

Novel Perspectives Regarding CD34 Immunoexpression Patterns in Gangliogliomas

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ABSTRACT: Gangliogliomas are extremely rare central nervous tumors composed of an admixture of glial and neuroepithelial elements. Gangliogliomas mainly affect the temporal lobe and occur in the pediatric population. There are several controversies in the scientific literature regarding these tumors, which debuted with the exclusion of grade II gangliogliomas in the 2006 edition of the current World Health Organization (WHO) classification. The upcoming edition due in the last months of 2021 is not expected to include changes regarding the current classification of glio-neuronal tumors. This vision has led to a number of articles that have pushed for the reintroduction of this category. However, these articles support the reintroduction of this degree in terms of prognosis and evolution, without providing clear criteria for the inclusion of certain gangliogliomas in this category. On the other hand, there are uncertainties about the relationship of gangliogliomas with focal cortical dysplasia. The coexistence of the two entities, as well as their succession are occasionally encountered in practice and have led to numerous studies that have tried to clarify the relationship between them. The most common and most accessible element in routine practice is the immunoreactivity for CD34. Both entities express this marker, and dual lesions express the highest percentage of immunoreactivity for CD34. In this article, we study the expression of CD34 on a series of cases including both grade I gangliogliomas and anaplastic gangliogliomas diagnosed between 2011 and 2020 in a Neuropathology Unit in Bucharest Romania.

KEYWORDS: Gangliogliomas, CD34, immunohistochemistry, prognostic marker.

Introduction

Gangliogliomas are rare benign tumors, affecting primarily the temporal lobe, that frequently occur in children or young adults.

These tumors have a biphasic structure, being composed out of a neuronal and a glial component, the latter being represented mostly by a pilocytic astrocytoma.

Currently, several immunohistochemical markers can highlight both components: for the glial component GFAP is the preferred marker, while for the neuronal component, synaptophysin, chromogranin and NeuN are preferred.

New emerging immunohistochemical markers have been recently implemented in

routine practice, in order to highlight the neuronal component: CD34 and MAP2.

The former has encountered a great acceptance worldwide, mostly due to its high accessibility.

This marker has the great advantage of being able to distinguish dysplastic or dysmorphic neurons from normal entrapped or captive neurons.

Normal cerebral cortex shows immunoreactivity for this epitope only in the walls of the vessels and only certain neuronal precursors of the CNS present in the early stages of its development express CD34.

Gangliogliomas particularly show immunoreactivity in up to 80% of cases for this marker.

This finding motivated several authors to state that gangliogliomas develop secondary to a neural stem cell differentiation defect [1].

This theory in which gangliogliomas arise from a precursor lesion, either dysplastic or a developmental disorder [2], is also supported by the fact that gangliogliomas are frequently associated with cortical dysplasia [3,4].

CD34 is described as having several staining patterns in gangliogliomas: *isolated cells*-in which rare, solitary cells are positive, which can be classified as dysplastic neurons.

They are characterized by a prominent nucleus with obvious nucleolus and frequently has markedly branched processes [5]; *clustered/bushy*-in which immunoreactive cells are organized in the form of cell groups.

These groups often have a circular contour, but linear extensions in the cortex or in the white-gray matter can be observed [6].

The clustered pattern is the most common, being reported in 55.5% [7] to 74% [4] of CD34-positive cases; *diffuse*-in which large stretches of the tumor surface show immunoreactivity to CD34 and which frequently contain dystrophic calcifications [5].

It can be found in up to 55% of cases [6];

Deb et al. observed that although not all gangliogliomas show immunoreactivity for CD34, this immunohistochemical profile is more common in females with temporal lobe gangliogliomas [6].

Methods

The present study is a retrospective analysis performed on all cases of ganglioglioma diagnosed between 2011 and 2020 at the Bagdasar Arseni Emergency Clinical Hospital, including both WHO grade I gangliogliomas and anaplastic gangliogliomas (WHO grade III).

The total number of patients included in the study totals 51 cases.

In particular, 11 of the cases included in the study have had tumor recurrences and one of those cases recurred as a high-grade tumor, namely as an anaplastic ganglioglioma.

The expression of CD34 in the neuronal cells of gangliogliomas is reputed as being connected to the epileptogenic potential of these tumors.

The expression can be encountered in three different patterns of expression: as solitary/isolated cells, cell clusters (bushy pattern) or even as diffuse expression throughout the whole tumor [5].

As a rule, CD34 expression is sensitive for the detection of vascular structures, but in

gangliogliomas in particular, the expression of this marker can be observed in the neuronal component.

In this study we used a manual immunohistochemistry technique for detecting CD34, utilizing the QBEnd-10 clone (mouse, Biocare Medical, Pacheco, CA, USA) as 1:100 in a 0.05mol/l Tris-HCl, pH 7,2 with 15mmol/l NaN₃ solution.

Briefly, the sections were deparaffinized, rehydrated, processed for antigen retrieval by microwaving in 0.1M citrate buffer pH6 for 20 minutes, incubated in 1% hydrogen peroxide in distilled water for 30 minutes to block the endogenous peroxidase activity, and kept for another 30 minutes in 3% skimmed milk in PBS for blocking unspecific antigen sites.

The primary antibodies were incubated on the slides at 4°C for 18h, and the next day the signal was amplified for 60 minutes utilizing an anti-mouse peroxidase polymer-based system (MACH 2, Biocare Medical).

The signal was then detected with 3,3'-diaminobenzidine (DAB) (Biocare Medical) and the slides were coverslipped after a hematoxylin counterstaining.

For statistical analysis, Levene's testing was utilized to assess the equality of variances, and Cramer's V testing was utilized to calculate correlations on categorical rows/columns of data.

In all cases, $p < 0.05$ was used to indicate statistical significance.

Written informed consents were obtained from all patients, and the agreement of the Ethical Committee of Bagdasar Arseni Emergency Clinical Hospital was obtained before the publication of these data.

Results

Immunohistochemical expression of CD34 was observed in 94.12% (48) of all cases (Table 1).

Among those, 35.42% showed positivity only in isolated neural cells (Figure 1 and Figure 2), 35.42% expressed positivity in cell clusters (Figure 3 and Figure 4) and 29.17% expressed diffuse positivity (Figure 5 and Figure 6).

In benign gangliogliomas, the distribution of expression types was as follows: 34.69% expressed CD34 in isolated neuronal cells, 34.69% expressed CD34 in cell clusters, and 30.61% expressed CD34 diffusely.

Table 1. CD34 immunoexpression pattern type in gangliogliomas.

CD34 immunoexpression pattern	Frequency	Percent	Cumulative percent
Negative	3	5.8	5.8
Isolated cells	17	32.7	38.5
Clusters	17	32.7	71.2
Diffuse	15	28.8	100.0
Total	52	100.0	

Table 2. CD34 immunoexpression pattern in relation to tumor location.

CD34	Tumor location in temporal lobe	Tumor location in other lobes	Total
Negative	0	3	3
Isolated cells	4	13	17
Clusters	4	13	17
Diffuse	9	5	14
Total	17	34	51

Analyzing the data presented in table II, we have observed a strong, statistically significant connection between temporal lobe location and the CD34 positivity (Cramer value $V=0.420$, value $P=0.029$).

Also noteworthy is the close association between diffuse expression pattern and temporal lobe location of the tumor.

Thus, 64.29% of all tumors that had diffuse expression pattern were located in the temporal lobe.

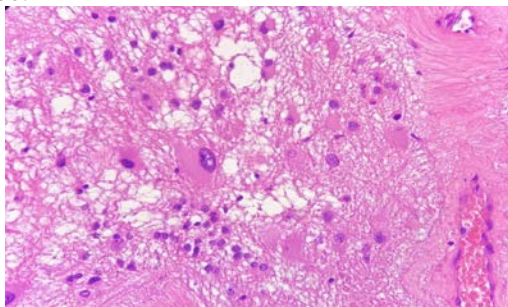


Figure 1. High power field showing isolated dysmorphic neuronal cells within ganglioglioma (H.E., 400x).

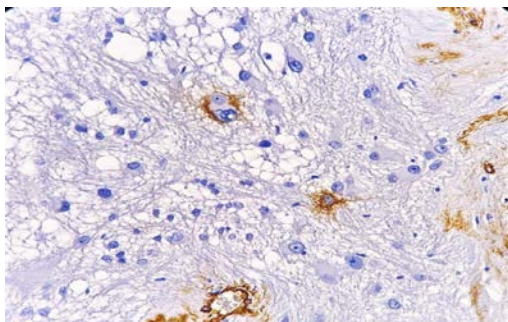


Figure 2. CD34 immunostaining revealing isolated dysmorphic neuronal cells within ganglioglioma (IHC stain with DAB chromogen, 400x).

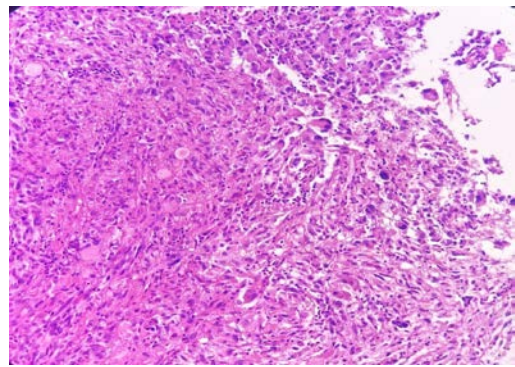


Figure 3. Medium-power field showing biphasic glio-neuronal proliferation (H.E., 200x).

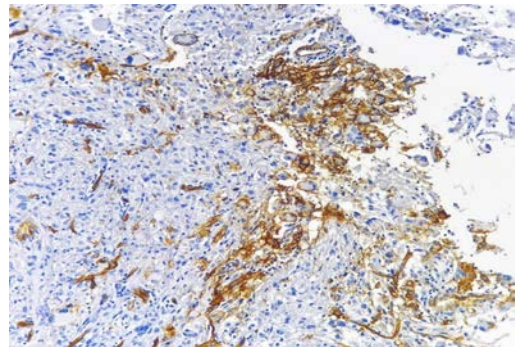


Figure 4. CD34 immunostaining showing multiple reactive neurons with a bushy/cluster-like pattern (IHC with DAB chromogen, 200x).

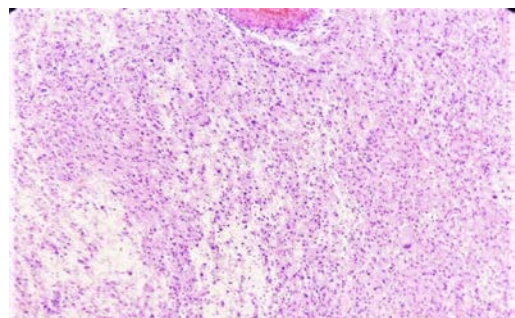


Figure 5. Medium-power field showing a low-grade ganglioglioma (H.E., 200x).



Figure 6. CD34 immunostaining showing diffuse reactivity within the entire ganglioglioma proliferation (IHC with DAB chromogen, 200x).

Immunohistochemical expression of CD34 was observed in 83.33% of anaplastic gangliogliomas.

While only 16.67% of all anaplastic gangliogliomas expressed CD34 in isolated neuronal cells, 33.33% expressed it as cell clusters and another 33% of cases expressed CD34 under a diffuse pattern.

Analyzing the dispersion of CD34 expression types based on the cellularity level of the

tumors, no statistically significant associations could be established (Cramer V=0.198, p value=0.679) (Table 3 and 4).

Similarly, the relationship between the glial components (pilocytic, diffusely infiltrative or glioblastoma) was analyzed, in relation to the types of CD34 expression.

No statistically significant association links were observed.

Table 3. CD34 immunoexpression in relation to tumor cellularity.

Cellularity	CD34				Total
	Negative	Isolated cells	Clusters	Diffuse	
Reduced	1	7	8	9	25
Moderate	0	3	4	2	9
High	2	7	5	3	17
Total	3	17	17	14	51

Table 4. Cramer analysis for data in table 3.

		Value	p-value
Nominal/Nominal	Phi	0.279	0.679
	Cramer's V	0.198	0.679
N of Valid Cases		51	

The dispersion of CD34 expression types in relation to the neural component (which could dominate or be found only focally) was analyzed and the following observations were noted: in cases where the neuronal component dominated, the solitary pattern CD34 expression was found in 23.53% of cases, the cell clusters pattern was found in 47.06% of cases, and the diffuse one in 23.53% of cases.

Similarly, in the cases with rare dysmorphic/atypical neurons, 38.24% presented only isolated cells positive for CD34, 26.47% of the cases presented groups of immunoreactive cells for CD34, and 29.41% of the cases presented the diffuse expression of this marker.

Among the cases without neuronal mitoses, 36.17% showed immunoreactivity for CD34 only in isolated cells, 34.04% in groups of cells, and diffuse expression was observed in 25.53% of cases.

Similarly, in those with identifiable neural mitoses on usual HE staining, 50% expressed diffuse CD34, 25% expressed in CD34 in groups of tumor cell and 25% did not express any immunoreactivity for this marker.

In cases with perivascular and diffuse chronic inflammatory infiltrate, CD34 expression followed the following distribution: 31.25% expressed CD34 only in isolated cells,

50% expressed CD34 in cell clusters, and 12.50% expressed diffuse immunoreactivity.

Among cases that presented only perivascular chronic inflammatory infiltrate, the immunoreactivity for CD34 was observed as follows: 34.48% in the form of solitary cells, 27.59% in the form of cell clusters, and 31.03% in the form of diffuse expression.

In the group of patients who did not clinically present epileptic seizures, 33.33% expressed CD34 in isolated cells, 42.86% expressed CD34 in cell clusters, and 19.05% expressed CD34 diffusely.

In the group of patients with epileptic manifestations, 33.33% expressed immunoreactivity to CD34 in isolated cells, 26.67% expressed CD34 in cell clusters, and 33.33% expressed CD34 diffusely.

No statistically significant associations were observed between the presence of epileptic symptoms and CD34 expression (Cramer V=0.196, p value=0.579).

The t-test analysis, which aimed to identify the differences in the Ki67 values between the immunoreactive gangliogliomas for CD34 and the ones that featured no immunoreactivity for this marker, showed statistically significant differences between the two groups (Table 5).

The mean difference was 17% between the Ki67 values of the cases featuring immunoexpression of any kind and those who did not feature any immunoreactivity for CD34 (p-value=0.0001).

Thus, high Ki67 values were more commonly encountered in gangliogliomas that did not show immunoreactivity for CD34.

Table 5. Variation of Ki67 values between the cases featuring immunoexpression towards CD34 and those showing no immunoreactivity for CD34.

	Levene Test		t-test for mean equalities		Sig. (2-tailed)	Mean difference
	F	Sig.	t	df		
Ki67 values	3.200	0.080	4.882	50	0.000	17.00000%

Additionally, analyzing the relationship between the mortality rate and the CD34 expression, it is observed that a large number of cases expressing immunoreactivity only in the form of solitary tumor cells eventually succumbed to the disease (47%).

Of the cases of CD34 immunoreactivity in the form of clustered cells, 25% died, while of those with diffuse CD34 immunoreactivity, 20% eventually passed away.

Cramer V analysis indicated that there exists a link between the presence of death and the absence of immunoreactivity for CD34 or the presence of immunoreactivity in the form of solitary cells (Cramer V=0.416, p value=0.03).

Discussion

CD34 expression has been reported in the literature in up to 80% of cases of benign gangliogliomas [8], while in the present study, the expression of this marker was observed in up to 94.2% of cases.

The patterns described in the literature (solitary, grouped and diffuse) have been reported with different prevalence in the literature.

The cluster pattern was reported in a percentage of cases varying between 55% [9] and 74% [10] of the total positive cases for CD34, while in the present study, only 34.69% had immunoreactivity in the form of this pattern.

Diffuse expression of the immunohistochemical marker CD34 has been reported in the literature in up to 55% of cases [11], while in our study only 30.61% of all cases with immunoreactivity for this marker were in the form of this pattern.

The solitary cell type of immunohistochemical expression of the CD34 marker was observed in 34.69% of the total cases that showed immunoreactivity for this marker.

In comparison, in the literature, no specific prevalence of the expression of this IHC staining pattern is reported.

In the present study, the association between diffuse expression of the IHC marker CD34 and the temporal location of the tumor is notable,

more exactly 62.29% of the cases that diffused CD34 were located at a temporal level.

Notable is the statistically significant difference between the values of Ki67 proliferation indices and the presence or absence of immunoreactivity for CD34, an aspect that has not been reported so far in the literature.

Thus, the mean value of Ki67 proliferation indices was 17% higher in gangliogliomas that did not show immunoreactivity to CD34, compared to those with this expression.

This observation could be in line with the observation made by some authors who have observed that anaplastic gangliogliomas are characterized by the absence of immunoreactivity for CD34, without confirming it, as our study also included cases of anaplastic gangliogliomas who showed reactivity towards this marker [12].

Analyzing the relationship between mortality and CD34 expression, it is observed that 47% of all cases expressing immunoreactivity only in the form of solitary tumor cells and 66% of those featuring no immunoreactivity towards CD34, eventually succumbed to the tumor.

Of the cases of CD34 immunoreactivity in the form of clustered cells, 25% suffered fatal events, while of those with diffuse CD34 immunoreactivity, 20% eventually died.

Cramer V analysis indicated a link between the mortality rate and the absence of immunoreactivity/immunoreactivity in the form of solitary cells against CD34 (Cramer V=0.416, p value=0.03).

This observation may indicate a prognostic role of CD34 expression in gangliogliomas.

This aspect contradicts the data currently present in the literature, in which the prognostic role of CD34 could not be demonstrated, possibly due to the small number of cases analyzed.

Conclusion

In conclusion, considering all the data above, the absence of immunoreactivity towards CD34 can emerge as a novel negative prognostic marker.

Immunoexpressing for CD34 revealed a statistically significant correlation between the

temporal location of gangliogliomas and diffuse expression of this marker.

The proliferation rate, revealed by the Ki67 index had significantly higher values (mean=17%) in those cases, which did not show immunoreactivity towards CD34 and in those who only showed immunoreactivity in the form of solitary cells.

This observation can be supported by the high mortality rate encountered in the patients who did not feature any expression for CD34 (47%), and in those who only had rare isolated CD34 reactive neurons (66%).

Another unresolved enigmatic connection that could be the subject of further research directions is the connection between CD34 immunoexpression and the presence of epileptic manifestations, but also their association with lesions of focal cortical dysplasia.

The present study could not demonstrate any statistically significant connection between the presence of epileptic seizures and CD34 expression, although diffuse CD34 expression is closely correlated with temporal lobe location.

Conflict of interests

None to declare.

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