Int J Clin Exp Pathol 2015;8(5):5318-5326
www.ijcep.com /ISSN:1936-2625/IJCEP0006943

Original Article

Mesothelial cell inclusions in pelvic and para-aortic lymph nodes: a clinicopathologic analysis

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Received February 10, 2015; Accepted April 12, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: Benign lymph node inclusions are commonly encountered during surgery for gynecologic neoplasms and are potential mimics of metastatic tumor. The presence of mesothelial cell inclusions in pelvic lymph nodes is extremely rare. We report the clinicopathologic features of 10 patients with ovarian tumors and mesothelial cell inclusions detected in the sinuses of pelvic and paraaortic lymph nodes. All patients had concurrent massive ascites and mesothelial cell hyperplasia at the time of lymph node dissection. Histologically, nodal mesothelial cells were identified predominantly within the subcapsular, trabecular and medullary sinuses. Moreover, intra- and extranodal lymphatics also contained mesothelial cells, confirming their mode of lymphatic transport to nodal sinuses. This finding, together with mesothelial cell hyperplasia and massive ascites suggest that mesothelial cells derive from reactive serosal mesothelium and are dislodged into draining lymphatics. This study indicated the pathogenic significance of the lymphatic transport mechanism. Nodal mesothelial cell inclusions should be distinguished from metastatic tumor to avoid inaccurate staging in a patient with a known tumor or the false negative diagnosis of an occult primary tumor. Recognition of this entity by immunohistochemical evaluation in addition to routinely stained sections is important to prevent a diagnosis of metastatic carcinoma or malignant mesothelioma.

Keywords: Mesothelial cell, inclusion, lymph node, ascites, metastatic carcinoma

Introduction

A large number of lymph node inclusions have been described in a variety of anatomical sites. They are usually epithelial and only rarely non-epithelial. They can give rise to an errorneous diagnosis of metastasis. In particular, the staging operation has recently become a frequent procedure in the management of female reproductive tract tumors, and involves pelvic and para-aortic lymph node dissection, inspection of the peritoneal surface, multiple biopsies and the resection of suspicious lesions. The occasional presence of benign inclusions in the resected specimens can mimic metastatic malignancy and lead to a diagnostic error. It is therefore necessary to be adequately informed about these inclusions, including their anatomic sites, histologic characteristics and their association with a tumor or an inflammatory process.

Benign inclusions of the lymph nodes include nevus cells, decidua, thyroid follicles, salivary gland tissue, breast tissue, squamous epithelium, Mullerian-type epithelium and mesothelial cells [1-4]. Mullerian-type epithelium in pelvic and para-aortic lymph nodes is commonly found in women [1]. Inclusions of mesothelial cells in the nodal sinuses reportedly occur mostly within mediastinal lymph nodes, and occasionally within cervical and abdominal lymph nodes [5-19]. On the other hand, localization of these inclusions in pelvic or para-aortic lymph nodes is an exceedingly rare event; to the best of our knowledge, only 5 cases have been previously reported [5, 14, 20].

We recently encountered 10 cases of mesothelial cell inclusions in pelvic and/or para-aortic lymph nodes associated with several different types of ovarian tumors. Because of the poten-

Table 1. Clinical features of 10 cases with mesothelial cell inclusions in pelvic and para-aortic lymph nodes

Case	Age/ Sex	Clinical presentation	Primary diagnosis	Large-volume ascites	Outcome	Follow-up (month)
1	52/F	Adnexal mass	Clear cell carcinoma of ovary	Present	NED	3
2	45/F	Abdominal distension	High-grade serous carcinoma of ovary	Present	AWD	38
3	75/F	Adnexal mass	High-grade serous carcinoma of ovary	Present	DOD	32
4	41/F	Abdominal distension	Struma ovarii	Present	NED	5
5	49/F	Adnexal mass	Proliferative struma ovarii	Present	NED	6
6	50/F	Adnexal mass	Proliferative struma ovarii	Present	NED	41
7	50/F	Bilateral adnexal masses	Serous borderline tumor of ovary	Present	NED	16
8	61/F	Adnexal mass	Mucinous carcinoma of ovary	Present	NED	53
9	50/F	Bilateral adnexal masses	Malignant Brenner tumor of ovary	Present	NED	5
10	52/F	Bilateral adnexal masses	Malignant Brenner tumor of ovary	Present	NED	2

NED indicates no evidence of disease; AWD, alive with disease; DOD, died of cancer-related disease.

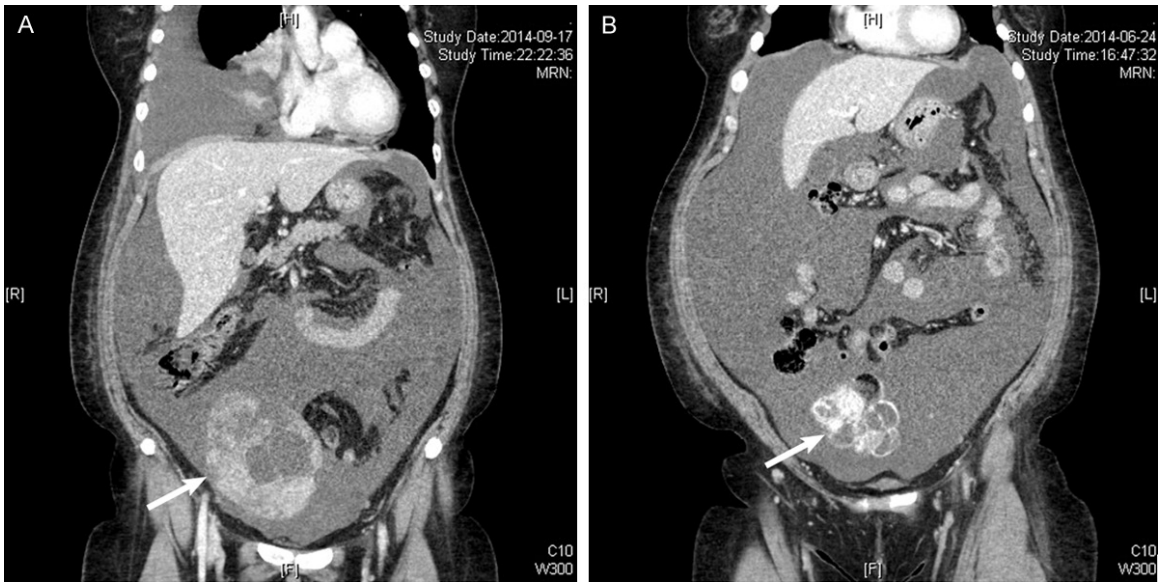

Figure 1. Representative imaging findings of A. Case 1 and B. Case 5. Abdominopelvic computed tomographic scan reveal massive ascites and adnexal masses (white arrows).

tial diagnostic problems associated with this finding, we provided a detailed description of clinicopathologic features and immunohistochemical results of these cases. We also discussed a possible pathogenic mechanism for nodal mesothelial cell inclusions.

Materials and methods

Patients and tissue specimens

We reviewed all hematoxylin and eosin-stained slides obtained from pelvic and para-aortic lymph node dissection specimens at the Samsung Medical Center (Seoul, Republic of Korea) from January 2009 to December 2014. Specimens with a final diagnosis of mesothelial cell inclusions in the pelvic and/or paraaortic lymph nodes were then selected for medical chart review. All specimens were reviewed by board-certified pathologists specializing in gynecologic pathology. The charts of these patients were reviewed for age, presenting symptoms, preoperative imaging findings, primary diagnosis, nodal status and follow-up data.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks were available for immunohistochemical staining in each case. Protein expression was

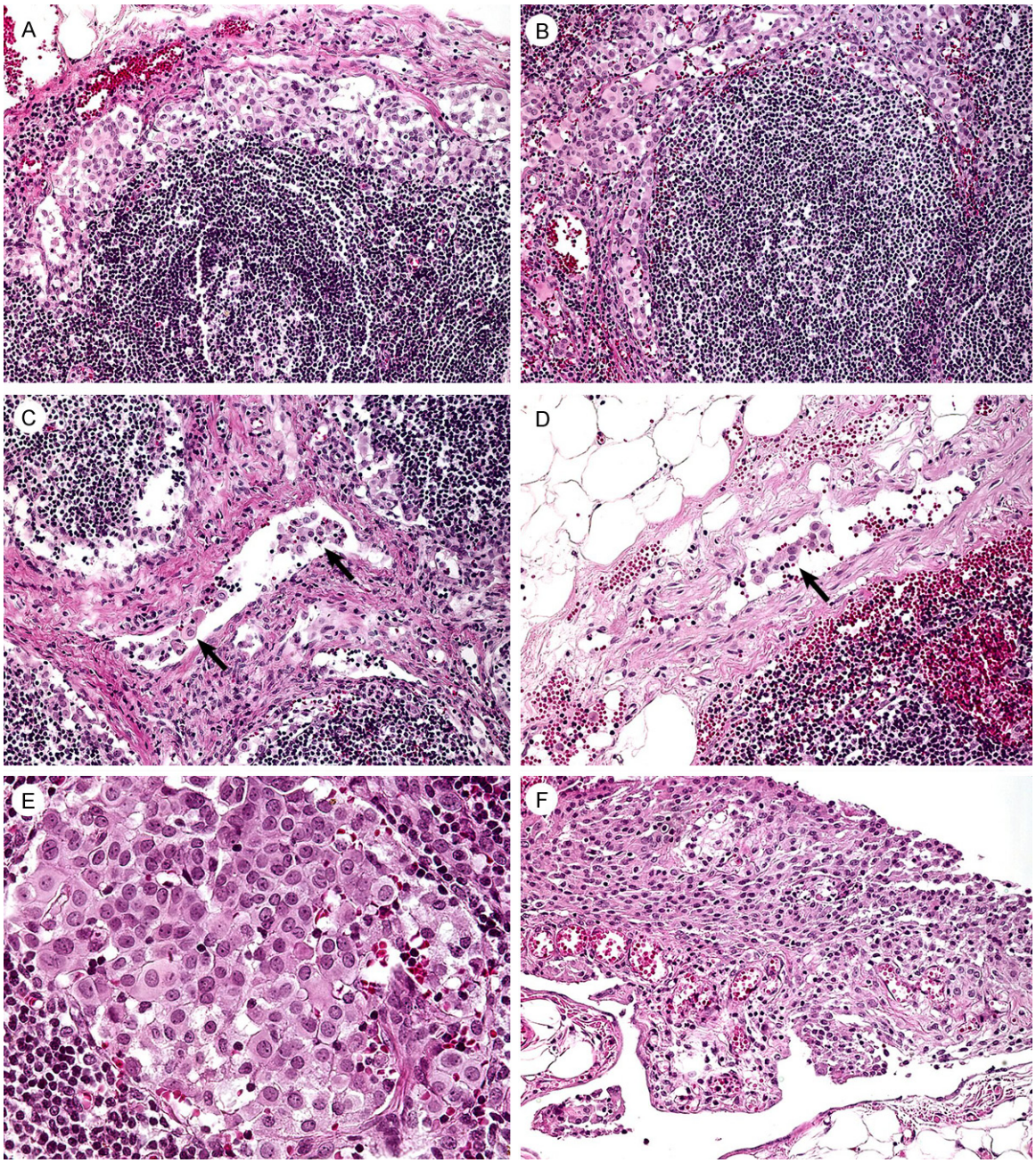

Figure 2. Histopathological findings. A. The mesothelial cells distend the subcapsular sinus. B. They form cuffs surrounding a lymphoid follicle. Both The C. Intra- and D. extranodal lymphatics contain mesothelial cells (black arrows). E. Clusters of mesothelial cells with polygonal shape and eosinophilic cytoplasm expand the medullary sinus. These cells show round or oval nuclei with inconspicuous nucleoli and no significant nuclear atypia. F. The omentum displays florid mesothelial cell hyperplasia.

evaluated using a Ventana BenchMark XT automated staining system (Ventana Medical Systems, Inc., Tucson, AZ, USA) with antibodies against calretinin (1:80, clone 5A5, Novocastra, Newcastle upon Tyne, UK), pan-cytokeratin (CK; 1:500, clone AE1/AE3, Dako, Glostrup, Denmark), D2-40 (1:100, clone D2-40, Dako), WT-1 (1:50, clone 6F-H2, Dako), CD68 (1:2,000, clone KP-1, Dako), carcinoembryonic antigen (CEA; 1:4,000, polyclonal, Dako), epithelial membrane antigen (EMA; 1:300, clone E29, Dako), p53 (1:5,000, clone DO-7, Dako) and thyroid transcription factor (TTF-1; 1:100, clone 8G7G3/1, Dako), according to the manufactur-

Table 2. Histopathological and immunohistochemical findings of 10 cases with mesothelial cell inclusions in pelvic and para-aortic lymph nodes

Case	Histopathological findings				Immunostaining results
	Location of involved lymph nodes	Microanatomical Location	Number of involved nodes	Association With MCH	
1	Pelvic, para-aortic	SCS, TS, ENL	3/37	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-
2	Pelvic	SCS, TS, MS, INL, ENL	6/82	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-/p53-
3	Pelvic	SCS, TS, MS, ENL	4/20	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-/p53-
4	Pelvic	SCS, TS, ENL	2/12	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-
5	Pelvic	SCS, TS, MS, INL	3/11	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-/TTF-1-
6	Pelvic, para-aortic	SCS, TS, MS, ENL	4/26	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-/TTF-1-
7	Pelvic, para-aortic	SCS, TS, MS, ENL	5/17	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68–/CEA-/EMA-
8	Pelvic	SCS, TS, ENL	4/11	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-
9	Pelvic, para-aortic	SCS, TS, ENL	2/21	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-
10	Pelvic	SCS, TS, INL, ENL	4/31	Yes	Calretinin+/pan-CK+/D2-40+/WT-1+CD68-/CEA-/EMA-

SCS, subcapsular sinus; TS, trabecular sinus; ENL, extranodal lymphatics; INL, intranodal lymphatics; MS, medullary sinus; CK, cytokeratin; WT-1, Wilms tumor-1; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; TTF-1, thyroid transcription factor-1.

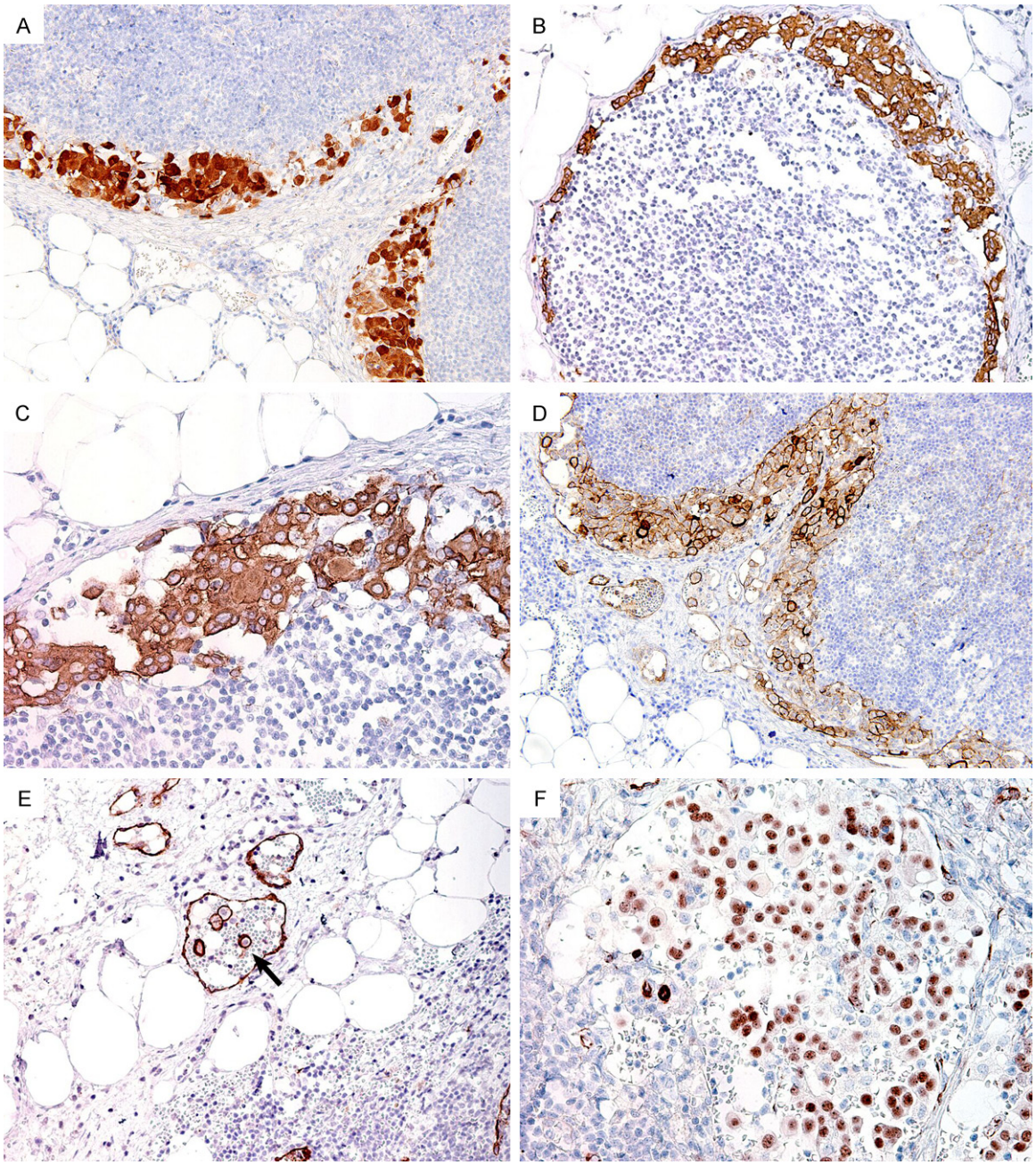

Figure 3. Immunohistochemical staining results. A. Calretinin intensely stains mesothelial cells within the subcapsular sinuses. B. Pan-CK highlights the presence of mesothelial cells that surround a lymphoid follicle. C. Pan-CK is strongly positive in the cytoplasm and membrane of mesothelial cells. D. Positive expression of D2-40 is also located in both the membrane and cytoplasm. E. The presence of mesothelial cells within extranodal lymphatics is confirmed by D2-40, which stains both lymphatic endothelial cells and mesothelial cells (black arrow). F. WT-1 immunostaining demonstrates uniform, strong nuclear expression in the mesothelial cells.

er's protocol. Briefly, 4-μm tissue sections were deparaffinized and rehydrated, and antigens were retrieved for 40 min in a citrate buffer (pH 6.1) at 95°C. 3,3'-diaminobenzidine was used as the chromogen, and the sections were counterstained with hematoxylin.

Results

Clinical findings

The clinicopathologic features of 10 patients with mesothelial cell inclusions in pelvic and/or

para-aortic lymph nodes were summarized in **Table 1**. The median age of patients was 50 years (range, 41-75 years). Eight patients presented with unilateral or bilateral adnexal masses, and 2 patients complained primarily of abdominal distension. Six patients received surgery for malignant ovarian tumors, including clear cell carcinoma (n=1), high-grade serous carcinoma (n=2), mucinous carcinoma (n=1) and malignant Brenner tumor (n=2). Three patients had struma ovarii; tumors in 2 of these patients showing histologic features of follicular adenoma of the thyroid gland were diagnosed as proliferative struma ovarii [21]. One patient received surgery for serous borderline tumor. Preoperative imaging studies revealed massive ascites ranging from 4-6 L in all cases. Representative computed tomographic scan findings were shown in **Figure 1**. Pelvic and/or para-aortic lymphadenectomy was performed in all cases. The median number of dissected lymph nodes was 20 (range, 11-82).

Pathologic findings

Grossly, the appearance of dissected lymph nodes was unremarkable, hence a minimal gross description of the involved lymph nodes was provided. The greatest dimension of the involved lymph node ranged from 0.2 cm to 3.2 cm. In 4 cases (Cases 2, 3, 8 and 9), pelvic lymph nodes harbored both metastatic tumor cells and mesothelial cells. In the remaining cases, non-tumorous lymph nodes contained mesothelial cells.

Microscopically, all cases had clusters of mesothelial cells confined to nodal sinuses. The median number of involved lymph nodes was 4 (range, 2-6). These were most prominent in the supcapsular and trabecular sinuses (**Figure 2A**), but in 5 cases they also packed the medullary sinuses. The sinuses containing mesothelial cells were more expanded than neighboring sinuses that contained histiocytes only. Some of these mesothelial cells formed cuffs surrounding lymphoid follicles (**Figure 2B**). Occasionally, cohesive nests or individual cells were identified within the lumina of dilated intranodal lymphatics (**Figure 2C**) or within lymphatics in the extranodal adipose tissue (**Figure 2D**). Cytologically, the mesothelial cells were polygonal, with abundant eosinophilic cytoplasm and bland vesicular nuclei (**Figure 2E**). Mesothelial cells were difficult to distinguish from sinus histiocytes due to their relatively indistinct cell borders, together with bland nuclear features. Mesothelial cells, however, exhibited a greater degree of cohesiveness, larger size, more eosinophilic cytoplasm, slightly better demarcated cell borders, epithelioid appearance and prominent nucleoli. Metastatic carcinoma was ruled out based on absence of significant nuclear atypia or hyperchromasia and condensed chromatin.

The outcome of patients was also summarized in **Table 1**. The median follow-up time was 15 months (range, 2-52). Eight patients are currently without evidence of disease after follow-up. One patient is alive with stable disease, and the remaining patient died of ovarian cancer. No patients developed complications or recurrent lesions associated with nodal mesothelial cell inclusions.

A summary of the immunohistochemical staining results was listed in **Table 2**. The findings clearly supported the mesothelial phenotype in each case. The mesothelial cells were strongly positive for calretinin (**Figure 3A**). Pan-CK immunostaining showed a membranous and cytoplasmic staining pattern (**Figure 3B**, **3C**). The distinction between intranodal mesothelial cells and histiocytes was facilitated by the pan-CK staining, which highlighted the presence of the strongly reactive mesothelial cells, in contrast to the nonreactive histiocytes. Positive expression of D2-40 was also located in both the membrane and cytoplasm (**Figure 3D**, **3E**). D2-40, which stains both the lymphatic endothelial cells and mesothelial cells, confirmed the presence of mesothelial cells within the extranodal lymphatics. WT-1 immunostaining revealed uniform, strong nuclear expression in the mesothelial cells (**Figure 3F**). The immunostained slides showed greater mesothalial cell involvement than could be detected on the hematoxylin and eosin-stained slides. The mesothelial cells were negative for CD68, CEA and EMA (in all cases) and p53 (in 2 cases). In addition, the absence of TTF-1 expression excluded the possibility of metastatic follicular carcinoma in two patients with proliferative struma ovarii.

In 4 cases with malignant ovarian tumors (Cases 2, 3, 8 and 9), biopsy samples of the pelvic peritoneum and omentum revealed both metastatic tumor and mesothelial cell hyper-

plasia. The other peritoneal biopsy specimens and omentum also showed a variable degree of inflammatory reaction and mesothelial cell hyperplasia (**Figure 2F**). Additionally, 2 cases had foci of endosalpingiosis within pelvic lymph nodes. In another case, 2 para-aortic lymph nodes contained ectopic decidua and decidualized intranodal endometriosis, respectively.

Discussion

This report documented the presence of mesothelial cells within pelvic and paraaortic lymph nodes that were removed as part of staging laparotomy in 10 women with ovarian tumors. In each case, we found massive ascites and hyperplasia of the peritoneal mesothelial cells, a common finding in patients with ovarian tumors. To the best of our knowledge, this is the largest single-center cohort of patients with nodal mesothelial cell inclusions occurring in association with massive ascites.

Benign mesothelial cells that involve the sinuses of mediastinal lymph nodes were originally described by Brooks et al. in 1990 [6]. They described mesothelial cell inclusions in 2 patients with pleuritis and pleural effusions. Rutty and Lauder reported another case of mesothelial cell inclusions within mediastinal lymph nodes, associated with both pleural and pericardial effusion [18]. In addition, mesothelial cell inclusions were reported in cervical, mediastinal, abdominal and pelvic lymph node sinuses [7, 13, 20, 22]. Most of these cases had concurrent serosal effusions. Nodal mesothelial cells are thought to originate from mesothelial surfaces disrupted by serosal effusions [11]. It is believed that mesothelial cell inclusions represent migration from the pleural or peritoneal cavity through preformed stomata that are located within the pleura or peritoneum [6, 23]. Lymphatic transport of dislodged mesothelial cells is the likely pathogenic mechanism for nodal mesothelial cell inclusions [6, 7, 18]. Peritoneal and pleural stomata were confirmed in animal studies [23]. These stomata link the peritoneal cavity with submesothelial lymphatics. Peritoneal inflammation and mesothelial reactions disrupt mesothelial stomata and distend lymphatics, allowing mesothelial cells to access submesothelial lymphatics and enter and drain into the nodal sinuses [6, 7, 18]. Their entry could be facilitated by the pressure gradient, secondary to massive ascites. The proposed pathogenesis was supported by the significant association of pleural, pericardial and peritoneal effusions with mesothelial cells in nodal sinuses [5-7, 9, 11, 12, 14, 16, 18-20, 22]. Consistent with previous data, it is possible that massive ascites present in this study led to distension of intraperitoneal lymphatics, allowing access of mesothelial cells to pelvic lymph node sinuses.

In addition to mesothelial cells in the nodal sinuses, there were mesothelial cell clusters within both intra- and extranodal lymphatics. The extranodal lymphatic permeation by mesothelial cells is confirmatory evidence of their lymphatic transport and strongly supports the lymphatic transport theory of pathogenesis of mesothelial cell inclusions within lymph nodes. To the best of our knowledge, there have been 3 previous reports of extranodal lymphatic involvement by mesothelial cells in the presence of nodal inclusions [7, 12].

Often mesothelial cell inclusions can present a diagnostic dilemma to the pathologist. The most important differential diagnosis of nonglandular nodal inclusions includes metastatic carcinoma and benign mesothelial cell inclusions. It is important not to misdiagnose mesothelial cells within pelvic lymph nodes as metastatic carcinoma, since this error could result in incorrect staging and overtreatment of patients with known intra-abdominal or pelvic malignant tumors or false negative diagnosis of occult primary tumors in patients without obvious tumor. Isotalo et al. [12] described a patient who received radiotherapy on misdiagnosis of mesothelial cells within the mediastinal lymph node sinuses as metastatic carcinoma. Benign mesothelial cell inclusions involve exclusively nodal sinuses and do not infiltrate the nodal parenchyma. Metastatic carcinoma, unlike benign inclusions, may demonstrate glandular differentiation and infiltration of the nodal capsule or cortex. The exclusively sinusoidal involvement and the characteristic appearance of the mesothelial cells including their bland nuclear features differentiate them from metastatic carcinoma. Furthermore, sinus mesothelial cells may also be confused with sinus histiocytes, metastatic melanoma and metastatic mesothelioma [6, 7, 22]. The benign histologic appearance of mesothelial cells and their unique immunophenotype are important identifying features [6, 7, 22]. Sinus histiocytosis

shows a proliferation of large histiocytes with abundant cytoplasm and bland nuclear features within the sinuses. The lack of nuclear atypia, positivity for CD68 and negativity for epithelial and mesothelial cell markers confirms the histiocytic origin. The majority of metastatic melanoma has an antecedent history of primary malignant melanoma on the skin or mucous membrane. Morphological diversity and/or lipofuscin granules can be seen in the cytoplasm. Melanoma cells exhibit diffuse positivity for S-100 protein, as well as positivity for melanocytic markers including HMB45 and melan-A. Malignant mesothelioma often presents a definite primary lesion, i.e., multiple, discrete, irregular nodules on the serosal surface. The patient usually has an obvious tumor mass. A high-grade nuclear atypia, formation of microtubules, tubulopapillary and alveolar patterns, destructive stromal infiltrates and sclerosis favor a diagnosis of malignant mesothelioma [7].

In summary, we demonstrated benign mesothelial cell inclusions in pelvic and para-aortic lymph nodes in patients with different types of ovarian tumors. In general, they consist of bland-appearing polygonal cells in the subcapsular and trabecular sinuses that are most likely overlooked or misinterpreted as histiocytes. Rarely, these cells are numerous and are suspected as metastatic carcinoma, melanoma or mesothelioma. The distinction from metastatic tumors is based on the exclusively sinusoidal pattern of involvement, bland nuclear features and immunophenotype. Mesothelial cell inclusions are likely to result from the dislodgement and subsequent lymphatic transport in the presence of serosal effusions. Even though these inclusions are benign, their identification may cause a diagnostic problem. Awareness of these mesothelial cells, their location and their similarity with malignant neoplasms, is crucial in preventing both misdiagnosis and the inappropriate therapeutic management of patients.

Acknowledgements

This paper was supported by a grant of the Korean Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI14C3418).

Disclosure of conflict of interest

None.

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