The role of immunohistochemistry in differentiating between peritoneal mesothelioma and ovarian serous carcinoma

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The concept of primary Müllerian tumors of the peritoneum is based on the putative existence of a secondary müllerian system.[1] The müllerian ductal system is derived embryologically from coelomic epithelium, and sub coelomic mesenchyme, and it is postulated that the adult derivatives of these structures, whether this be the surface epithelium of the ovary or the peritoneal lining, and its subjacent connective tissue, retain a potential for müllerian differentiation.[2] The differential diagnosis of a papillary tumor of serous type affecting the female peritoneum lies between a papillary serous carcinoma, either originating from the peritoneum or metastatic from the ovary, or an epithelioid peritoneal malignant mesothelioma. Primary papillary serous carcinoma of the peritoneum (PPSCP) is a controversial entity. These tumors are identical histologically to papillary serous carcinoma of the ovary as would be expected given their suggested origin.[3] The recognition of PPSCP as a distinct entity is based on the tumor being reported in the male peritoneum,[4] and the occurrence of these tumors in patients who have undergone prophylactic oophorectomy for a family history of ovarian carcinoma.[5] To diagnose PPSCP, examination of the ovaries is required to exclude a papillary serous carcinoma of the ovary with metastatic involvement of the peritoneum. The extra-ovarian involvement by the tumor must be greater than ovarian involvement. If the ovary is involved, the tumor must be present on the surface only or, if involving the stroma, less than 5x5mm in size.[6] Diffuse malignant mesothelioma of the peritoneum is far less common than pleural mesothelioma, accounting for less than 10% of all cases of malignant mesothelioma.[7] Peritoneal malignant mesothelioma does however account for 32% of all mesothelioma cases in females.[8] Malignant mesothelioma may of course arise from the peritoneum itself, or may arise from the ovary.[9] The histological differentiation of epithelioid peritoneal malignant mesothelioma, and papillary serous carcinoma involving the peritoneum in women is a well-recognized problem for pathologists, and can be extremely difficult. In some cases, it is not possible to make a definitive diagnosis even after extensive immunohistochemistry, and electron microscopy.[10] The accurate diagnosis of diffuse malignant mesothelioma is important for prognostic, and therapeutic reasons, and in relation to compensation claims following occupational asbestos exposure.[7] Previously, many studies have concentrated on the distinction between malignant mesothelioma of the pleura, and pulmonary carcinoma. However, several studies have recently compared the immunohistochemical findings in malignant mesotheliomas, and papillary serous carcinomas of the peritoneum.[6,11,12] There is no single antibody available that is 100% specific for these entities, and so a panel of antibodies is required. Previous studies have found the best positive markers for malignant mesothelioma to be calretinin, cytokeratin 5/6, and thrombomodulin, and the best positive markers for papillary serous carcinoma to include Ber-EP4, B72.3, Leu-Ml (CDI5), CA19-9, and MOC-31.[6,11] The aim of this study is to assess an immunohistochemical panel of antibodies for differentiating epithelioid malignant mesothelioma from ovarian serous carcinoma

The material included in this study was obtained in 2004 from the files of the Department of Pathology at Queen Alexandra Hospital, Portsmouth, UK, from 1990 to 1999 for mesothelioma cases, and from 1997 to 1998 for ovarian serous carcinoma. It consisted of 8 cases with a clinical, and histological diagnosis of peritoneal malignant mesothelioma (7 males, one female), and 15 cases of papillary serous carcinoma of the ovary. Immunohistochemical studies were performed using the Avidin-Biotin Peroxidase Complex method. Sections were cut 4 µm thick, deparaffinized in xylene, and rehydrated in descending grades (100-70%) of ethanol. Endogenous peroxidase activity was blocked by a 10-minute treatment with 3% hydrogen peroxide in absolute methanol. The primary antibodies used were monoclonal anti-human carcinoembryonic antigen (CEA) (Dako Ltd., Ely, Cambridgeshire, UK), monoclonal Leu-Ml (CDI5) (Dako Ltd., Ely, Cambridgeshire, UK), monoclonal anti-human epithelial antigen (Ber-EP4) (Dako Ltd., Ely, Cambridgeshire, UK), Polyclonal Calretinin (Zymed Laboratories, Inc., South San Francisco, USA), monoclonal CA-125 (Novocastra Laboratories Ltd., Newcastle-Upon-Tyne, UK), monoclonal TAG-72 (B 72.3) (Neomarkers Inc., Runcorn, UK), monoclonal MOC-31, and monoclonal thrombomodulin (Dako Ltd., Ely, Cambridgeshire, UK). A panel of immunohistochemical stains was used on sections from peritoneal mesotheliomas and ovarian serous carcinoma to demonstrate their reactivity. To evaluate the specificity of the antibodies, known positive and negative tissues were used as controls. The score of immunoreactivity was referred to as focal or weak, when <25% of the cells were positive, moderate reactivity, when 25-50% positive cells, and scored strong or diffuse

staining, when >50% of the cells were positive.

The result of the study revealed that 7 (46.7%) of the 15 serous carcinomas of the ovary showed focal and weak cytoplasmic staining with carcino-embryonic antigen (CEA). None (0%) of the 8 cases of peritoneal mesothelioma were stained with CEA. Focal, granular, and cytoplasmic reactivity with Leu-MI antibody (CD15) was observed in one (6.7%) of the 15 cases of serous carcinoma. None of the mesotheliomas reacted with this antibody. All 15 cases (100%) of serous carcinoma showed strong and diffuse staining (>50% of the cells) with anti-human epithelial antigen (Ber-EP4) antibody. Two (25%) of the 8 mesotheliomas showed focal staining in a limited number of cells. However, in both types of tumor, the staining occurred along the cell membranes. All 8 (100%) mesotheliomas showed strong, diffuse staining with calretinin in both the cytoplasm, and the nucleus of the cells. Six (40%) of the 15 cases of ovarian serous carcinoma showed focal cytoplasmic staining with calretinin. Positive staining with CA-125 was seen in 14 (93.3%) of the 15 cases of ovarian serous carcinoma, and 5 (62.5%) of the 8 peritoneal mesotheliomas. In both types of tumor, the reactivity occurred mainly along the cell membranes. Seven (46.7%) of the 15 cases of serous carcinomas showed strong, diffuse, granular, cytoplasmic staining with TAG-72 (B 72.3) monoclonal antibody. None of the mesotheliomas reacted with this antibody. Reaction with MOC-31 antibody was observed in 9 (60%) of the serous carcinomas. In most cases, the staining was weak, cytoplasmic, and along the cell membrane. None of the mesotheliomas reacted with this antibody. Six (75%) of the cases of peritoneal mesothelioma were positive for thrombomodulin. The reactivity was strong and along the cell membrane. None of the 15 serous carcinoma cases showed reactivity for this marker.

The differentiation of peritoneal epithelial mesothelioma from carcinoma diffusely involving the serosal membrane in the absence of a known primary tumour can be facilitated by the use of immunohistochemical methods. The primary focus of most studies currently available, however, has been on differentiating epithelial pleural mesothelioma, and metastatic carcinoma of the lung within the pleura.[13] Some of the markers that have been proven to be useful in separating pleural mesotheliomas from lung carcinomas have a different value in differentiating between epithelioid peritoneal mesotheliomas, and papillary serous carcinomas.[6] The main objective of this study was to determine the best discriminatory markers that could be in a routine diagnostic panel to separate these tumors. Carcinoembryonic antigen is expressed in the large majority of cases of lung carcinoma, but not in mesotheliomas. Therefore, anti-CEA antibodies are commonly used to differentiate pleural mesotheliomas from lung carcinomas.[13,14] Carcinoembryonic antigen reactivity has been reported in up to 69% of serous carcinomas of the ovary in different series,[6,12] and 0% of the mesothelioma. We were able to demonstrate reactivity in 7 (46.7%) of the 15 serous carcinomas, but none of the mesotheliomas included in this study. This difference could be resulting from difference in the type of anti-CEA antibody used. The results of our study support the findings of previous studies showing CEA is a valuable marker for separating epithelial peritoneal mesotheliomas from papillary serous carcinomas of the ovary. Although previous studies have shown Leu-M1 (CDI5) to be valuable in differentiating pleural mesothelioma, and pulmonary carcinoma,[13,15] only a few were available on the presence of this antigen in papillary serous carcinomas.[13,16,17] This marker has been reported to be positive in 2-8% of cases of epithelial mesothelioma in some series,[14,18] but other studies have shown no staining in mesotheliomas with this marker.[19,20] Leu-M1 staining has been observed in 30-80% of serous carcinomas. These results suggest that if Leu-M1 staining is present, the tumor is unlikely to be a mesothelioma, and may therefore be helpful, but if staining is absent, this antibody cannot be used to differentiate epithelial peritoneal mesotheliomas from serous carcinomas due to low sensitivity as supported by a study of Attanoos et al.[21]

Monoclonal anti-human epithelial antigen Ber-EP4 can assist in differentiating epithelial mesothelioma from metastatic carcinoma within the serosal membranes.[10,19] The studies that have reported Ber-EP4 reactivity in epithelioid peritoneal mesotheliomas have described the staining in these cases as being focal or confined to infrequent isolated cells.[22] In our study, 2 cases of epithelioid mesothelioma (25%) and all of the serous carcinomas showed Ber-EP4 reactivity. The staining in the serous carcinomas was however, strong and diffuse, whereas the epithelioid peritoneal mesothelioma cases showed only focal staining, often limited to a few cells. It is concluded that Ber-EP4 immunostaining may be useful in separating these 2 types of tumors.[21,23,24]

Doglioni et al[25] reported strong diffuse staining with calretinin in all 44 (100%) of mesotheliomas, but only focal staining in 28 (10%) of the 294 carcinomas of various origins including one (6%) of 16 serous carcinomas of the ovary. Also, another study by Ordonez et al[26] reported positive reaction to calretinin in 40 (100%) cases of mesothelioma, whereas, 13% of serous carcinoma cases reacted to it. In our study, with the use of polyclonal antibody from a different commercial source (Zymed Laboratories Inc., South Francisco, USA), all 8 (100%) of the mesotheliomas in this study demonstrated calretinin expression, whereas only 6 (40%) of the serous carcinomas exhibited reactivity for this marker. In the mesotheliomas, the

staining was diffuse, and within both the cytoplasm and the nuclei, whereas the staining in the serous carcinomas was limited to only a few cells and was cytoplasmic. Therefore, this confirms observations made in previous, and following studies indicating that calretinin is a very useful immunohistochemical marker for distinguishing epithelioid mesotheliomas from serous carcinomas.[23,24] In a later study by Ordonez et al,[26] this marker was also found to have a little or no diagnostic rule in establishing the differential diagnosis between the 2 conditions. CA-125 reactivity was demonstrated in the majority of serous carcinomas and many mesotheliomas, which confirms the results of previous reports indicating that this marker is not helpful in the diagnosis of peritoneal mesotheliomas.[20] The mouse monoclonal antibody B72.3 recognizes a tumor associated glycoprotein (TAG-72) that is present in a wide variety of carcinomas, including those of pulmonary, gastrointestinal, mammary, pancreatic, endometrial, and ovarian origin.[5] In the present study, 46.7% of the serous carcinomas, but none of the mesotheliomas showed B72.3 immunostaining. Other investigators have reported positive staining in 72-100% of carcinomas.[6,13,16,23] In some studies, none of the mesotheliomas showed staining with this antibody,[6,16,23] whereas others reported focal positivity in 2-48% of these tumors.[13,18]

We conclude that, as B72.3 staining tends to be mostly focal in mesothelioma, but strong and diffuse in serous carcinoma. This marker appears to be one of the valuable negative markers for mesotheliomas.[26] Delahaye et al[27] were able to obtain MOC-31 staining in 18 (58%) of 31 carcinomas of various sites (including 5 of 8 originating in the ovary), and in 2 (8%) of 24 mesotheliomas. These authors concluded that MOC-31 immunostaining had a very limited value in differentiating mesothelioma from carcinoma, due to a relatively low percentage of positive carcinoma cases. In another study, 44 (98%) of the 45 serous carcinomas stained with MOC-31 antibody, whereas only one of the 35 of the mesotheliomas showed positive staining in a limited number of cells.[19] These results suggest that MOC-31 immunostaining may be helpful in discriminating epithelioid mesotheliomas from papillary serous carcinomas. In our study, 9 cases (60%) of the serous carcinomas stained with MOC-31, but all the mesotheliomas were negative. These results support the observation, that MOC-31 may have value in differentiating mesotheliomas from carcinoma. Collins et al[28] reported thrombomodulin expression in all 31(100%) pleural mesotheliomas, and only 4 (8.3%) of 448 lung carcinomas. As only one carcinoma showed strong reactivity for thrombomodulin, the authors concluded that immunostaining for this marker could assist in distinguishing between mesothelioma, and lung carcinomas. Thrombomodulin expression has been reported in 60-100% of mesotheliomas, and in 8-77% of lung carcinomas. Another 2 studies have assessed thrombomodulin staining in peritoneal mesotheliomas, and carcinomas of the ovary. The first reported positive staining in 8 out of 24 examples of ovarian carcinomas.[29] The other study showed a positive result in 74% of the 35 peritoneal mesotheliomas, and only one (2%) of the papillary serous carcinomas.[11] The fact that in the present study, 75% of the mesotheliomas, and none of the papillary serous carcinomas showed thrombomodulin expression suggests that thrombomodulin immunostaining can assist in discriminating between both types of tumors.

The results of this study suggest that, calretinin, thrombomodulin, and Ber-EP4 are the best markers for distinguishing between epithelial malignant peritoneal mesotheliomas, and papillary serous carcinomas. Among other antibodies currently available, MOC-31, B72.3, and CEA appear to be additional helpful diagnostic discriminators. Immunostaining for CA-l25 has little or no practical diagnostic utility in differentiating between epithelial mesotheliomas, and serous carcinomas, and Leu-Ml has only limited value.

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