British Journal of Medicine & Medical Research
4(1): 95-103, 2014

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Malignant Mesothelioma vs. Reactive Mesothelial Proliferations: Immunohistochemical Profile

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Authors' contributions

All the authors concur with the submission. All the authors of this protocol have made a substantial contribution to its conception and design, data collection, analysis and interpretation. All authors read and approved the final manuscript.

Research Article

Received 6th February 2013
Accepted 20th May 2013
Published 14th September 2013

ABSTRACT

Aims: To examine the distribution of immunohistochemical markers GLUT-1, EMA (membrane epithelial antigen) and Ki-67 in benign and malignant mesothelial lesions. Thus, the sensitivity, specificity, positive and negative predictive value of these markers, used alone or in conjunction, was established.
Study Design: Observational, retro-prospective and non-randomized study.
Place and Duration of Study: Department of Pathology, Center for Medical Education and Clinical Research "Norberto Quirno" (CEMIC), between 2004 and 2011.
Methodology: A total of 53 cases diagnosed as mesothelioma (n=15) or reactive mesothelial hyperplasia (n=38) were selected. Routine techniques using hematoxylin-eosin and immunostaining with EMA, GLUT-1, and Mib-1 were performed.
Results: Mesotheliomas cohort was immunoreactive for GLUT-1 in 11/15 (73%) cases, and for EMA in 13/15 (87%) cases. The group of reactive lesions was positive for GLUT-1 in 2/38 cases (5%), and positive for EMA in 7/38 (18%) cases. The median proliferation rate was 1% in benign lesions and 3% in mesotheliomas. The sensitivity and specificity for EMA was 87% and 82% respectively, with a positive predictive value of 65% and a

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negative predictive value of 94%. The sensitivity and specificity for GLUT-1 was 73% and 95% respectively, with a positive predictive value of 85% and a negative predictive value of 90%.

Conclusion: EMA and GLUT-1 are sensitive and specific markers that express more frequently in mesothelioma than in benign mesothelial lesions with higher specificity in the case of GLUT-1 for the detection of malignant proliferations. In a mesothelial proliferation without invasion criteria, EMA and GLUT-1, including histopathology, may be sensitive and specific markers to define malignancy. Thus, a morphologically doubtful mesothelial proliferation with positive staining (diffuse and intense), for these antibodies could be a mesothelioma. However, the evidence of underlying tissue infiltration by mesothelial cells currently remains the gold standard for diagnosis of mesothelioma. Both markers should be included in the immunohistochemical panel to distinguish benign from malignant mesothelial lesions.

Keywords: Mesothelioma; GLUT-1; EMA; mesothelial hiperplasia; immunohistochemistry.

1. INTRODUCTION

Malignant mesothelioma is an aggressive tumor of serosal surfaces, such as the pleura or peritoneum [1], with short survival [2]. Although this is a rare tumor, its incidence is increasing worldwide inter alia, by exposure to asbestos. Patients undergoing no treatment are most likely to die within 6 to 18 months [3]. The response to chemotherapy is modest, yielding the best results through the combination of therapies, which offer a median survival of 23 months. Surgery (pleuroneumonectomy) is only performed on certain occasions at qualified health centers [4], in patients with early stages, good performance status and favorable histology. Depending on imaging studies, chemoterapy with pemetrexed and cisplastin prior to surgery are added as well as radiotherapy to reduce symptoms.

Patients with epithelioid subtype experience longer survival than those with other subtypes (sarcomatoid, biphasic). However, a strong variability in survival and response to therapy has been observed, which is not only associated with stage and histologic subtype, but with biological variability [5]. Reactive mesothelial proliferations can mimic the appearance of mesothelioma. High cellularity, presence of mitotic figures, cytologic atypia, necrosis, papillary group formation and mesothelial cells entrapment in the fibrous tissue simulating invasion often make it difficult the differential diagnosis between mesothelioma and reactive mesothelial hyperplasia, especially in biopsies, small surgical specimens or cytologic samples. On the other hand, the obvious invasion of surrounding tissues by tumor cells is the gold standard for mesothelioma diagnosis [6-8]. Cagle et al. [9] have made a good revision of criteria used to distinguish between these lesions. According to them and in addition to the features named previously, the presence of capillaries that is parallel to each other and perpendicular to the pleural surface favor a benign diagnosis. They also state that benign reactive mesothelial proliferations tend to demonstrate zonation; that is, the proliferating cells are found toward the pleural surface and the deeper tissues are more fibrotic. Finally, they recommend the use of the term *atypical mesothelial proliferation* or *atypical mesothelial hyperplasia* be used for the diagnosis and additional tissue obtained if clinically indicated [9].

Several studies analyzed the utility of immunohistochemical markers such as desmin, EMA, and p53 protein to discriminate between mesothelioma and reactive hyperplasia. Although none of these markers have been shown to make a clear discrimination [8], desmin and

EMA proved to be the most useful ones. Reactive mesothelial cells are positive for desmin and negative for EMA, while the reverse pattern would be observed in malignant mesothelioma cells. However, these markers have a sensitivity and specificity lower than 90% [10-11]. EMA staining with intense membranous pattern is highly suggestive of mesothelioma [12-13]. Of the most commonly available anti-EMA antibodies, E29 has a significantly higher specificity than MC-5 (100% vs. 40%) [13].

It has been recently suggested that glucose transporter type 1 (GLUT-1) could serve as an immunohistochemical marker for malignant transformation of mesothelial cells [11]. GLUT-1 is undetectable in normal epithelial tissue and benign tumors, but is expressed in a variety of malignant tumors, including carcinomas of breast, head and neck, bladder, kidney and lung [8]. GLUT-1 is widely distributed in normal tissues such as erythrocytes, endothelial cells of the blood-brain barrier, perineurium, renal tubular cells and activated lymphocytes of the germinal centers. Several studies have shown a close relationship between GLUT-1 expression and carcinogenesis, tumor development and a poor prognosis in some tumors. Differential expression in malignant and premalignant or benign ovarian, endometrial, pancreatic and mammary gland lesions has also been demonstrated [14]. In recent years there has been a growing interest in the search of an antibody that is highly sensitive and specific for the diagnosis of mesothelioma, especially in small samples or paraffin-embedded cytologic material. In this regard, EMA and GLUT-1 are reported as two of the most effective markers for the differentiation between malignant and benign mesothelial cells. Recently, a paper published by Lee at al. [15] evaluated the performance of GLUT-1 and IMP3 (a marker highly expressed in lung carcinomas and mesotheliomas) alone or in combination and found that the coexpression of both markers helped them to identify malignant mesotheliomas with a good specificity. They found that IMP3 was positive in 53% of malignant and 27% of benign cases (P=0.03), whereas GLUT-1 was positive in 60% of malignant and 13% of benign cases (P=0.0003). Fortythree percent of malignant cases, but only 4% of benign cases, were positive for both IMP3 and GLUT-1 (P=0.00003) [15].

Assessment of proliferation index has also been used to discriminate between benign and malignant pleural lesions, with a significantly higher expression of MCM2 and Ki-67 in mesotheliomas than in reactive mesothelial hyperplasia, and better results obtained with MCM2 [11-16]. Taheri et al. [17], assessed Ki-67 and other proliferation marker called repp86, and demonstrated that used in combination they are useful to discriminate between malignant mesothelioma and reactive mesothelial cells. Using a cut-off of 9% they could differentiate both lesions with a sensitivity and specificity of about 90% [17].

2. MATERIALS AND METHODS

Paraffin blocks of biopsy or surgical resection specimens diagnosed as reactive mesothelial cells, benign mesothelial hyperplasia and malignant mesothelioma between 2004 and 2011 were selected from the Pathology Laboratory registries at the Center for Medical Education and Clinical Research "Norberto Quirno" (CEMIC). The study group consisted of 53 subjects: 38 men (72%) and 15 women (28%). A total of 38 cases were diagnosed as benign, reactive mesothelial hyperplasia (72%), and 15 as mesotheliomas (28%). Enough representative material was obtained in all cases. Mesotheliomas were all from pleural origin and were classified into epithelioid type (11), sarcomatoid (2), desmoplastic (1), and biphasic (1). Mean age was 61 years (range 22-74). Regarding benign lesions only, mean age was 61 years, with 27 (71%) males and 11 (29%) women. Mesotheliomas were reported in 9 (60%) males and 6 (40%) women, mean age 62 years. In all cases, clinical records and histopathological diagnoses of benignity or malignancy were confirmed by patient

examination. Diagnoses were all made based on the morphology observed in hematoxylin-eosin (HE) stained sections, using the immunohistochemical techniques currently available. The mesothelial origin of both malignant and benign proliferations was confirmed by the immunohistochemical panel, including antibodies to calretinin, cytokeratin 5/6, WT-1, Ber-EP4, thyroid transcription factor (TTF-1) and carcinoembryonic antigen (CEA). Four-to-five micron sections underwent HE staining, while 3-4 micron sections underwent immunohistochemistry. The tissue was mounted on previously silanized slides and was gradually rehydrated with ethanol. Endogenous peroxidase was blocked with an inhibitor solution of methyl alcohol and hydrogen peroxide Vol 30, for 20 minutes, then held antigen retrieval in a microwave oven, using citrate buffer (pH = 6) for 15 minutes at maximum power and 15 minutes at 50% power. After cooling the solution, sections were rinsed with distilled water and placed in TBS solution. Then, normal horse serum (MP detection kit 7500-VECTOR LABORATORIES INC.-Burligame, CA 94010, USA) was applied for 30 minutes. Then, the appropriate primary antibody was incubated for 2 hours in a humid chamber. After TBS / Triton washings, a visualization reagent consisting of a dextran polymer and peroxidase-conjugated anti-rabbit immunoglobulins mouse was used for 30 minutes. Immunoreactions were detected by the labeled streptavidin biotin method, and visualized with 3,30-diaminobenzidine, followed by counterstaining with hematoxylin.

The primary antibodies used were GLUT-1 (polyclonal, Abcam), EMA (E29, Dako) and MIB1 (Ki67, Biogenex). Immunostaining was interpreted in each case according to previous descriptions, using appropriate positive and negative controls, and including red blood cells as internal positive control for GLUT-1. A cut-off higher than 5% of mesothelial cells / tumor was required to define positive staining [18]. The area of positive staining for GLUT-1 and EMA was evaluated using a semiquantitave scale: negative (<5%), focal positive (5-50%) and diffuse positive (50-100%), as a representation of the percentage of cells with positive membranous staining among the population of mesothelial cells, as described by others [2].

3. RESULTS AND DISCUSSION

Positive staining for GLUT-1 was observed in 11/15 cases of malignant mesothelioma. Of these 11 cases, 8 were diffuse and 3 were focal. The immunoreactivity pattern was membranous, with moderate to severe intensity. Weak staining for this antibody was observed in only one case. The 4 negative cases were diagnosed as epithelioid mesothelioma, one of them being well-differentiated. Furthermore, immunostaining for EMA was positive in 13/15 cases, focal in 4/13 cases and diffuse in 9/13. The type of labeling observed was similar to that of GLUT-1. The two cases were negative for EMA and positive for GLUT-1, while 4 patients negative for GLUT-1 expressed EMA. In 9/15 cases positive staining was observed for both antibodies. No cases were negative with both markers. Regarding benign lesions, 2/38 cases were positive for GLUT-1 (one focal and one diffuse), and 7/38 cases showed staining for EMA. Of these, 4/7 were focal and 3/7 diffuse (Fig. 1).

In cases with more severe reactive mesothelial hyperplasia, staining with GLUT-1 was negative (0/4), whereas EMA was positive, at least focal and of variable degree, in 2 cases. Both markers were positive in 2/38 cases. Thirty one cases were negative for both markers, and 5 had positive staining for at least one of the antibodies (EMA) (Table 1).

Regarding positive staining for GLUT-1, sensitivity was 73% and specificity 95%, positive predictive value 85% and negative predictive value 90% (Table 2). Considering only those cases with diffuse positive staining, sensitivity was 60% and specificity 97%, positive predictive value 90%, and negative 86%.

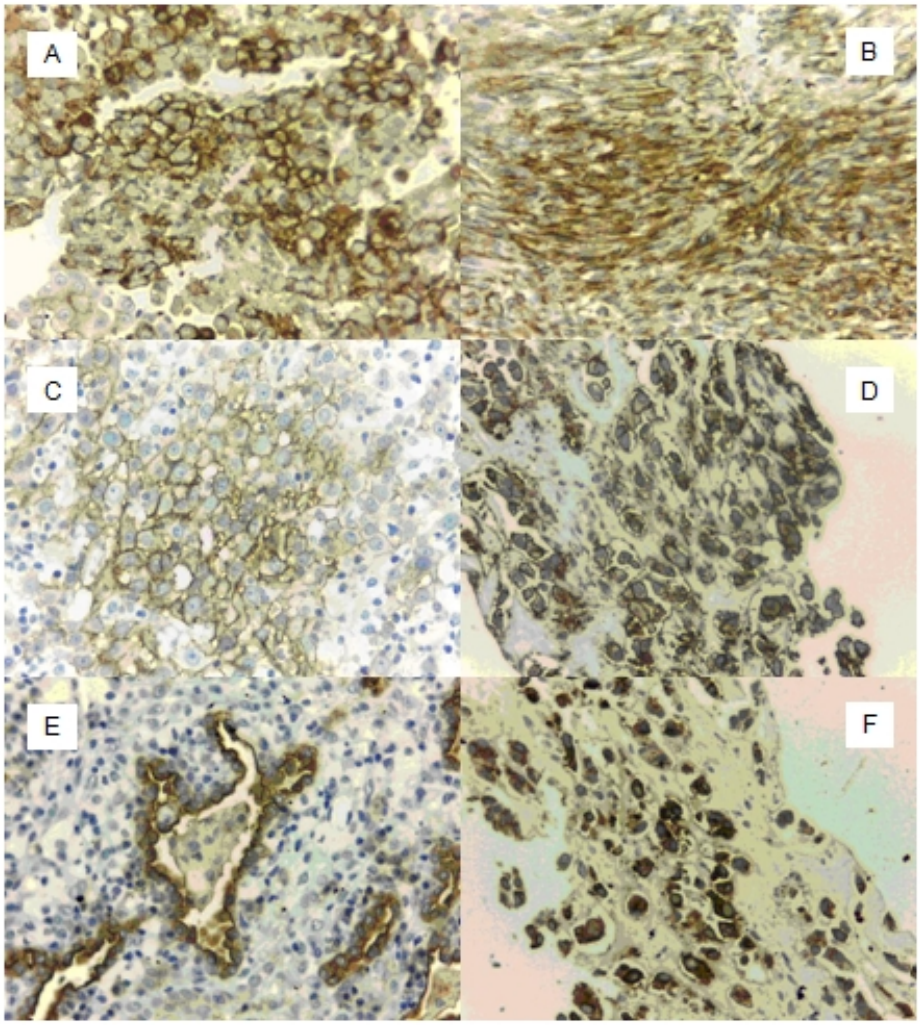

Fig. 1. Immunostaining with GLUT-1 (400X): Diffuse positive: A) Epithelioid malignant mesothelioma. B) Sarcomatoid mesothelioma. C) Reactive mesothelial hyperplasia. Focal positive: D) Epithelioid malignant mesothelioma. Immunostaining with EMA (400X): Diffuse positive: E) Epithelioid malignant mesothelioma. F) Reactive mesothelial hyperplasia.

Table 1. Staining pattern with GLUT-1 and EMA

		Mesotheliomas (n=15)	**Hyperplasias (n=38)**
Staining pattern with GLUT-1	*Positive diffuse*	9/15 (60%)	1/38 (3%)
	Positive focal	2/15 (13%)	1/38 (3%)
	Negative	4/15 (27%)	36/38 (95%)
Staining pattern with EMA	*Positive diffuse*	9/15 (60%)	2/38 (5%)
	Positive focal	4/15 (27%)	5/38 (13%)
	Negative	2/15 (13%)	31/38 (82%)

Table 2. Statistical Parameters of Immunostaining

	EMA	GLUT-1
Sensitivity	87 %	73 %
Specificity	82 %	95 %
Positive predictive value	65 %	85 %
Negative predictive value	94 %	90 %

Regardless of type of staining (either diffuse or focal), EMA had a sensitivity and specificity of 87% and 82%, respectively, with a positive predictive value of 65% and a negative predictive value of 94% (Table 2). Considering only those cases with diffuse positive staining, sensitivity decreased to 60% but specificity increased to 95%, with a positive predictive value of 82% and a negative predictive value of 86%.

The 2 cases of sarcomatoid were positive for EMA and negative for GLUT-1. On the other hand, the case of desmoplastic mesothelioma showed reactivity for GLUT-1 only.

From small biopsies with mesothelial hiperplasia that do not shown tissue invasion, 5% were positive for both markers. Because most of the malignant lesions are positive for these markers, a diagnosis of benignity should be made with caution. We believe that in such cases it should be recommended to obtain new tissue samples in order to exclude tissue invasion.

Regarding positive staining with at least one of the markers and any proportion of positive cells, sensitivity was 100% and specificity 82%. The median proliferation index (Ki-67) was 1% in benign lesions (p25 = 1%, p75 = 2%), with a range from 1 to 10% (mean 2%), and 3% in mesotheliomas (p25 = 2%, p75 = 5%), with a range from 1 to 20% (mean 5%), showing a statistically significant difference between groups ($P < .001$) (Wilcoxon test, Mann-Whitney).

GLUT-1 is a transmembrane glucose transporter protein that contributes to the facilitated glucose diffusion into cells, and is normally expressed in tissues that rely primarily on this substrate for the production of metabolic energy. Immunoreactivity for GLUT-1 has been shown to have diagnostic value in benign changes, premalignant and malignant endometrium. It has been demonstrated that endometrial hyperplasia is always negative, while atypical hyperplasias are positive in 71% of the cases [14]. In recent years, researchers have focused on molecular immunohistochemical markers for the diagnosis of mesothelioma. Some studies have shown that GLUT-1 expression is useful for the differential diagnosis between benign and malignant mesothelial lesions. Using immunohistochemistry, Godoy et al. [19], analyzed the coexpression of GLUT-1 and other isoforms (GLUT-2 to 6 and GLUT-9) in various benign and malignant tumors, and showed that 2 out of 4 cases of malignant mesothelioma were positive for GLUT-1 [19]. Kato et al. [8], evaluated the utility of GLUT-1 detection for the differential diagnosis between benign reactive mesothelium and malignant mesothelioma. They concluded that it is a sensitive and specific marker that can differentiate the two lesions, with 100% of the studied cases of malignant mesothelioma being positive for GLUT-1 (40/40), and all the cases of reactive mesothelium being negative (0/40) [8]. In a study on the expression profile of desmin, p53, EMA and GLUT-1 in normal hyperplastic mesothelium and mesothelioma, Acurio et al. [20], concluded that EMA and GLUT-1 had a similar sensitivity and specificity to detect malignant cells. However, they found 34% and 20% of malignant lesions that were negative with the respective markers, with 100% of benign lesions being negative for GLUT-1 [20]. In general, published studies indicate that positive GLUT-1 is a useful marker for malignant

mesothelioma, which is not the case of negative GLUT-1. In a study performed in paraffin blocks from pleural, pericardial and peritoneal fluid, Hasteh et al. [10], observed that EMA staining was useful for differential diagnosis, with results similar to those in the literature. On the other hand, GLUT-1 showed a sensitivity and specificity much lower than that reported by Kato et al. [8], in tissue specimens [10]. In an immunocytochemical study, Ikeda et al. [21], showed that antibodies specific for IMP3, GLUT-1 and EMA for pleural effusion and ascites are a useful tool to differentiate in these samples malignant cells from reactive mesothelial elements, with high sensitivity and specificity [21]. Most reactive lesions are negative for GLUT-1, while mesotheliomas are positive for this marker. In atypical hyperplasia with diffuse positive staining for EMA, GLUT-1 was negative. Thus, GLUT-1 needs to be added in the panels, since it also detects a greater number of malignant lesions. In our work we have found a high rate of EMA and GLUT-1 expression in malignant mesothelial proliferations, similar to that reported in previous publications. Both markers have high sensitivity and specificity, with greater specificity observed in the case of GLUT-1. As of immunostaining type of evaluation, we deem it convenient to discriminate between focal and diffuse positive staining, since it allows good sensitivity with acceptable specificity. Our work shows that there is a more frequent expression of the immunohistochemical markers GLUT-1 and EMA in malignant lesions, and that would be potentially useful as an adjuvant method to morphology to discriminate between mesothelioma and hiperplasia if there are not found all the invasion criteria. Further studies are required to confirm or refuse ours and others findings.

Atypical mesothelial hyperplasias, which are the lesions most difficult to diagnose, were positive for EMA and negative for GLUT-1. Therefore, the immunohistochemical panel is of diagnostic value because these lesions are the most difficult to interpret.

4. CONCLUSION

EMA and GLUT-1 are sensitive and specific markers that are frequently expressed in mesothelioma and may serve as a complement technique to discriminate between benign mesothelial lesions and mesothelioma. In a mesothelial proliferation without invasion criteria, co-expression of EMA and GLUT-1, including histopathology, could be sensitive and specific markers to suggest malignancy.

Thus, a morphologically doubtful mesothelial lesion with positive staining (diffuse and intense), for these antibodies could be a mesothelioma. However, the evidence of underlying tissue infiltration by mesothelial cells currently remains the gold standard for diagnosis of mesothelioma. Both markers should be included in the immunohistochemical panel to distinguish benign from malignant mesothelial lesions. We emphasize that no therapeutic decisions should be made in the absence of tumor invasion and that EMA and GLUT-1 can be used for screening and to support requests for a more representative biopsy or, alternatively, close follow-up of positive cases. We believe that the information apported by an immunohistochemistry panel may help to make a diagnosis of malignancy but it should be interpreted cautiously in and adecuate morphological and clinical context.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

We thank Myrthala Duguid (Director CEMIC Reference Library), Juan A. Gili for statistical advice, and Valeria Melia for language revision.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=215&id=12&aid=2003