Key words: BDNF; HLA-G; TREM-1; IL-13; glioma

Gliomas belong to the most frequent primary brain tumors that have the third highest mortality and morbidity rates among cancers in humans. More than half of glioma patients suffer from the most frequent, aggressive, and lethal form of glioma – glioblastoma multiforme (GBM; glioma of grade IV) [1]. In 2019, Cantrell et al. showed that despite the improvements in the median and short-term overall survival, the percentage of patients with glioblastoma achieving the 5-year overall survival remains very low 4.6% [2]. The majority of the central nervous system (CNS) tumors are diagnosed after the clinical symptoms become apparent. The markers of survival prognosis in patients with gliomas are still evaluated. Serum or plasma biomarkers have been detected by the analysis of gene expression profiles of non-brain tumors with promising results [3–5], but none of these proteins alone was sufficiently specific and sensitive to serve as a diagnostic marker.

There are two main factors influencing tumor growth – the state of adaptive cell-mediated immunity and inflammation, which is one of the hallmarks of cancer [6]. At the beginning of tumor formation, the Th1 immunity and the inflammation as a part of the first phase of the immunoediting (phase of tumor elimination) have a positive antitumor activity. Later, the Th1 immunity is downregulated by the immunosuppressive activity of growing tumor cells and immunosuppressive cells and factors. Finally, in cancer patients, the immunity in the peripheral blood is rather suppressed, especially in advanced stages.

Inflammation is a defense mechanism that develops to any damage caused by different infectious or non-infectious stimuli [7]. Acute inflammation is beneficial, helps to eliminate the pathologic threat, and promotes tissue repair. However, if the cause of the initial inflammatory response is not resolved, the acute inflammation can shift to a chronic one that is protumorigenic [8–11].

We decided to investigate more biomarkers of immunity and inflammation, interferon-gamma (IFN-γ) as the main sustaining cytokine of the Th1 pathway of immunity with pro-inflammatory activity, and IL-4 and IL-13 from the Th2 pathway of immunity. Also, pro-inflammatory cytokine IL-6, pro-inflammatory molecule – soluble triggering receptor expressed on myelocytes (sTREM-1), TGF-β (transforming

growth factor beta), IL-10, and sHLA-G (soluble human leukocyte antigen-G) with anti-inflammatory and immune suppressive activities, later also as an immune checkpoint molecule.

Besides these biomarkers, we investigated also other factors playing an important role in tumor formation and its growth, such as angiogenic factor VEGF (vascular endothelial growth factor), the neurotrophin BDNF (brain-derived neurotrophic factor), which is critical for neuronal survival, differentiation, and plasticity, and chemokine fractalkine (CX3CL1 or neurotactin).

The majority of central nervous system tumors are diagnosed after clinical symptoms become apparent. We tried to find molecules that could eventually help diagnose patients with gliomas in the early stages.

Patients and methods

The study group included 63 patients older than 18 years with partial or complete resection of the CNS tumor. Patients with a primary diagnosis and with a relapse of the tumor were analyzed. In our cohort, only patients with histologically proven gliomas of grades II, III, and IV were enrolled, all other histological types of tumors or other diagnoses were excluded. Tumors of grade II with signs of grade III were taken as grade III, and one tumor of grade III with signs of glioblastoma was taken as grade IV. The diagnosis was approved by two neuropathologists according to the most recent WHO classification criteria. Blood samples were obtained from the patients on day 0, before surgical treatment.

The reference cohort in our case-control study comprised 26 healthy volunteers (13 males and 13 females from 26 to 75 years). All control subjects were without any personal or family history of cancer or acute inflammatory disease and they were randomly recruited from a larger population sample. All patients and controls were Caucasians of Slovak descent. The study was conducted in accordance with the International Ethical Guidelines and Declaration of Helsinki, approved by the Ethical Committee of the Faculty of Medicine, Comenius University, and University Hospital in Bratislava (project identification code: 17/2015 (16 April 2015)). Written informed consent for enrolling in the study and for personal data management was obtained from all examined cases.

The blood was obtained in the period between the years 2015 and 2018. The serum levels of soluble sTREM-1, HMGB1, and plasma levels of IL-6, IL-10, IL-4, IL-13, sHLA-G, IFN gamma, TGF beta, VEGF, BDNF, and CX3CL1 were analyzed by a sandwich ELISA test (Human Elisa tests; all Fine Tests; Wuhan Fine Biotech Co., Ltd., Wuhan, China; except sHLA-G from EXBIO Olomouc, Czech Republic) precisely according to the instruction of the manuscripts.

Statistics. Programs InStat and SAS were used for statistical analysis. The Student´s t-test and the Mann-Whitney test were employed. The results were expressed as the median and the interquartile range (IQR), the mean ± standard deviation (SD) depending on the Gaussian distribution. The statistical analysis included the ROC curves with the corresponding AUC parameters. The p-value <0.05 was considered to indicate statistical significance.

Results

Characteristics of glioma patients and candidate molecules. Characteristics of patients and selected immune molecules are summarized in Tables 1 and 2.

Table 1. Characteristics of glioma patients.

Patients	No	Mean age ± SD
All gliomas	63	53.29±14.98
Sex (male/female)	38/25	
Grades (male/female)		
G. II	14/5	14/5 (40.47±12.30)
G. II-III	2/0	2/0 (30.5)
G. III	7/4	7/4 (48.55±12.57)
G. III-IV	0/1	0/1 (55)
G. IV	15/15	15/15 (64.07±8.70)
Primary diagnosis	49	
Relapse	13	
Unknown	1	
Diagnosis		
Diffuse glioma II	4	
Oligodendroglioma II	7	
Oligoastrocytoma II	1	
Astrocytoma II	7	
Oligodendroglioma II-III	1	
Astrocytoma II-III	1	
Anaplastic astrocytoma	11	
Anaplastic astrocytoma with signs of GBM	1	
Primary GBM	28	
Unknown GBM	2	
Completely resected		
G. II	4	
G. II-III	0	
G. III	3	
G. III-IV	0	
G. IV	4	
IDH1/2 mutated	25	
G. II, III	24	
G. IV	1	
Steroid treated/untreated		
G. II	7/12	
G. III	9/4	
G. IV	28/3	

Abbreviations: No-number of patients; G-grade; GBM-glioblastoma multiforme; IDH-isocitrate dehydrogenase

Comparison of selected markers between different grades of gliomas. No significant differences in the serum/plasma levels of selected biomarkers were observed during the comparison of different grades of gliomas (Table 3).

Comparison of selected markers between gliomas of different grades and healthy controls. Serum levels of sTREM-1 in glioma patients in comparison with healthy people were significantly decreased in all grades of gliomas: grade II: p=0.0051, grade III: p=0.02, grade IV: p=0.01 (Tables 4, 5, and 6; Figure 1A). Glioma patient's levels of IL-10 in comparison with healthy people were also significantly increased in all grades of gliomas: grade II: p=0.0017, grade III: p=0.03, grade IV: p=0.007 (Tables 4, 5, and 6; Figure 1B).

In grade IV glioblastoma, five more markers showed different levels in comparison with healthy controls. Soluble HLA-G was significantly higher in grade IV glioma patients than in healthy controls: p=0.04 (Table 4; Figure 1C). Plasma levels of BDNF, VEGF, fractalkine, and IL-13 were significantly lower in grade IV patients than in healthy controls: p=0.001, p=0.05, p=0.05, p=0.02, respectively (Figures 1D–1G). Only box plots with significant markers in glioblastoma and healthy controls' levels are demonstrated in Figures 1A–1G.

Markers with a good capability to discriminate glioma patients from healthy subjects. In grade IV glioma patients, two sets of markers showed promising results in distinguishing patients from healthy people. For the first set consisting of four selected markers, sTREM-1, sHLA-G, BDNF, and IL-13, the ROC (receiver operating characteristic) curves indicate a good discriminatory capability for glioblastoma patients (AUC=0.9510; Figure 2A). The best discriminatory capability for glioblastoma patients (AUC=0.9534) was found for the second set consisting of three selected markers: sTREM-1, sHLA-G, and BDNF with 79.2% sensitivity and 94.1% specificity (Figure 2B). A combination of these markers was obtained in 24 glioblastoma patients and 17 healthy controls (see suppl. material). We did not find any combination of tested markers with good sensitivity and specificity in grades II and III glioma patients to discriminate them from healthy controls.

Differences between steroid-treated and untreated patients. When we compared the concentrations of selected molecules between steroid-treated and untreated patients, none of these molecules was significantly affected by the corticosteroid treatment.

Differences between primary and relapsed tumors. No differences in plasma levels of the selected markers were observed when primary and relapsed tumors from the group of all glioma patients were compared. When we looked

Table 2. Functions of candidate molecules.

Candidate molecule	Function
Soluble HLA-G	Immune suppression
	Anti-inflammatory
Soluble TREM-1	Inflammatory biomarker
	Potential anti-inflammatory activity
Interferon gamma	Pro-Th1 immunity
	Pro-inflammatory
HMGB1	Pro-inflammatory
BDNF	Nerve growth-factor
TGF beta	Anti-inflammatory
	Immune suppressive
VEGF	Pro-angiogenic
Fractalkine	Chemotactic
IL-6	Pro-inflammatory
IL-10	Anti-inflammatory
IL-4	Pro-Th2 immunity
IL-13	Pro-Th2 immunity

Abbreviations: BDNF-brain-derived neurotrophic factor; HMGB-high mobility group box; HLA-G-human leukocyte antigen G; IL-interleukin; TGF-transforming growth factor; VEGF-vascular endothelial growth factor

Table 3. Diagnostic candidate proteins selected for serum/plasma profiling in different glioma grades.

Candidate molecule	Mean/median concentration G. II (SD, IQR)	Mean/median concentration G. III (SD, IQR)	Mean/median concentration grade IV (SD, IQR)
Soluble HLA-G (plasma, U/ml)	28.38	28.65	27.25
Soluble TREM-1 (serum, pg/ml)	24.85	33.2	31.58
Interferon gamma (plasma, pg/ml)	18.29	5.66	22.38
HMGB1 (Z-score) (serum)	–0.34	–0.40	–0.42
BDNF (plasma, pg/ml)	1021.3	863.6	621
TGF beta (plasma, pg/ml)	522.74	504.5	561.3
VEGF (plasma, pg/ml)	292.70	89.1	53.69
Fractalkine (plasma, pg/ml)	939.6	740.3	551
IL-6 (plasma, pg/ml)	7.39	0.92	2.538
IL-10 (plasma, pg/ml)	186.07	144.5	194.87
IL-4 (plasma, pg/ml)	206	209.85	216.6
IL-13 (plasma, pg/ml)	46.1	4.27	27.28

Abbreviations: BDNF-brain-derived neurotrophic factor; HLA-G-human leukocyte antigen G; HMGB-high mobility group box; IL-interleukin; SD-standard deviation; TGF-transforming growth factor; VEGF-vascular endothelial growth factor

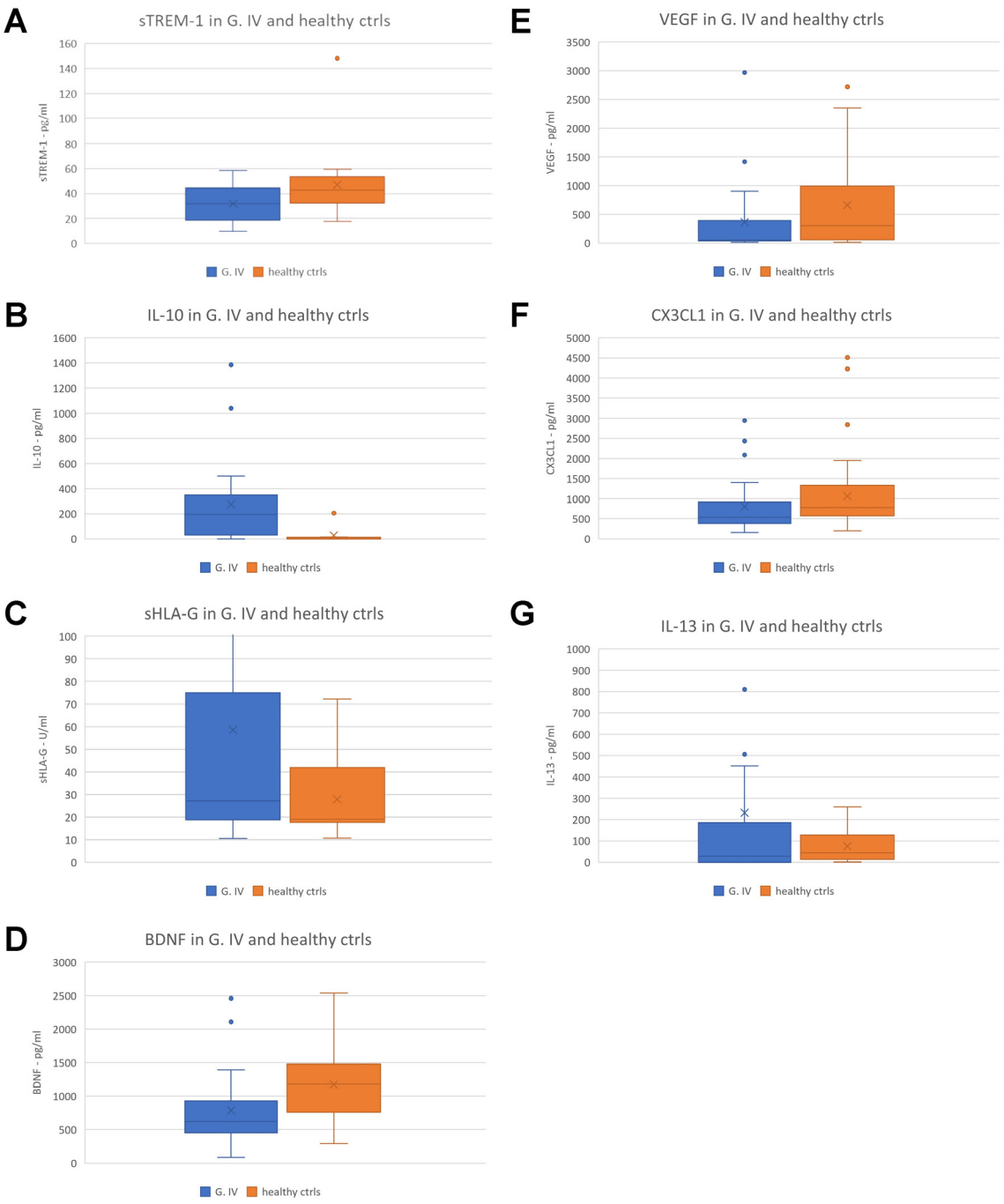

Figure 1. A) Serum levels of sTREM-1 in grade IV glioma patients and healthy controls. Serum levels of sTREM-1 are significantly lower in glioblastoma patients (N = 30) than in healthy controls (N=26) (p=0.01; mean 31.58 pg/ml ± SD 14.90 vs. 42.95 pg/ml ± SD 11.80). B) Serum levels of IL-10 in grade IV glioma patients and healthy controls. Plasma levels of IL-10 are significantly higher in glioblastoma patients (N=24) than in healthy controls (N=26) (p=0.007; median 194.87 pg/ml, IQR 319.4 vs. median 0 pg/ml, IQR 12.35). C) Serum levels of sHLA-G in grade IV glioma patients and healthy controls. Plasma levels of sHLA-G (N=30) are significantly higher in glioblastoma patients than in healthy controls (N=24) (p=0.04; median 27.25 U/ml, IQR 62.78 vs. median 19.1 U/ml, IQR 12.85). D) Serum levels of BDNF in grade IV glioma patients and healthy controls. Plasma levels of BDNF are significantly lower in glioblastoma patients (N=30) than in healthy controls (N=17) (p=0.001; median 621 pg/ml, IQR 473.2 vs. median 1265 pg/ml, IQR 681.77). E) Serum levels of VEGF in grade IV glioma patients and healthy controls. Plasma levels of VEGF are significantly lower in glioblastoma patients (N=30) than in healthy controls (N=26) (p=0.05; median 53.69 pg/ml, IQR 353 vs. median 303.3 pg/ml, IQR 927.77). F) Serum levels of CX3CL1 in grade IV glioma patients and healthy controls. Plasma levels of fractalkine are significantly lower in glioblastoma patients (N=24) than in healthy controls (N=26) (p=0.02; median 551 pg/ml, IQR 473 vs. median 967 pg/ml, IQR 634). G) Serum levels of IL-13 in grade IV glioma patients and healthy controls. Plasma levels of IL-13 are significantly lower in glioblastoma patients (N=26) than in healthy controls (N=17) (p=0.05; median 27.28 pg/ml, IQR 185.14 vs. median 122.76 pg/ml, IQR 222.4).

Table 4. Diagnostic candidate proteins selected for serum/plasma profiling in healthy and glioblastoma subjects.

Candidate molecule	Mean/median concentration grade IV (SD, IQR)	Mean/median concentration healthy controls (SD, IQR)	p-value
Soluble HLA-G (plasma, U/ml)	27.25 (IQR 62.78)	19.1 (IQR 12.85)	**0.04**
Soluble TREM-1 (serum, pg/ml)	31.58 (SD 14.90)	42.95 (SD 11.80)	**0.01**
Interferon gamma (plasma, pg/ml)	22.38	25.80	0.68
HMGB1 (Z-score) (serum)	–0.42	–0.24	0.18
BDNF (plasma, pg/ml)	621 (IQR 473.20)	1265 (IQR 681.77)	**0.001**
TGF beta (plasma, pg/ml)	561.3	489.6	0.22
VEGF (plasma, pg/ml)	53.69 (IQR 353)	303.3 (IQR 927.77)	**0.05**
Fractalkine (plasma, pg/ml)	551 (IQR 473)	967 (IQR 634)	**0.02**
IL-6 (plasma, pg/ml)	2.538	0	0.91
IL-10 (plasma, pg/ml)	194.87 (IQR 319.4)	0 (IQR 12.35)	**0.007**
IL-4 (plasma, pg/ml)	216.6	273.61	0.57
IL-13 (plasma, pg/ml)	27.28 (IQR 185.14)	122.76 (IQR 222.4)	**0.05**

Abbreviations: BDNF-brain-derived neurotrophic factor; HLA-G-human leukocyte antigen G; HMGB-high mobility group box; IL-interleukin; SD-standard deviation; TGF-transforming growth factor; VEGF-vascular endothelial growth factor

Table 5. Diagnostic candidate proteins selected for serum/plasma profiling in healthy and grade III gliomas subjects.

Candidate molecule	Mean/median concentration G. III (SD, IQR)	Mean/median concentration healthy controls (SD, IQR)	p-value
Soluble HLA-G (plasma, U/ml)	28.65	19.1	0.06
Soluble TREM-1 (serum, pg/ml)	33.2 (IQR 12.52)	42.95 (IQR 20.88)	**0.02**
Interferon gamma (plasma, pg/ml)	5.66	25.80	0.30
HMGB1 (Z-score) (serum)	–0.40	–0.24	0.17
BDNF (plasma, pg/ml)	863.6 (SD 510)	1270.3 (SD 525)	**0.05**
TGF beta (plasma, pg/ml)	504.5	489.6	0.85
VEGF (plasma, pg/ml)	89.1 (IQR 183.62)	303.3 (IQR 927.77)	0.18
Fractalkine (plasma, pg/ml)	740.3	978	0.11
IL-6 (plasma, pg/ml)	0.92	0	0.76
IL-10 (plasma, pg/ml)	144.5 (IQR 114)	0 (IQR 12.35)	**0.03**
IL-4 (plasma, pg/ml)	209.85	273.61	0.47
IL-13 (plasma, pg/ml)	4.27 (IQR 57.24)	122.76 (IQR 222.4)	**0.02**

Abbreviations: BDNF-brain-derived neurotrophic factor; HLA-G-human leukocyte antigen G; HMGB-high mobility group box; IL-interleukin; SD-standard deviation; TGF-transforming growth factor; VEGF-vascular endothelial growth factor

Table 6. Diagnostic candidate proteins selected for serum/plasma profiling in healthy and grade II gliomas subjects.

Candidate molecule	Mean/median concentration G. II (SD, IQR)	Mean/median concentration healthy controls (SD, IQR)	p-value
Soluble HLA-G (plasma, U/ml)	28.38	19.1	0.43
Soluble TREM-1 (serum, pg/ml)	24.85 (IQR 15.31)	42.95 (IQR 20.88)	**0.0051**
Interferon gamma (plasma, pg/ml)	18.29	25.80	0.58
HMGB1 (Z-score) (serum)	–0.34	–0.24	0.14
BDNF (plasma, pg/ml)	1021.3	1270.3	0.18
TGF beta (plasma, pg/ml)	522.74	489.6	0.62
VEGF (plasma, pg/ml)	292.70	303.3	0.54
Fractalkine (plasma, pg/ml)	939.6	978	0.71
IL-6 (plasma, pg/ml)	7.39	0	0.17
IL-10 (plasma, pg/ml)	186.07 (IQR 206.3)	0 (IQR 12.35)	**0.0017**
IL-4 (plasma, pg/ml)	206	273.61	0.38
IL-13 (plasma, pg/ml)	46.1	122.76	0.17

Abbreviations: BDNF-brain-derived neurotrophic factor; HLA-G-human leukocyte antigen G; HMGB-high mobility group box; IL-interleukin; SD-standard deviation; TGF-transforming growth factor; VEGF-vascular endothelial growth factor

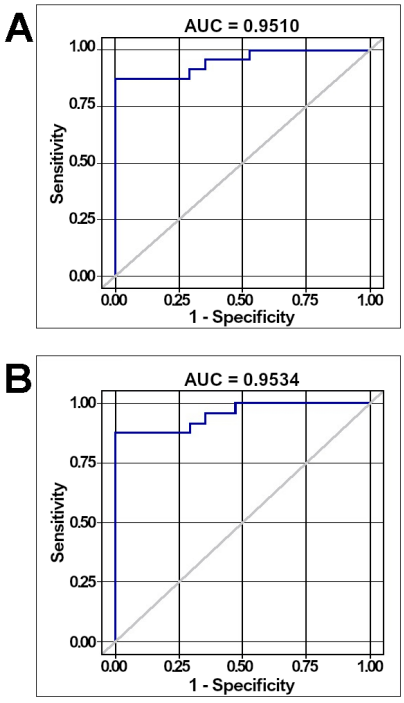

Figure 2. A) ROC curve analysis with four markers (sTREM-1, sHLA-G, BDNF, and IL-13) for the diagnosis between glioblastoma patients and the healthy control group. ROC curves for four selected markers sTREM-1, sHLA-G, BDNF, and IL-13. The AUC of the ROC curve is 0.9510, indicating that it possessed good discriminatory capability for glioblastoma patients. B) ROC curve analysis with three markers (sTREM-1, sHLA-G, and BDNF) for the diagnosis between glioblastoma patients and the healthy control group. ROC curves for three selected markers sTREM-1, sHLA-G, and BDNF. The AUC of the ROC curve is 0.9534, indicating that it possessed the best discriminatory capability for glioblastoma patients.

at relapsed tumors, methylation of the MGMT promotor, and serum/plasma levels of selected markers, we could not make any conclusion. MGMT methylation status was performed only in GBM. We have four relapsed tumors in this subgroup, three of them are unmethylated and one is methylated, though we cannot compare serum/plasma levels between these relapsed patients. We suppose the high effect of oncological therapy on selected markers at the time of tumor regression or therapy-mediated necrosis, but we can speculate, that at the time of developed relapse (blood collection was performed just before surgical intervention), these markers had risen again.

Differences between IDH mutated and IDH wild-type glioblastomas. Only one patient from the GBM subgroup was IDH mutated, so we did not compare serum/plasma levels of selected markers between IDH wild type and IDH mutated GBMs.

Differences between gliomas and other CNS malignancies. Because our primary project is focused on gliomas, we have only 6 patients with other types of CNS malignancies: 3 meningiomas, 1 chondroma, 1 metastasis of solid tumor, and 1 CNS lymphoma. Comparing selected markers, we did not find significant differences between gliomas and other types of CNS tumors, probably due to the small number of patients with other types of CNS malignancy.

Discussion

Single molecular markers are not sufficient to follow up cancer patients and will be replaced by multiple marker profiles [12]. Currently, circulating tumor cells (CTCs) and a few secreted proteins expressed by astrocytomas have been proposed as potential serum markers, for example, cell-free tumors (ctDNAs), circulating cell-free microRNAs (cfmiRNAs), circulating extracellular vesicles (EVs), and others are under investigation [13–15]. We evaluated the serum/plasma concentrations of 12 proteins, which are known to modulate anti-cancer immunity or are associated with angiogenesis or nerve function.

sHLA-G. HLA-G is an immune checkpoint molecule with immunosuppressive and anti-inflammatory activities [16]. In our cohort of investigated subjects, significantly increased levels of sHLA-G were observed in comparison with healthy controls. We assume that soluble HLA-G could be released from tumors to help them escape from immune surveillance of the body. HLA-G induces tolerance by inhibiting different cells. This main function is mediated by the binding of both the soluble and the membrane-bound HLA-G to inhibitory receptors [17–19]. Consistent evidence in the literature showed that HLA-G represents an important factor in determining the diagnosis and prognosis of various types of cancer. Elevated plasma or serum levels of sHLA-G were found in breast, lung, gastrointestinal, and urogenital cancer [20–30]. Furthermore, the HLA-G protein expression was found in 65 of 108 samples and its mRNA in 20 of 21 samples by Wastowski et al. [31]. The absence of HLA-G protein expression was associated with a better long-term survival rate [31].

VEGF. In our study group, we observed significantly lower plasma levels of VEGF in glioblastoma patients than in healthy controls. VEGF, an endothelial-cell-specific mitogen, is abundantly expressed in glioma cells that reside along necrotic areas. In 1996, Takano et al. reported that VEGF concentrations of glioblastoma cyst fluid were 200–300-fold higher than those of serum in the patients [32]. The VEGF concentration in the tumors was significantly correlated with the vascularity measured by counting vessels stained with von Willebrand factor antibody. VEGF immunopositivity was well reflected in the VEGF concentration determined by ELISA. The VEGF ELISA method demonstrated a time-dependent increase of the VEGF concentration in the serum-free conditioned medium of various glioma cell lines. The conditioned medium with high VEGF concentration induced endothelial cell migration.

These observations suggest that VEGF represents a useful marker and measurable element of glioblastoma angiogenesis [32]. In 2000, Stockhammer et al. [33] measured the concen-

tration of VEGF in matched samples of the aspiration fluid from tumor cysts and serums. Samples were collected from 14 patients with primary brain tumors of various histologies and two patients with solitary cystic brain metastases from adenocarcinomas of the lung. Aspiration fluids of tumor cysts from all patients revealed high VEGF levels which were from 2 to >2,000 times higher than the corresponding serum levels. Serum VEGF levels did not differ from the serum levels in 145 healthy volunteers. These findings indicate that immunoreactive VEGF is produced at the tumor site and abundantly released into the cyst fluid of primary and metastatic brain tumors. Interestingly, this abundant local release is not reflected in serum VEGF levels [33].

In our study group, we suppose that similarly to the results of Stockhammer et al. [33], the levels of VEGF do not correspond with the levels of VEGF in the brain, and might be even lower than in healthy subjects. Combining anti-angiogenic therapies with radiation therapy, cytotoxic drugs, immunotherapy, and targeted molecular agents may greatly enhance treatment strategies for high-grade gliomas. However, until now durable responses and survival benefits to these therapies are lacking.

BDNF. In the present study, a significantly lower plasma concentration of BDNF was observed in patients with high-grade tumors. Only a few data on serum/plasma BDNF in cancer patients are available. Although the major source of BDNF in the adult brain appears to be neurons, BDNF can also be detected in oligodendrocytes, astrocytes, and microglia [34]. There are two forms of the brain-derived neurotrophic factor (BDNF), the proBDNF and mature BDNF, which exert opposite effects. In 2013, Brierley et al. described significantly lower serum levels of BDNF in colorectal cancer patients when compared to a control population [35]. According to the present data, the levels of BDNF in combination with the levels of sHLA-G and sTREM-1 indicate that these molecules may serve as promising markers to distinguish grade IV glioma patients from healthy subjects. The majority of the BDNF release by neurons in the adult brain appears to be triggered by neuronal activity. In gliomas, the neuronal activity is eventually suppressed due to the tumor process, though, production of BDNF could be lower than under healthy conditions.

Fractalkine. Significantly lower concentrations of chemokine fractalkine (CX3CL1) were observed in our study in glioblastoma patients than in healthy controls. We suppose that a smaller amount of fractalkine could be the consequence of a very aggressive tumor. Accumulating evidence has shown that fractalkine/CX3CL1, a member of the CX3C chemokine subfamily, is involved in the pathogenesis of different types of cancer [36, 37]. Its soluble form originates from extracellular proteolysis by proteases, such as tumor necrosis factor-α converting enzyme, where ADAM10 mediates the cleavage and shedding of fractalkine [38]. The secreted form induces chemotaxis of natural killer cells, cytotoxic T-lymphocytes, and macrophages. An increase in sCX3CL1 in the cancer microenvironment allows the chemotaxis of all the aforementioned cells with CX3CR1 expression towards the cancer niche, where they exert an anti-cancer effect. NK cells and $CD8^+$ T cells, and macrophages are most significant in the direct anti-cancer action of CX3CL1 [39–41].

As was shown in HIV infections and various cancer diseases with impaired local and systemic immune responses, fractalkine can induce potent anti-tumor and tissue-protective effects. In HIV patients an increased expression of fractalkine protects neurons from neurotoxins, which have a key role in neural apoptosis in the brain [42, 43]. Ohta et al. showed that a higher level of expression of fractalkine in patients with colorectal cancer correlates with a higher density of tumor-infiltrating immune cells and results in a better prognosis than in those with a weak expression [44]. Similar results were confirmed by Vitale et al. in 2007. They showed that the secreted form of fractalkine reduces the metastatic potential of C26 colon cancer cells in the liver and lungs. Even both molecular forms exhibited their anti-tumor potential depending on the target tissue [45].

Important functions are also reported in the CNS. As described by Lauro et al., fractalkine protects against cerebral ischemia modulating the activation state of the microglia and its metabolism in order to restrain inflammation and organizes a neuroprotective response against the ischemic insult [46]. The study of Sciumé et al. analyzed the expression and function of the chemokine CX3CL1 and its receptor CX3CR1 in human glioma cells. Their results indicate that the blockade of CX3CL1 activity provokes a strong increase in glioma cell invasion that correlates with delayed cell aggregation, suggesting that the adhesive properties of CX3CL1 counteract the invasive phenotype [47]. Therefore, the expression of fractalkine may be considered to be an essential biomarker for predicting prognosis and for the identification of those patients who might benefit most from additional immunomodulating therapy.

IL-13. The levels of IL-13 in high-grade glioma patients in comparison with healthy controls were significantly lower in the present study. We suppose that IL-13 binds to its receptors in gliomas, although lower levels are detectable in peripheral blood. In a series of studies, the overexpression of IL-4 and IL-13 receptors on cancer cells was described [48, 49]. In addition, both these cytokines and their receptors have been shown to play important roles in modulating the immune system for tumor growth. The IL-4, IL-13, and their receptors seem to play a role in cancer stem cells and provide unique pathways to eradicate these cells. Debinski et al. demonstrated that the vast majority of high-grade gliomas bind interleukin-13 and are highly susceptible to the cytotoxicity of IL-13-based cytotoxins [50]. Kioi et al. discovered that 60–80% of malignant brain tumors have an overexpressed high-affinity plasma membrane receptor for IL-13. The IL-13 cytotoxin was found to mediate a remarkable efficacy in animal models of human brain tumors. Later, the four phase 1/2 clinical trials

in adult patients with recurrent malignant glioma have been completed. These clinical trials involved a convection-enhanced delivery (CED) of IL-13 cytotoxin. The resection of the tumor revealed that the CED mechanism was either intratumoral or intraparenchymal [51].

The IL-4 and IL-13 tethered exotoxins have already led to clinical trials. Similarly, IL-13Rα2 (interleukin 13 receptor alfa 2) as an immunogen is the basis of a DIPG (diffuse intrinsic pontine glioma)/high-grade glioma vaccine trial for children [https://beta.clinicaltrials.gov/study/NCT01130077]. Thus, targeting overexpressed glioma-specific receptors or receptor tyrosine kinases in combination with targeted antibodies may further improve the survival rate. A case report of *IL13RA2*-targeted chimeric antigen receptor T-cell therapy showed regression of glioblastoma in a human patient [52]. Berlow et al. have identified IL-4Rα, IL-13Rα1, and especially IL-13Rα2 as potential therapeutic targets in DIPG [53].

sTREM-1. Our glioma patients had significantly lower serum levels of sTREM-1 than healthy controls. The TREM-1 molecule occurs in two isoforms – as a membrane-bound and as a soluble molecule. These isoforms may arise by splitting from the membrane surface or by monocyte production, respectively. The sTREM-1 is an inflammatory biomarker and also a decay receptor able to bind ligands for the membrane-bound TREM-1. We assume that a smaller amount of this soluble decoy receptor supports systemic inflammation, though it could promote tumor growth. There are few studies concerning serum sTREM-1 levels in cancer patients. Soluble TREM-1 as a form of TREM-1 serves as a decoy receptor, by binding its ligands prevents their binding to membrane-bound TREM-1 (pro-inflammatory), and exerts this way anti-inflammatory activity [7, 54].

Elevated serum/plasma levels of sTREM-1 were found in inflammatory diseases of both infectious and non-infectious origin [54, 55]. Both increased and decreased serum levels were detected in some types of cancers [56]. In a study from 2008, Karapanagiotou et al. observed high levels of sTREM-1 in 50% of breast cancer patients, in 33.3% of small cell lung carcinomas (SCLC), 26.7% of colorectal cancers, and 13.3% of non-small cell lung carcinoma patients (NSCLC). Higher concentrations were observed in the absence of lung metastases [57]. In 2018, Kuemmel et al. published a study in which they demonstrated that sTREM-1 was a marker of short survival in patients with NSCLC [58]. In 2008, Huang et al. compared values of sTREM-1 in pleural effusions from various diagnoses. They found significantly lower concentrations of sTREM-1 in malignant, tuberculous, and transudate groups than in bacterial effusions [59].

IL-10. In our study group, all grades of gliomas had significantly higher levels of IL-10 than healthy controls, but we did not observe any correlation with the survival time. We suppose that elevated levels of IL-10 downregulate the systemic inflammation, however, it could also point out suppression of immunity in the peripheral blood, so it could facilitate tumor growth.

Many studies concerning IL-10 function in gliomas were performed. The role of IL-10 in cancer is controversial. It is considered immunosuppressive, so it could promote tumor growth. On the other hand, it suppresses the excessive inflammation that is protumorigenic, so it may inhibit the development of the tumor. It has been even argued that many of the attributes of IL-10 associated with immune suppression are equally likely to enhance immune activation [60]. In gliomas, Huettner et al. in 1997 demonstrated that IL-10 was involved in the progression of glial tumors, especially in the enhancement of tumor cell proliferation and migration, which promoted the infiltration of the surrounding tissue [61]. Similarly, two decades later, Zhang et al. showed that IL-10 promotes glioma progression via the upregulation of KPNA2 (karyopherin subunit alpha 2) [62]. Qi et al. have first shown that IL-10 from M2 macrophage promoted the proliferation of glioma through interaction with JAK2 (Janus kinase) [63]. The concentration of IL-10 in the blood as well as in the tumor microenvironment would be crucial in the process of tumorigenesis.

We realize that our study has some limitations. We obtained sera/plasma when the tumors were diagnosed (after clinical symptoms) and their values at the time of preclinical stages are unknown. These markers could be altered also in other tumors, but for some cancer types, there exist serum/plasma markers with relatively good sensitivity and specificity.

Various histological subtypes of gliomas exist and the biological behavior of each subtype could influence the processes in peripheral blood. Moreover, comorbidities of patients may also have an impact on the state of the immune system. At the time of the planned operation, all patients were free of clinical and laboratory signs of acute infection, but comorbidities such as hypertension, type 2 diabetes, and others can also cause some type of inflammatory process, we cannot guarantee the influence of our markers by non-tumor processes. In our study, we had both relapsed and primary glioma patients, and the inflammatory process could be different in these subgroups because relapsed patients were treated previously. However, no differences in these markers between the primary and the relapsed tumors were observed.

In conclusion, multiple biomarkers associated with inflammation and anti-tumor immunity to identify profiles associated with gliomas were analyzed in this study. We found decreased serum levels of sTREM-1 and increased levels of IL-10 in all, even grade II glioma patients, in comparison with healthy controls. In grade IV glioma patients, two sets of markers showed promising results in distinguishing patients from healthy people. For the first set consisting of four selected markers, sTREM-1, sHLA-G, BDNF, and IL-13, the ROC curves indicate a good discriminatory capability for glioblastoma patients (AUC=0.9510). The best discriminatory capability for glioblastoma patients (AUC=0.9534) was found for the second set consisting of three selected markers sTREM-1, sHLA-G, and BDNF with 79.2% sensitivity and 94.1% specificity.

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