

## Mobilization of lymphatic endothelial progenitor cells and lymphatic neovascularization in primary Sjögren's syndrome

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Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder characterized by chronic lymphocytic infiltration of exocrine glands leading to progressive functional impairment. Lymphangiogenesis is a common finding in chronic inflammatory diseases; however, its role in pSS remains to be elucidated. Inflammation induces the production of growth factors for lymphatic vessels, such as vascular endothelial growth factor-C (VEGF-C). Recent growing evidence indicates that bone marrow-derived lymphatic endothelial progenitor cells (LEPCs) may differentiate into lymphatic endothelial cells via VEGF-C/VEGFR-3 signaling contributing to lymphangiogenesis. This study was designed to investigate the circulating levels of LEPCs and the occurrence of lymphangiogenesis in pSS. Peripheral blood mononuclear cells were collected from 10 female pSS patients and 11 healthy females. LEPCs, defined as CD34+CD133+VEGFR-3+ cells, were identified by FACS using CD34-FITC, CD133-APC and VEGFR-3-PE antibodies. Results were expressed as percentage of CD133+VEGFR-3+ cells among CD34+ cells. Labial minor salivary gland (MSG) biopsies were obtained from 12 female pSS patients and 16 sicca non-pSS control females. MSGs were evaluated by haematoxylin-eosin and immunofluorescence for CD3/CD20 and CD21 to assess focus score, Tarpley biopsy score, T/B cell segregation and germinal center-like structures. Lymphatic vessels were identified by immunohistochemistry for podoplanin (D2-40), a mucin-type transmembrane protein expressed by lymphatic endothelial cells but not by blood vessels. VEGF-C/VEGFR-3 expression in MSGs was investigated by immunofluorescence. An average ten-fold increase in circulating levels of LEPCs was found in pSS ( $35.2 \pm 2.7\%$ ) compared with controls ( $3.4 \pm 0.8\%$ ) ( $p=0.0003$ ). In control MSGs, lymphatic vessels were only detected around excretory ducts in the interlobular connective tissue. In pSS MSGs, the number of lymphatic vessels was increased around interlobular excretory ducts and a newly formed lymphatic capillary network was found within inflammatory foci. A strong expression of VEGF-C was detected in ductal cells, vessels and inflammatory cells in pSS MSGs. VEGFR-3 expression was observed in a subset of vessels and infiltrating mononuclear cells. Our findings suggest that LEPC mobilization and MSG lymphatic vessel reorganization may take center stage in the chronic inflammatory process of pSS.

### Keywords

Sjögren's syndrome; lymphangiogenesis; minor salivary glands.