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EXPLORING THE MESENTERIC LYMPHATIC APPARATUS: A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION WITH CLINICAL CORRELATIONS

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ABSTRACT

Modern immunohistochemical techniques allow a detailed study of the lymphatic system in many organs and areas of the body. We performed an in-depth study on lymphatic vessels of the ileal and colonic mesenteries, together with the greater omentum where they appear particularly numerous and mainly represented by capillaries interconnected among themselves and with lymph nodes. The capillary wall consists of a fine single sheath of endothelial cells wrapped around by a subtle collagen membrane and deprived of valves. The progression of lymph flow is promoted by external forces acting on the capillary walls. Only at the mesenteric roots can pre- and post-lymph nodal collector vessels be observed. Our observations help to explain different patho-physiological correlations and the possible presence of skip lymph node metastases.

Keywords: lymphatic vessels, lymphatic apparatus, mesentery, immunohistochemistry, podoplanin, D2-40, skip lymph node metastasis

Until today, the highest interest of pathologists and surgeons has been focused on the number and location of colonic lymph nodes in order to accomplish radical dissection and to precisely stage the colon tumor. It has been widely accepted that the lymph node spatial arrangement follows the main arterial arcades of each colonic or ileal segment (1). In the past, no particular attention was directed at the mesenteric lymphatic capillaries in humans primarily because the histological techniques were not adequate to investigate this fine lymphatic network. Recently, immunohistochemical improvements have made possible a more precise study of these small lymphatic vessels (including the capillaries) (2-5), and we can now revisit this area and its pathophysiological implications.

MATERIALS AND METHODS

We utilized surgical specimens of colon and ileal segments from adult patients (mean age: 67 years) operated on for different inflammatory or neoplastic conditions. Cases were excluded with evident mesenteric lesions such as peritoneal inflammation or carcinomatosis, acute or chronic vascular lesions, portal hypertension, etc. We have focused our studies on 20 right colonic resections for adenocarcinoma, 30 left colonic

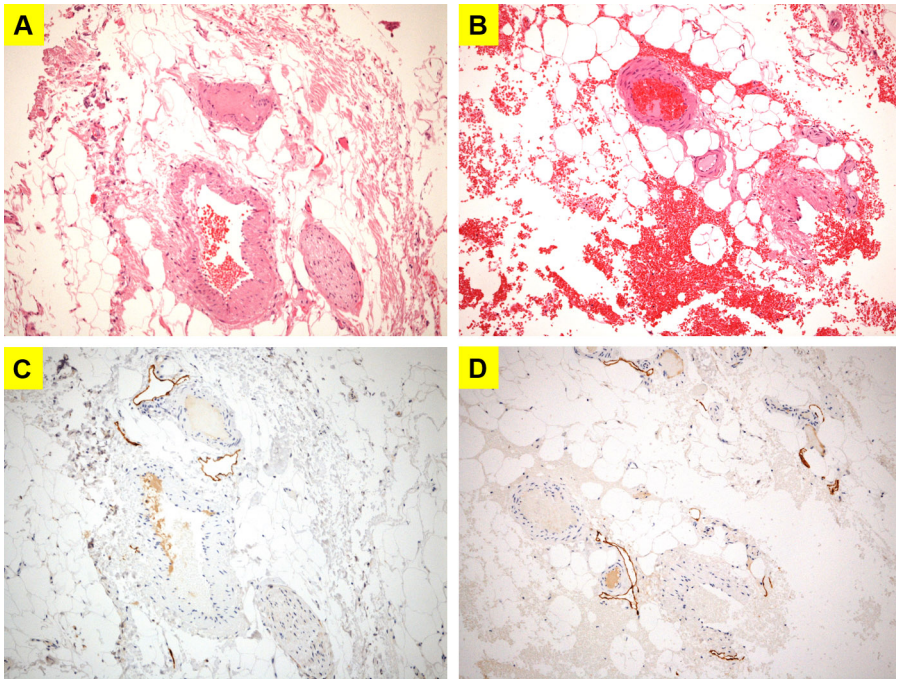

Fig. 1. In the mesentery (A, H&E, x10) and omentum (B, H&E, x10), the lymphatic apparatus is constituted by thin capillaries, which run along the neurovascular bundles, brown-stained with anti-podoplanin (C and D, x10).

resections (15 performed for adenocarcinoma and 15 for non-acute diverticulitis), and 20 ileal resections for chronic adhesions. Tissues were collected following surgical specimens with the first sample proximal to the intestinal wall, a second in the central part of the mesentery, and a third corresponding to the more distal region of the mesenteric root. The location of these samples corresponded to that of the equivalent lymph node stations: para-colic or para-ileal, intermediate, and proximal. Those from the colonic specimens can be considered just proximal to the retroperitoneal lymphatic channels. In order to compare the results, equivalent experiments were carried out on samples from the parietal peritoneum (10 cases), the greater epiploon (10 cases), and the retroperitoneum (10 cases). All surgical specimens were fixed in 4% neutral buffered formalin, paraffin embedded, and submitted for routine haematoxylin/eosin (H&E) staining and immunohistochemical characterization.

After deparaffinization, hydration, endogenous peroxidase blocking, and heat-induced antigen retrieval, tissue sections were incubated for 30 minutes at room temperature with anti-podoplanin (clone D2-40, prediluted; Ventana, Tucson, AZ, USA), anti-CD31 (clone JC70, prediluted; Ventana) and anti-collagen type IV (clone CIV22, prediluted; Ventana). Biotinylated secondary antibody was applied and the staining product detected with an avidin-biotin complex displayed against a hematoxylin counterstain. Detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers from Roche Ventana Medical Systems, with 3-3'diaminobenzidine tetrahydrochloride as chromogen. We adopted current magnification techniques, taking care to study the lymphatic vessels with both longitudinal and transversal sections.

RESULTS

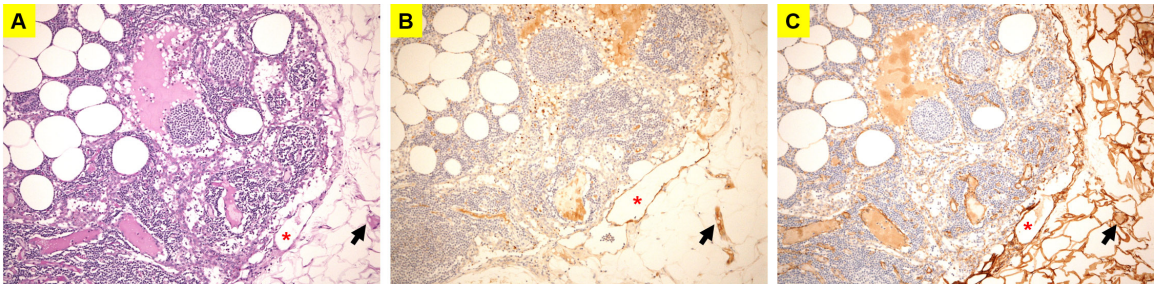

Fig. 2. The perinodal (arrow) and subcapsular (asterisk) lymphatic capillaries (A, H&E, x10), brown-stained by anti-podoplanin (B, asterisk, x10), show also immunoreactivity for anti- collagen IV, which testifies the presence of a basal membrane (C, asterisk, x10).

The lymphatic systems of the ileal and colonic mesenteries and the greater epiploon show overlapping features that fit well with their common embryological origin. The mesenteries and the epiploon have a particularly rich lymphatic network compared to that of parietal peritoneum and are highlighted well by D2-40 immunohistochemistry (*Fig. 1*). It mainly encompasses capillaries and lymph nodes without the collecting channels, both pre- and post-lymph nodal in the distal, para-intestinal, and intermediate parts. In particular, in the middle and para-intestinal part of the ileal or colonic mesenteries, the lymphatic system afferent to and efferent from the lymph nodes is only represented by capillaries. Their wall consists of a simple endothelium wrapped around and along their entire course by a basal membrane which is electively stained by collagen IV antibody (*Fig. 2*). No pericytes or smooth muscle fibers have been observed around the lymphatic capillaries. Among the single capillaries, we have noticed multiple and branched interconnections, which increase the surface of exchange with the interstitial space. We did not detect histological structures referable to valves in the capillaries. Only in the roots of the colonic mesenteries from patients operated on with total mesocolic excision and central ligation of the vascular pedicles have we observed the presence of collecting lymphatic channels – those with a larger caliber, a more complex wall, and demonstrating smooth muscle connective components and pericytes. Topographically, they accompany the feeding arteries of different ileal or colonic segments and are connected with retroperitoneal lymphatic channels. In the retroperitoneal tissue, we found pre- and post-lymph nodal D2-40 positive collectors with a more complex wall consisting of fibromuscular fibers, distinct valves, and a connective basement membrane (*Fig. 3*). In these sections the capillary network, while still present, is less abundant. In the parietal peritoneum, the lymphatic structures are represented by scattered capillaries, present under the mesothelial surface and histologically equivalent to those observed in the colonic or ileal mesentery (*Fig. 4*). None of the investigated lymphatic structures were immunoreactive for CD31, a well-known marker for hematic endothelia.

DISCUSSION

The great abundance of lymphatic vessels in the ileal and colonic mesenteries and in the greater omentum identify them not as simple ‘ligaments’ but also as structures providing a large lymphatic network with a consequent extensive function of exchange in addition to that of simple transport. This is interrelated with the activity of the corresponding intestinal segments (6-8). The term ‘lymphangion” refers to the functional unit

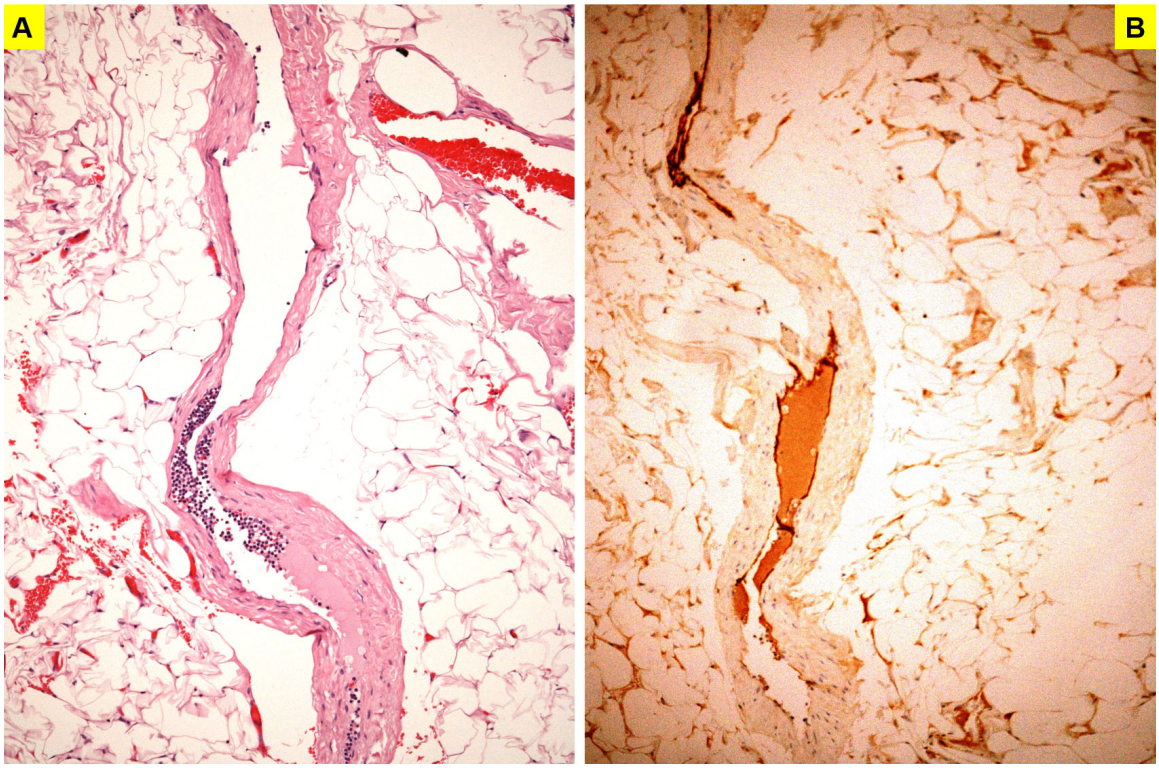

Fig. 3. The lymphatic collectors of the retroperitoneum are provided with a thick fibro-muscular wall and are engulfed by lymph together with lymphocytes (A, H&E, x10); the D2-40 immunoreactivity has been realized (B, x10).

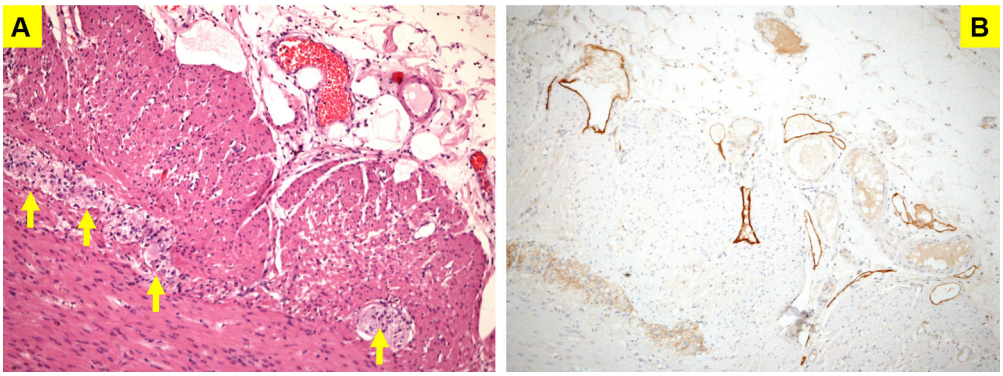

Fig. 4. Sub-peritoneal lymphatic capillaries: the arrows point to the myenteric nervus plexus of Auerbach (A, H&E, x10). The ectasic thin lymphatics are demonstrated by immunoreactivity for anti-podoplanin (B, x10).

of a lymph vessel that lies between two semilunar (half-moon shaped) valves, capable of contracting on its own due to the presence of smooth muscle (9). A histological arrangement of the mesenteric capillaries in lymphangions cannot be expected considering the absence of real valves and the simple constitution of the wall. Lymph propulsion in these simple capillaries is promoted by forces acting externally on their wall such as the

positive pressure present in the mesenteric interstitial spaces and in the whole peritoneal cavity, the respiratory movements of the diaphragm, the pulsations of the accompanying arterial vessels, and the peristaltic activity of the bowel, etc. In this phase, as already demonstrated with ultrastructural magnification techniques, the basal membrane has an important adjuvant role through its actomyosin filaments which anchor the capillary walls to the adjacent interstitial structures and make more efficient the action of the aforementioned external forces (10-15).

On the contrary, lymphatic collectors of the retroperitoneum show specific propensity for lymph transport and propulsion with their walls equipped with valves and smooth muscle fibers intermingled with a connective tissue component. Clearly, the lymphatic vessels located at the proper mesenteric roots have intermediate features between the simple capillaries and the more complex retroperitoneal lymphatic structures since their function is not of absorption or exchange but mainly of transport.

These findings all agree with the different embryogenesis of these structures: the ileal and colonic mesenteries together with the greater omentum develop from the primitive enteric tube and become the most important functional pathways for their nervous, vascular, and lymphatic connections. Inversely, the parietal peritoneum, which originates from the walls of the primitive coelomic cavity, is supplied by common somatic blood and lymph vessel, and it has no particular function of active absorption or lymphatic transport under normal conditions (16).

In light of these observations, we can explain some aspects of mesenteric pathology. For example, during the course of peritonitis, active bidirectional exchanges among the peritoneal cavity, the mesenteric interstitial space, and the lymphatic capillaries occur. In this case, local and general humoral agents, especially inflammatory mediators, easily increase the permeability of the lymphatic capillaries. Similarly, the mesenteric capillary network, when congested from a downstream obstruction, or overfilled by lymph overproduction as in liver cirrhosis, favors through a mechanism of ultrafiltration the development of ascites (17-20) that becomes chylous when the lymph is particularly rich in lipids.

The numerous lymph nodes interrelated with the capillary lymphatic network assure an immunological function to the lymphatic apparatus of the intestinal mesentery (21). Moreover, our morphological data reflect a further functional observation: lymph flow in the rich capillary lymphatic mesenteric network can follow not only the most direct way among the lymph node chains but promoted by different mechanical forces, can run through other routes especially in pathological conditions (6).

CONCLUSION

In the human colonic and ileal mesenteries, the lymphatic vascular apparatus is exclusively represented by a fine and largely extended network of simple capillaries. This confirms a similar observation in primates (22,23) and, for the first time, underlines the important role of lymphatic capillaries in different pathological conditions such as ascites or peritonitis. Moreover, this finding contributes to support the concept of skip lymph node metastasis. Our observations agree with the policy of 'complete mesocolic excision,' performed in surgery of colonic cancers with central ligation of the feeding arteries following embryological planes to allow good results (24). In particular, the mesenteric excision must be adapted not only to the lymph node topography but also to the related capillary lymphatic network.

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CONFLICT OF INTEREST AND DISCLOSURE

All authors declare that no competing financial interests exist.

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