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CHARACTERISTICS AND QUANTITATIVE ANALYSIS OF MYOCARDIAL LYMPHATIC ARCHITECTURE IN PATIENTS WITH DIFFERENT TYPES OF END-STAGE HEART FAILURE

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ABSTRACT

This study explored differences in morphology and distribution of myocardial lymphatic drainage ducts in patients with dilated cardiomyopathy (DCM), ischemic cardiomyopathy (ICM), and hypertrophic cardiomyopathy (HCM) in end-stage heart failure to reveal the relationship between the morphological distribution characteristics of myocardial lymphatic drainage ducts and different etiologies and these disease courses. Hearts of 24 patients (transplant recipients) who underwent heart transplantation and 1 normal heart were collected. Myocardial tissue from the anterior wall of the ventricle was taken and quickly frozen. Immunohistochemical and Western blotting techniques were used to detect the expression of LYVE-1, Podoplanin, and VEGFR-3 in cardiac tissue. Ink injection, immunohistochemical staining, and immunofluorescence double labeling techniques were used to detect the co-localization of three antibodies in human heart tissue. Masson staining was used to observe the distribution and content of collagen fibers in the heart tissue of transplant recipients. Compared with the normal group, expression levels of LYVE-1 ($p<0.05$) and VEGFR-3 ($p<0.001$) in the DCM group were significantly increased. Expression level of LYVE-1 ($p<0.05$) was significantly increased in the ICM and HCM groups, but there was no significant change in the expression level of VEGFR-3. The expression level of Podoplanin in the normal group was higher than that in the heart failure groups, but the protein expression results were the opposite. The expression levels of LYVE-1 and VEGFR-3 in the DCM and ICM groups showed an increasing trend with the progression of the disease, while the expression levels of Podoplanin showed a decreasing trend. The immunofluorescence results showed that on larger lymphatic vessels, LYVE-1 and VEGFR-3 were expressed on the inner side of the lymphatic lumen, while Podoplanin was expressed on the outer side, and there was co-localization between the two layers. Masson's staining results showed that the degree of myocardial fibrosis in the DCM group ($p<0.01$) and HCM group ($p<0.001$) increased, while there was no significant change in the ICM group ($p<0.05$). Positive expression intensity of LYVE-1, Podoplanin, and VEGFR-3 does not have a consistent quantitative relationship with different types of heart failure and the duration of the disease. The expression of three biomarkers confirms that DCM, ICM, and HCM have increased lymphatic vessel architecture compared to normal hearts, and the number of lymphatic vessels increases with prolongation of disease course.

Keywords: LYVE-1; VEGFR-3; Podoplanin; Lymphatic capillary; Lymphatic vessels; End stage heart failure; Human heart

INTRODUCTION

Terminal heart failure (THF) refers to the inability of patients to improve heart function through conventional drug therapy, leading to the terminal stage of heart function and disease progression. The clinical types of THF include dilated cardiomyopathy, ischemic cardiomyopathy, restrictive cardiomyopathy, hypertrophic cardiomyopathy, etc., and the first two are more common among them. The ultimate outcome of occurrence and development of heart failure is cardiac structural remodeling. Cardiac remodeling includes a decrease in myocardial cells, an increase in non myocardial cells, proliferation of extracellular matrix, angiogenesis, and lymphatic vessel remodeling. At present, there is more research on myocardial microvasculature in patients with heart failure, and relatively less research on lymphatic vessels. There are studies showing that insufficient or maladaptive remodeling of the cardiac lymphatic vessels can lead to lymphatic fluid accumulation, resulting in myocardial edema (1). Long term cardiac edema activates fibroblasts and triggers myocardial interstitial fibrosis through inflammatory factors (2,3). Some studies have also found that myocardial edema causes changes in extracellular matrix pressure, which promotes the transformation of fibroblasts into myofibroblasts and the secretion of more collagen (4). Myocardial fibrosis can cause the deposition of type I collagen on the walls of lymphatic capillaries, leading to lymphatic obstruction (5) and a decrease in lymphatic drainage efficiency, thereby making heart failure difficult to reverse. The in-depth study of lymphatic vessels benefits from the discovery of lymphatic specific endothelial markers such as LYVE-1, Podoplanin, and VEGFR-3 (6-8). Previous investigators have used these lymphatic endothelial markers to study the morphology of lymphatic vessels in human myocardial tissue (9), atherosclerosis (10), and lymphatic remodeling after myocardial infarction (11). Jiang et al. found that the severity of myocardial interstitial fibrosis in hypertrophic cardiomyopathy is closely related to lymphatic microvascular density (12). From this, it can be seen that increasing the density of cardiac lymphatic vessels and improving lymphatic drainage can provide new treatment ideas for restoring normal function after heart injury. The main problems currently existing in this field include: 1) The structural effects of different types of THF on myocardial lymphatic vessels are not yet clear; 2) Is the neogenesis of THF lymphatic vessels related to the length of the disease course; and 3) The mechanism of action of lymphatic endothelial markers in THF is still unclear. This article aims to explore the pathological changes of different types of THF lymphatic vessels using immunostaining and molecular biology techniques, providing a morphological basis for the clinical diagnosis and treatment of heart failure.

MATERIAL AND METHODS

1. General clinical data

Twenty-four heart transplant recipients and 1 normal donor heart were collected from Zhengzhou Seventh People's Hospital. Among them were 12 cases in the DCM group, which were divided into three groups according to the disease course, with 4 cases in each group: DCM1 group (disease course 0-5 years), DCM2 group (disease course 6-10 years), and DCM3 group (disease course>10 years). There were 8 cases in the ICM group, which are divided into two groups according to the disease course, with 4 cases in each group: ICM1 group (disease course 0-5 years) and ICM2 group (disease course>5 years). In addition, there were 4 cases in the HCM group which were not sub-grouped due to the small number of specimens. The patients were between 35 and 60 years old and all research specimen use was approved by the Ethics Committee of Zhengzhou Seventh People's Hospital with the informed consent of patients.

2. Ink injection technique for displaying cardiac lymphatic vessels

Five fresh hearts of surgically excised recipients were obtained. Indian ink was slowly injected into the endocardium of the left ventricle with the syringe needle moving slowly and uniformly towards the epicardium until the needle tip reaches the epicardium completing the injection process. Then, further injections at 3-5 sites again approximately 1 centimeter from the first injection point was performed. This multi-point injection is performed on each heart. Gentle massage and application of pressure was applied to the injection area (13) to promote ink entry into the lymphatic capillaries. At the center of the injection site, myocardial tissue with a volume of approximately 2 cm3 was obtained. This tissue was frozen and sections obtained for both HE and immunohistochemistry with observation under a light microscope.

3. Tissue staining

All tissue sections from myocardial tissue near the interventricular septum of the left ventricular anterior wall was washed 3-5 times with pre cooled physiological saline. Then, tissue was embedded in OCT and 0.125 μm sections were obtained. All sections were collected and stored in a -80 ℃ freezer for the following staining purposes.

Immunohistochemical staining

Sections were fixed with 4% paraformaldehyde and antigen blocked according to the instructions of the reagent kit. Rabbit anti-human primary antibody LYVE-1 (Affinity, 1:500), VEGFR-3 (Affinity, 1:500), and Podoplanin (Affinity, 1:500) antibody solutions were added dropwise, and incubated overnight at 4 ℃. Goat anti-rabbit secondary antibody (Zhongshan Jinqiao, 1:1000) was then added and incubated at room temperature for 1 hour. Finally, sections were treated with DAB colorimetric solution and counter stained with hematoxylin for 3 minutes. After gradient dehydration, sections were covered with neutral gel and observed and photographed under a light microscope.

Immunohistochemical fluorescence staining

Frozen sections were fixed with acetone at 4 ℃, and 0.5% Triton X100 was added dropwise for membrane penetration. After blocking with goat serum, rabbit anti-human primary antibodies LYVE-1 (Affinity, 1:500), VEGFR-3 (Affinity, 1:500), and Podoplanin (Affinity, 1:500) solutions were added dropwise. The negative control utilized PBS and all were incubated overnight at 4 ℃. Then Cy3 labeled goat anti-rabbit antibody (Beyotime, 1:500) and FITC labeled goat anti rabbit antibody (Beyotime, 1:500) were added separately under dark conditions. These were incubated at room temperature for 2 hours. Then cell nucleus was stained with DAPI and anti-fluorescence quenching agent was added dropwise before observation under a fluorescence microscope.

Masson staining

Mason staining followed instructions of the reagent kit (Solarbio, G1346). After staining, the sections were dehydrated and cleared, sealed with neutral gum, and observed and photographed under a light microscope.

4. Detected protein expression by western blotting technique

Myocardial tissue was placed on ice box, cut into small fragments, fully lysed, and centrifuged at 12,000 rpm 4 ℃ for 5 minutes. Supernatant was collected and protein concentration was measured using the BCA method. FiveX loading buffer was added to the remaining supernatant and boiled at 99 ℃ for 10 minutes to prepare the protein sample. SDS-PAGE electrophoresis was performed with loading of 20μg per lane. The membrane was transferred and incubated with skim milk powder. Rabbit anti-human primary antibodies LYVE-1 (Affinity, 1:1000), VEGFR-3 (Affinity, 1:1000), Podoplanin (Affinity, 1:1000), and DAPDH (Affinity, 1:5000) solutions were applied and incubated overnight at 4 ℃. After washing, HRP labeled goat anti-rabbit secondary antibody (Affinity, 1:1000) was added dropwise and incubated for 2 hours. Then the membrane was washed developer solution added for imaging.

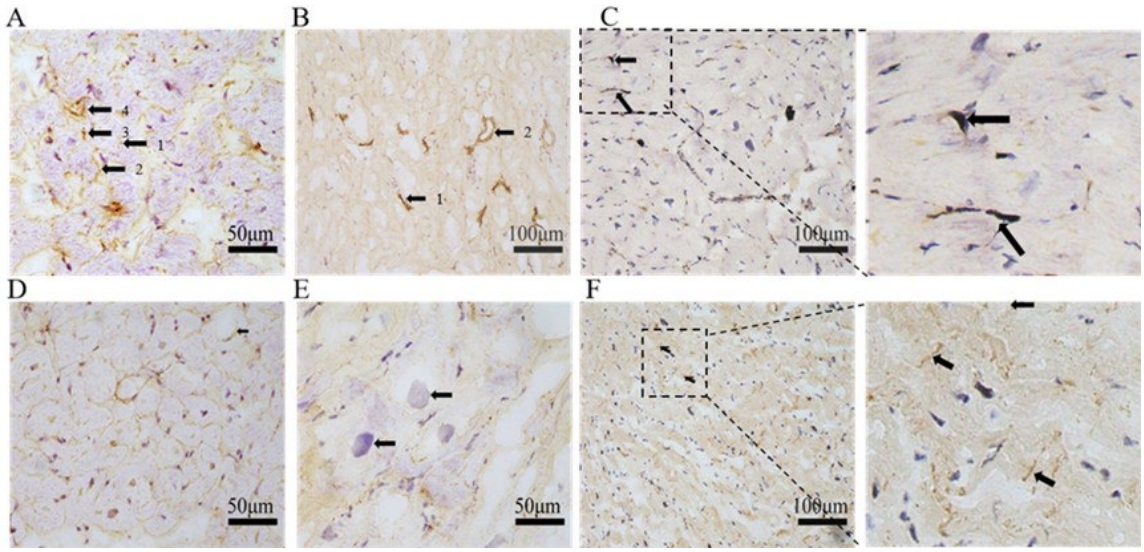

Fig. 1. *Morphological distribution of lymphatic vessels and capillaries in cardiac tissue of hypertrophic cardiomyopathy and ischemic cardiomyopathy. A and B: Podoplanin immunohistochemical staining of myocardial tissue in patients with hypertrophic cardiomyopathy; C: Injection of ink particles and immunohistochemical staining of VEGFR-3 into lymphatic capillary and vessels of patients with hypertrophic cardiomyopathy (with zoomed image); D and E: Podoplanin immunohistochemical staining of cardiac tissue in patients with ischemic cardiomyopathy and heart failure; F: LYVE-1 immunohistochemical staining is mainly expressed in the intercalated discs of the myocardium in patients with dilated cardiomyopathy(with zoomed image).*

5. Statistical methods

Image J was used for grayscale measurement, and results analyzed and statistically examined using Graphpad Prism8. Intergroup comparison was conducted using one-way analysis of variance, and results expressed as mean ± standard deviation. Statistical significance was set at $p \leq 0.05$.

RESULTS

1. Morphological distribution of lymphatic capillaries and lymphatic vessels in myocardium

Immunohistochemical results demonstrated positive sites specifically labeled with LYVE-1, Podoplanin, and VEGFR-3 were brownish yellow in color, and their morphology presented as dots (Fig. 1A1), stripes (Fig. 1A2), plaques (Fig. 1A3), and irregular rings (Fig. 1A4). These markers are mainly distributed in the interstitial space of myocardial tissue. The larger lumens are lymphatic vessels (Figs. 1B1 and 1B2), while the smaller ones are lymphatic capillaries. The lymphatic vessels have visible lumens, while lymphatic capillaries are punctate (transverse section) (Fig. 1A1) and linear (longitudinal section) (Fig. 1A2). Lymphatic capillaries are not observed with lumens. Due to injection of Indian ink into the cardiac muscle, ink particles can only enter the lymphatic capillaries and cannot enter the blood capillaries and following ink injection, only lymphatic capillaries are filled with black carbon particles. After immunohistochemical staining with VEGFR-3, the periphery containing ink particles showed positive expression, and the combination of these two techniques demonstrated the objective and reliable lymphatic vessels shown in this study (Fig. 1C). On the transverse section of ischemic cardiomyopathy tissue, lymphatic vessels exhibit a network like distribution (Fig. 1D); Heart failure patients have a strip-shaped distribution on the longitudinal section of the myocardium, with enlarged lymphatic lumens. Occasionally, inflammatory cell infiltration

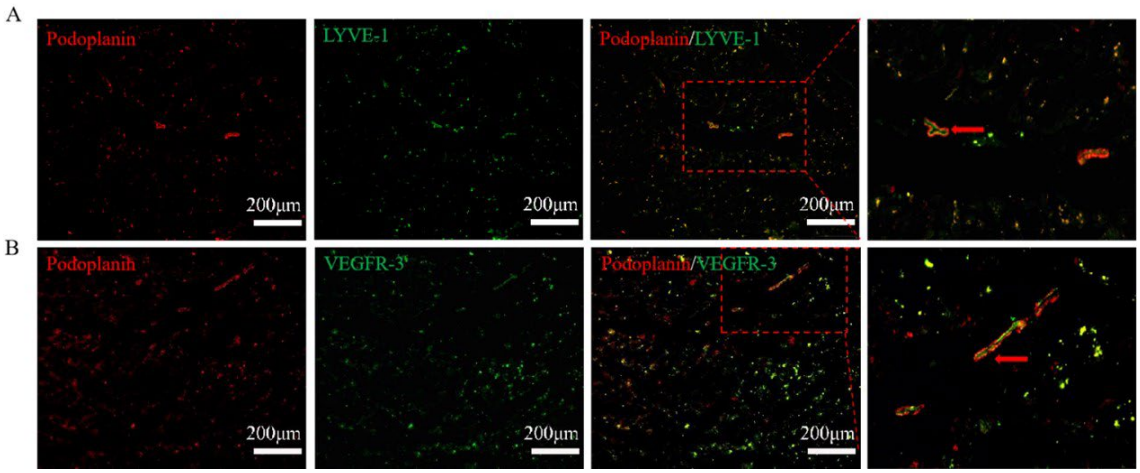

Fig. 2. *Immunofluorescence staining of co-localization of Podoplanin with LYVE-1 or VEGFR-3 in myocardial tissue. A (top row): Podoplanin (red) and LYVE-1 (green) and co-localization (with zoomed section); B: Podoplanin (red) and VEGFR-3 (green) and co-localization (with zoomed section).*

was observed in the myocardial tissue of patients with heart failure. Unlike VEGFR-3, LYVE-1 is expressed in a granular distribution, mainly in the intercalated discs of myocardial cells (Fig. 1F); However, in patients with ischemic heart disease and heart failure, Podoplanin is highly expressed in the lymphatic vessels of myocardial tissue.

2. Immuno- localization of LYVE-1, VEGFR-3, and Podoplanin

LYVE-1 was labeled with green fluorescence, and Podoplanin with red fluorescence. After merging the images, it was found that most of the small lymphatic capillaries co-expressed both (in yellow) (Fig. 2A). The larger lymphatic vessels expressed green fluorescence on the inner membrane side of the lumen, red fluorescence on the outer membrane side of the lumen, and regional co expression (in yellow) was observed at the inner and outer membrane junctions or within the outer membrane (Fig. 2A).

VEGFR-3 was labeled with green fluorescence and Podoplanin with red fluorescence. Results showed green fluorescence expressed on the inner membrane side of the lumen, and red fluorescence was expressed on the outer membrane side of the lumen. Co-expression was observed at the junction of the inner and outer membranes (yellow) (Fig. 2B). Therefore, most lymphatic capillaries express three marker proteins. On larger lymphatic vessel walls, LYVE-1 and VEGFR-3 are expressed on the inner membrane side of the lumen, while Podo-planin is expressed on the outer membrane side of the lumen, and there is a co-expression region between the two.

3. LYVE-1, Podoplanin, and VEGFR-3 protein expression in the myocardium of three groups of patients

Western blotting results showed that compared with normal myocardium, expression of LYVE-1 ($p<0.05$), Podoplanin ($p<0.001$), and VEGFR-3 ($p<0.001$) were significantly increased in the DCM group. The expression of LYVE-1 and Podoplanin proteins in the ICM group and HCM group were significantly higher than those in the normal group ($p<0.001$), while there was no significant difference in the expression of VEGFR-3. Among them, there was no significant difference in the expression contents of LYVE-1 between the DCM group for either the 0-5 year and 6–10 year disease course, while the expression contents in the >10 year group was significantly higher than the previous two groups ($p<0.001$) demonstrating an increase during disease course. Expression of LYVE-1 in the

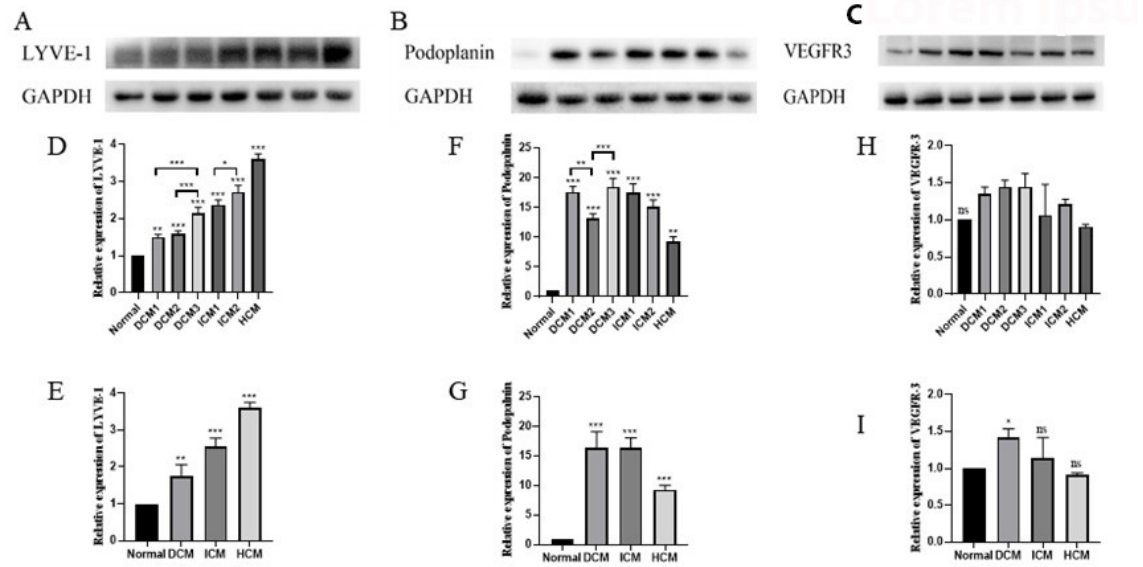

Fig. 3. *Western blotting expression and quantitative analysis of lymphatic endothelial marker proteins in cardiac tissue of three groups of patients. Expression (A) and quantitative analysis (D) of LYVE-1 content in cardiac tissue of patients in each group. Expression (B) and quantitative analysis (F) of Podoplanin content in cardiac tissue of patients in each group. Expression (C) and quantitative analysis H) of VEGFR-3 contents in cardiac tissue of patients in each group. (E) Analysis of LYVE-1 expression contents in cardiac tissue of normal and three different types of end-stage heart failure patients; (G) Analysis of Podoplanin expression contents in cardiac tissue of normal and three different types of end-stage heart failure patients; (I) Analysis of VEGFR-3 expression levels in cardiac tissue of normal and three different types of end-stage heart failure patients. ns p>0.05, * p<0.05, * * p<0.01, * * * p<0.001.*

two groups (p<0.001) demonstrating an increase during disease course. Expression of LYVE-1 in the 0-5 year and >5 year groups of the ICM group were statistically significant (p<0.01), and also showed an upward trend with disease progression. However, there was no statistically significant difference for Podoplanin and VEGFR-3. Expression of Podoplanin in the ICM group decreases with duration of disease course, while expression of LYVE-1 and VEGFR-3 in the DCM group and ICM group increased with increased disease course. In addition, compared with DCM, ICM, and HCM, the expression contents of LYVE-1 and VEGFR-3 showed an increasing trend, while the expression contents of Podoplanin showed a decreasing trend (Fig. 3).

4. Immunohistochemical expression of LYVE-1, Podoplanin, and VEGFR-3 in three groups of patients

Immunohistochemistry showed that compared with normal myocardial tissue, expression levels of LYVE-1 (p<0.05) and VEGFR-3 (p<0.001) in the DCM group increased, while expression of Podoplanin (p<0.001) decreased. Expression of LYVE-1 (p<0.05) in both the ICM and HCM groups were higher than those in the normal group, while expression contents of Podoplanin (p<0.001) were lower. There was no significant difference in expression of VEGFR-3. Expression of Podoplanin in the DCM group with a 0–5 year disease course was significantly higher than that in the other two groups (p<0.001) with expression showing a decreasing trend with the increase of disease course. There was no significant difference in expression of VEGFR-3 between the DCM group in the 0-5 and 6-10 year disease course groups, but expression in the >10-year group was significantly higher than the previous two groups (p<0.001, p<0.05) with expression

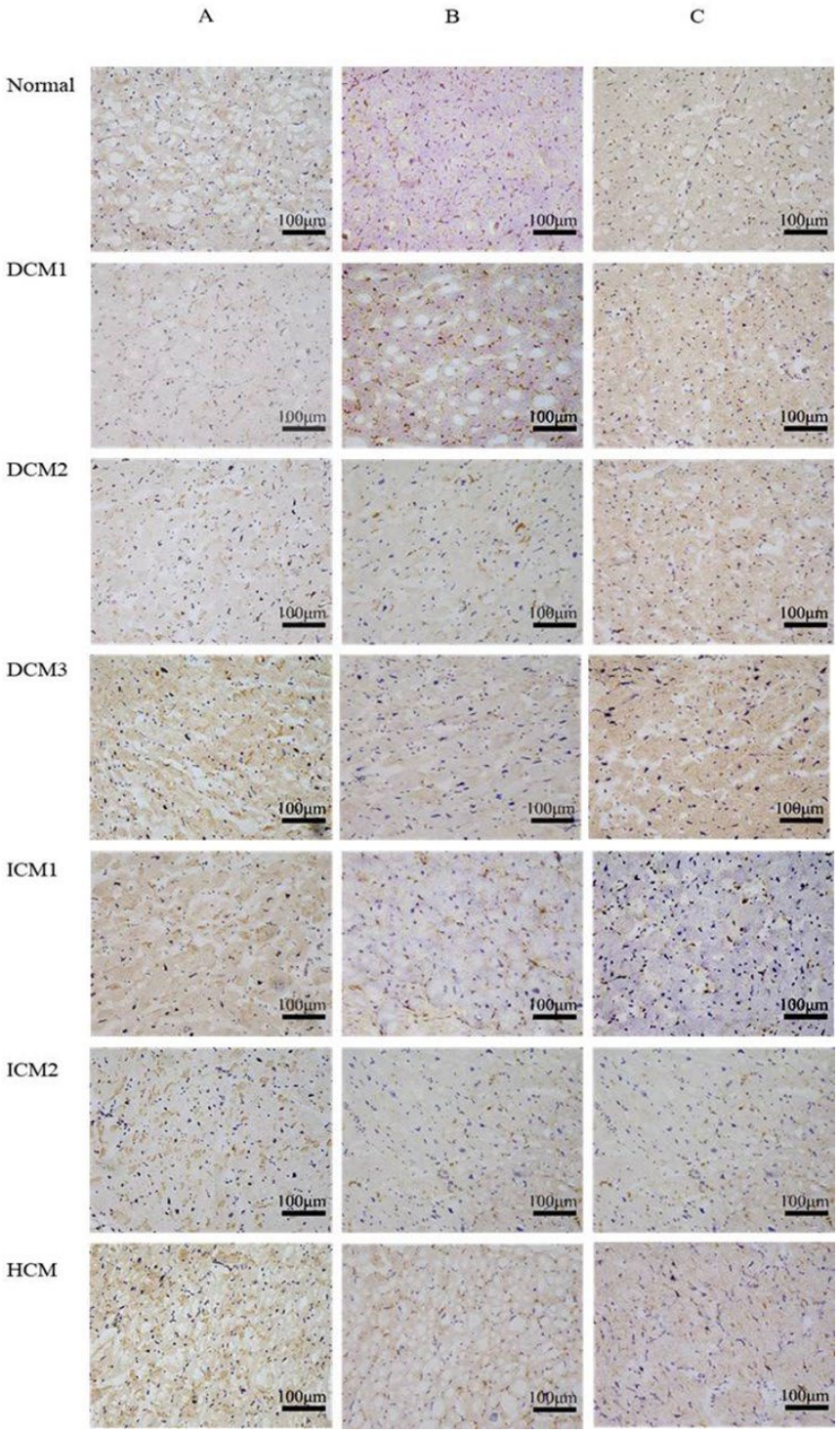

Fig. 4. *Immunohistochemical staining results comparing expression results of LYVE-1 (A), Podoplanin (B), and VEGFR-3 (C) lymphatic endothelial markers in cardiac tissues of the different groups of patients.*

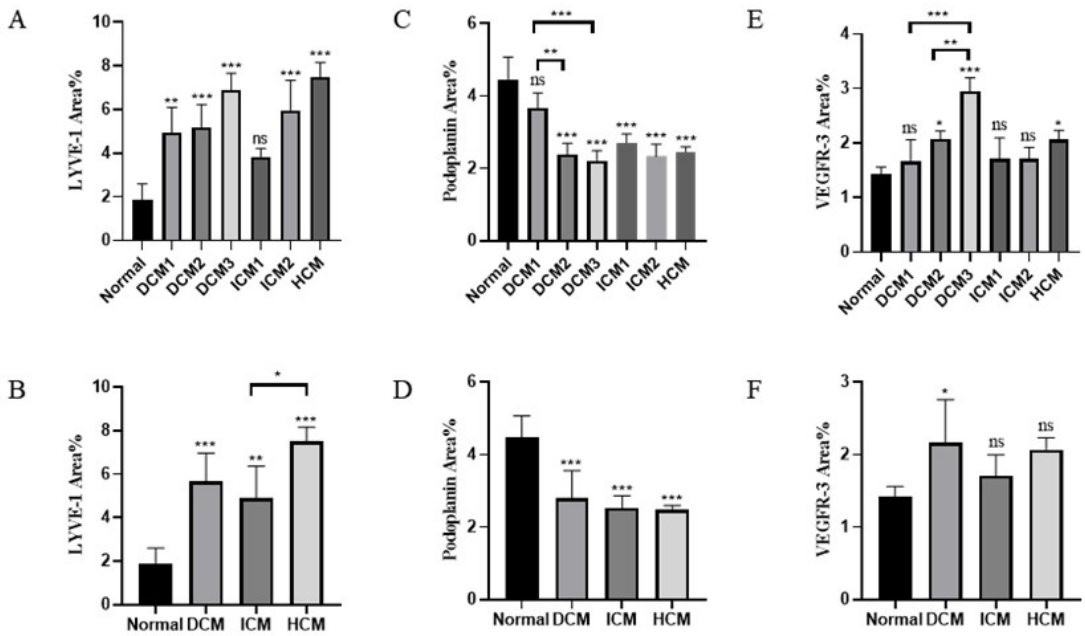

Fig. 5. *Quantitative analysis of immunohistochemical staining results for LYVE-1, Podoplanin, and VEGFR-3 in cardiac tissue of patients in each group. (A) Analysis of LYVE-1 content in cardiac tissue of patients in sub-groups. (B) Analysis of LYVE-1 content in cardiac tissue of normal and three different types of end-stage heart failure patients. (C) Analysis of Podoplanin content in cardiac tissue of patients in sub-groups. (D) Analysis of Podoplanin content in cardiac tissue of normal and three different types of end-stage heart failure patients. (E) Analysis of VEGFR-3 content in cardiac tissue of patients in sub-groups. (F) Analysis of VEGFR-3 content in cardiac tissue of normal and three different types of end-stage heart failure patients. ns p>0.05, * p<0.05, * * p<0.01, * * * p<0.001.*

showing an upward trend with increase of disease course. Expression of LYVE-1 and VEGFR-3 in both DCM and ICM groups showed an increasing trend with the progression of the disease, while expression of Podoplanin showed a decreasing trend with disease progression. Finally, expression of Podoplanin in myocardium of DCM, ICM, and HCM patients showed a decreasing trend (Figs. 4 and 5).

5. Comparison of pathological changes in myocardial interstitial collagen fibers

Masson staining results depicted myocardial cells as red and collagen fibers as blue. Collagen fibers are distributed between myocardial cells and interweave into a network. The thickness of collagen fiber bundles varies. Compared with the normal group, the collagen fiber content in DCM ($p<0.05$) and HCM ($p<0.001$) increased, while there was no significant difference in collagen fiber content in the ICM group. The DCM group showed no significant change in collagen fiber content as the disease progressed and there was no statistically significant difference in collagen fiber content between the 0-5 year group and the normal group in the ICM group, while the collagen fiber content increased in the >5 year group ($p<0.05$). Collagen fiber content in the HCM group significantly increased, indicating significant myocardial fibrosis (Fig. 6).

DISCUSSION

End stage heart failure is the final stage of heart disease caused by a variety of disease factors and development processes, but final pathological manifestations are approximately the same. Three diseases (DCM, ICM, , and

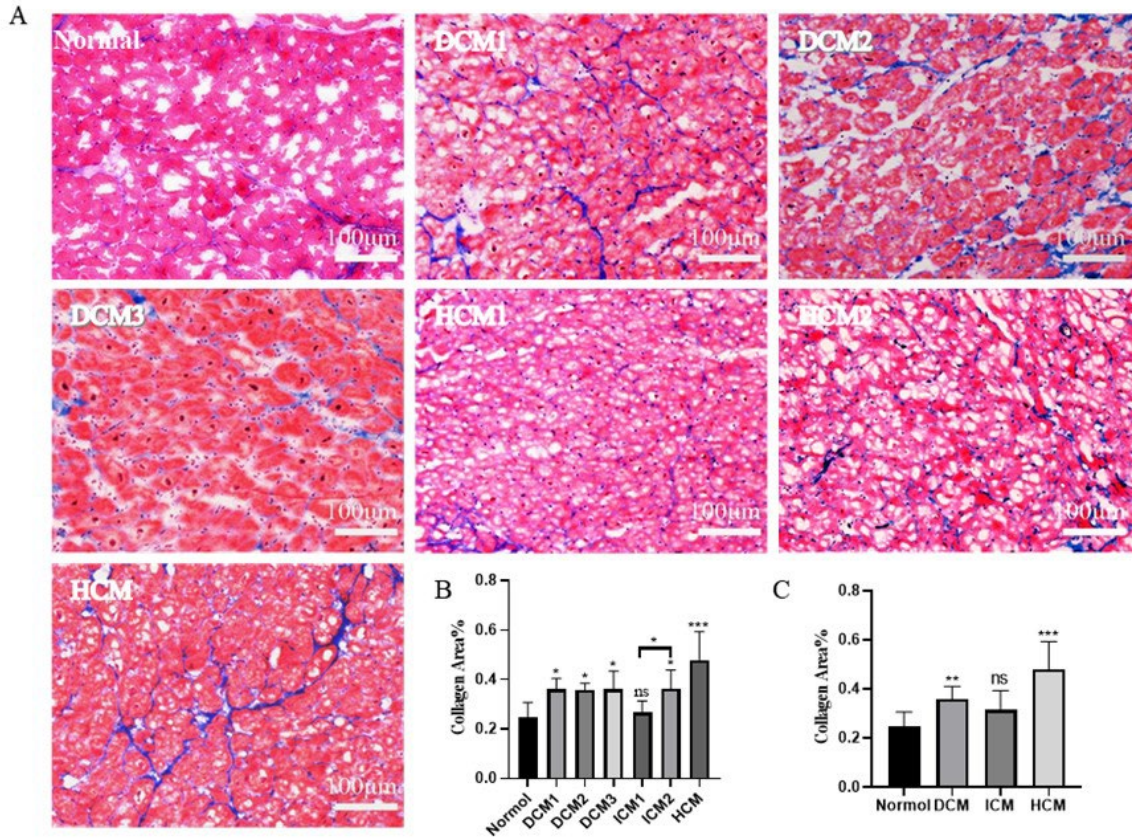

Fig. 6. *Comparison of collagen fiber distribution and content in cardiac tissue of patients in each group with Masson staining. (A) Distribution of collagen fibers in cardiac tissue of patients in each group and sub-group. (B) Quantitative analysis of collagen fibers in cardiac tissue of patients in each group and sub-group. (C) Quantitative analysis of collagen fibers in myocardial tissue of patients with normal myocardium and three types of end-stage heart failure groups. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001.*

HCM) ultimately result in cardiac structural remodeling, including myocardial cell hypertrophy, abnormal microcirculation system function and structure, and collagen proliferation. Previous studies have mainly focused on myocardial cells, blood vessels, and myocardial fibrosis, with limited research on the pathological changes of lymphatic vessels in different stages of cardiomyopathy. Due to the fact that lymphatic vessels are one of the important pathways for myocardial metabolites and tissue fluid reflux, changes in the myocardium may lead to pathological changes in the myocardial lymphatic vessels. Ultimately, these can impact heart function and exacerbate heart failure. There is currently a lack of research studies on whether myocardial lymphatic vessels undergo morphological changes in the state of heart failure.

Study of morphology and distribution of lymphatic capillaries and lymphatic vessels is mostly based on the expression site and intensity of markers in the lymphatic vessel endothelium. Common lymphatic endothelial markers include LYVE-1, Podoplanin, and VEGFR-3. Expression of these on the surface of these cells are is usually used to describe the morphology and structure of lymphatic vessels. The results of this study showed that the expression level of LYVE-1 in dilated cardio-

myopathy significantly increased, and the expression level showed an upward trend with the progression of the disease. LYVE-1 is a homolog of hyaluronic acid, involved in hyaluronic acid penetrating the lymphatic vessel wall (14). Therefore, the significant increase in LYVE-1 expression may be related to an increase in extracellular matrix. LYVE-1 is also a marker of lymphatic endothelial cells and its increase may indicate neogenesis of lymphatic capillaries. In addition, expression levels of VEGFR-3 is higher than the normal (control) group and also demonstrating an upward trend with progression of disease. This may be due to increase in myocardial interstitial components stimulating lymphatic endothelial cells and activating VEGFR-3 signaling to promote lymphatic endothelial cell proliferation (15). The longer the course of disease, the more interstitial fluid retention causing greater tension on the lymphatic endothelial cells which may result in expansion of lymphatic capillaries. Therefore, dilated cardiomyopathy not only increases drainage volume through the regeneration of lymphatic capillaries, but also reduces myocardial edema by expanding and draining more interstitial fluid through lymphatic capillaries, ultimately achieving a new tissue fluid balance. Ischemic cardiomyopathy is heart failure caused by myocardial ischemia caused by coronary atherosclerosis (16). Its most common pathological change is diffuse large areas of myocardial cell degeneration, necrosis, and fibrosis seen among myocardial cells all leading to serious myocardial dysfunction. Partial ischemic cardiomyopathy can further develop into dilated cardiomyopathy. This study demonstrates that the expression level of LYVE-1 is increased in ischemic cardiomyopathy, which may be the result of the combined effect of capillary lymphangiogenesis and increased extracellular matrix.

Myocardial fibrosis is an important pathological change in heart failure. For example, most hypertrophic cardiomyopathy presents with a large amount of myocardial cell hypertrophy, abnormal myocardial microcirculation, exacerbation of relative myocardial ischemia, myocardial cell death, and an increase in collagen cells between myocardial cells, ultimately leading to myocardial fibrosis (17). Results of this study showed that the expression of LYVE-1 was highest in the hypertrophic cardiomyopathy group, with an increase in collagen fibers (see Masson staining results). This result suggests that there may be a correlation between heart failure complicated by hypertrophic cardiomyopathy and lymphatic vessels and collagen components (12). In the state of heart failure, the number of lymphatic capillaries increases, collagen synthesis increases, and extracellular matrix secretion of myocardial cells increases, ultimately leading to worsening of myocardial fibrosis.

Podoplanin, LYVE-1, and VEGFR-3 are currently recognized endothelial markers of lymphatic vessels, and their expression can represent the presence of lymphatic vessels. However, there are differences in the expression results of different markers on larger lymphatic vessel walls. The immunofluorescence results of this study showed that LYVE-1 and VEGFR-3 were expressed on the inner layer side of larger lymphatic vessels, while Podoplanin was expressed on the outer layer side of the lumen. There was co-localization between the inner and outer layers, which is an interesting finding.

Based on the results of disease analysis combining immunohistochemistry and western blotting, comprehensive analysis shows that the three marker proteins have highest expression levels in dilated cardiomyopathy, followed by ischemic cardiomyopathy, while the expression levels in hypertrophic cardiomyopathy show a decreasing trend, but there is no statistical significance. It should be noted that immunohistochemical results of this study showed that expression level of Podoplanin in the normal group's myocardium was higher than that in the diseased group's myocardium, but the western blotting results showed that the expression level in the normal group was lower than that in the diseased group. This contradictory phenomenon may be related to the insufficient sample of normal specimens (only 1 case).

Due to the relative specificity of lymphatic vessel antibodies, research progress on lym-

phatic vessels is severely limited. At present, no lymphatic vessel specific marker has been found to be expressed on the endothelium of all lymphatic vessels. It has been shown through triple immunofluorescence staining that some lymphatic vessels can simultaneously express LYVE-1, Prox-1 (Prospero related hemeobox gene-1), and Podoplanin antibodies. Some lymphatic vessels can express any two of these antibodies, while others only express one (18). LYVE-1 is mainly expressed in adult lymphatic endothelial cells (6,19), but it is also expressed in myocardial macrophages and endocardial cells (20). In this study, it was found that it is also expressed in intercalated discs. In addition, LYVE-1 is not a stable expression marker, for example, its expression level is downregulated during acute inflammation. In adult tissues, VEGFR-3 is mainly expressed in the lymphatic vessel endothelium, but there is also a small amount of expression in the vascular endothelium (21). Podoplanin is specifically expressed in lymphatic vessels and not in vascular endothelium, with strong specificity (22). The expression of LYVE-1 and Podoplanin is limited to the lymphatic capillaries, while they are not expressed in the collecting lymphatic vessels with smooth muscle structures (23,24). Therefore, the density quantitative analysis results of lymphatic capillaries and vessels are relative. In addition, the calculation of lymphatic vessel density is influenced by differences in myocardial cell size and extracellular matrix components in different disease states. For example, myocardial edema and the degree of myocardial fibrosis can affect the density of lymphatic capillaries and lymphatic vessels.

In summary, the overall trend of results of this study shows an increase in lymphatic vessel density in the myocardial tissue of patients with DCM, ICM, and HCM. However, there are differences in the expression of lymphatic markers among the three types of heart failure. The expression level of LYVE-1 increased in all three types of patients, but VEGFR-3 only increased in the DCM group. The expression levels of LYVE-1 and VEGFR-3 in the DCM and ICM groups showed an increasing trend with the progression of disease, while the expression levels of Podoplanin in the myocardium of the three types of heart failure showed a decreasing trend with the progression of disease. End-stage heart failure cardiomyopathy results in imbalanced myocardial metabolism, increased metabolic products, increased intercellular lymphatic fluid, and the need for more lymphatic vessels to reflux lymphatic fluid. These factors may all promote an increase in number of lymphatic vessels.

DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

AUTHOR CONTRIBUTIONS

Guo Zhikun designed and supervised the project. Wang Yiqi and Dou Jintao performed the experiments and analyzed the data. Zhang Xiangli and Fu Zhikun providing the patient's myocardial tissue and data analysis. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

All authors declare no relevant financial or non-financial interests exist.

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