CYTOKERATIN AE1/AE3 MIMICRY IN GLIOBLASTOMA

George St. Stoyanov, Deyan Dzhenkov, Peter Ghenev

Department of General and Clinical Pathology, Forensic Medicine and Deontology, Faculty of Medicine, Medical University of Varna

ABSTRACT

INTRODUCTION: The diagnosis and treatment of intracranial tumors requires a multidisciplinary approach. A key moment in this process is the pathological verification of the tumor type. This process, although aided by immunohistochemistry (IHC), can often be difficult and misleading.

MATERIALS AND METHODS: Ten histologically confirmed cases of glioblastoma multiforme (GBM) were reviewed for their IHC reaction with the anti-glial fibrillary acidic protein (GFAP) glial marker and the CK AE1/AE3 antibody cocktail, whose main use in neuropathology is to either prove or rule out metastatic cancer of epithelial origin, the primary location of which may not be known or even suspected.

RESULTS: All ten pathologically verified cases of GBM were diagnostically positive for GFAP, with eight of them also revealing CK AE1/AE3 expression with variable intensity. Out of the CK AE1/AE3 positive cases, five (50% in total) gave a low to intermediate non-diagnostic positive reaction, while the other three cases (30% in total) gave a strong positive reaction with possible diagnostic value. Cells, across all GBM cases, that tested positive for CK AE1/AE3, regardless of the strength of the reaction, were also positive for GFAP on neighboring IHC serial slides.

CONCLUSION: The presented results reveal CK AE1/AE3 expression in a great portion of GBM cases, which may be caused by three-dimensional mimicry between the CK AE1/AE3 and GFAP target molecules. This therefore necessitates the need for a careful interpretation of the results. CK AE1/AE3, however, remains a useful tool in neuropathology, regardless of the possibility of false positivity in GBM cells.

Keywords: *glioblastoma, IHC, CNS tumor, antigene mimicry*

Address for correspondence:
George St. Stoyanov
Department of General and Clinical Pathology, Forensic Medicine and Deontology
Faculty of Medicine
Medical University of Varna
55 Marin Drinov St
9002 Varna
e-mail: georgi.geesh@gmail.com

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INTRODUCTION

Glioblastoma multiforme (GBM) is a World Health Organization (WHO) grade IV malignant astrocytoma and is widely considered as the most malignant primary intracranial tumor and the second most common after meningioma (1-2). According to some studies the total incidence of GBM outweighs that of central nervous system metastatic disease (3-5).

GBM has an extremely poor prognosis, with the 5-year survival rate being around 3% (6). Some studies, however, estimate that this low frequency is due to improper evaluation of the WHO grade (7). Another consideration could be attributed to the existence of distinct molecular subtypes, which share the same histomorphological picture as GBM but have the biological potential of lower WHO grade astrocytoma (8).

GBM is a challenge for the neuropathologist and has historically earned the name multiforme as on H&E it may be presented by a wide spectrum of histological subtypes and even mimic other tumors along with its classical pattern (1) (Fig. 1). In the age of immunohistochemistry (IHC), the set of markers for GBM continues to expand, although some of them identify proteins that are not present in neuroglial cells and are characteristic in cells from a different tissue type and embryonic origin (9-15).

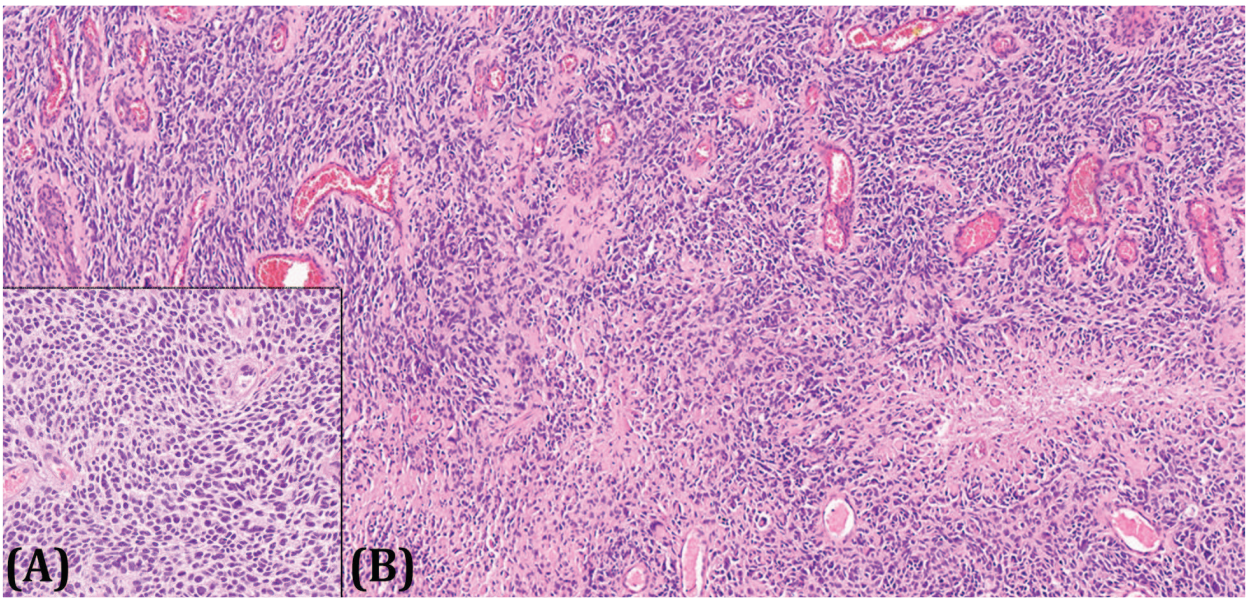

Fig. 1. Cellular and nuclear atypia of GBM – original magnification x400 (A). Classical manifestation of GBM with Scherer formations or geographical necrosis with pseudopalisadic arrangement of tumor cells around them and neovascularization with immature blood vessels – original magnification x200 (B)

This presents a further challenge for the neuropathologist as the IHC results may sometimes mislead and interfere with the correct diagnosis. Glial specific markers, such as glial fibrillary acidic protein (GFAP), give a constant positive IHC reaction and are used as a discriminating factor in the diagnostic process (1). Other less cell-specific markers such as Vimentin and S-100 also give strong positive reactions and can often be used in the diagnostic process (1). On the other hand, some non-glial markers that do not react in healthy astrocytes may give positive IHC reactions in GBM cells. One such IHC marker is the pan-epithelial cytokeratin (CK) AE1/AE3 antibody cocktail (12-15).

MATERIALS AND METHODS

Ten pathologically verified cases of GBM registered at the St. Marina University Hospital, Varna, Bulgaria in the period July-November, 2015 were retrieved from the central pathology archive. All cases were reviewed on H&E specimens. IHC slides were prepared using formalin-fixed, paraffin-embedded tissue sections on a DAKO AUTOSTAINER Link 48 using DAKO catalogue ready-to-use primary monoclonal mouse anti-human CK AE1/AE3 and polyclonal rabbit anti-GFAP, secondary antibodies and chromogen. Digital images of the slides were obtained using a Leica Aperio AT2 automated digital slide scanner, using the pre-calibrated settings.

The IHC slides were then reviewed based on the intensity of the reaction with GFAP, used as a pos-

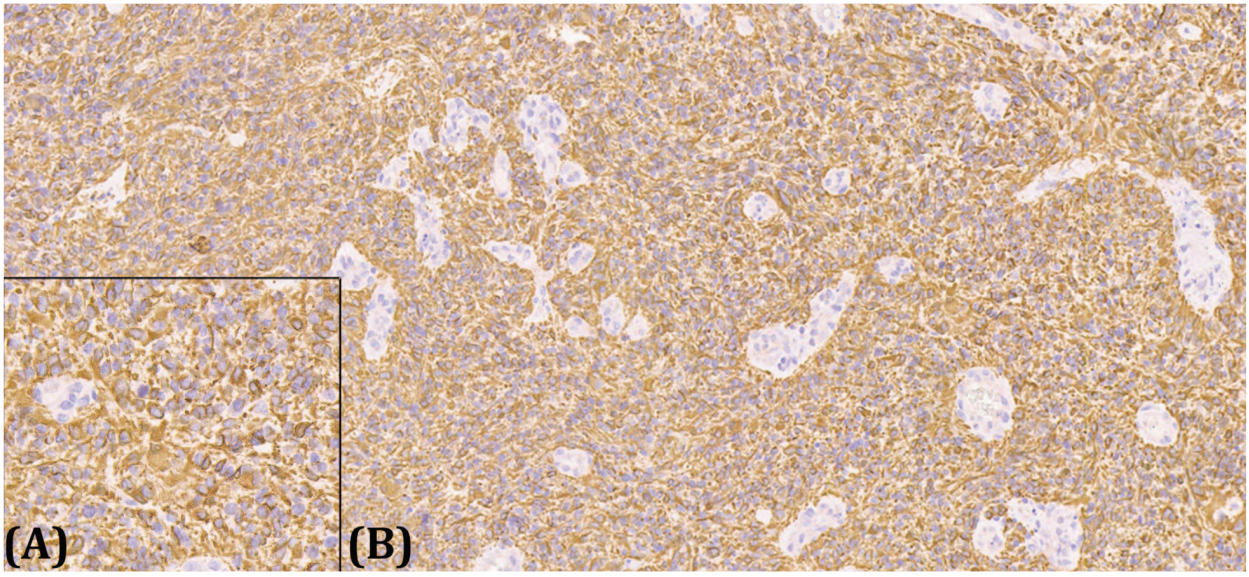

Fig. 2. Expression of GFAP in GBM – original magnification x400 (A) and original magnification x200 (B)

itive control. Their CK AE1/AE3 profiles and were later compared.

RESULTS

As expected, all ten of the GBM cases showed intensively positive cytoplasmic reaction for GFAP (1) (Fig. 2). Only two GBM, however, remained completely negative for the CK AE1/AE3 antibody mixture.

Eight out of the ten GBM cases revealed a varying in intensity reaction with the pan-epithelial CK AE1/AE3 antibody cocktail, which is non-reactive in normal astrocytes. Five of those eight cases gave a weak diffuse positive reaction across all tumor cells or a patchy reaction in individual tumor cells with the CK AE1/AE3 antibody, which would normally carry no diagnostic value if reviewed by a pathologist with experience in working with IHC (Fig. 3).

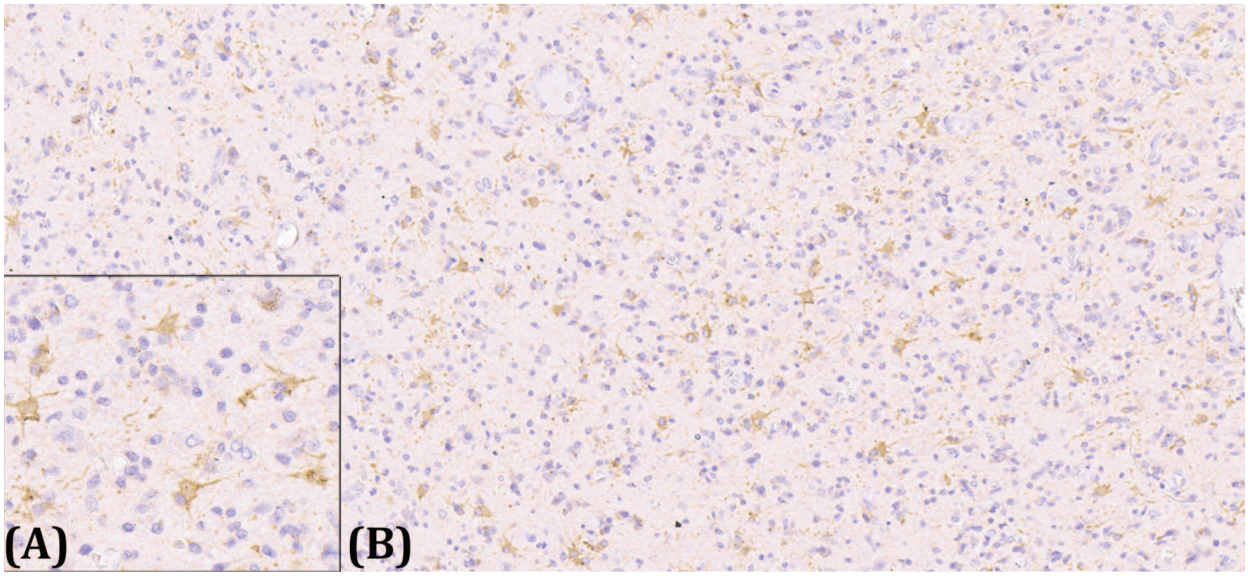

Fig. 3. Expression of CK AE1/AE3 in individual GBM cells, without diagnostic significance – original magnification x400 (A) and original magnification x200 (B)

The three remaining cases of GBM, however, gave a strong positive reaction with CK AE1/AE3 that would carry possible diagnostic significance, if reviewed out of the context of GFAP (Fig. 4). In these cases, the positive reaction with CK AE1/AE3 was weaker when compared to that with GFAP (Fig. 5).

On neighboring IHC it was well visible that the cells positive for CK AE1/AE3 were also positive for GFAP (Fig. 5).

DISCUSSION

The main questions that arise from the results are whether neoplastic astrocytes in GBM start expressing CK molecules, or if there is some other reason for GBM to react with the CK AE1/AE3 antibody cocktail. Also, as IHC is not the most specific immunology-based diagnostic test, do other immunology-based protein tests confirm these results?

Cytokeratins are a type I - acidic and type II - basic intermediate cytoskeletal filaments found in all epithelial cells but are not expressed in healthy brain tissue. CK AE1/AE3 is a pan-cytokeratin antibody cocktail, used in pathology for the detection of these molecules and therefore used for the IHC detection of all cells with epithelial origin and the differential diagnosis between epithelial and non-epithelial tumors. The AE1 fraction of the antibody detects type I cytokeratins – the high molecular weight 10, 14, 15, 16 and the low molecular weight 19, while the AE3 fraction detects type II cytokeratins – the high molecular weight 1, 2, 3, 4, 5, 6 and the low molecular weight 7 and 8 (16).

GFAP is type III intermediate cytoskeletal filament found in all neuroglial cells. All central and peripheral nervous system glial tumors express GFAP and therefore the antibody is used as a discriminat-

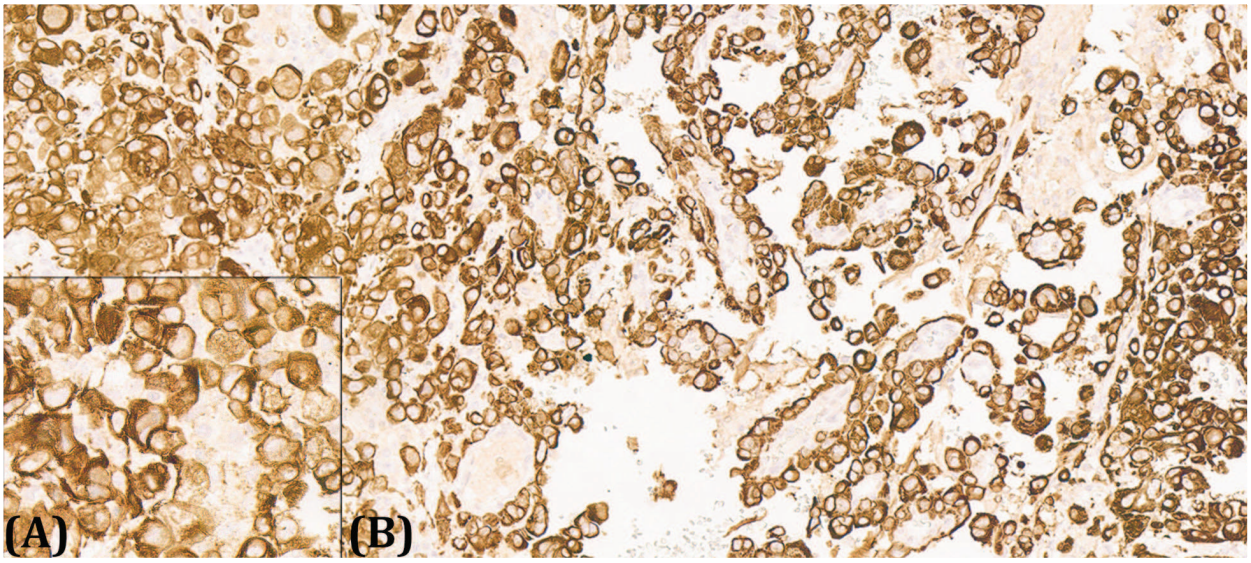

Fig. 4. Expression of CK AE1/AE3 with diagnostic significance in GBM – original magnification x400 (A) and original magnification x200 (B).

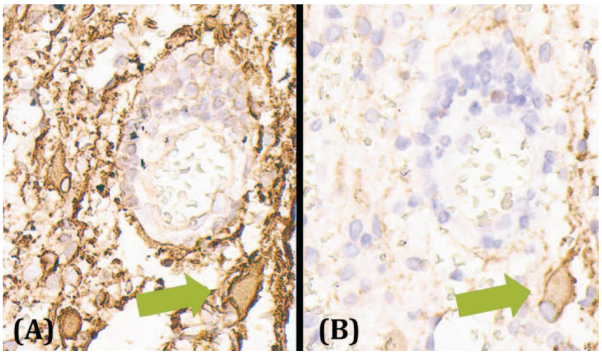

Fig. 5. Co-expression for GFAP (A) and CK AE1/AE3 (B) in the same cell (arrows), on successive IHC sections– original magnification x400. The reaction with CK AE1/AE3 although positive is weaker when compared to the GFAP reaction.

ing factor for tissue of glial origin and differential diagnosis between glial and non-glial tumors (17).

Although some studies state that up to 96% of GBM cases have positive reactions with CK AE1/AE3, these studies do not supplement detailed photographic materials and instead focus only on individual cells. Up to 96% of GBM cases might have positive reactions with CK AE1/AE3 in individual cells, but the reaction itself has no diagnostic value (13-15).

Although some research teams agree that the results are based on the production of cytokeratin molecules by neoplastic astrocytes, they are based only on IHC of a large number of GBM cases and are only a guess, not supported by results from other, more specific tests (13-15).

Evidence, based on immunoblot tests, showed that neoplastic astrocytes in GBM do not produce cytokeratin molecules and that the results on IHC are based on cross reactivity between the GFAP produced by the neoplastic astrocytes and the AE3 fraction of the CK AE1/AE3 antibody cocktail (12).

Furthermore, GBM has been reported to have a large number of molecular subtypes, with different clinical manifestations and prognosis (8,18-20). It is yet unknown if these molecular subtypes have an effect on the continuously expanding IHC profile of GBM.

CONCLUSION

IHC alone does not replace the need for an experienced neuropathologist, who has been trained to work in the field and interpret the results. IHC should never be interpreted out of context, based on the results of one marker alone, regardless of the data from H&E, other classical stains and the clinical manifestations of the disease. Interpretation of the results is an indispensable part of the process.

Many GBM cases may have a positive reaction with CK AE1/AE3. These reactions, however, are reportedly based on the AE3 antibody fraction of the CK AE1/AE3 cocktail recognizing and reacting with the GFAP molecules produced by the neoplastic astrocytes in GBM and not on their production of CK molecules. A similar phenomenon may be observed with some other epithelial markers that have variable IHC reactions with GBM cells (11).

The value of the reported results is key in the evasion of a pathological misdiagnosis of GBM with central nervous system metastatic disease from epithelial origin in cases of CK AE1/AE3 IHC cross reactivity. This would prevent the inadequate use of medical resources such as ultrasound, X-ray, CT, MRI, PET-CT, endoscopy, tumor markers, additional biopsies and others routinely used to pinpoint the primary location of epithelial tumors, while such location is non-existent (21).

Therefore, CK AE1/AE3, while still an extremely valuable tool in clinical pathology, should not be used out of the context of GFAP when verifying intracranial and other suspected glial tumors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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