CEOG Clinical and Experimental Obstetrics & Gynecology

Tertiary lymphatic organs of the female pelvis: not negligible structures

L. Roncati, A. Manenti

Departments of Diagnostic and Clinical Medicine and of Surgery, University of Modena and Reggio Emilia, Modena (Italy)

Summary

Introduction: The lymphatic organs are subdivided in primary, secondary, and tertiary. The latter are scantly known structures, typically containing far fewer lymphocytes, imported from blood and lymph, which can be found theoretically in any abdominal or extra-abdominal site. The present authors' intent was to search them in the mesentery and peritoneum of the female pelvis, in order to trace a possible line of evolution towards more mature lymphatic organs, and to evaluate their immune arrangement. *Materials And Methods:* The authors also investigated the normal mesentery and peritoneum, obtained from ten surgical specimens of hysterectomy with bilateral salpingo-oophorectomy, performed for symptomatic uterine prolapse, in patients aged between 60 and 70 years. After routine procedures of diagnosis and staging, the representative lymphatic tissue was submitted to immunohistochemical characterization for CD20, CD3, CD4, CD8, CD56, CD68, CD138, and podoplanin. *Results:* The authors were able to recognize in a simple aggregate of lymphocytes, gathered around a capillary or arteriola, the primordial nucleus of the tertiary lymphatic organ. Over time, this microaggregate increases in size and volume for the accumulation of lymphocytes, until it reaches the final conformation of a small lymph node, equipped with a thin capsule and a proper hilus. Its core is represented by CD20+ B lymphocytes, while CD3+ T lymphocytes are less numerous and almost exclusively represented by CD4+ T-helper lymphocytes, being the CD3+ T-cytotoxic reduced to a minimum percentage. At its periphery, some CD68+ histocytes can be also observed; no CD56+ NK lymphocytes, neither CD138+ plasma cells were detected. Moreover, the immunohistochemistry for D2-40 did not reveal significant neolymphangiogenesis. *Conclusion:* Tertiary lymphatic organs can be considered a reserve system, ready to replace the lymph nodes, when they become aged or inefficient, or when their increase in number is requested. Therefore, the tertiary lymphatic organs of the female pelvis can be thought as potential defenders against infections or cancer. However, they can be involved by metastases at any stage of their development, an event that testifies an intrinsic immune immaturity. Also, this secondary neoplastic colonization can be suspected as a key step towards peritoneal carcinomatosis, because the tertiary lymphatic organs can integrate in the lymph dynamics of the female pelvis and peritoneal cavity.

Key words: Tertiary lymphatic organs; Female pelvis; Immunohistochemistry.

Introduction

The lymphatic system consists of lymphatic organs, lymphatics, and circulating lymph [1-6]. The lymphatic organs are further subdivided in primary lymphatic organs, corresponding to thymus and bone marrow, as secondary lymphatic organs, namely spleen, lymph nodes or mucosa-associated lymphatic tissue (MALT), and as tertiary lymphatic organs (TLO), which today are attracting the attention of many researchers [7, 8]. The latter are scantly known lymphatic structures, typically containing far fewer lymphocytes, imported from blood and lymph, which can be found theoretically in any abdominal or extra-abdominal site. Inside the peritoneal cavity, especially in the greater omentum, TLO have been described by the figurative term "milky spots"; however, they are also present in other parts of the peritoneal serosa, as microscopic structures not completely studied [9]. The present authors' aim was to search them in the mesentery and peritoneum of the female pelvis, to trace a possible line of evolution towards more mature lymphatic organs, and to evaluate their immune arrangement.

Materials and Methods

For this study, the authors excluded autoptic material, unsuitable for immunohistochemical investigations, preferring segments of normal mesentery and peritoneum, obtained from ten surgical specimens of hysterectomy with bilateral salpingo-oophorectomy, performed for symptomatic uterine prolapse, in patients aged between 60 and 70 years. They adopted the following exclusion criteria: concomitant or earlier peritoneal inflammation, previous abdominal surgery, lymph node metastatization, peritoneal carcinomatosis, neoadjuvant chemoradiotherapy, immunological or endocrine disorders, steroid or immunosuppressive therapy, ascites, portal hypertension, malnutrition, obesity. After the routine procedures of diagnosis and staging, mainly based on hematoxylin and eosin (H&E) staining method, the tubal and ovarian mesenteries, exempt from any inflammatory or neoplastic pathology, have been submitted to further immunohistochemical investigations. After deparaffinization, hydration, endogenous peroxidase blocking, and heat-induced antigen retrieval, the tissue sections

Revised manuscript accepted for publication June 8, 2017

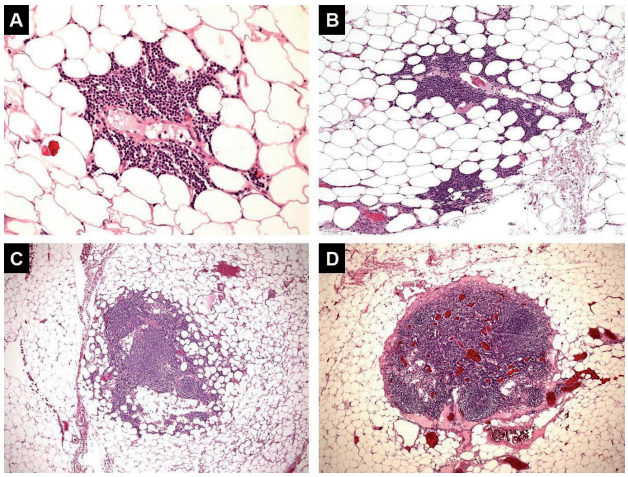

Figure 1. — The photographed TLO originates as a primordial microaggregate of lymphocytes around a capillary in the adipose tissue (A: H&E, ×20); over time, it increases in size (B: H&E, ×10) and volume (C: H&E, ×5), until to reach the final conformation of a small lymph node (D: H&E, ×5).

were incubated for 30 minutes at room temperature with anti-CD20 (clone L26), anti-CD3 (clone 2GV6), anti-CD4 (clone SP35), anti-CD8 (clone SP57), anti-CD56 (clone SP35), anti-CD68 (clone KP1), anti-CD138 (clone B-A38) and anti-podoplanin (clone D2-40). Biotinylated secondary antibody was applied and the staining product detected with avidin-biotin complex against a hematoxylin counterstain; detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers, with 3-3'diaminobenzidine tetrahydrochloride as chromogen.

Results

At first, the authors recognized in a simple aggregate of lymphocytes, gathered around a capillary or arteriola, the primordial nucleus of TLO (Figure 1, panel A). Over time, this nucleus increases in size for the accumulation of lymphocytes (Figure 1, panel B), then it evolves towards a more defined lymphatic structure, provided with an adequate connective-vascular framework (Figure 1, panel C), maintaining unchanged its original cellular arrangement. More in detail, this distribution pattern is characterized by a prevalent CD20+ B lymphocytic population, centrally located, and less numerous CD4+ T-helper lymphocytes. Only few and scattered CD8+ T-cytotoxic lymphocytes and marginal CD68+ histiocytes have been detected; no CD56+ NK lymphocytes or CD138+ plasma cells have been observed. At this stage, lymphatic capillaries were neither found inside (Figure 2). When the cellular component further increases in volume, the structure acquires an oval shape, equipped with a thin capsule, and a proper lymph node hilus, where small vessels, arterial and venous, and an efferent lymphatic duct become visible (Figure 1, panel D). In absence of inflammatory or immunological reaction, as in the present selected cases, TLOs appear to be "dormant" structures.

Discussion

TLOs can be considered a "reserve" system, ready to replace the lymph nodes, when they become aged or inefficient, or when their increase in number is requested. In these cases, they can modify their cellular pattern, according to the contingent situation. In more favorable circum-

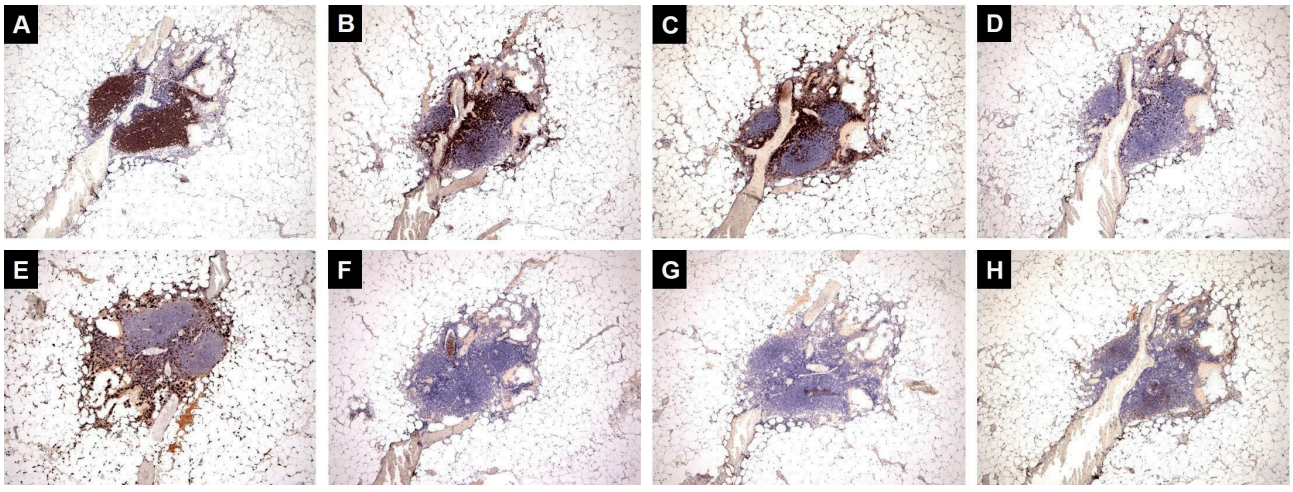

Figure 2. — The photographed TLO develops around an arteriola: its core is represented by CD20+ B lymphocytes (A: ×5), while CD3+ T lymphocytes are less numerous (B: ×5) and almost exclusively represented by CD4+ T-helper lymphocytes (C: ×5), being the CD8+ T-cytotoxic reduced to a minimum percentage (D: ×5). At the periphery, some CD68+ histocytes can be observed (E: ×5). No CD56+ NK lymphocytes (F: ×5), neither CD138+ plasma cells are noticeable (G: ×5); moreover, a neolymphangiogenesis is not evident by D2-40 immunohistochemistry (H: ×5).

stances, TLOs can in fact become an immunologically active system, representing a defence mechanism against neoplasms [10-16]. Therefore, the TLOs of the female pelvis can be thought as potential defenders against infections or cancer. Exactly as in other districts (lung, intestine, peripheral arteries, *etc.)*, they can become the targets of humoral agents, in particular cytokines, released during pathological processes. Cytokines act as hematopoietic inducers, favoring the proliferation of specific lymphocytic clones, but also as stromal organizers, promoting the development of a complete interstitial stroma, supplied by blood vessels [17-23]. On the other hand, at different stages of their development, TLOs can be involved by metastases, which can find here an adequate supply of oxygen and nutritional substances [24]. This colonization is accompanied by the development of vascular structures, the high endothelial venules, and newly formed lymphatic capillaries, whose walls are easily permeable to the neoplastic cells. In this event of neoangiogenesis, the action of the vascular endothelial growth factor is prominent [25-27]. Therefore, the aforementioned neoplastic proceeding can be interpreted as the final result of two opposite trends, supported by protumoral and anti-tumoral factors, inducing tolerance towards the neoplastic cells, even where a dense population of lymphocytes is present, but devoid of significant cytotoxic attitude [28-32]. Also, this same neoplastic colonization can be supposed as a key step towards peritoneal carcinomatosis.

Conclusion

TLOs of the female pelvis can integrate in the lymph dynamics of the peritoneal cavity and in the immunological and inflammatory mechanisms of the peritoneal serosa. Considering the speculative, but also the practical value of these observations, the authors encourage further morphological and functional studies assessing pelvic TLOs in course of different gynecological diseases.

References

[1] Roncati L., Barbolini G., Pusiol T., Piscioli F., Maiorana A.: "New advances on placental hydrops and related villous lymphatics". *Lymphology.* 2015, *48*, 28.

[2] Manenti A., Roncati L., Barbolini G.: "The role of lymphatics in portal biliopathy". *J. Visc. Surg*., 2016, *153*, 81.

[3] Roncati L., Manenti A., Zizzo M., Farinetti A.: "Re: Anatomy of hepatic lymphatics and its implications in hepatic malignancies". *ANZ J. Surg.,* 2017, *87*, 103.

[4] Manenti A., Pavesi E., Farinetti A., Roncati L.: "For a clearer comprehension of the lymphatic system in perihilar cholangiocarcinoma". *Am. J. Surg*., 2016, *211*, 493.

[5] Roncati L., Manenti A., Sighinolfi P.: "Immunohistochemical improvement in the analysis of the lymphatic metastases from lung carcinoma". *Ann. Thorac. Surg*., 2014, *97*, 380.

[6] Roncati L., Manenti A., Pavesi E., Piscioli F., Farinetti A., Pusiol T.: "Surgicopathologic correlations regarding lymphatics in hilar cholangiocarcinoma". *Surgery,* 2016, *159*, 1483.

[7] Buettner M., Lochner M.: "Development and function of secondary and tertiary lymphoid organs in the small intestine and the colon". *Front. Immunol*., 2016, *7*, 342.

[8] Liu J.Y., Yuan J.P., Geng X.F., Qu A.P., Li Y.: "Morphological study and comprehensive cellular constituents of milky spots in the human omentum". *Int. J. Clin. Exp. Pathol*., 2015, *8*, 12877.

[9] Krishnan V., Clark R., Chekmareva M., Johnson A., George S., Shaw P., *et al*.: "In vivo and ex vivo approaches to study ovarian cancer metastatic colonization of milky spot structures in peritoneal adipose". *J. Vis. Exp*., 2015, *105*, e52721.

[10] Di Caro G., Castino G.F., Bergomas F., Cortese N., Chiriva-Internati M., Grizzi F., *et al*.: "Tertiary lymphoid tissue in the tumor microenvironment: from its occurrence to immunotherapeutic implications". *Int. Rev. Immunol*., 2015, *34*, 123.

[11] Roncati L., Manenti A., Piscioli F., Pusiol T., Barbolini G.: "The immune score as a further prognostic indicator in carcinoid tumors". *Chest*, 2017, *151*, 1186.

[12] Roncati L., Manenti A., Pusiol T., Piscioli F., Barbolini G., Maiorana A.: "Testosterone aromatization to estradiol in course of ovarian functioning Brenner tumor associated with endometrial carcinoma and endometriosis (Roncati-Manenti triad)". *Int. J. Gynecol. Cancer*, 2016, *26*, 1461.

[13] Roncati L., Manenti A., Piscioli F., Pusiol T., Barbolini G.: "Immunoscoring the lymphocytic infiltration in carcinoid tumours". *Histopathology*, 2017, *70*, 1175.

[14] Roncati L., Barbolini G., Sartori G., Siopis E., Pusiol T., Maiorana A.: "Loss of CDKN2A promoter methylation coincides with the epigenetic transdifferentiation of uterine myosarcomatous cells". *Int. J. Gynecol. Pathol*., 2016, *35*, 309.

[15] Roncati L., Manenti A., Farinetti A., Pusiol T.: "The association between tumor-infiltrating lymphocytes (TILs) and metastatic course in neuroendocrine neoplasms". *Surgery*, 2016, *160*, 1709.

[16] Roncati L., Pusiol T., Piscioli F., Barbolini G., Maiorana A.: "Undetermined cervical smear due to angiomyofibroblastoma of the cervix uteri". *J. Obstet. Gynaecol.* 2017, *11*, 1.

[17] van de Pavert S.A., Mebius R.E.: "New insights into the development of lymphoid tissues". *Nat. Rev. Immunol*. 2010, *10*, 664.

[18] Hwang J.Y., Randall T.D., Silva-Sanchez A.: "Inducible bronchus-associated lymphoid tissue: taming inflammation in the lung". *Front. Immunol*. 2016, *7*, 258.

[19] McNamee E.N., Rivera-Nieves J.: "Ectopic tertiary lymphoid tissue in inflammatory bowel disease: protective or provocateur?" *Front. Immunol*., 2016, *7*, 308.

[20] Pitzalis C., Jones G.W., Bombardieri M., Jones S.A.: "Ectopic lymphoid-like structures in infection, cancer and autoimmunity". *Nat. Rev. Immunol*., 2014, *14*, 447.

[21] Corsiero E., Nerviani A., Bombardieri M., Pitzalis C.: "Ectopic lymphoid structures: powerhouse of autoimmunity". *Front. Immunol*., 2016, *7*, 430.

[22] Yadava K., Bollyky P., Lawson M.A.: "The formation and function of tertiary lymphoid follicles in chronic pulmonary inflammation". *Immunology*. 2016, *149*, 262.

[23] Yin C., Mohanta S.K., Srikakulapu P., Weber C., Habenicht A.J.: "Artery tertiary lymphoid organs: powerhouses of atherosclerosis immunity". *Front. Immunol*., 2016, *7*, 387.

[24] Reggiani Bonetti L., Manenti A., Domati F., Farinetti A.: "Mesocolic micro-skip metastases". *Clin. Res. Hepatol. Gastroenterol*. 2017, *41*, 341.

[25] Ruddle NH.: "High endothelial venules and lymphatic vessels in tertiary lymphoid organs: characteristics, functions, and regulation". *Front. Immunol*., 2016, *7*, 491.

[26] Ruddle NH.: "Lymphatic vessels and tertiary lymphoid organs". *J. Clin. Invest*., 2014, *124*, 953.

[27] Coles M., Kioussis D., Veiga-Fernandes H.: "Cellular and molecular requirements in lymph node and Peyer's patch development". *Prog. Mol. Biol. Transl. Sci*., 2010, *92*, 177.

[28] Hirakawa S., Brown L.F., Kodama S., Paavonen K., Alitalo K., Detmar M.: "VEGF-C-induced lymphangiogenesis in sentinel lymph

nodes promotes tumor metastasis to distant sites". *Blood*, 2007, *109*, 1010.

[29] Watanabe M., Tanaka H., Ohira M., Yoshii M., Sakurai K., Toyokawa T., *et al*.: "Intranodal lymphangiogenesis precedes development of lymph node metastasis and accelerates progression of gastric cancer". *J. Gastrointest. Surg*., 2014, *18*, 481.

[30] Dieu-Nosjean M.C., Giraldo N.A., Kaplon H., Germain C., Fridman W.H., Sautès-Fridman C.: "Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers". *Immunol. Rev*., 2016, *271*, 260.

[31] Dieu-Nosjean M.C., Goc J., Giraldo N.A., Sautès-Fridman C., Fridman W.H.: "Tertiary lymphoid structures in cancer and beyond". *Trends Immunol.,* 2014, *35*, 571.

[32] Yeo K.P., Angeli V.: "Bidirectional crosstalk between lymphatic endothelial cell and T cell and its implications in tumor immunity". *Front. Immunol*., 2017, *8*, 83.

Corresponding Author:
L. RONCATI, M.D., PHD
Department of Diagnostic and Clinical Medicine and of Public Health, Institute of Pathology, University of Modena and Reggio Emilia, Policlinico Hospital
Viale del Pozzo 71,
I-41124 Modena (Italy)
e-mail: emailmedical@gmail.com