

# THE GREAT IMITATOR – EMA POSITIVE GLIOBLASTOMA MULTIFORME

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## ABSTRACT

**INTRODUCTION:** Glioblastoma multiforme (GBM) has always been a diagnostic challenge for pathologists. As a rare oncological entity with astrocytic differentiation, it can manifest itself in a variety of histomorphological forms, mimic other tumors and it often has varying immunohistochemical (IHC) profiles, further challenging the process of its verification.

**MATERIALS AND METHODS:** Four pathologically verified cases of GBM, registered at the St. Marina University Hospital, Varna, Bulgaria were retrieved from the central pathological archive. The cases were tested and reviewed based on their hematoxylin and eosin (H&E) profiles and IHC reactions with GFAP used as a glial differentiation marker, Vimentin - as a positive IHC control and EMA, an epithelial marker, non-reactive in healthy brain tissue.

**RESULTS:** As expected all GBM cases had the histomorphological hallmarks of the tumor on the H&E stain. They were diagnostically positive for GFAP and had a strong positive IHC reaction with Vimentin. Three out of the four cases also revealed a varying in intensity reaction with EMA, with one case having a weak reaction in individual cells that could not be considered diagnostic and the other two cases having a diffuse positive reaction in most of the tumor cells.

**CONCLUSION:** In the age of immunohistochemistry, GBM continues to expand the set of IHC markers that react with it, although several of them such as Cytokeratin AE1/AE3 and EMA, as demonstrated in this study, should be non-reacting as they react with proteins normally present only in epithelial cells and absent in healthy brain tissue. This can often be misleading and, in certain cases, lead to histopathological misdiagnosis.

**Keywords:** GBM, IHC, EMA, CNS tumor, pathology, antibody mimicry

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## INTRODUCTION

Glioblastoma multiforme (GBM) is a World Health Organization (WHO) grade IV malignant astrocytoma, widely considered as one of the most malignant primary intracranial tumors and the most malignant one with astrocytic differentiation (1). GBM is also one of the most common entities in neuro-oncology and per some studies its incidence even outweighs that of the central nervous system (CNS) metastatic disease (2-8). GBM presents itself as a

challenge for the neuropathologist as on the hematoxylin and eosin (H&E) stain it can present itself in a wide variety of histomorphological forms, mimic other primary and even CNS metastatic tumors (9).

Even in the age of immunohistochemistry GBM remains a diagnostic challenge due to the varying immunohistochemical (IHC) profiles it often has (10-12). Further on, GBM continues to expand the set of IHC markers that react positively with it, although some of the target proteins are not found in healthy astrocytes and are hallmark proteins of other tissue and cell types, with different embryonic origin (10-12).

These specifics of GBM further increase the difficulty of distinguishing between primary and metastatic lesions in some cases and present a diagnostic dilemma for untrained pathologists.

The aim of the study was to evaluate the distinguishing capabilities of epithelial membrane antigen (EMA) for GBM and metastatic epithelial lesions, as false positive results for some other epithelial markers such as cytokeratin (CK), mainly CK AE1/AE3, in GBM tissue samples have already been reported (10-12).

## MATERIALS AND METHODS

Four cases of histologically verified GBM, registered in the St. Marina University Hospital, Varna, Bulgaria were retrieved from the central pathological archive. Their histomorphological profile and IHC reactions with glial fibrillary acidic protein (GFAP) and vimentin (Vim) were reevaluated and new slides were IHC stained for EMA, prepared from the original paraffin-embedded tissue sections.

All IHC slides were prepared using ready-to-use Dako catalogue primary and secondary antibodies and chromogen on a Dako Autostainer Link48, with the preprogrammed IHC protocols from paraffin-embedded tissue sections.

GFAP is a class III intermediate cytoskeletal filament with a 50kD molecular weight, found in all astrocytes, other glial cells, and some pericytes. The GFAP IHC stain, performed with a polyclonal rabbit antibody, was used in the context of this study, as a glioma control stain (13-14).

Vim is also a class III intermediate cytoskeletal filament with a 57kD molecular weight, found in

all cells with a mesenchymal origin, astrocytes and others, normally not expressed in most types of epithelial cells. In the context of this study, due to its wide range of positive reactions, the IHC Vim stain, performed with a murine monoclonal antibody, was used as a positive control for IHC (15).

EMA is a surface glycoprotein found on the outer cellular membrane of most epithelial cell types and some hematopoietic cells, but it is non-reacting in healthy brain tissue. As such the EMA antibody is often used in neuropathology to distinguish between epithelial metastases and primary intracranial tumors, except for ependymoma. The IHC stain was performed with a murine monoclonal antibody (16).

Digital images of the H&E and IHC slides were obtained on a Leika Aperio AT2 automated slide scanner using the pre-calibrated scanner settings.

## RESULTS

As expected all four GBM cases had a strong, diagnostically positive reaction with GFAP and Vim, very characteristic of GBM (Fig. 1).

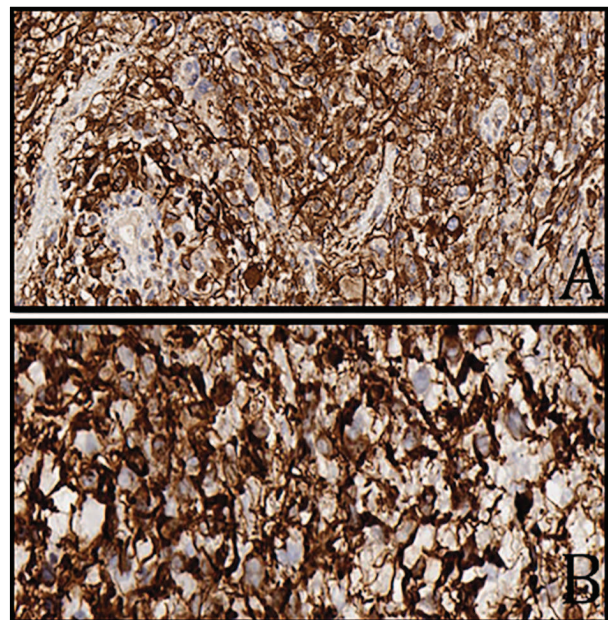


Fig. 1. IHC reaction of GBM with GFAP (A) and Vim (B). Original magnification x400

However, only one case had a completely negative IHC reaction with EMA and one of the remaining three had a patchy weak positive reaction in individual cells. The remaining two cases had a dif-



fuse weak positive reaction with EMA, which if interpreted out of the context of the H&E and other IHC stains would carry a diagnostic weight (Fig. 2). The two cases with an EMA-positive IHC reaction were however weaker when compared to the IHC reaction with GFAP and Vim on neighboring slides of the same tissue samples and had an uncharacteristic cytoplasmic reaction compared to the surface nature of the EMA molecule (Fig. 3).

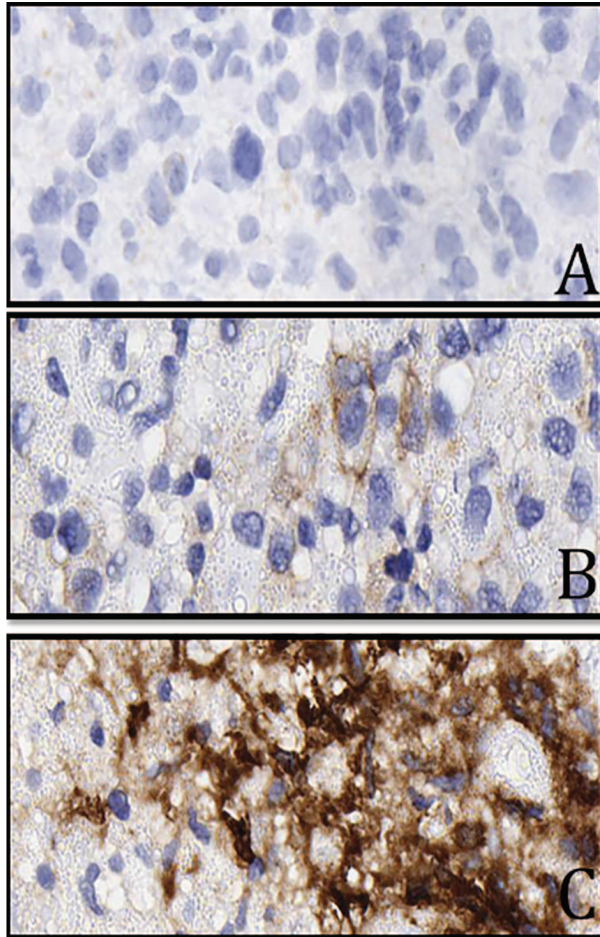


Fig. 2. IHC reaction of GBM with EMA – negative (A), weak positive (B) and strong (C). Original magnification x400

The reposted results give rise to the question why some GBM tissue samples react positively with antibodies targeting proteins normally not present and non-reacting in healthy astrocytes, which are hallmark proteins of cell of a different tissue type and embryonic origin.

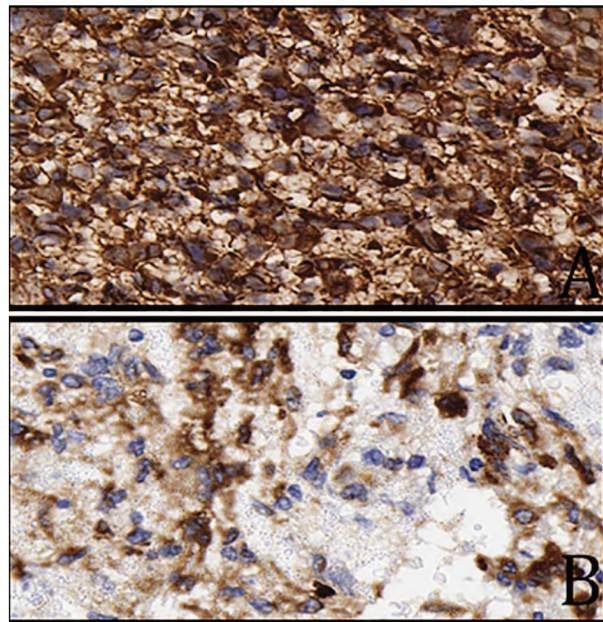


Fig. 3. Comparison between IHC reaction of GBM with GFAP (A) and EMA (B). Note the reaction with EMA is weaker than that of GFAP. Original magnification x400

## DISCUSSION

IHC, although a specific *in vitro* immunology based test, has been known to give false positive reactions in cases of three-dimensional conformational similarity between specific sequences (*epitomes*) of cellular antigens and the target antibody. In GBM, such a phenomenon has already been established between the AE3 fraction of the CK AE1/AE3 antibody cocktail (a pan-epithelial marker) and the GFAP produced from neoplastic astrocytes (10-12, 17).

Such a correlation has not yet been established for EMA. However, based on the similarity of the reported results, some similar reports, specifics of the targeted membrane molecules and cytoplasmic IHC reactions in GBM tissue samples, it seems to be highly likely that EMA is another candidate for a GBM false positive IHC reaction, not based on the true expression of EMA in GBM (18-20).

GBM has been known to have a great variety of molecular types and subtypes, which have only very recently been demonstrated to be associated with different histomorphological forms of the tumor (21-28). It is yet unknown whether these mutations would play a role in the IHC profile, but such a correlation does not seem unlikely.

## CONCLUSION

Due to the specifics of IHC, it is important to never interpret the result of one marker out of the context of H&E and other IHC markers. The IHC reaction with EMA is another candidate reaction to be included into the expanding non-diagnostic spectrum of GBM IHC reactions, which requires increased attention when interpreting the results and pathological differential diagnosis between a CNS metastatic epithelial tumor and GBM.

The reported results are important for the evasion of a pathological misdiagnosis of GBM and CNS metastatic lesions of epithelial origin and the prevention of an inadequate use of medical resources targeted at the search for a primary locus of the metastatic lesion (X-ray, CT, MRI, PET-CT, etc.), when such tumor is in fact non-existent (29).

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