

# Quantification of Lymphatic Vessels in Dilated and Chronic Chagasic Cardiomyopathy

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## Introduction

The recent development of specific antibodies for the lymphatic endothelium has allowed its detection by the immunohistochemical technique<sup>1</sup>. In spite of this methodological advancement, the morphological study of the myocardial lymphatic vessels has been neglected and very little is known about this in the different cardiopathies.

The congestive heart failure causes interstitial edema in different organs and tissues, including the myocardium, and this excess fluid must be drained by the lymphatic system. The myocardial edema is involved in the development of interstitial fibrosis and impairs the heart function<sup>2</sup>.

The objective of this study is to compare the myocardial lymphatic vessels of patients with congestive heart failure secondary to idiopathic dilated cardiomyopathy or chronic chagasic cardiomyopathy with normal myocardial lymphatic vessels.

## Methods

Some morphological characteristics of endomyocardial biopsies from patients with congestive heart failure functional class III or IV, secondary to idiopathic dilated cardiomyopathy (IDC) or chronic chagasic cardiomyopathy (CCC) were previously evaluated; the latter was characterized by positive serological tests for Chagas disease and by the presence of chronic lymphohistiocytic myocarditis<sup>3,4</sup>. In this study, the biopsies of 31 patients with IDC (mean age 40.65 ± 12.14 years; 22 males) and 24 patients with CCC (mean age: 43.71 ± 9.29 years; 21 males) were re-evaluated, which supplied new histological sections that presented at least 1.25 mm<sup>2</sup> of analyzable area (five microscopic fields of 400 ×). As the control group, we collected small samples of normal right ventricular endomyocardium of 11 individuals that died due to non-cardiovascular causes and were submitted to necropsy (mean age 50.82 ± 15.39;

## Key words

Chagasic cardiomyopathy; dilated cardiomyopathy; lymphatic vessels.

3 males). These samples were routinely processed and paraffin-embedded. Serial 4- $\mu$ m sections were stained by hematoxylin and eosin, Masson's trichrome or submitted to the immunohistochemical technique for the detection of lymphatic vessels.

## Immunohistochemistry

Histological sections were incubated with the monoclonal antibody D2-40 (Dako Corporation, CA) diluted 1:100, overnight, at 4°C. The reactions were developed with the EnVision peroxidase labeled polymer (Dako Corporation, CA) for 30 minutes at room temperature and visualized with 3,3' diaminobenzidine. Histological sections of normal skin were used as positive and negative controls, omitting the primary antibody in the latter.

## Histopathology and morphometry

The mean diameter of the myocytes, the fractional area of collagen, the fractional area of the lymphatic vessels and the diameter of the largest-caliber lymphatic vessel of each sample were analyzed. Myocarditis was characterized by the presence of mononuclear inflammatory infiltrate associated to degeneration and/or necrosis of myocytes, according to the Dallas criteria<sup>5</sup>. The diameter of myocytes and the fractional area of collagen were assessed as previously described<sup>3,4</sup>. The total area occupied by the histological section, the total area occupied by the lymphatic vessels and the diameter of the largest-caliber lymphatic vessel were measured with the help of a computerized image analysis system. The fractional area of the lymphatic vessels was calculated through the ratio between the total area occupied by the lymphatic vessels and the total area occupied by the histological section, multiplied by 100.

## Statistical analysis

The morphometric data of the three groups were compared using the Kruskal-Wallis analysis of variance by ranks. A p value  $\leq 0.05$  was considered statistically significant.

## Results

The lymphatic vessels were present among the myocytes, around the blood vessels or, more rarely, in the endocardium. The lymphatic vessels were sparse and at a lower number than the blood vessels, and their density varied greatly from sample to sample and even in different areas of the same sample. Occasionally, there were several collapsed, ramified and

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grouped lymphatic vessels, particularly around the arterioles (Figure 1). Patients from the IDC and CCC groups presented myocytes with larger diameters ( $p < 0.001$ ) and higher fractional area of collagen ( $p < 0.001$ ) than the individuals from the control group, with no differences between the two first groups. There was no statistical difference among the groups regarding the fractional area of the lymphatic vessels ( $p = 0.075$ ) and it is noteworthy the fact that the median in the control group was the highest among the three groups. The diameter of largest-caliber lymphatic vessel was smaller in the IDC group, when compared to the control group, but not when compared with the CCC group ( $p = 0.035$ ), with no differences between the last two groups (Figure 2). Lymphohistiocytic myocarditis was present in 20/24 (83%) patients from the CCC group and in none of the patients from the IDC or control groups.

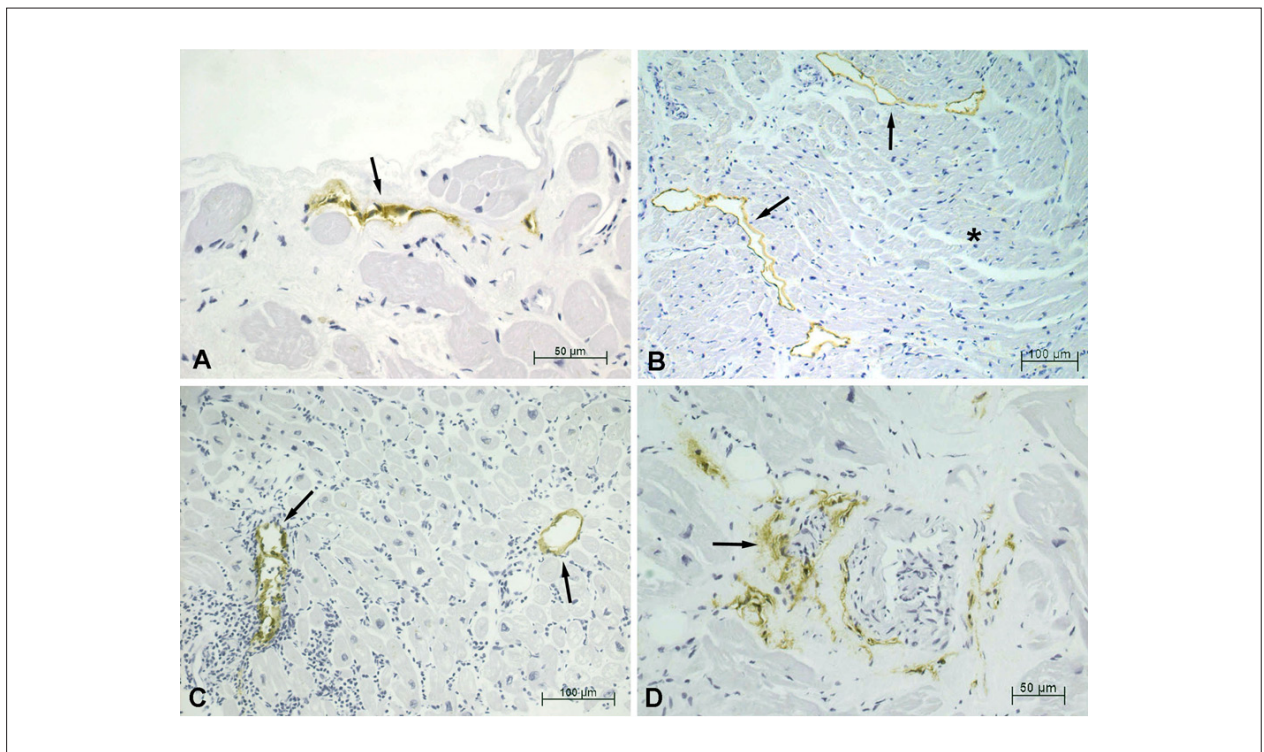
## Discussion

The investigation of the lymphatic vessels in the several human cardiopathies has been neglected. This is greatly due to the difficulty in differentiating these vessels from blood vessels, which until recently was only possible through electron microscopy. However, the recent development of monoclonal antibodies capable of specifically recognizing the lymphatic endothelium<sup>1</sup> has changed this scenario and some morphological studies on the alterations in the lymphatic vessels in ischemic heart disease and heart transplantation have been reported<sup>6,7</sup>.

The heart of the mammals presents a subendocardial lymphatic plexus, lymphatic vessels in the myocardium (at much lower numbers than blood capillaries) and a subepicardial lymphatic plexus, the latter containing larger caliber collecting vessels that follow the epicardial branches of the coronary arteries<sup>2,8</sup>.

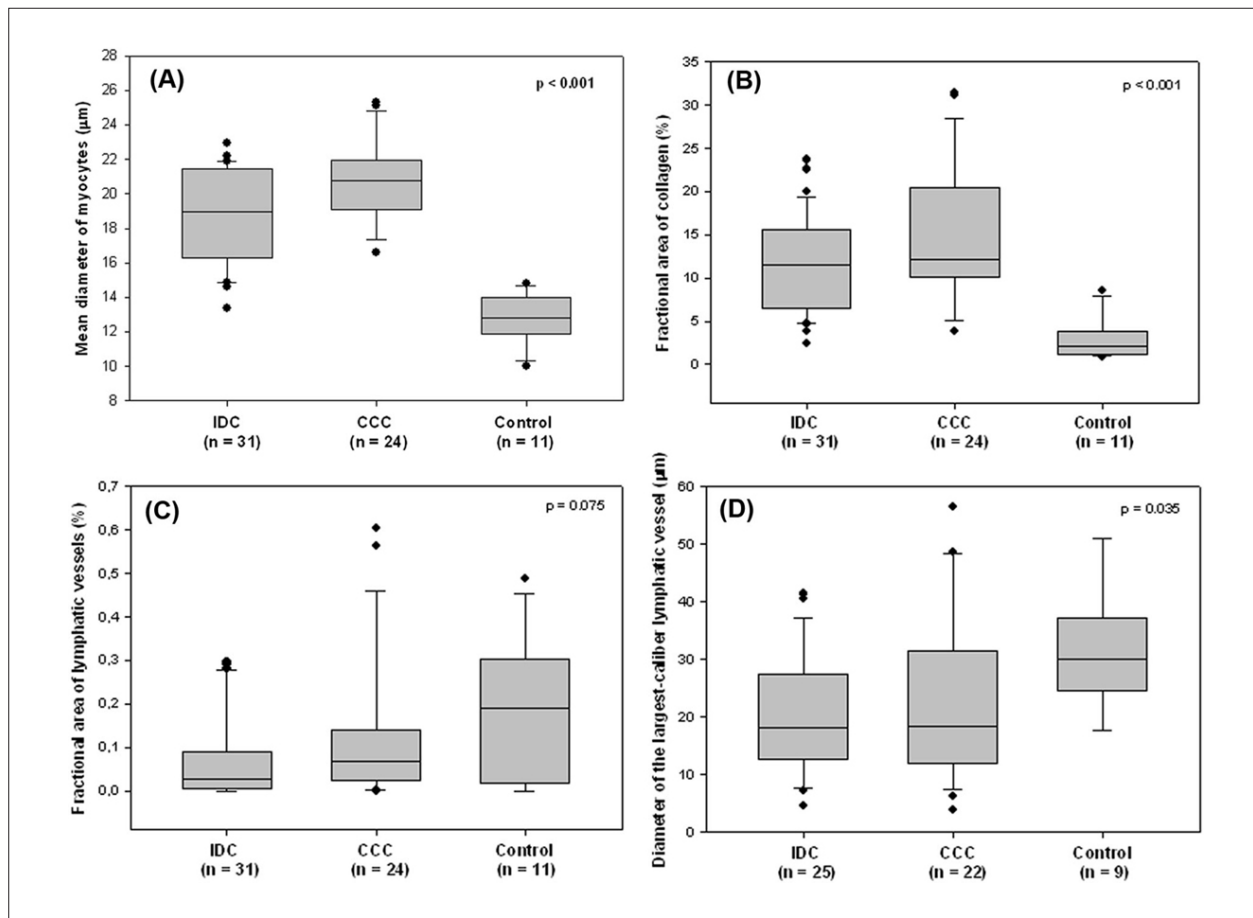
In our samples, the lymphatic vessels appeared focally collapsed, ramified and grouped, preventing their accurate counting. Thus, we chose to assess their fractional area, which differently from the absolute number, is under the influence of the state of vascular distension.

To the best of our knowledge, there has been only one report on the lymphatic vessels of the myocardium in cardiomyopathies: analyzing samples of the heart of patients with IDC through electron microscopy, the authors reported dilation of lymphatic vessels in areas of interstitial edema and their rarefaction and irregularity in areas of interstitial fibrosis<sup>9</sup>. The present study is the first to perform a morphometric immunohistochemical assessment of the lymphatic vessels of the myocardium in cardiomyopathies. Although we expected the increase in their fractional area, caused by the congestive heart failure presented by the patients and also by the chronic inflammatory process (lymphohistiocytic myocarditis) in the CCC group, the morphometric measurements did not confirm this hypothesis. On the contrary, although statistically non-significant, the patients from the IDC and CCC groups presented a smaller fractional area of lymphatic vessels than the control group.



**Figure 1** - Lymphatic vessels of the myocardium (arrows) detected by the immunohistochemical technique, located in the endocardium (panel A, IDC group), myocardial interstitium among the myocytes (panels B and C, control and CCC groups, respectively) and around the arteriole (panel D, IDC group). Observe the presence of areas without lymphatic vessels (asterisk, panel B) and the collapsed, ramified and grouped aspect of the vessels in panel D.

## Brief Comments



**Figure 2** - Mean diameter of the myocytes (A), fractional area of collagen (B), fractional area of the lymphatic vessels (C) and diameter of the largest-caliber lymphatic vessel (D) in the IDC, CCC and control groups (box-plots).

Additionally, the patients from the IDC group presented a smaller diameter of the largest-caliber lymphatic vessels when compared with those from the control group. It is possible that such unexpected results might be related to the larger fractional area of collagen (fibrosis) in the IDC and CCC groups, which would result in lymphatic vessel rarefaction and atrophy, as previously reported<sup>9</sup>.

The present study results are preliminary ones, as the relative scarcity and the great regional variability of the lymphatic vessels in the myocardium make it difficult to achieve a conclusive interpretation of the data obtained through the assessment of small samples, such as the ones obtained at endomyocardial biopsies.

Our results must be confirmed by further studies, using larger samples of hearts of patients submitted to heart transplant or necropsy.

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## Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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## Study Association

This study is not associated with any post-graduation program.

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