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*From Cell Biology to Tissue Engineering*

# Benign mesothelial cells in lymph nodes and lymphatic spaces associated with ascites

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**Summary.** Intra-nodal mesothelial cells are assumed to be indicative of metastatic mesothelioma. The invasion of benign mesothelial cells into lymph nodes is an extraordinary complication of different (mostly inflammatory) disorders involving the serosal cavities. In a cirrhotic patient with recurrent ascites, this report describes the first case of mesothelial cell spreading into lymphatic vessels, coexisting with non-malignant inclusions of mesothelial cells in multiple abdominal lymph nodes.

**Key words:** Intra-nodal mesothelial cells, Benign mesothelial implants, Cirrhosis, Ascites

## Introduction

The histological differentiation between malignant mesothelioma and mesothelial hyperplasia is a major surgical pathology challenge, which basically relies on both morphology and immunohistochemical profiling. As in the present case, however, the clinico-pathological correlations remain of paramount importance to conclusively establish the diagnosis (Husain et al., 2013).

Overall, the histological detection of mesothelial aggregates in lymph nodes and/or within the lumen of lymphatic vessels is considered indicative of malignant mesothelioma. In rare cases, however, intra-nodal non-neoplastic mesothelial cells have been documented even in inflammatory and neoplastic disorders involving the serosal cavities (mostly associated with pleural, pericardial and/or ascitic effusions) (Brooks et al., 1990; Clement et al., 1996; Parkash et al., 1999; Colebatch et al., 2001; Turner et al., 2003; Moonim et al., 2011; Peng et al., 2013).

Benign mesothelial cell clusters have been described in the subcutaneous lymphatics of a cirrhotic patient (González-Navajas et al., 2007), but no cases of intra-nodal benign mesothelial cell spreading have ever been reported.

This report describes the first case of coexisting lymphatic vessel invasion and nodal inclusions of benign mesothelial cells in a cirrhotic patient with recurrent ascites. The clinical, radiological, and histological features of this extremely rare complication of liver cirrhosis are outlined here. How a differential diagnosis between malignant and benign mesothelial proliferations was achieved is also discussed.

## Materials and methods

### *Clinical and laboratory data*

Clinical and laboratory data were retrieved from the patient's clinical chart. The following clinical, radiological and laboratory parameters were assessed: (i) symptoms at presentation; (ii) physical examination at admission; (iii) cytochemical findings of the ascitic fluid; (iv) PET-TC findings (total body examination).

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The patient's written informed consent was obtained, according to the institutional regulation for clinical research.

### *Histological evaluation*

Formal-fixed, paraffin-embedded tissue sections were stained with Hematoxylin and Eosin (H&E) for histological evaluation. Immunohistochemical analysis was performed on representative tissue samples (peritoneal and lymph node tissue samples), using a fully automated platform (Bond-maX; Leica, Newcastle Upon Tyne, UK) (Pizzi et al., 2015). The following primary antibodies were used: pan-cytokeratin (clone MNF116, Dako), Calretinin (polyclonal, Cell Marque), EMA (clone E29, Thermo Scientific), HMBE-1 (clone HMBE-1, Cell Marque), CK5/6 (clone DS/16B4, Invitrogen), WT-1 (clone BC.6F-H2, Biocare Medical), CK7 (clone OV-TL 12/30, Cell Marque), CEA (clone CEA31, Cell Marque), CK20 (KS20.8, Cell Marque), Desmin (clone D33, Dako), p53 (clone D07, Cell Marque), S100 (polyclonal, Genemed), TTF1 (clone 8G7G3/1, Genemed), p16 (clone E6H4, CINtec) (Table 1).

### *Cytological and microbiologic evaluation of the ascitic fluid*

Ascitic fluid was submitted for both cytology and microbiology evaluations. Cytologic assessment was performed on Giemsa-stained smears. Microbiologic tests included: i) standard bacterial cultures for pathogens commonly associated with spontaneous bacterial peritonitis (*Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*); ii) PCR analysis for the detection of bacterial DNA, using the Mastermix 16S Complete kit (Molzym, Bremen, Germany), as described by S. Krohn and colleagues (Krohn et al., 2014).

## Results

In January 2014, a 66-year-old Caucasian male with an established history of exotoxic cirrhosis and ascites (two episodes in 2012 and 2013) presented at Padova Teaching Hospital with symptoms of abdominal discomfort and dyspnea. Physical examination revealed mild hypotension, muco-cutaneous jaundice, and abdominal swelling. Cytochemical analysis of the ascitic fluid uncovered a neutrophil count of 180 cells/mm$^3$; the microbiological assessment of the peritoneal fluid failed, however, to identify any bacterial infection (no cultural/molecular evidence of *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumoniae* or other bacteria infections). The concurrent cytological assessment of the serous effusion featured clusters of atypical mesothelial cells, "suspicious for abdominal mesothelioma" (Fig. 1A).

Both the cytological findings and the lack of improvement of clinical symptoms led to further clinical investigations. A total body PET-CT scan excluded any thoracic and abdominal lesions suspicious for neoplasia. Laparoscopic exploration of the abdominal cavity uncovered peritoneal hyperemia with no evidence of neoplastic lesions and/or intestinal perforation. Random biopsies of the peritoneum and four enlarged mesenteric lymph nodes were obtained and submitted for histological evaluation.

The biopsy samples obtained from the peritoneal surface disclosed florid mesothelial hyperplasia and (low-grade) sub-serosal inflammation. Superficial mesothelial cells were prominent, and occasionally enlarged with increased nuclear-to-cytoplasmic ratio; they did not feature, however, overt cytological atypia. None of the available biopsy samples showed "infiltrating" mesothelia in the sub-mesothelial fat tissue layer. Occasional aggregates of mesothelial cells (see immunophenotyping results) were present in the enlarged sub-serosal lymphatic vessels. In 3 out of the 4 mesenteric submitted lymph nodes, cohesive clusters of mesothelial cells were also documented within both the sub-capsular and the medullary sinuses; these nodal "mesothelial implants" were not associated with nodal architecture derangement, desmoplastic reaction, or

**Table 1.** Immunohistochemical profile of the intra-vascular/intranodal mesothelial cells

| Antibody | Antibody source | Working dilution | Immunostain |
|---|---|---|---|
| Calretinin | Polyclonal (Cell Marque, Rocklin; CA-USA) | 1:100 | Positive |
| MNF-116 | MNF116 (Dako, Glostrup; DENMARK) | 1:200 | Positive |
| EMA | E29 (Thermo Scientific, Waltham; MA-USA) | 1:200 | Positive |
| HMBE-1 | HBME-1 (Cell Marque, Rocklin; CA-USA) | 1:100 | Positive |
| CK5/6 | DS/16B4 (Invitrogen, Milan; ITALY) | 1:100 | Positive |
| WT-1 | BC.6F-H2 (Biocare Medical, Concord; CA-USA) | 1:50 | Positive |
| CK7 | OV-TL 12/30 (Cell Marque, Rocklin CA-USA) | 1:200 | Weakly positive |
| CEA | CEA31 (Cell Marque, Rocklin; CA-USA) | 1:100 | Negative |
| CK20 | KS20.8 (Cell Marque, Rocklin CA-USA) | 1:200 | Negative |
| Desmin | D33 (Dako, Glostrup; DENMARK) | 1:50 | Negative |
| p53 | D07 (Cell Marque, Rocklin; CA-USA) | 1:50 | Negative |
| S100 | Polyclonal (Genemed, San Francisco; CA-USA) | 1:50 | Negative |
| TTF1 | 8G7G3/1 (Genemed, San Francisco; CA-USA) | 1:50 | Negative |
| p16 | E6H4 (CINtec, Tucson; AZ-USA) | pre-diluted | Weakly positive |

angiogenic activity. Mesothelial cells were confined within the nodal structure, with no invasion of the nodal capsule (Fig. 1B-D).

At immunohistochemical profiling, both the intra-lymphatic and the intra-nodal cells consistently featured Calretinin, MNF-116, EMA, CK7, HBME-1, CK5/6, WT-1, and scattered expression of p16 protein. Immunostaining for Desmin, p53, S100, and other epithelial cell markers (CK20, TTF-1, CEA) were consistently negative (Table 1; Fig. 1E-H).

The histological features were considered consistent with endo-lymphatic spreading of benign mesothelial cells, with nodal "implants" of benign mesothelia; coexisting mesothelial peritoneal hyperplasia, and low-grade subserosal inflammation were also reported.

A year later, the patient was referred to the same hospital for worsening of a preexisting inguinal hernia and the lesion was surgically treated. In view of the previous suspicion of mesothelial malignancy, the abdominal cavity was carefully explored (laparoscopy), but no evidence of any gross lesion suspicious for malignancy was found.

At his last clinical examination (July 2015), the patient did not refer any clinical signs/symptoms besides those expected in a cirrhotic patient.

## Discussion

The spreading of mesothelial cells into lymphatic vessels and/or in lymph nodes is usually considered as evidence of malignancy; both these situations, however, may extraordinarily occur in either inflammatory, or neoplastic disorders involving serosal cavities (Brooks et al., 1990; Clement et al., 1996; Parkash et al., 1999; Colebatch et al., 2001; Turner et al., 2003; Moonim et al, 2011; Peng et al., 2013). The single case associated with cirrhosis simply featured mesothelial vascular embolization (Suarez-Vilela and Izquierdo-Garcia, 2000), and no cases have ever been described of nodal mesothelial benign implants.

The pathophysiology of this rare clinico-biological situation is poorly understood. Electron microscopy studies demonstrate that normal subserosal lymphatic vessels are structured in terminal *lacunae* in close proximity to the serosal surface. In view of this peculiar anatomic situation, any damage to the serosal barrier (due to both mechanical or inflammatory situations) may theoretically permit mesothelial cells to gain access to the lymphatic system, potentially resulting in benign mesothelial implants in lymph nodes (Wang, 1975; Brooks et al., 1990).

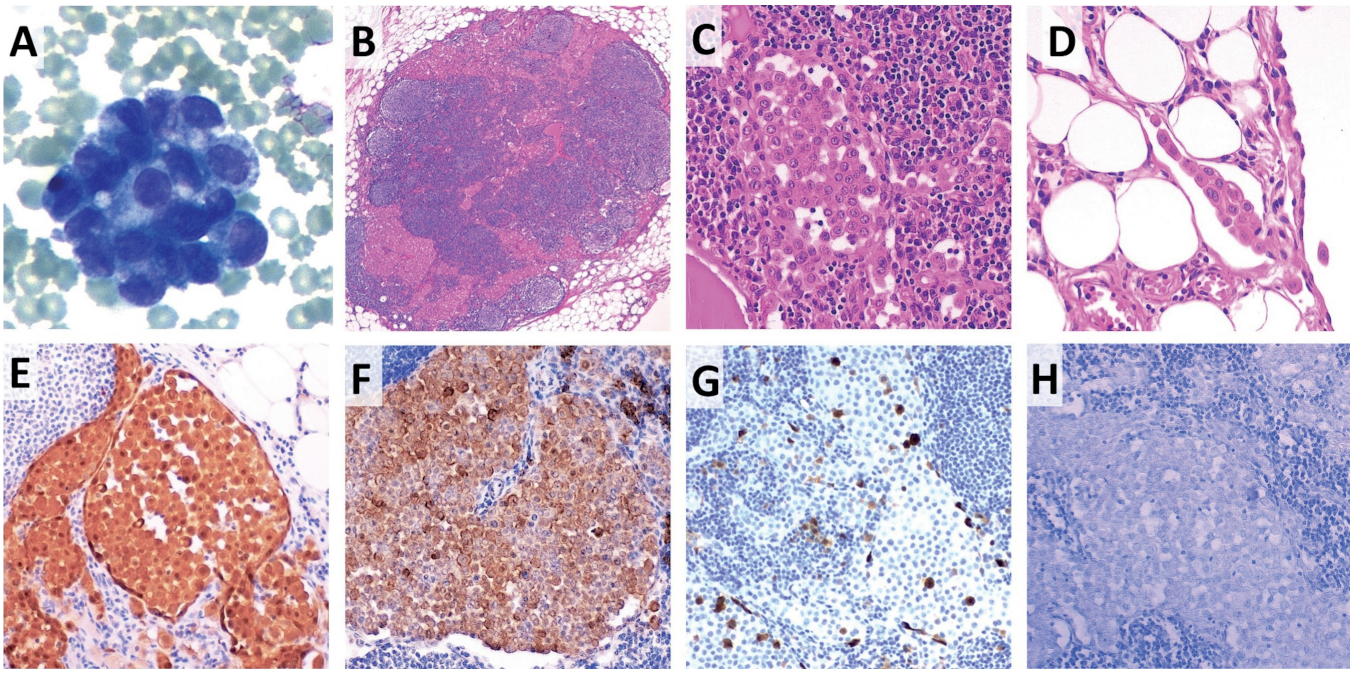


**Fig. 1.** Cytological, histological and immunohistochemical features of the peritoneal, intra-vascular and intra-nodal mesothelial clusters. **A.** The ascitic fluid smear showed occasional clusters of mesothelial cells with enlarged nuclei. **B.** Intra-nodal clusters of mesothelial cells in the sub-capsular and medullary sinuses. **C.** Mesothelial cells were confined within the ectatic sinuses, with no isolated mesothelial cells infiltrating the surrounding nodal parenchyma. **D.** Mesothelial micro-emboli in peritoneal subserosal lymphatic vessels. **E-F.** Intranodal mesothelial cells expressing Calretinin **(E)**, EMA **(F),** and p16 **(G)**; no immunostain for Desmin is documented **(H)**. Giemsa, H&E and immunoperoxidase stains; original magnification x 4, x 10, x 20 and x 40

In the present case, the mesothelial profile of the nodal inclusions was consistently confirmed by immunohistochemistry; additionally, the expression of p16 protein argued against those p16 derangements, which have been mostly associated (at both genetic, and protein level) with mesothelial malignancies (Chiosea et al., 2008; Takeda et al., 2010; Hwang et al., 2014). Hence, in spite of the alarming histological features of the "misplaced" mesothelia, the conclusive diagnosis of non-neoplastic disease was primarily based on the "benign phenotype" of the nodal implants: preserved nodal architecture, lack of infiltrating growth pattern, the absence of any angiogenic activity and desmoplasia (Attanoos et al., 2003; Mocanu et al., 2007; Hasteh et al., 2010; Husain et al., 2013). The non-malignant nature of the intra-vascular/intra-nodal mesothelial spreading has been further supported by the long-term follow up, which consistently excluded any neoplastic disease, as reliably assessed by both PET-CT, and by two laparoscopic inspections of the abdominal cavity.

We report the first case of a cirrhotic patient in whom a benign mesothelial embolization to the lymphatic vessels coexisted with multiple nodal mesothelial implants. Both of these features should be included among the rare, non-neoplastic complications of recurrent ascites associated with cirrhosis.

## References

Attanoos R.L., Griffin A. and Gibbs A.R. (2003). The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium: A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. Histopathology 43, 231-238.

Brooks J.S., LiVolsi V.A. and Pietra G.G. (1990). Mesothelial cell inclusions in mediastinal lymph nodes mimicking metastatic carcinoma. Am. J. Clin. Pathol. 93, 741-748.

Chiosea S., Krasinskas A., Cagle P.T., Mitchell K.A., Zander D.S. and Dacic S. (2008). Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. Mod. Pathol. 21, 742-747.

Clement P.B., Young R.H., Oliva E., Sumner H.W. and Scully R.E. (1996). Hyperplastic mesothelial cells within abdominal lymph nodes: mimic of metastatic ovarian carcinoma and serous borderline tumor--a report of two cases associated with ovarian neoplasms. Mod. Pathol. 9, 879-886.

Colebatch A., Clarkson A. and Gill A.J. (2011). Benign mesothelial cells as confounders when cytokeratin immunohistochemistry is used in sentinel lymph nodes. Hum. Pathol. 42, 1209-1210.

González-Navajas J.M., Francés R. and Such J. (2007). Bacterial DNA in patients with cirrhosis and sterile ascites. Its role as a marker of bacterial translocation and prognostic tool. Rev. Esp. Enferm. Dig. 99, 599-603.

Hasteh F., Lin G.Y., Weidner N. and Michael C.W. (2010). The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. Cancer Cytopathol. 118, 90-96.

Husain A.N., Colby T., Ordonez N., Krausz T., Attanoos R., Beasley M.B., Borczuk A.C., Butnor K., Cagle P.T., Chirieac L.R., Churg A., Dacic S., Fraire A., Galateau-Salle F., Gibbs A., Gown A., Hammar S., Litzky L., Marchevsky A.M., Nicholson A.G., Roggli V., Travis W.D. and Wick M.; International Mesothelioma Interest Group. (2013). Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. Arch. Pathol. Lab. Med. 137, 647-667.

Hwang H., Tse C., Rodriguez S., Gown A. and Churg A. (2014). p16 FISH deletion in surface epithelial mesothelial proliferations is predictive of underlying invasive mesothelioma. Am. J. Surg. Pathol. 38, 681-688.

Krohn S., Böhm S., Engelmann C., Hartmann J., Brodzinski A., Chatzinotas A., Zeller K., Prywerek D., Fetzer I. and Berg T. (2014). Application of qualitative and quantitative real-time PCR, direct sequencing, and terminal restriction fragment length polymorphism analysis for detection and identification of polymicrobial 16S rRNA genes in ascites. J. Clin. Microbiol. 52, 1754-1757.

Mocanu L., Cimpean A.M. and Raica M. (2007). Expression of cytokeratin MNF116 and vimentin in pleural serous effusions. Rom. J. Morphol. Embryol. 48, 291-294.

Moonim M.T., Ng W.W. and Routledge T. (2011). Benign metastasizing mesothelial cells: a potential pitfall in mediastinal lymph nodes. J. Clin. Oncol. 29, 546-548.

Parkash V., Vidwans M. and Carter D. (1999). Benign mesothelial cells in mediastinal lymph nodes. Am. J. Surg. Pathol. 23, 1264-1269.

Peng L., Shen Q., Liu X., Wang J., Shi S., Yu B. and Zhou X. (2013). Diffuse hyperplastic mesothelial cells in multiple lymph nodes: case report with review of the literature. Int. J. Clin. Exp. Pathol. 6, 926-931.

Pizzi M., Piazza F., Agostinelli C., Fuligni F., Benvenuti P., Mandato E., Casellato A., Rugge M., Semenzato G. and Pileri S.A. (2015). Protein kinase CK2 is widely expressed in follicular, Burkitt and diffuse large B-cell lymphomas and propels malignant B-cell growth. Oncotarget 6, 6544-6552.

Suarez-Vilela D. and Izquierdo-Garcia F.M. (2000). Hyperplastic mesothelial cells in mediastinal lymph node sinuses. Arch. Pathol. Lab. Med. 124:1749.

Takeda M., Kasai T., Enomoto Y., Takano M., Morita K., Kadota E. and Nonomura A. (2010). 9p21 deletion in the diagnosis of malignant mesothelioma, using fluorescence in situ hybridization analysis. Pathol. Int. 60, 395-399.

Turner R.R., Nora D.T., Trocha S.D. and Bilchik A.J. (2003). Colorectal carcinoma nodal staging. Frequency and nature of cytokeratin-positive cells in sentinel and nonsentinel lymph nodes. Arch. Pathol. Lab. Med. 127, 673-679.

Wang N. (1975). The preformed stomas connecting the pleural cavity and the lymphatics in the parietal pleura. Am. Rev. Respir. Dis. 111, 12-20.

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