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**The influence of endothelial hyperplasia on pseudoprogression  
development in patients with glioblastoma**

Утицај ендотелијалне хиперплазије на појаву псеудопрогресије код  
пацијената са глиобластомом

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## The influence of endothelial hyperplasia on pseudoprogression development in patients with glioblastoma

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### SUMMARY

#### Introduction/Objective

Pseudoprogression represents an enlarging contrast enhancing lesion that occurs after chemoradiation and stabilizes with time without any changes in the therapeutic procedure. This phenomenon is highly significant, because it can have influence on further therapeutic procedures, however precise criteria for pseudoprogression diagnosis have not yet been defined. The main goal of this study is to examine the endothelial hyperplasia influence on pseudoprogression.

**Methods** We analyzed a group of 106 patients with glioblastoma who had undergone surgical treatment from 2010–2012, at the Clinic of Neurosurgery, Clinical Center of Serbia, who received Stupp protocol. Pre- and post-treatment imaging was evaluated using RANO criteria. Lesions that improved or stabilized were defined as pseudoprogression, and lesions that progressed were defined as true progression. Endothelial hyperplasia was identified based on the hematoxylin eosin pathohistological examination.

**Results** Thirty-two (30.2%) of the patients were diagnosed with pseudoprogression. Endothelial hyperplasia was observed in 51 (48.1%) of glioblastoma tissue samples, and 28 (87.5%) of all the patients with pseudoprogression were found to have endothelial hyperplasia. The group of 51 (68.9%) patients without pseudoprogression did not show the presence of endothelial hyperplasia. Statistical analysis showed significantly higher incidence of pseudoprogression in patients with endothelial hyperplasia. ( $\chi^2 = 26.269$ ,  $r < 0.01$ )

**Conclusion** Taking into account that there are no precise diagnostic methods that could determine the presence of endothelial hyperplasia with certainty, it could be an indicator, as a pathohistological entity, of a higher likelihood of pseudoprogression which could be used in everyday clinical practice. In order to reach definite conclusions – we believe it is necessary to conduct prospective controlled studies with larger sample sizes.

**Keywords:** glioblastoma; endothelial hyperplasia; pseudoprogression

### САЖЕТАК

**Увод/Циљ** Псеудопрогресија представља појаву лезије која се пребојава контрастом након хемоирадијације и која не прогредира или се повлачи без промене терапије. Сам феномен има изразит клинички значај зато што у великој мери утиче на даље терапијске поступке али критеријуми за њено дијагностиковање још увек нису јасно дефинисани. Циљ овог истраживања је испитивање утицаја ендотелијалне хиперплазије на настанак псеудопрогресије.

**Метод** Испитивано је 106 пацијената који су оперисани због глиобластома у Клиничком центру Србије у периоду од 2010.–2012. године, и који су након тога лечени Ступовим протоколом. Снимање пре и након лечења је утврђено RANO критеријумом. Промене које су биле стабилне или су се повукле су дефинисане као псеудопрогресија, док су лезије које су прогредирале окарактерисане као праве прогресије. Ендотелијална хиперплазија је утврђена након патохистолошког прегледа хематоксилином еозином.

**Резултати** Псеудопрогресија је регистрована код 32 (30.2%) пацијената. Код 51 (48.1%) узорка ткива глиобластома је уочена ендотелијална хиперплазија. Од свих пацијената код којих је регистрована псеудопрогресија, њих 28, (87.5%), је имало ендотелијалну хиперплазију. Код пацијената који нису имали псеудопрогресију, њих 51 (68.9%) није имало ни ендотелијалну хиперплазију. Код пацијената који су испољили псеудопрогресију било је значајно више пацијената који су у патохистолошком налазу имали описану и ендотелијалну хиперплазију, док код пацијената без псеудопрогресије има више случајева без ендотелијалне хиперплазије. ( $\chi^2 = 26.269$ ,  $p < 0.01$ ).

**Закључак** С обзиром да за сада не постоји прецизна дијагностичка метода која би са сигурношћу утврдила да ли се ради о псеудопрогресији, присуство ендотелијалне хиперплазије би могло да укаже на већу вероватноћу појаве псеудопрогресије, што би се могло користити у свакодневном клиничком раду. Ипак, ради доношења дефинитивних закључака, сматрамо да је неопходно спровести проспективне контролисане студије на већем узорку.

**Кључне речи:** глиобластом; ендотелијална хиперплазија; псеудопрогресија

## INTRODUCTION

Pseudoprogression represents new or enlarging contrast enhancing lesion after chemotherapy within the radiation field that stabilizes with time without any changes in the therapeutic procedure [1]. This phenomenon is highly significant, because it mimics true tumor progression and, if it is misinterpreted as such, it can have a negative influence on the further therapeutic procedures. Pseudoprogression is commonly seen in asymptomatic patients. However, some patients present with clinical deterioration. These complications can include worsening of pre-existing symptoms, transient cognitive decline, subacute rhombencephalitis or somnolence syndrome [2].

The pathophysiological basis of pseudoprogression remains poorly understood. There are certain indications that it is part of the spectrum of radiation-induced changes ranging from subacute radiation-induced changes to late radiation necrosis [3]. It is assumed that there are two components of pseudoprogression: vascular injuries and treatment-related cell toxicity [4]. It is thought that transient breakdown of the blood brain barrier can cause the edema and contrast enhancement seen on the magnetic resonance imaging (MRI) [5]. The cells most sensitive to radiation are oligodendrocytes, endothelial cells and neural precursors. Cellular damage can lead to cell death including p53 and p53-independent mechanisms of apoptosis.

Pathohistological examination of patients operated because of pseudoprogression showed distinct characteristics. On the microscopic level, tumor recurrence is usually characterized by microvascular proliferation and highly cellular tumor tissue. This is contradictory to the histological characteristics of the contrast enhancing tissue of pseudoprogression that usually has a low cellularity. Typically, pleomorphic tumor cells can also be found in these lesions with the low mitotic index. In addition, elements of coagulative necrosis can be found, and it appears eosinophilic on light microscopy. Hyalinization of the wall of blood vessels and fibrinoid necrosis is frequent. Telangiectatic blood vessels may be seen, though they are less specific. Fibrillary and gemistocytic astrocytes may be observed as well. Scattered pleomorphic astrocytes are mostly associated with the tumor exposed to radiation and are often present in the tissue obtained from pseudoprogression [6].

The accurate diagnosis of pseudoprogression from the true tumor progression is of great significance in planning of further treatment [7]. Modern techniques and MR sequences are developed with the goal of differentiating pseudoprogression and true tumor progression. Diffusion-Weighted Imaging, Diffusion-Tensor Imaging, Perfusion-Weighted Imaging and

MR spectroscopy can be helpful, but are not conclusive. Positron Emission Tomography combined with other specific biomarkers is also used along with MRI. However, neither one of the abovementioned methods can be used for diagnosing any pseudoprogression-related changes with certainty.

In this paper we have examined if endothelial hyperplasia, being one of pathohistological features of glioblastoma, affects the development of pseudoprogression in patients with glioblastoma.

## **METHODS**

We used a retrospective analysis of prospectively collected data in order to analyze the patients with glioblastoma who had undergone surgical treatment during the 3-year period (2010–2012), at the Clinic of Neurosurgery, Clinical Center, Serbia and who received Stupp protocol after the surgery. The patients were monitored by a series of MRI scans. While making the final diagnosis, we used the RANO criteria, taking into consideration the fact, established after the numerous literature data, that pseudoprogression may even occur 12 weeks after the chemoradiation has been completed.

### **Statistical methods**

IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for all calculations in the study. Specific measures of central tendency and variability measures were found for continual variability whereas the frequency of the separate categories was specified for the categorical variables. A chi-square test was used for examining the factors that have an influence on pseudoprogression development. The maximum level of acceptability of the null hypothesis probability used in our study is 0.05. The conducted study was approved by the Ethics Committee of the Clinical Centre of Serbia.

## **RESULTS**

A total of 106 patients with glioblastoma who underwent surgery in the period from January 1, 2010 to December 31, 2012 and who underwent Stupp protocol were included in this study. 32 (30.2%) of the patients were diagnosed with pseudoprogression, whereas 74 (69.8%) of the patients showed no signs of pseudoprogression. On average, pseudoprogression was observed after 4.64 months.

In the group of the patients with pseudoprogression, 59.4% of them were men and 40.6% were women, whereas in the group of the patients with no pseudoprogression 54.1% of

them were men and 45.9% were women. In the group of the patients with pseudoprogession, the average age of patients was  $53.03 \pm 10.14$  years, while in the group of the patients with no signs of pseudoprogession the average age was  $53.99 \pm 12.53$  years. The highest number of patients with developed pseudoprogession was found in the age group ranging from 51–60 years (53.1%), whereas the least number was found in the age group ranging from 71–80 years (3.1%). The greatest number of patients had a radical operation: 74 (70.2%), and out of them 19 patients (17.2%) had subtotal resection, 12 (11.5%) patients had the tumor reduction, whereas there was only one biopsy and that patient was excluded from further analysis (1.1%). Pseudoprogession occurred in 15 (46.7%) of the patients who had received the radical surgery, it occurred in 11 cases (33.3%) of the patients who had had subtotal tumor resection, whereas 6 (20%) of the patients with pseudoprogession had the tumor reduction. The influence of a degree of tumor resection on the incidence of pseudoprogession was not significant ( $\chi^2 = 5.493$ ,  $p = 0.139$ ).

Endothelial hyperplasia was observed in 51 (48.1%) of glioblastoma tissue samples. Twenty-eight (87.5%) of all patients with pseudoprogession were found to have endothelial hyperplasia, whereas four patients (12.5%) were without it. Fifty-one (68.9%) patients who were not diagnosed with pseudoprogession did not show the presence of endothelial hyperplasia either, whereas it was shown that 23 (31.1%) of the patients who were not diagnosed with pseudoprogession had endothelial hyperplasia. In the group of patients who developed pseudoprogession, the number of patients who had endothelial hyperplasia described in their pathohistological findings was statistically significantly higher, whereas there were more patients who were not diagnosed with pseudoprogession and who did not have endothelial hyperplasia. ( $\chi^2 = 26.269$ ,  $p < 0.01$ )

## DISCUSSION

Pseudoprogession occurred in 32 patients (30.2%). When we compared our results with the literature data, we found the heterogeneity in the data related to the incidence of pseudoprogession [8, 9, 10]. The study undertaken in 2017, which included the papers written in the period from 2005 to October 8, 2014 due to the meta-analysis, published that pseudoprogession was present in 36% of patients [11]. The reason for this may be found in various criteria for its defining along with the fact that the results of a specific number of papers were based on small sample sizes. In our study, we used the RANO criteria for defining the pseudoprogession (Figure1). However, we carefully approached one specific piece of information related to the incidence of pseudoprogession occurring even within 12 weeks after

completing chemo-irradiation, because of various literature data claiming that pseudoprogression can be observed much later and that it can be observed even later than 40 weeks and more [12, 8]. Our research demonstrated that pseudoprogression occurred on an average of 4.64 months. The highest number of patients with pseudoprogression was found in the age group ranging from 51–60 years, and then in the age group ranging from 61–70 years. In a study published by Chu et al. [13], the average age of the patients with pseudoprogression was  $46.66 \pm 15.34$  years, which is similar to the results obtained in our study. Endothelial hyperplasia, commonly characterized by the formation of glomeruloid structures, represents one of the main characteristics of glioblastoma (Figure 2). It is usually located in the vicinity of necrosis and appears directionally oriented to it (Figure 3). After the analysis of all the scientific papers published so far, we have not been able to find the results dealing with the influence of endothelial hyperplasia on pseudoprogression development. The results obtained in our study demonstrated that pathohistological characteristics such as endothelial hyperplasia, are statistically significantly higher in the patients developing pseudoprogression. This phenomenon could be primarily explained in terms of disturbing the integrity and normal functions of the blood-brain barrier (BBB). Namely, one of the pathophysiological mechanisms explaining the incidence of pseudoprogression is increased permeability of the BBB [5]. The BBB is an extremely important structure which maintains the balance of the central nervous system (CNS) microenvironment and maintains the normal functioning of the brain. The BBB is constituted of endothelial cells, astrocytes, peripheral cells, macrophage, fibroblasts, neuronal cells, basement membranes, microglia and other cell types. There are many transporters on the BBB, including P-glycoprotein (P-gp). Astrocytes are involved in nerve signal transmission, nutrient transport, maintaining the balance of brain microenvironment and extracellular matrix ion balance buffering. Peripheral cells are multifunctional cells, with immune function in the CNS neurovascular unit. Peripheral cells surround the endothelial cells and play an important role in the BBB microenvironment and in maintaining the BBB function by secreting growth factors and extracellular matrix. Microglia are a kind of long-standing immune cell in the human brain. They can stimulate the opening of BBB, leukocyte extravasation, and angiogenesis. Fibroblasts, when co-cultured with glioblastoma cells, can induce production and activation of matrix metalloproteinase MMP2, and its activators membrane type 1 metalloproteinase (MT1-MMP) and MT2-MMP, which affect the growth progression of gliomas. Other cells, like endothelial cells, in the BBB microenvironment maintain the normal function and integrity of the BBB by forming tight junctions that limit transcytosis. CNS neurons bind chemicals and convey electrical signals. They can regulate the

ionic microenvironment of the synaptic and axonal regions of the nerve cell, which are essential to the nerve signal transduction. The basement membrane is attached as a support tissue to the neurovascular unit cells. The endothelial cell is the most important structural component of the BBB. Changes in the phosphorylation state of the tight junction protein (ZO-1 or occluding) are critical to the control of BBB vascular permeability. In areas of tumor environment, the endothelial cells connection is very loose and almost lacks integrity [14]. Endothelial hyperplasia is a frequent finding in glioblastoma and it is connected with an increase in nonselective transport through the BBB [15]. The major changes reflect in an increased number of endothelial cells, endothelial hyperplasia leading to function loss and volume reduction in the endothelial cells, cell form changes, tight junction damage, an increased number of vesicles, caveolae and fenestrations, the basement membrane thickening, perivascular space expansion, and the necrosis of capillary endothelial cells [16–19]. All the above-mentioned changes lead to BBB degradation. A significant degradation of the integrity and an increase in the BBB permeability in patients with endothelial hyperplasia may be the cause of contrast leaking the blood vessels which may eventually induce radiology changes described under the notion of pseudoprogression.

## CONCLUSION

Pseudoprogression is a phenomenon of great clinical significance. Distinguishing pseudoprogression from true tumor progression has significant influence on further treatment of patients with glioblastoma. Considering the fact that no accurate diagnostic method has been found so far due to which it would be possible to undoubtedly confirm the presence of pseudoprogression, the presence of endothelial hyperplasia as a pathohistological entity could be an indicator of a higher likelihood of pseudoprogression which could be used in everyday clinical practice. Nevertheless, in order to reach definite conclusions – we believe it is necessary to conduct prospective controlled studies with larger sample sizes.

## NOTE

This paper is based on Dr Marko Petrović's PhD thesis.

**Conflict of interest:** None declared.

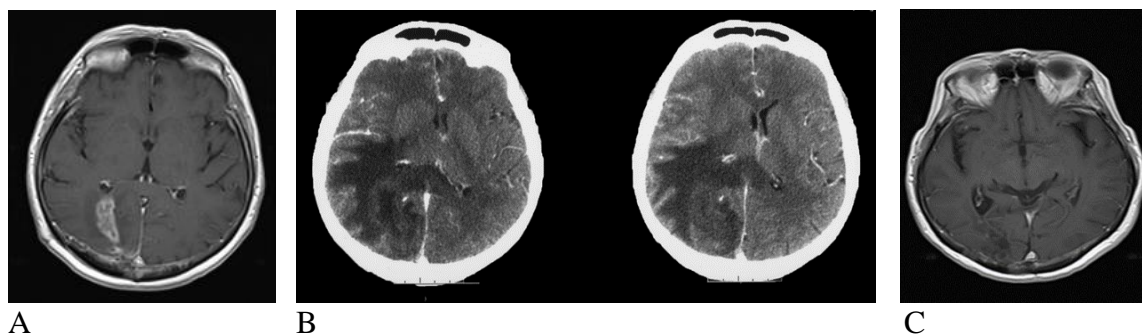
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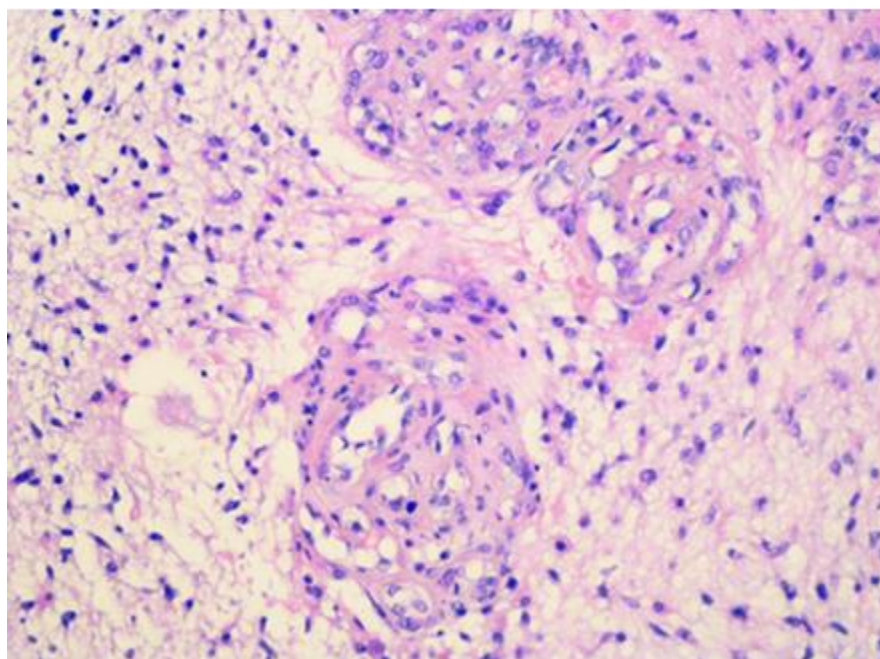
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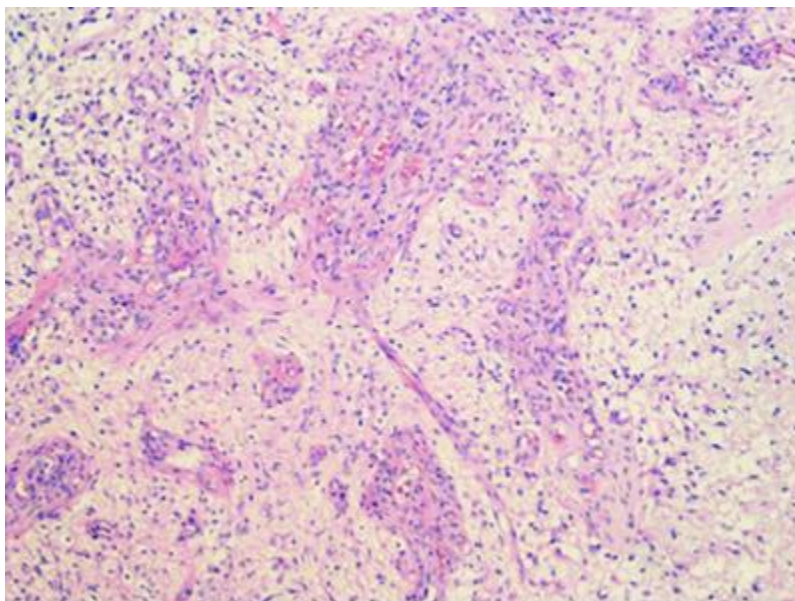
**Figure 1.** A – The first endocranial MR after treatment, radiological progression without clinical deterioration; B – endocranial CT after two months, extensive edema followed by clinical deterioration; C – complete regression after corticosteroid therapy and continuing with chemotherapy

Paper accepted



**Figure 2.** Endothelial hyperplasia, formation of glomeruloid structures (HE)

Paper accepted



**Figure 3.** Endothelial hyperplasia and necrosis (HE)

Paper accepted