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# Serum Gangliosides in Patients with Brain Tumors

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## ABSTRACT

*In order to determine possible differences in serum gangliosides content and composition before and after surgical removal of tumor, gangliosides isolated from preoperative and postoperative sera of patients with brain tumors were analyzed. Serum samples were collected from patients with glioblastoma, meningioma, acoustic neurinoma, haemangioma, oligodendroglioma and astrocytoma, one week before and one week after surgical removal of the tumor. Serum gangliosides were qualitatively and quantitatively analyzed by high performance thin layer chromatography and laser densitometry. Results showed changes of total gangliosides concentrations in analyzed postoperative sera comparing to preoperative sera. There was not a significant difference in ganglioside pattern of preoperative vs. preoperative sera. However, a postoperative decreased proportion of ganglioside GD3 was observed in sera derived from patients with complete tumor removal. The results of this study indicate that comparative quantitative and compositional analysis of both preoperative and postoperative serum gangliosides may provide useful information concerning tumor progression, surgical success and prognosis.*

**Key words:** serum, gangliosides, brain tumors

## Introduction

Gangliosides (GG) are important lipid constituents of cell membranes<sup>1</sup>. Chemically, gangliosides are glycosphingolipids containing sialic acids (SA) and are particularly abundant in membrane microdomains considered as highly organized units with various specific functions<sup>2</sup>. Gangliosides are involved in many significant cellular processes such as cell growth, cell proliferation, differentiation, oncogenic transformation, etc<sup>3</sup>. Animal brain tissue is highly enriched in gangliosides – phylogenetic and ontogenetic differences in brain ganglioside biosynthesis have been evidenced as well as regional distribution and specific ganglioside patterns characterizing neuronal and glial cells during brain development, aging and neurodegeneration<sup>4–7</sup>. Specific changes of gangliosides pattern in brain tumors reflecting tumor histopathological origin, malignancy and progression, have been extensively studied<sup>8–12</sup>. Also, it has been observed that tumor cells of neuroectodermal origin may shed their gangliosides into circulation, resulting in higher ganglioside concentrations in serum<sup>13,14</sup>. This shedding of gangliosides into interstitial spaces and blood of oncological patients has

been suggested to be involved in increased tumor cell growth and lack of immune cell recognition<sup>14</sup>. Although many studies have consistently reported higher concentrations of serum gangliosides in oncological patients comparing to healthy individuals, there have been no data on comparison of ganglioside concentrations in serum before and after surgical removal of tumor in individual patients<sup>13,15,16</sup>. The aim of this study was to trace possible differences between preoperative and postoperative concentrations of serum gangliosides in patients with brain tumors and to estimate a potential prognostic value of these differences.

## Material and Methods

### Material

Blood samples (5–10 mL) were collected from individuals with no evidence of neurological or oncological disease (N=10), and patients with diagnosis of glioblastoma (N=3), meningioma (N=3), acoustic neurinoma (N=1),

haemangioma (N=1), oligodendroglioma (N=1) and astrocytoma (N=1), one week before and one week after surgical removal of the tumor. In control group of 10 volunteers, there were 4 females and 6 males in age range from 23 to 75 years. Group of patients consisted of 4 males and 6 females in age range from 22 to 76 years. Clinical diagnosis and localization of brain tumors were determined using computerized tomography (CT) and magnetic resonance (MR), followed by final pathological analysis of postoperative tumor tissue. Serum was isolated from the whole blood by routine laboratory method, *i.e.* blood was collected in tubes containing anti-coagulants and serum was finally separated from plasma and blood cells by centrifugation.

#### Biochemical analysis of serum gangliosides

Ganglioside extraction and purification was performed according to the method of Svennerholm<sup>17</sup>. Extraction and reextraction steps used chloroform (C), methanol (M) and water (W) in a final volume ratio 1:1:0.8 (C:M:W, v/v/v). Upper phases containing polar gangliosides were collected and air-dried, and obtained dry ganglioside extract was finally purified by dialysis (overnight, 4 °C). Purified ganglioside extract was used for quantitative and qualitative analysis of gangliosides.

Qualitative analysis of serum gangliosides was performed by high performance thin layer chromatographic (HPTLC) separation of individual gangliosides on silica gel plates. The samples of purified ganglioside extracts derived from patients and pooled control sera were dissolved in chloroform:methanol (1:1) and applied to the HPTLC plates. Also, three standard mixtures of gangliosides were applied to the HPTLC plates: (a) a range of concentrations of Cronassial ganglioside mixture (3, 5 and 9 micrograms of GG-SA per lane); (b) GM2 ganglio-

side from the human lung tissue; (c) ganglioside mixture derived from the human cerebellar tissue, prepared and purified in our laboratory. The plates were developed in a solvent system containing chloroform, methanol and 12 mM MgCl<sub>2</sub> (58:40:9, v/v/v). After drying, plates were sprayed with resorcinol reagent and heated during 30–45 minutes until the visualization of ganglioside fractions which appeared as bluish bands. Finally, HPTLC plates with separated and visualized ganglioside fractions were analyzed by laser densitometry (LKB 2202 Laser Ultrascan) at 580 nm. Laser densitometry enabled direct quantification of total gangliosides, according to the method of Trbojević-Čepe *et al.*<sup>18</sup>. The total concentrations of gangliosides were expressed as nanomoles of ganglioside-bound sialic acids (GG-SA) in milliliter of serum. Densitometric quantification of individual gangliosides in each sample was expressed as their relative proportion (%) in a sample. Abbreviations used for designation of the individual ganglioside species are in accordance with the accepted system of Svennerholm<sup>19</sup> and the recommendations of IUPAC-IUB Commission on Biochemical Nomenclature<sup>20,21</sup>.

## Results and Discussion

#### Total ganglioside concentrations in sera derived from patients with brain tumors

In this study, total ganglioside concentrations were determined in serum samples derived from 10 patients with different types of brain tumors and 10 individuals with no evidence of oncologic disease, as shown in Table 1. Determined values were in a range from 1.68 to 14.37 nmol GG-SA/mL. Such a high variation of total serum ganglioside concentrations among individuals has been also

TABLE 1  
COMPARISON OF GANGLIOSIDE CONCENTRATIONS IN PREOPERATIVE AND POSTOPERATIVE SERA DERIVED FROM PATIENTS WITH BRAIN TUMORS

Diagnosis	Surgery outcome	Preoperative serum gangliosides (nmol GG-SA/mL)	Postoperative serum gangliosides (nmol GG-SA/mL)
Meningioma 1		9.45	2.23
Meningioma 2	Complete removal of the tumor	13.91	4.92
Meningioma 3		3.07	2.04
Acoustic neurinoma		7.57	7.47
Haemangioma		14.37	10.16
Glioblastoma 1		1.68	4.24
Glioblastoma 2	Partial removal of the tumor	2.81	7.96
Glioblastoma 3		2.59	n. d.
Oligodendroglioma		3.14	n. d.
Astrocytoma		2.13	3.46
	Control (range:	serum nmol GG-SA/mL)	gangliosides
Healthy individuals (N=10)		5.8 – 6.3	

GG-SA, ganglioside-bound sialic acid; n. d., not determined.

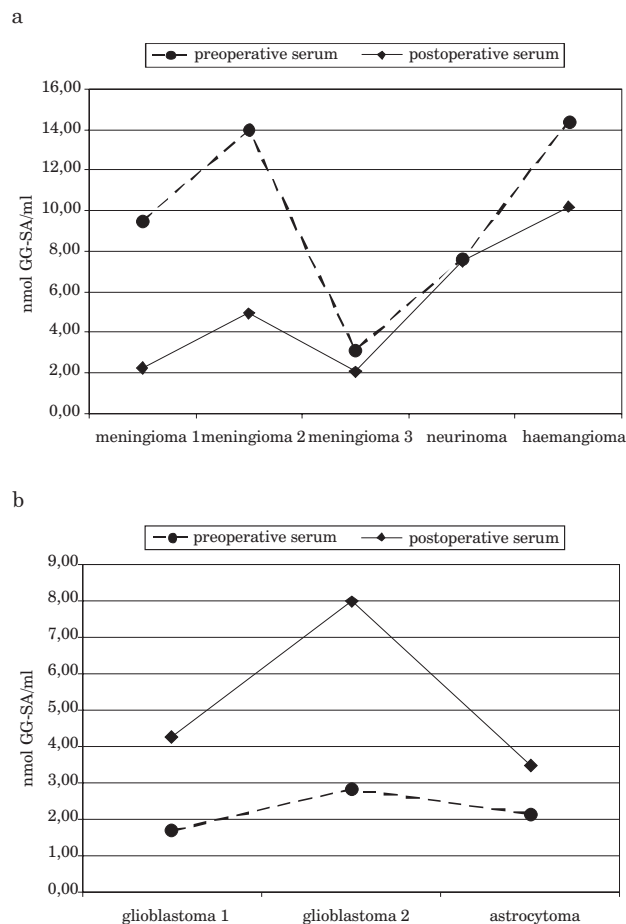


Fig 1. Comparison of total gangliosides concentrations in preoperative and postoperative sera in cases of a) complete tumor removal and b) partial tumor removal. GG-SA, ganglioside-bound sialic acid.

reported by other authors<sup>22</sup>. Although total ganglioside concentrations in patients' sera were within a control range, clear differences between preoperative and postoperative sera were observed. Moreover, it seems that surgical outcome i.e. complete or partial tumor removal influenced the trend of postoperative ganglioside concentration changes. Figure 1a shows postoperative decrease of serum ganglioside concentrations in patients in which tumor was completely removed (meningioma, acoustic neurinoma, haemangioma) (mean values: 9.67 nmol GG-SA/mL preoperatively vs. 5.36 nmol GG-SA/mL postoperatively). This finding is in accordance with generally accepted explanation that increase of total ganglioside concentrations in serum of oncological patients originates from ganglioside production and secretion/shedding by tumor tissue<sup>13,14</sup>. On the other hand, in the case of partial tumor removal (glioblastoma, astrocytoma, oligodendroglioma), concentrations of serum gangliosides increased postoperatively (mean values: 2.47 nmol GG-SA/mL preoperatively vs. 5.22 nmol GG-SA/mL postoperatively), as represented in Figure 1b. It has to be pointed out that all these partially removed tumors were of much higher malignancy comparing to the group of

completely removed tumors. Thus, patients bearing more malignant types of brain tumors were treated by aggressive radiotherapy which, apart from having immunosuppressive effects, could also influence a liver metabolism of circulating gangliosides. This intriguing result indicates potential relevance of gangliosides as biomarkers of a surgical outcome.

According to our knowledge, this is the first study including the analysis of postoperative sera in order to compare differences between serum ganglioside concentrations before and after surgical treatment in individual patients. This study, showing clear differences between preoperative vs. postoperative sera, indicates the potential prognostic value of serum ganglioside determination in patients with brain tumors. However, a larger study and longer postoperative follow-up is needed in order to characterize the exact relation of serum ganglioside concentrations to surgical outcome.

#### *Ganglioside composition in preoperative and postoperative sera derived from patients with brain tumors*

Qualitative analysis of ganglioside pattern in patients' and control sera, using high performance thin layer chromatography, showed in all tested samples the fractions migrating as GT1b, GD1a, GD3, GM2 and GM3 (Figure 2). GD3 and GM3 were found to be the most abundant ganglioside species in all analyzed serum samples, which is common finding for ganglioside pattern of extraneural tissues<sup>23</sup>. Several unknown minor fractions with migrating properties of disialoganglioside and monosialoganglioside structures were also observed in both patients' and control sera. Possible candidate structures with HPTLC-migration properties of monosialylated species are GM1a, GM1b, LM1 and nLM1, while those migrating as a reference disialo-species are GD1a, LD1 and nLD1 (and their isomers). Since ganglioside pattern in normal human serum has not yet been systematically and reliably analyzed, our further investigation will focus on the elucidation of serum ganglioside structures using highly sensitive mass spectrometry methods.

Chromatographically separated ganglioside patterns of normal serum as well as preoperative and postoperative serum did not reveal significant differences (Figure 2). However, a quantification of individual serum gangliosides by laser densitometric analysis showed that in all patients with completely removed tumor (meningioma, acoustic neurinoma, haemangioma) there was a postoperative decrease of GD3 proportion and increase in GM3 proportion (Figure 3). The most prominent postoperative decrease of GD3 proportion was observed in the case of haemangioma and meningioma, which is consistent with the postoperative decrease of corresponding total serum ganglioside concentrations (Table 1). In the case of partial tumor removal (glioblastoma and astrocytoma), such a change in postoperative GD3 and GM3 proportion was not so obvious. Since GM3 is a direct biosynthetic precursor of GD3, change in GM3/GD3 ratio in serum directly reflects alteration of tumor ganglioside

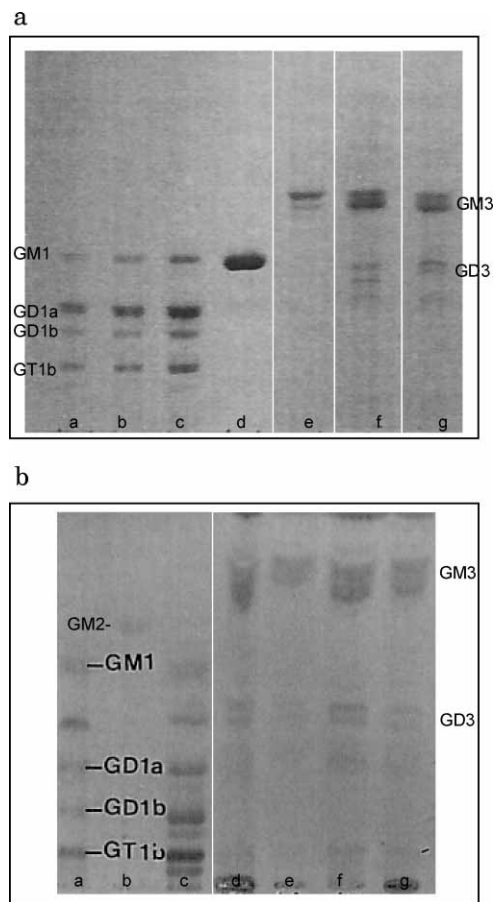


Fig. 2. Ganglioside composition of analyzed serum samples as revealed by high performance thin layer chromatographic separation of individual gangliosides. a) Gangliosides in control human serum; a, b and c – Cronassial standard mixture of gangliosides; d – GM1 ganglioside; e – GM3 ganglioside; f and g – control serum samples. b) Serum gangliosides in patients with brain tumors; a – Cronassial standard mixture of gangliosides; b – GM2 ganglioside; c – gangliosides extracted from human cerebellum; d – glioblastoma preoperative serum; e – glioblastoma postoperative serum; f – meningioma preoperative serum; g – meningioma postoperative serum.

metabolism. In addition, analyzing the ganglioside patterns of corresponding tumor tissues of here selected patients we indeed observed higher proportion of GD3 and GD2 as well as several additional fractions which need to be structurally characterized; detailed structural identification is being currently conducted using reliable instrumental methods (data to be published). Simplification of ganglioside pattern and increased GD3 proportion in both tumor tissue and serum has been previously reported and observed to be in relation with glioma malignancy<sup>13</sup>. Also, increased GD3 concentrations were found in serum and cerebrospinal fluid of patients with meningioma, medulloblastoma and astrocytoma<sup>24,25</sup>.

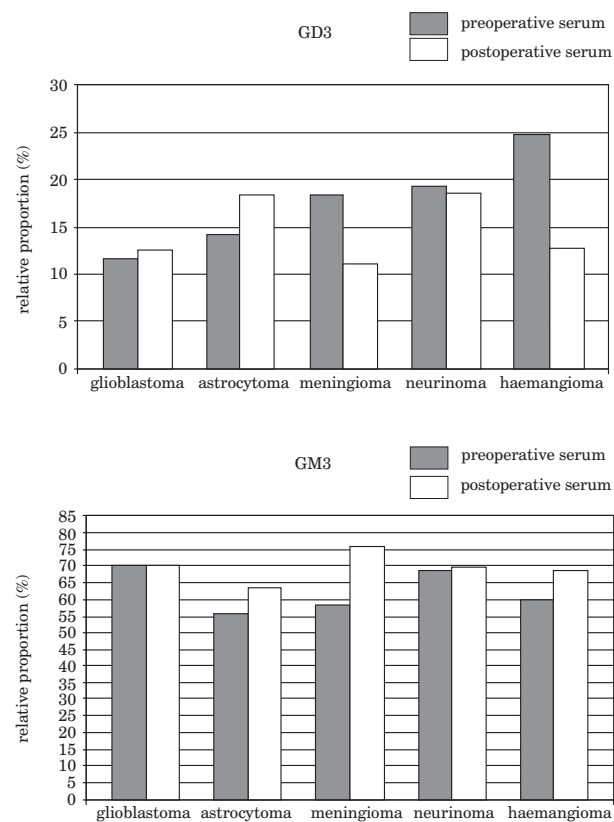


Fig. 3. Relative proportion of GD3 and GM3 in preoperative vs. postoperative sera derived from patients with brain tumors, as quantified by laser densitometric analysis of gangliosides separated by high performance thin layer chromatography. Data for glioblastoma and meningioma represent mean values of three analyzed samples.

The results of this study demonstrate that compositional analysis of serum gangliosides could provide additional data on serum ganglioside alterations with potential diagnostic relevance. However, employment of more sensitive methods such as mass spectrometry would ensure much deeper insight into compositional changes of serum gangliosides particularly concerning minor species not detectable by conventional analysis.

In conclusion, the results of this study indicate that comparative quantitative and compositional analysis of both preoperative and postoperative serum gangliosides may provide useful information concerning tumor progression, surgical success and prognosis.

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## SERUMSKI GANGLIOZIDI U PACIJENATA S MOŽDANIM TUMORIMA

### SAŽETAK

U ovom radu analizirani su gangliozidi izolirani iz preoperativnih i postoperativnih seruma pacijenata s moždanim tumorima, kako bi se utvrdile moguće razlike u sadržaju i sastavu serumskih gangliozida prije i poslije kirurškog odstranjenja tumora. Uzorci seruma sakupljeni su od pacijenata s dijagnozom glioblastoma, meningeoma, neurinoma, hemangioma, oligodendroglioma i astrocitoma, jedan tjedan prije i jedan tjedan nakon kirurškog uklanjanja tumora. Kvalitativna i kvantitativna analiza serumskih gangliozida provedena je tankoslojnom kromatografijom visoke moći razlučivanja i laserskom denzitometrijom. Rezultati su pokazali razlike ukupnih koncentracija gangliozida u analiziranim postoperativnim serumima u usporedbi s preoperativnim serumima. Gangliozidni obrazac preoperativnih i postoperativnih seruma nije se značajno razlikovao. Međutim, uočeno je postoperativno smanjenje udjela gangliozida GD3 u serumima pacijenata u kojih je tumor potpuno uklonjen. Rezultati ovog istraživanja ukazuju da poredbeno kvantitativno-kvalitativna analiza preoperativnih i postoperativnih serumskih gangliozida može pružiti korisne podatke o tumorskoj progresiji, uspješnosti kirurškog postupka i prognozi.