Immunohistochemical detection of Glut-1 helps to distinguish between reactive mesothelium and malignant mesothelioma

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Abstract
Objective: The distinction between benign mesothelial cells and malignant mesothelial cells in fluid cytology is a common scenario facing pathologists. This study aims to examine the usefulness of Glut-1 as a tool in making this distinction when applied to cell block material. Glut-1 staining was interpreted in conjunction with desmin. Glut-1 staining in mesothelioma was compared to epithelial membrane antigen staining.

Methods: 34 cytology cases (17 benign and 17 mesothelioma) reported at Labplus between 2002 and 2009 were reviewed. Glut-1, desmin and epithelial membrane antigen immunohistochemistry were applied to the cell blocks. All cases in both categories had the diagnoses confirmed histologically. The staining results were then divided into negative, focal positive (<30% staining) and diffuse positive (>30% staining).

Results: Glut-1 positivity was seen in 82.4% of malignant mesotheliomas. Only one case of mesothelioma showed focal weak positivity for desmin while the other 16 cases were completely negative. All 17 cases of benign mesothelial proliferation were negative for Glut-1. Of the 17 cases of benign mesothelial proliferation, two showed focal positivity for desmin, four were negative, while the rest were all strongly positive.

Conclusion: Glut-1 immunohistochemistry was proven to be a useful adjunct to routine fluid cytology in patients with possible mesothelioma. Negative staining with Glut-1 does not rule out mesothelioma, especially when clinical suspicion is high. Glut-1 is best interpreted together with other markers including epithelial membrane antigen and desmin.

Keywords: mesothelioma, mesothelial hyperplasia, Glut-1, desmin, epithelial membrane antigen.

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Introduction
The finding of atypical mesothelial cells is a common encounter in fluid cytology. However, distinguishing reactive mesothelial cells from malignant mesothelial cells is often difficult in cytology. Until recently, histochemical stains and electron microscopy were the standard tools used for the diagnosis of malignant mesothelioma. With the advent of immunohistochemistry, pathologists now rely on immunohistochemical markers as adjuncts to routine histology when it comes to diagnosing malignant mesothelioma. Unfortunately, no absolutely specific or exclusive antibodies have been yet identified for such a diagnosis. Epithelial membrane antigen (EMA) and desmin are two antibodies frequently used for the purpose. Recent literature has examined the reliability of Glut-1 in discriminating between reactive and malignant mesothelium in histological sections. This study evaluated the usefulness of Glut-1 when applied to cytology samples.

Materials and methods
Case selection

The materials for the present study were extracted from cases reported by the Labplus cytology department, Auckland, New Zealand, between 2002 and 2009. The benign mesothelial cell group comprised of six cases of pleuritis/pleural plaque, two cases of pericarditis, one case of multiloculated benign peritoneal inclusion cyst and eight cases of reactive mesothelial proliferation in the context of gynaecological malignancy staging (peritoneal washing). The malignant group was composed of 17 cases of malignant mesothelioma, one of which was sarcomatoid and one showing mixture of sarcomatoid and epithelioid patterns. The diagnosis in all cases was confirmed on the basis of conventional histopathologic features evident in subsequent hematoxylin and eosin stained tissue sections.

Immunohistochemistry
All the cases in this study had cellblocks done at the time of initial processing. In our laboratory, fluid samples were spun down into cell buttons by centrifugation. Human derived serum was added to the cell buttons and left standing for 2-3 minutes. Thrombin was then added to the mix and this produced clots containing the cells of interest. The clots then went through routine automated procedures to get paraffinised.

The aim of this study was to examine the usefulness of Glut-1 when applied to cellblock material when used in conjunction with application of EMA and desmin. The staining intensity of Glut-1 was compared to that of EMA in each case of mesothelioma.

5-µm-thick sections were deparaffinised and treated with 3% hydrogen peroxide to block endogenous peroxidase activity, followed by washing in deionised water. Heat induced epitope retrieval with target retrieval solution (Dako) was performed. The slides were stained with primary antibody against GLUT-1 (1:200, polyclonal, Dako). Immunoreactions were detected by the labeled streptavidin-biotin method and visualized with 3, 3-diaminobenzidine, followed by counterstaining with hematoxylin. Red blood cells were used as positive control. The staining results were then divided into negative, focal positive (<30% staining) and diffuse positive (>30% staining). Staining for EMA and desmin for each case were reviewed and graded negative, focal positive (<30% staining) and diffuse positive (>30% staining). EMA was not performed on 11 of the 17 reactive cases.

Results
The results of immunohistochemistry are summarised in Table 1. Glut-1 expression was demonstrated by membranous staining with or without faint cytoplasmic staining. Positive Glut-1 staining was seen in 14 cases (82.4%) of malignant mesothelioma. One case of biphasic mesothelioma was included. However, the cytology sample of this case contained epithelioid mesothelial cells only. The single sarcomatoid mesothelioma in our series was negative for Glut-1. The two cases of Glut-1 negative epithelioid mesothelioma were strongly positive with EMA. Of the six cases of mesothelioma...
showing focal Glut-1 positivity, three were only focally positive for EMA and one was negative for EMA. Only one case of mesothelioma showed focal weak positivity for desmin while the other 16 cases were completely negative. All 17 cases of benign mesothelial proliferation were negative for Glut-1. Of the 17 cases of benign mesothelial proliferation, 11 were strongly positive for desmin, two showed focal positive staining, and four were negative (including the multiloculated benign peritoneal inclusion cyst).

**Table 1. Results of Glut-1 staining**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Glut-1 positive (%)</th>
<th>Negative</th>
<th>Focal</th>
<th>Diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesothelioma</td>
<td>17</td>
<td>14 (82.4%)</td>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>15</td>
<td>13 (86.7%)</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Biphasic</td>
<td>1</td>
<td>1 (100%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>1</td>
<td>0 (0%)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benign</td>
<td>17</td>
<td>0 (0%)</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritoneal</td>
<td>1</td>
<td>0 (0%)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pleural</td>
<td>6</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>8</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>2</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

Malignant mesothelioma is a solid tumour of the pleura. It is locally aggressive and usually presents with dyspnoea and chest pain (1). Without treatment the prognosis is poor, with median survival ranging from 6 to 18 months (2). In Australia, the malignant mesothelioma-associated mortality has increased since 1965; with 6,129 cases of mesothelioma reported to the Australian mesothelioma register (3). In New Zealand, 554 cases of asbestos related disease were reported to the Asbestos Disease Register between 1992 and 1997. These include 96 cases of mesothelioma, 47 cases of lung carcinoma, 118 cases of asbestosis and 293 cases of pleural abnormalities (4).

Many risk factors have been identified as contributors to malignant mesothelioma including asbestos, radiation and SV40 virus. Among all, asbestos is the environmental factor that is most frequently associated with mesothelioma. Asbestos belongs to the fibrinous silicate group of minerals. These can be structurally divided into two main groups, the serpentine and amphibole groups. The serpentine fibers (mainly chrysotile) are long and flexible. They are mainly used in the textile applications (1). They are soluble and therefore clear faster in the lung. Amphibole fibers (crocidolite, tremolite, anthophyllite and amosite) are mainly used as fire resistant application. They are short and stiff. Their physical structure allows them to penetrate deep into the respiratory tracts where they have a half life of about seven years (very difficult to clear from the lung) (5). Asbestos is thought to induce mutations in mesothelial cells by damaging the mitotic spindle (6,7). It also leads to the formation of reactive oxygen species. Almost 95% of asbestos used internationally is chrysotile. Yarborough concluded in his review that the role of chrysotile fibers in mesothelioma pathogenesis is weak although he suggested the possibility of a threshold for chrysotile (8,9). A discussion of the role of radiation and SV40 poliomavirus in the pathogenesis of mesothelioma is beyond the scope of this article.

The examination of fluid cytology is now part of the daily routine of many pathologists. Better understanding of the morphology of cells in cytologic preparations and the use of various immunohistochemical markers, has enabled distinction between malignant epithelial cells and mesothelial cells in most cases. However, distinguishing reactive mesothelial cells from malignant mesothelial cells in cytology remains challenging. Some say there is limited role of cytology in the primary diagnosis of malignant mesothelioma, but this is debated between cytopathologists and surgical pathologists. The diagnosis of malignant mesothelioma has to be made with certainty, and the International Mesothelioma Interest Group panel recommends that a cytologic suspicion of malignant mesothelioma be followed by tissue confirmation, supported by clinical and radiologic data (10).

Many of the cytologic features of mesothelial cells such as scalloped borders of cell clusters, intercellular windows, biphasic cytoplasm and low nuclear to cytoplasmic (NC) ratio are shared by reactive and malignant mesothelial cells. This makes diagnosis very difficult. Features suspicious for malignant mesothelioma include hypercellular smears with many large clusters of mesothelial cells, usually more than 50 cells in each cluster. The cells in these clusters may be larger than benign mesothelial cells but generally maintain a normal NC ratio (11). The nuclear membranes of malignant mesothelial cells often appear thicker than those of reactive mesothelial cells in cytologic preparations (11). Some authors use macronucleoli and nuclear atypia as criteria for malignancy. However, large nucleoli are often seen in reactive mesothelial cells. Reactive mesothelial cells in the setting of cirrhosis, pulmonary infarct, pancreatitis and uraemia can be highly atypical in appearances (11-13). It is important to remember that there may be a mixture of reactive and malignant mesothelial cells in the same sample. In some cases, especially sarcomatoid mesothelioma, one will expect more reactive mesothelial cells to be exfoliated into the fluid than the malignant cells. In difficult cases, a conservative diagnostic approach should be used. It is often useful to request repeat samples as malignant effusions are likely to reaccumulate quickly and subsequent collections may actually yield a more cellular sample.

**Histology** is the most reliable way of diagnosing malignant mesothelioma. Mesothelial proliferation that infiltrate into fat, muscle or lung is diagnostic of mesothelioma. While histology is the gold standard, there are several immunohistochemical markers that can assist in the cytological assessment of fluid when mesothelioma is suspected. EMA and desmin are the two most commonly used markers. Most malignant mesothelioma show membranous staining for EMA. While EMA expression is generally expected to be negative in reactive mesothelium, the author has seen cases of histology proven reactive mesothelial proliferation with weak EMA staining. However, in these reactive cases the staining is usually weak and cytoplasmic, unlike the strong and crisp membranous positivity seen in malignant mesothelial cells (Figure 1). Desmin is generally negative in malignant mesothelioma and positive to a certain degree in reactive mesothelial lesions. Desmin positivity in the latter context is cytoplasmic in location. In a study conducted by Attanoos et al, 80% of malignant mesothelioma cases were positive for EMA while only 20% of reactive cases were positive (14). In the same study, 10% of malignant cases were desmin positive while 85% of reactive ones were desmin positive.

**Figure 1.** Epithelial membrane antigen (EMA) showing strong membranous positivity in the malignant mesothelial cells (cell block).

“Glut” stands for glucose transporter, a family of 14 receptors (Glut-
1 to Glut-14) found in mammals and responsible for transporting glucose down a concentration gradient. The human Glut-1 gene has been localized to the short arm of chromosome 1 (1p34.2). Glut-1 is generally not detectable in benign or normal human tissue with the exceptions of erythrocytes, testicular germinal cells, renal tubules, perineurium of peripheral nerve, endothelial cells of blood-brain-barrier and placental trophoblasts (15). In general, malignant cells are found to have higher levels of Glut-1 expression than their benign counterparts. This is hardly surprising considering that malignant cells are expected to consume more energy and therefore require more glucose transporters. High levels of Glut-1 expression have been documented in various malignancies including tumours of the breast, pancreas, cervix, endometrium, lung, mesothelium, colon, bladder, thyroid, bone and soft tissue (16-20). Some authors believe that Glut-1 expression is associated with increased malignant potential and invasiveness of tumours (21-24). Kato et al conducted a study comprised 40 cases of reactive mesothelial proliferation and 48 cases of malignant mesothelioma (15). All 48 cases of malignant mesothelioma were positive for Glut-1 while all the reactive cases were negative.

In our series, Glut-1 positivity was seen in 82.4% of malignant mesothelioma cases (Figure 2). Two epithelioid mesothelioma and one sarcomatoid mesothelioma were negative for Glut-1. Red blood cells were present on all of the slides and were used as internal controls. These showed satisfactory staining. The tendency for sarcomatoid mesothelial cells to stain only weakly for Glut-1 was also documented by Kato et al (15). Glut-1 reactivity in our benign (reactive) group is comparable to that of Kato’s study (0% staining with Glut-1). The desmin staining pattern in our study (6% in malignant mesothelioma and 76% in reactive mesothelioma) is comparable to that reported by Attanoos et al (14).

**Figure 2.** Strong membranous staining with Glut-1 in the same cell block

In conclusion, Glut-1 has proven itself as a useful adjunct to routine fluid cytology in the investigation of patients with possible malignant mesothelioma. However, it is important to recognize the fact that reactive mesothelial cells are often exfoliated at the same time as neoplastic cells, contributing to a variable staining pattern within the same sample. Negative staining with Glut-1 does not rule out the possibility of malignant mesothelioma, especially when clinical suspicion is high. Another limitation of the use of Glut-1 in cytology is the frequent heavy background blood staining of the samples, making assessment difficult. Glut-1 is best interpreted in conjunction with other markers such as EMA and desmin. At the end of the day, clinical and radiological correlation is crucial.

**Acknowledgements**

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**References**


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In regard to the macrocytosis in COPD paper by Wu and colleagues, the Editor of the Journal, Rob Siebers, is a co-author. In order of transparency and avoidance of conflict of interest the handling of this article was independently done by the Deputy Editor who oversaw the peer review process and made the final editorial decision of acceptance. The Journal’s Editor had no input in the editorial process and was treated the same as any other author submitting to the Journal.

Each year the NZIMLS Council invites a member, who has made a significant contribution to medical laboratory science, to deliver the TH Pullar Memorial Address at the Annual Scientific Meeting. This prestigious address is in honour of Dr TH Pullar, a pathologist who was a champion and great friend of New Zealand medical laboratory scientists. Thos Pullar was involved in their training and welfare, drafting conditions of employment and preparing syllabi and examinations for their professional training. This year Christine Pry, from Abbott Diagnostics, was honoured to deliver the TH Pullar Memorial Address. Her address, entitled “Believe in yourself, and anything is possible” is in this issue.

In this issue is another Journal questionnaire. You may have noticed that over time the Journal questionnaire has required more in-depth or multiple answers for the questions. Despite this, some members write very sparse answers. For instance, question one from the August 2010 questionnaire asked members to name the three types of von Willebrand disease. Quite a few just simply answered “Type 1, Type 2 and Type 3”. The fuller and correct response should have been “Type 1 is a partial quantitative deficiency in vWF, Type 2 is a qualitative defect and Type 3 is an extreme quantitative defect (where vWF is absent). Similarly, question 6 asked for the principles of measurement of the vWF:RCo and HaemosIL methods. Just answering “by turbidometry” meant that those members only got a fraction of the mark for that question as they did for the above simplistic answer to question one. Thus some members, because they also missed out answers on other questions, did not achieve the minimum of eight (full) correct answers and were not awarded their 5 CPD points. Please read the questions and articles carefully before answering the questions in detail.