Pathology - Research and Practice 215 (2019) 152462

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Signet ring cell mesothelioma; A diagnostic challenge

Hui Wang*, Chaturika Herath

Department of Pathology and Laboratory Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N0W8, Canada

ARTICLE INFO

Keywords:
Signet ring cell mesothelioma
Diagnostic pitfall
Differential diagnosis
Immunohistochemistry
Electron microscopy
Molecular study

ABSTRACT

Signet ring cell mesothelioma is a rare variant of epithelioid mesothelioma with limited cases published. It has a male predilection and most commonly occurs on pleura; it can also arise in the peritoneal cavity. The signet ring cell morphology can pose a challenge leading to a potential diagnostic error. A variety of benign and malignant diseases, including reactive histiocytic hyperplasia, adenocarcinoma, melanoma, and lymphoma with signet ring cell morphology should be considered in the differential diagnosis. In signet ring cell mesothelioma work up, mucin stains are of limited value. Even though immunohistochemistry is routinely used in mesothelioma diagnosis, there is no sole specific mesothelial marker. Hence, a panel of mesothelial and epithelial markers are used; these should be interpreted with caution especially in this variant. Electron microscopy and genetic testing can be very helpful in distinguishing signet ring cell mesothelioma from its mimickers.

1. Introduction

Malignant mesothelioma (MM) is a neoplasm occurring on serosal surfaces, such as pleura, peritoneum, pericardium, ovary, and tunica vaginalis of testes. According to the 2015 World Health Organization classification [1], MM can be subtyped into four categories: epithelioid, sarcomatoid, desmoplastic, and mixed type (biphasic), the most common which is epithelioid. A few vacuolated cells with signet ring like morphology are not uncommon in epithelioid mesothelioma; however, excessive signet ring like cells in epithelioid mesotheliomas are extremely rare and have been sub-classified as signet ring cell mesothelioma [2]. In 2003, Cook et al. published the first case report on signet ring cell mesothelioma. Thus far due to rarity, signet ring cell variant mesothelioma has had limited cases reported [3–7].

There are well established cytomorphological features to differentiate mesothelial cells, including reactive and malignant types from carcinoma cells and macrophages [8]. However, mesothelioma with signet ring cell features can be a diagnostic challenge since it morphologically mimics many benign and malignant diseases, predominantly signet ring cell adenocarcinoma from lung, stomach, breast, colon, and urinary bladder. Herein, we provide an overview of malignant signet ring cell mesothelioma, its differential diagnosis, and ancillary studies with emphasis on diagnostic pitfalls.

2. Clinical and pathological features of signet ring cell mesothelioma

Mesothelioma with signet ring cell variant is a rare tumor that most commonly seen on pleura; can also arise from the peritoneum. Signet ring cell mesothelioma demonstrates a male predominance and has been seen in patients with asbestos exposure and/or smoking history [3]. However, there is no clear evidence showing occupational asbestos exposure or smoking increases the risk of its development. To date, only 27 cases of signet ring cell mesotheliomas are reported in the English literature, with 23 cases [3–5] being pleural and four cases [3,6,7] being of peritoneal origin. 23 patients were men [3–5,7], and four patients were women [3,6]. 13 patients [3,7] had a history of asbestos exposure and 17 patients were smokers [3,7]. The most common clinical manifestations of signet ring cell mesothelioma are unexplained recurrent pleural or peritoneal effusion, chest pain, and weight loss. Common radiological findings include pleural or peritoneal plaques and calcification. Similar to other variants of MM, combined surgery with chemotherapy and/or radiation therapy is the common management of signet ring cell mesothelioma. Extra-pleural pneumonectomy, decortication, and tumor debulking are usual surgical procedures, followed by chemo- and/or radiation therapy [3,5,6]. Prognosis of signet ring cell mesothelioma is poor. The median survival is 15 months post-diagnosis [3].

Grossly, pulmonary signet ring cell mesothelioma diffusely involves visceral and parietal pleura. Invasion of adjacent structures, such as lung parenchyma, diaphragm, chest wall, and pericardium, can be

* Corresponding author.
E-mail address: huw546@usask.ca (H. Wang).

https://doi.org/10.1016/j.prp.2019.152462
Received 2 March 2019; Received in revised form 5 May 2019; Accepted 21 May 2019
0344-0338/ © 2019 Elsevier GmbH. All rights reserved.

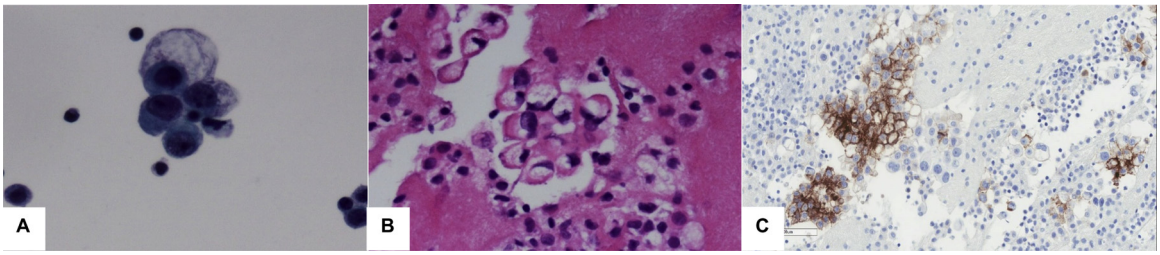

Fig. 1. Signet ring cell mesothelioma in effusion cytology (A, ThinPrep, objective magnification ×40) and cell block (B, hematoxylin-eosin, objective magnification ×40). The epithelioid cells contain large intra-cytoplasmic vacuoles and displaced nuclei. Tumor cells with aberrant BerEP4 expression (C, objective magnification ×20).

observed [3]. Lymph node metastasis is common [3]. Signet ring cell mesothelioma in the peritoneum may involve omentum, mesentery, and pelvic organs [3,6,7].

Exfoliated cytology is usually the initial specimen for signet ring cell mesothelioma workup. The smears are cellular and show a relatively monomorphic population of dis-cohesive epithelioid cells. The cells have large clear intracytoplasmic vacuoles and distorted nuclei displaced to the periphery of the cells showing irregular crescent form. The cytoplasm shows thick cyanophilic, finely vacuolated endoplasm and thin ectoplasm with lacy borders giving a two-tone appearance (Fig. 1A, B). Multinucleation and mitosis can be prominent. Occasionally cell in cell and cell hugging can be noted.

Biopsies show infiltrative tumor cells arranged in sheets, cords, tubules, and pseudopapillae usually in a myxoid background (Fig. 2A). The tumor cells are with clear to eosinophilic cytoplasmic vacuoles and displaced nuclei. The nuclei are often distorted and variably indented and frequently exhibiting sickle or crescent shape (Fig. 2B). On occasion, cells may have two or more nuclei. Most signet ring cells contain clear vacuoles but some of the vacuoles contain bluish granular material.

3. Differential diagnosis and ancillary studies

The signet ring cells indicate cells with intracytoplasmic vacuoles

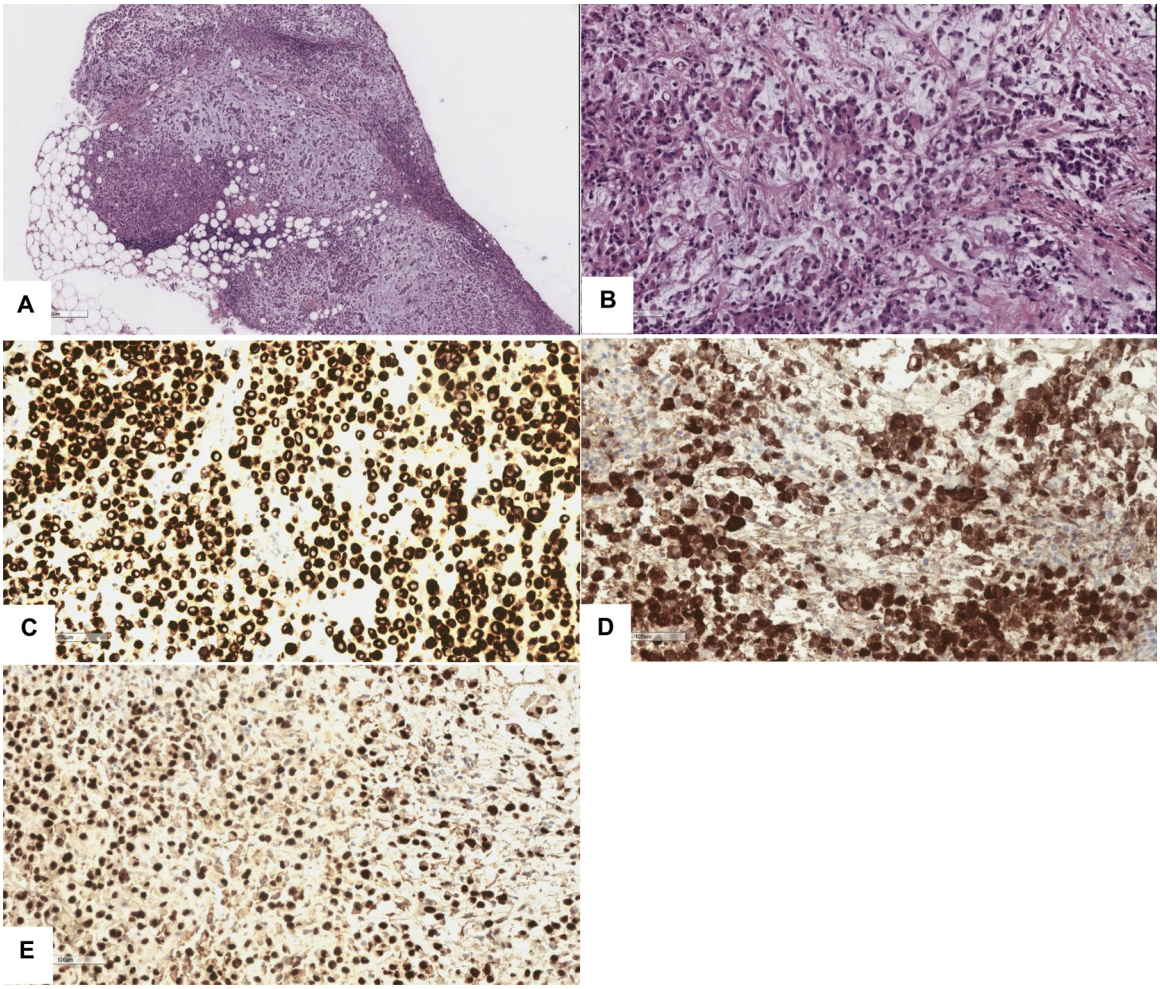

Fig. 2. Signet ring cell mesothelioma with myxoid background infiltrates omentum (A, hematoxylin-eosin, objective magnification ×4; B, hematoxylin-eosin, objective magnification ×20). Tumor cells with positive CK7 (C, objective magnification ×20), Calretinin (D, objective magnification ×20) and WT-1 (E, objective magnification ×20).

and eccentric nuclei. Those cells can be benign or malignant. The benign signet ring cells in effusion cytology are most often macrophages and degenerate mesothelial cells [6]. In contrast, the malignant signet ring cells can be adenocarcinoma or malignant mesothelial cells. Malignant signet ring cells usually have distorted hyperchromatic nuclei with crescent or sickle shape.

The commonest benign differential for signet ring cell mesothelioma is reactive histiocytic proliferation due to its site of origin and cellular morphology. Reactive histiocytic proliferation is a rare benign lesion that has been reported in mesothelial and non-mesothelial lined sites, with the most common sites are pleura, pericardium, peritoneum and hernia sac [8–10]. Morphologically this lesion contains an aggregation of histiocytes with moderate to abundant cytoplasm, lobulated nuclei and small prominent nucleoli. Mitosis can be seen [11]. It was previously described and considered as nodular mesothelial proliferation or mesothelial cells with malignant potential. Negative stain with cytokeratin and strong positivity for macrophage lineage markers CD68 and/or CD163 confirm the signet ring cells in this lesion are histiocytes, instead of mesothelial cells or any other signet ring cell malignancy [8,9].

Further differential diagnoses of signet ring cell mesothelioma include a group of neoplasms with signet ring cell morphology. Metastatic signet ring cell adenocarcinomas, which can be originated from lung, breast, urinary bladder, ovary, and gastrointestinal (GI) tract such as stomach, pancreaticobiliary and colon, are at the top of the differential list. Unfortunately, among reported cases, three signet ring cell mesothelioma cases were initially misdiagnosed as metastatic adenocarcinoma [5–7]. In the case reported by Cook et al. [7], the patient presented with abdominal pain and ascites with ultrasound showing a thickened gastric wall. Even though gastric adenocarcinoma was suspected, multiple gastric biopsies showed no evidence of malignancy. Eventually, the diagnosis of signet ring cell mesothelioma was made at the post-mortem examination [7]. Hence, pathologists should be aware of the morphological and ancillary study pitfalls of signet ring cell mesothelioma in order to avoid diagnostic errors.

Mucin histochemistry can be the initial attempt to differentiate MM from adenocarcinoma. Usually, the large cytoplasmic vacuoles present in signet ring mesothelial cells are negative for mucicarmine or Alcian blue [7]. However, the vacuoles in mesothelial cells may contain hyaluronic acid, which is positive for mucicarmine and Alcian blue. Therefore, mucicarmine and Alcian blue are not recommended for distinguishing mesothelioma from adenocarcinoma. Diastase-resistant periodic acid-Schiff (PAS-D), which highlights the neutral mucin, is another well-accepted mucin stain. PAS-D positivity strongly supports the diagnosis of adenocarcinoma, while the negativity indicates the diagnosis of mesothelioma. Nevertheless, “mucin-positive” epithelioid mesothelioma in both pleura and peritoneum has been reported [7,13,14]. The aberrant PAS-D positivity in mesothelioma is a potential cause of misdiagnosis. Thus, the utility of mucin histochemistry is limited in mesothelioma diagnosis and usually only applicable when IHC is indeterminate.

IHC panel including at least two epithelial markers and two mesothelial markers are regularly applied in mesothelioma diagnosis. The commonly used epithelial markers are BerEP4, B72.3, and claudin 4; and mesothelial markers are calretinin, WT-1, podoplanin (D2-40), and CK5/6 [12]. Literature show that signet ring cell mesothelioma is positive for calretinin, CK5/6, CK7, mesothelin, WT-1, and podoplanin; negative for BerEP4, MOC-31, CEA, B72.3, and additional specific adenocarcinoma markers, such as TTF-1, Napsin A, keratin 20, or CDX2 [3–7].

BerEP4 is an epithelial membrane glycoprotein and a sensitive marker for carcinoma. About 95–100% of lung adenocarcinoma and greater than 98% of pancreatic and gastric carcinoma are strongly positive for BerEP4. However, it cross reacts with mesothelial cells and stains about < 20% of pleural MM and 9–13% of peritoneal MM [12]. The mesothelial cells may have strong positive membranous stain with BerEp4 (Fig. 1C) and cause misdiagnosis. B72.3 labels tumor-associated glycoprotein 72 and is positive in 75–85% of lung adenocarcinoma and 84–98% of pancreaticobiliary and GI adenocarcinoma. Very few MM (0–3% of peritoneal MM) have an expression of B72.3 [12]. It is notable that Claudin-4, a tight junction–associated protein, is expressed in most epithelial cells; but has not been reported to react with mesothelial cells. It is a highly sensitive and specific IHC marker distinguishing metastatic carcinoma from epithelioid mesotheliomas [15,16]. Claudin-4 should be used as the primary marker in effusion cytology and small biopsies that contain limited material to avoid extensive IHC workup.

CK7 is positive in mesothelioma (cytoplasmic pattern, Fig. 2C) and signet ring cell carcinoma from lung, breast, stomach, urinary bladder, and ovary. Thus, CK7 is a non-specific IHC marker differentiating mesothelioma from carcinoma. Calretinin is a very useful mesothelial marker and demonstrates in almost all epithelioid mesotheliomas (both nuclear and cytoplasmic pattern, Fig. 2D). But it is also expressed in 15% of breast adenocarcinomas and 4–18% of pulmonary adenocarcinoma [17]. WT-1 is positive in 75–95% of pulmonary MM and 43–93% of peritoneal MM (nuclear pattern, Fig. 2E). It has no cross-reaction with lung and pancreatic carcinoma and expressed in up to 3% of gastric adenocarcinoma [12]. Podoplanin is a highly sensitive mesothelial marker that demonstrates positivity in 90–100% of pleural MM and 93–96% of peritoneal MM. It is expressed in less than 15% of lung adenocarcinoma [12]. CK5/6 is a useful marker due to its positivity in 75–100% of pleural MM and 53–100% of peritoneal MM [12]. Nevertheless, up to 20% lung adenocarcinoma and 38% of pancreatic adenocarcinoma have an expression of CK5/6 [12]. Therefore, no single mesothelioma marker should be used alone due to the limitation. The panel of epithelial and mesothelial staining can be confusing and misleading and should be interpreted with caution.

GATA3 is a common marker used in breast and urothelial carcinoma diagnosis. It is important to be aware that GATA3 is positive in the majority (50%–89%) of MM, with some cases showing strong and diffuse positivity [18]. Thus, the utility of GATA3 is limited when the differential diagnosis includes breast or urothelial carcinoma.

Signet ring cell melanoma, even though rare, is a great mimicker of signet ring cell mesothelioma. S-100, Melan A, and HMB45 are markers labeling melanoma. In the study done by Zenali et al., all epithelioid mesothelioma cases (12/12) demonstrated strong positive Melan-A expression [19]. Melan A should be used with caution in differentiating MM from metastatic melanoma to avoid the potential diagnostic pitfall.

Signet ring cell lymphoma, a rare variant of non-Hodgkin lymphoma, occurs in broad anatomic sites and can be present in effusion cytology and fine needle aspiration (FNA) smears. It contains malignant lymphocytes with intra-cytoplasmic vacuoles and displaced nuclei. The nature of intra-cytoplasmic vacuoles is uncertain. It may be immunoglobins that forming eosinophilic globules or membrane bound spaces derived from multivesicular bodies [20]. Signet ring cell lymphoma can be distinguished by being negative for cytokeratin, mucin stains, and mesothelial markers, and positive for lymphocytic markers [20]. Immunofluorescence technique can demonstrate immunoglobulins in FNA smears and is a rapid method distinguishing signet ring cell lymphoma from mesothelioma [21].

Electron microscopic study exhibits that signet ring cell morphology in mesothelioma is due to single or multiple intra-cytoplasmic lumina, which is lined by delicate microvilli or a layer of electron-dense material [3,7]. The size of the cytoplasmic lumina increases gradually and displaces the nucleus to the periphery of the cell [3]. Interestingly, somesignet ring cells do not contain cytoplasmic lumina, but markedly dilated intercellular space between two adjacent cells. In that case, binucleation can be observed if two nuclei are in the same plane of section [3]. On the other hand, signet ring cell adenocarcinoma consists of many mucin granules in the cytoplasm, with or without intra-cytoplasmic lumina [3]. Hence, electron microscopy is very helpful in signet ring cell mesothelioma diagnosis.

The most common genetic alteration of MM is homozygous deletion

of the 9p21 locus within a cluster of genes that includes cyclin-dependent kinase inhibitor (CDKN)-2A, CDKN2B, and methylthioadenosine phosphorylase (MTAP) [22,23]. Studies demonstrated that p16/CDKN2A deletions were detected in up to 70% of primary pleural epithelioid mesotheliomas and up to 20% of peritoneal mesothelioma [24,25]. To date, the presence of this homozygous deletion is highly specific in MM as it has not been reported in any of the benign lesions. The fluorescence in situ hybridization (FISH) assay is a reliable method of detecting p16/CDKN2A deletions. IHC is not recommended since the loss of p16 protein expression does not always correlate with p16 deletion. The presence of p16 protein may be observed in p16/CDKN2A deletion, while the absence of p16 protein may be found with intact p16/CDKN2A [26].

The tumor suppressor gene BRCA-associated protein 1 (BAP1) is located at 3p21 and modulates calcium-mediated apoptosis. BAP1 mutation results in the accumulation of DNA-damaged cells and greater susceptibility to develop malignancy [27]. Germline or sporadic BAP1 mutation is commonly identified in MM [27–29]. Loss of nuclear staining of BAP1 by IHC is the most reliable method of evaluating BAP1 mutation [27]. The study showed negative BAP1 staining in 57% mesotheliomas in effusion cytology [29]. Therefore, the absence of nuclear stain of BAP1 can be supportive evidence of mesothelioma diagnosis in effusion cytology.

4. Conclusion

Signet ring cell mesothelioma is an entity that may cause a diagnostic error because it morphologically mimics reactive histiocytic hyperplasia, signet ring cell adenocarcinoma, melanoma, and lymphoma. Hence, pathologists should be aware of the diagnostic pitfalls of signet ring cell mesothelioma. Due to its rarity, only limited cases have been reported and ancillary studies play an important role in its diagnosis. Mucin stain is of limited value as mucin-positive malignant mesothelioma has been observed. All mesothelial cell markers cross-react with other malignancies and should be interpreted with caution. Claudin-4 is highly recommended since it is positive in most epithelial cells and does not react with mesothelial cells. Furthermore, electron microscopy study demonstrates that signet ring cell mesothelioma contains single or multiple intra-cytoplasmic lumina; differenced from adenocarcinoma that has intra-cytoplasmic mucin granules. FISH assay of detecting p16/CDKN2A deletion and IHC of the absence of BAP1 nuclear stain can be used as supportive evidence in mesothelioma diagnosis.

Competing interests

None declared.

References

[1] W.D. Travis, E. Brambilla, A.P. Burke, A. Marx, A.G. Nicholson (Eds.), WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, fourth ed., IARC Press, Lyon, France, 2015, pp. 154–168.
[2] S.P. Hammar, D.W. Henderson, S. Klebe, R.F. Dodson, Neoplasms of the pleura, in: J. Tomashefski, P.T. Cagle, C.F. Farver, A.E. Fraire (Eds.), Dail and Hammar's Pulmonary Pathology: Volume II: Neoplastic Lung Disease, third ed., Springer Link, 2008, pp. 602–604.
[3] N.G. Ordóñez, Mesothelioma with signet-ring cell features: report of 23 cases, Mod. Pathol. 26 (2012) 370–384.
[4] M.M. Mishra, C.F. Farver, D.J. Chute, Fine needle aspiration biopsy of metastatic malignant mesothelioma with myxoid change and signet ring cells: a case report and review of the literature, Cytojournal 13 (2016) 4.
[5] S. Beder, A. Yalcin, F. Ulger, S. Percinel, Malignant mesothelioma with signet ring cell formation: a very rare presentation, Lung Cancer 54 (2006) S59.
[6] B. Rekhi, S. Pathuthara, D. Ajit, S.V. Kane, "Signet-ring" cells-A caveat in the diagnosis of a diffuse peritoneal mesothelioma occurring in a lady presenting with recurrent ascites: an unusual case report, Diagn. Cytopathol. 38 (2009) 435–439.
[7] D.S. Cook, R.L. Attanoos, S.S. Jalloh, A.R. Gibbs, 'Mucin-positive' epithelial mesothelioma of the peritoneum: an unusual diagnostic pitfall, Histopathology 37 (2000) 33–36.
[8] N.G. Ordóñez, J.Y. Ro, A.G. Ayala, Lesions described as nodular mesothelial hyperplasia are primarily composed of histiocytes, Am. J. Surg. Pathol. 22 (1998) 285–292.
[9] J. Rosai, L.P. Dehner, Nodular mesothelial hyperplasia in hernia sacs: a benign reactive condition simulating a neoplastic process, Cancer 35 (1975) 165–175.
[10] S. Rao, N. Khurana, A. Mohta, Nodular histiocytic proliferation in hernial sac: a potential diagnostic pitfall, Indian J Pathol Micr. 55 (2012) 126.
[11] F. Mir, A. Naumaan, H. Alnajar, et al., Reactive histiocytic proliferation in the pleural fluid mimicking metastatic signet ring adenocarcinoma, Diagn. Cytopathol. 46 (2018) 525–527.
[12] A.N. Husain, T.V. Colby, N.G. Ordóñez, et al., Guidelines for pathologic diagnosis of malignant mesothelioma 2017 update of the consensus statement from the international mesothelioma interest group, Arch. Pathol. Lab. Med. 142 (2018) 89–108.
[13] D.B. MacDougall, S.E. Wang, B.L. Zidar, Mucin-positive epithelial mesothelioma, Arch. Pathol. Lab. Med. 116 (1992) 874–880.
[14] S.P. Hammar, D.E. Bockus, F.L. Remington, K.A. Rohrbach, Mucin-positive epithelial mesotheliomas: a histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing pulmonary adenocarcinomas, Ultrastruct. Pathol. 20 (1996) 293–325.
[15] V.Y. Jo, E.S. Cibas, G.S. Pinkus, Claudin-4 immunohistochemistry is highly effective in distinguishing adenocarcinoma from malignant mesothelioma in effusion cytology, Cancer Cytopathol. 122 (2014) 299–306.
[16] N.G. Ordóñez, Value of claudin-4 immunostaining in the diagnosis of mesothelioma, Am. J. Clin. Pathol. 139 (2013) 611–619.
[17] B. Thapa, M. Walkiewicz, C. Murone, et al., Calretinin but not caveolin-1 correlates with tumour histology and survival in malignant mesothelioma, Pathology 48 (2016) 660–665.
[18] J.L. Sauter, S.E. Kerr, GATA3 expression in malignant mesothelioma is a potential pitfall in effusion cytology, Mod. Pathol. 29 (2016) 114A–115A.
[19] M. Zenali, M.T. Deavers, N. Kalhor, V.G. Prieto, Malignant mesothelioma expresses Melan-A (A-103), a possible diagnostic pitfall, Mod. Pathol. 24 (2011) 429A–430A.
[20] J. Wang, R.L. Katz, J. Stewart, G. Landon, M. Guo, Y. Gong, Fine needle aspiration of lymphoma with signet ring features: potential pitfalls and solutions, Cancer Cytopathol. 12 (2013) 525–532.
[21] C.R. Gore, N.K. Panicker, H. Kumar, A. Buch, S.S. Chandanwale, A rare case of signet ring cell lymphoma: diagnosis aided by immunofluorescent staining, J. Cytol. 34 (2017) 56–58.
[22] J.B. Prins, K.A. Williamson, M.M. Kamp, et al., The gene for the cyclindependent-kinase-4 inhibitor, CDKN2A, is preferentially deleted in malignant mesothelioma, Int. J. Cancer 75 (1998) 649–653.
[23] J.Q. Cheng, S.C. Jhanwar, W.M. Klein, et al., p16 Alterations and deletion mapping of 9p21-P22 in malignant mesothelioma, Cancer Res. 54 (1994) 5547–5551.
[24] Q. Arif, A.N. Husain, Malignant mesothelioma diagnosis, Arch. Pathol. Lab. Med. 139 (2015) 978–980.
[25] M. Takeda, T. Kasai, Y. Enomoto, et al., 9p21 deletion in the diagnosis of malignant mesothelioma, using fluorescence in situ hybridization analysis, Pathol. Int. 60 (2010) 395–399.
[26] S. Chiosea, A. Krasinskas, P.T. Cagle, K.A. Mitchell, D.S. Zander, S. Dacic, Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas, Mod. Pathol. 21 (2008) 742–747.
[27] M. Cheung, J. Talarchek, K. Schindeler, et al., Further evidence for germline BAP1 mutations predisposing to melanoma and malignant mesothelioma, Cancer Genet. 206 (2013) 206–210.
[28] M. Bott, M. Brevet, B.S. Taylor, et al., The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma, Nat. Genet. 43 (2011) 668–672.
[29] J. Andrici, A. Sheen, L. Sioson, et al., Loss of expression of BAP1 is a useful adjunct, which strongly supports the diagnosis of mesothelioma in effusion cytology, Mod. Pathol. 28 (2015) 1360–1368.