

Adventitial lymphatics of internal carotid artery in healthy and atherosclerotic vessels

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Abstract: Objectives. Presence of lymphatics in adventitia of major arteries remains controversial. Presence of lymphatics in adventitia of internal carotid artery was not documented and its relation to atherosclerosis was not studied. The aim of our study was to evaluate presence of lymphatic vessels in adventitia of internal carotid artery in healthy and atherosclerotic arteries. Methods. Fragments of arterial wall of internal carotid artery were obtained during the surgical eversion endarterectomy in 15 patients with internal carotid artery stenosis and 2 healthy organ donors. 21 arteries were studied. Patients age ranged from 56 to 77 years. Fragments of arterial wall were embedded in paraffin. Lymphatics of arterial adventitia were visualized with immunohistochemistry using LYVE-1 and anti-podoplanin antibodies. Results. The lymphatic vessels were visualized in adventitia of 20 carotid arteries. The serial sections have revealed that both LYVE-1 and podoplanin have identical specificity for lymphatic endothelium. Number of lymphatics in adventitia significantly correlated with thickness of intima ($p < 0.046$). Conclusions. Lymphatics are present in adventitia of internal carotid artery. Number of adventitial lymphatics increases with severity of atherosclerosis measured as intimal thickness.

Key words: vasa vasorum, lymphangiogenesis, lymphatic system

Introduction

The atherosclerosis is currently seen as a chronic inflammatory process with infiltration of mononuclear lymphocytes into the intima, local expansion of vascular smooth muscle cells, and accumulation of extracellular matrix [1]. Vascular adventitia for a long time was considered mainly a supportive tissue providing nutrients for the vascular wall, however its function in arterial pathology was suspected [2,3]. Recently many studies point at the role of the adventitia in initiation and development of atherosclerosis [4,5]. Adventitial vasa vasorum (VV) were found to proliferate in response to arterial injury and progress of atherosclerosis [6,7]. Lymphatic vessels are present in arterial adventitia, but their exact function remains unclear. Recently Xu and coworkers suggested that adventitial lymphatics play an important role in development of

atherosclerosis enhancing activation and accumulation of inflammatory cells [8].

The lymphatic system regulates interstitial fluid balance, takes part in lipid absorption and lipid metabolism, and is crucial for development immune response. Adventitial lymphatics might be involved in reverse cholesterol and lipid transport from the arterial wall [9,10] and in transport immune cells to the regional lymph nodes. In response to inflammation lymphatic vessels can proliferate. Lymphangiogenesis was detected in corneal inflammation [11], inflammatory neoplasms [12] and inflammatory bowel diseases [13]. Lymphangiogenesis in adventitia may accompany inflammation in atherosclerotic arteries.

The aim of our study was to detect lymphatic vessels in adventitia of internal carotid arteries and to analyse possible relationship with severity of atherosclerosis.

Materials and Methods

Patients. The fragments of internal carotid artery were collected during internal carotid surgery (eversion endarterectomy) in 15 patients (3 women and 12 men, 4 patients had bilateral endarterec-

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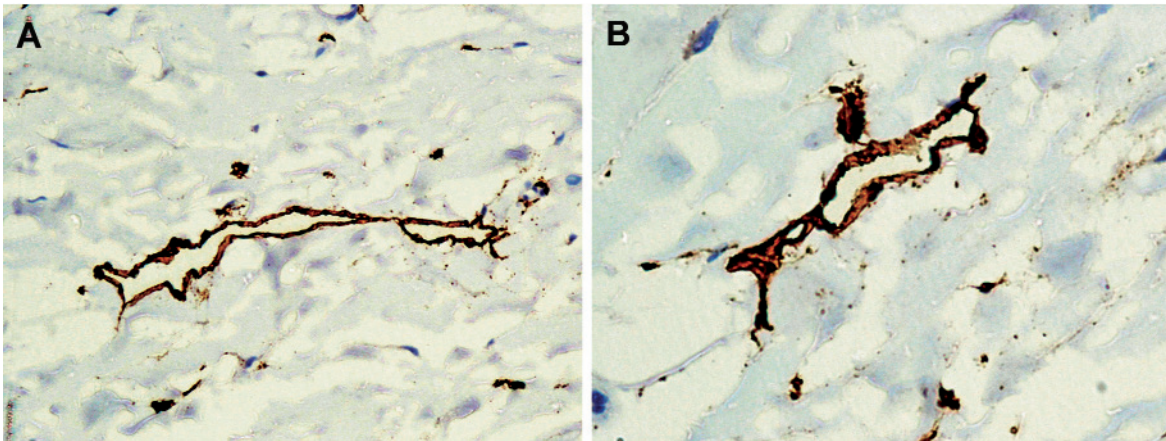


Fig. 1. Adventitial lymphatic vessels of internal carotid artery (anti-LYVE-1 Ab). Magnification $\times 200$ (A) and $\times 400$ (B).

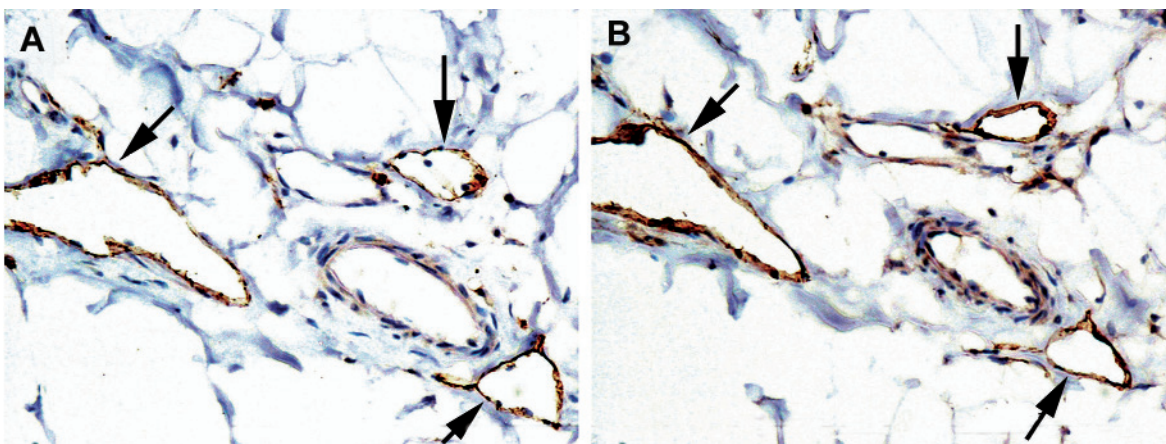


Fig. 2. Serial sections of internal carotid artery adventitia with lymphatic vessels. A. anti-LYVE-1 staining; B. anti-podoplanin staining. Black arrows point at lymphatics (magnification $\times 200$).

tomy) with symptomatic carotid artery stenosis over 70%. Patients age ranged from 56 to 77 years. Additionally fragments of healthy internal carotid artery were harvested from 2 young (24 and 27 years old) organ donors. Altogether, samples of 21 carotid arteries were obtained for further studies. The study was approved by the Bioethical Committee of the Wrocław Medical University. The surgeries were performed at the Department of Vascular, General and Transplantation Surgery, Wrocław Medical University.

Tissue preparation. All fragments of arterial wall were initially fixed in 10% buffered formaldehyde solution, then embedded in paraffin and cut into 5 μm sections. Paraffin sections from all 21 arteries were stained with hematoxylin and eosin for histological evaluation.

Immunohistochemistry. Immunohistochemical staining of lymphatic vessels was performed using specific antibodies for lymphatic endothelium. Paraffin sections were deparaffinized, dehydrated, and pre-treated with Target Retrieval Solution (DakoCytomation) at 95°C for 20 min. After wash in Tris-buffered saline (TBS), the sections were treated with 3% H_2O_2 for 10 min. Subsequently after wash in distilled H_2O (10 min) and PBS (5 min) the sections were incubated with mouse monoclonal antibodies against LYVE-1 and podoplanin (RELIATech GmbH, Germany), diluted 1:200, for 60 min at room temperature. The serial sections were made for both podoplanin and

the LYVE-1 antibodies. For all slides, a wash in TBS was followed by treatment with peroxidase-labeled polymer conjugated to goat anti-rabbit or anti-mouse immunoglobulins (Envision+kit; DakoCytomation, Denmark) for 30 min at room temperature. The immunostaining was visualized with diaminobenzidine tetrahydrochloride (DAB) and then counterstained with hematoxylin. In each case the negative control was included with Primary Negative Control (DakoCytomation, Denmark).

Light microscopy. The thickness of intima was measured under magnification $\times 100$, with Olympus BX 41 light microscope using the visual mode analySIS 3.2 software for computer. Intimal thickness served as a marker severity of atherosclerosis. For evaluation of lymphatic vessel number, slides were scanned with the Olympus BX 41 light microscope at $\times 200$ and then at $\times 400$ magnification by two different investigators. The morphological hallmarks for lymphatic vessel were: positive reaction with LYVE-1 and podoplanin antibodies, a thin vessel wall with irregular or collapsed lumen and no red blood cells and inward handed nucleus. We have examined the cross section of internal carotid artery and we have counted all lymphatic vessels in each cross section. The pictures were taken of each found lymphatic vessel.

Statistical analysis. Statistical analysis was performed with Statistica 5.1 PL software (StatSoft, Cracow, Poland). Mann-Whitney,

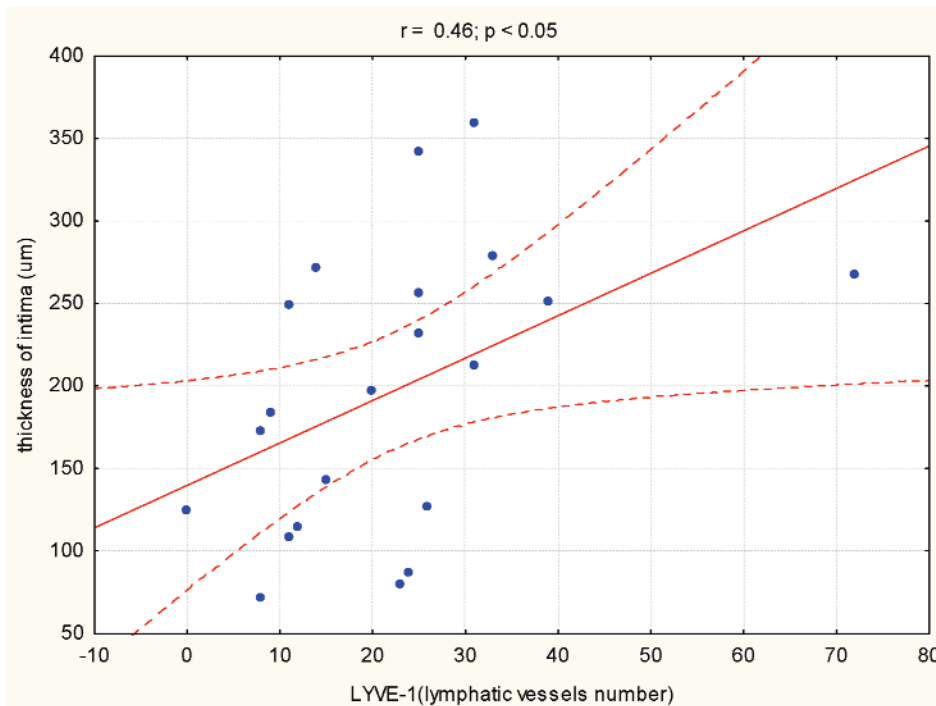


Fig. 3. Correlation between thickness of intima and number of lymphatic vessels in adventitia of internal carotid artery; $n = 21$, $r = 0.46$, $p < 0.05$.

F-Cox, Chi-square, and Spearman's correlation tests were performed. The differences were considered significant at $p < 0.05$.

Results

The lymphatic vessels were visualized by immunohistochemical reactions in 20 samples. There was one sample without visualized lymphatics. The lymphatics were found mainly in adventitia but also in periadventitia (Fig. 1). The serial sections have revealed that both LYVE-1 and podoplanin have identical specificity for lymphatic endothelium (Fig. 2). Degree of atherosclerosis severity was reflected by increased number of lymphatics in arterial adventitia (Fig. 3).

Discussion

The salient findings of our study are: confirmation of lymphatic vessels presence in adventitia of internal carotid arteries in humans and significant increase in adventitial lymphatic vessels number with progression of atherosclerosis.

Lymphatic vessels were detected in arterial adventitia of animal and human arteries by several authors [10,14-17]. However, there are reported differences between human and animal arteries. Adventitial lymphatics were found in coronary arteries in dogs [18] but not in humans [19]. Also different arteries may have different characteristics of adventitial vasa vasorum [20]. Therefore our finding of lymphatic vasa vasorum in human internal carotid adventitia is new

and important. We have not detected adventitial lymphatics in one studied sample, where the specimen was almost completely lacking adventitia.

We have also found that increased thickness of arterial intima – a marker of atherosclerosis severity – is accompanied by increased number of lymphatics in adventitia. We believe that it might reflect inflammatory lymphangiogenesis in atherosclerotic arteries. Inflammation is a potent stimulus of lymphangiogenesis [21,22] and inflammatory reaction described in adventitia of atherosclerotic arteries [5,23].

Our findings indicate that adventitial lymphatics play an important role in arterial wall metabolism and pathogenesis of atherosclerosis.

Further studies are necessary to elucidate function of adventitial lymphatics in atherosclerotic process.

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