

Initial lesions of the elastic fibers and extracellular matrix in varicose veins: an immunohistochemical and confocal microscopy study

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INTRODUCTION

Varicose veins are an important cause of morbidity with a prevalence of 10 to 50%.

Risk factors: advanced age, female gender, diets, obesity, physical activity, standing occupations, connective tissue alterations, genetic predisposition.

The aetiology and pathogenesis: Two hypothesis have been proposed:

1. Valvular dysfunction causing venous reflux in early state of pathology
2. A primary change in varicose vein wall (structural and biochemical changes).

We hypothesize that the formation of varicose vein is secondary to defects in cellular and extracellular matrix components, causing wall weakness and altered tone.

METHODS

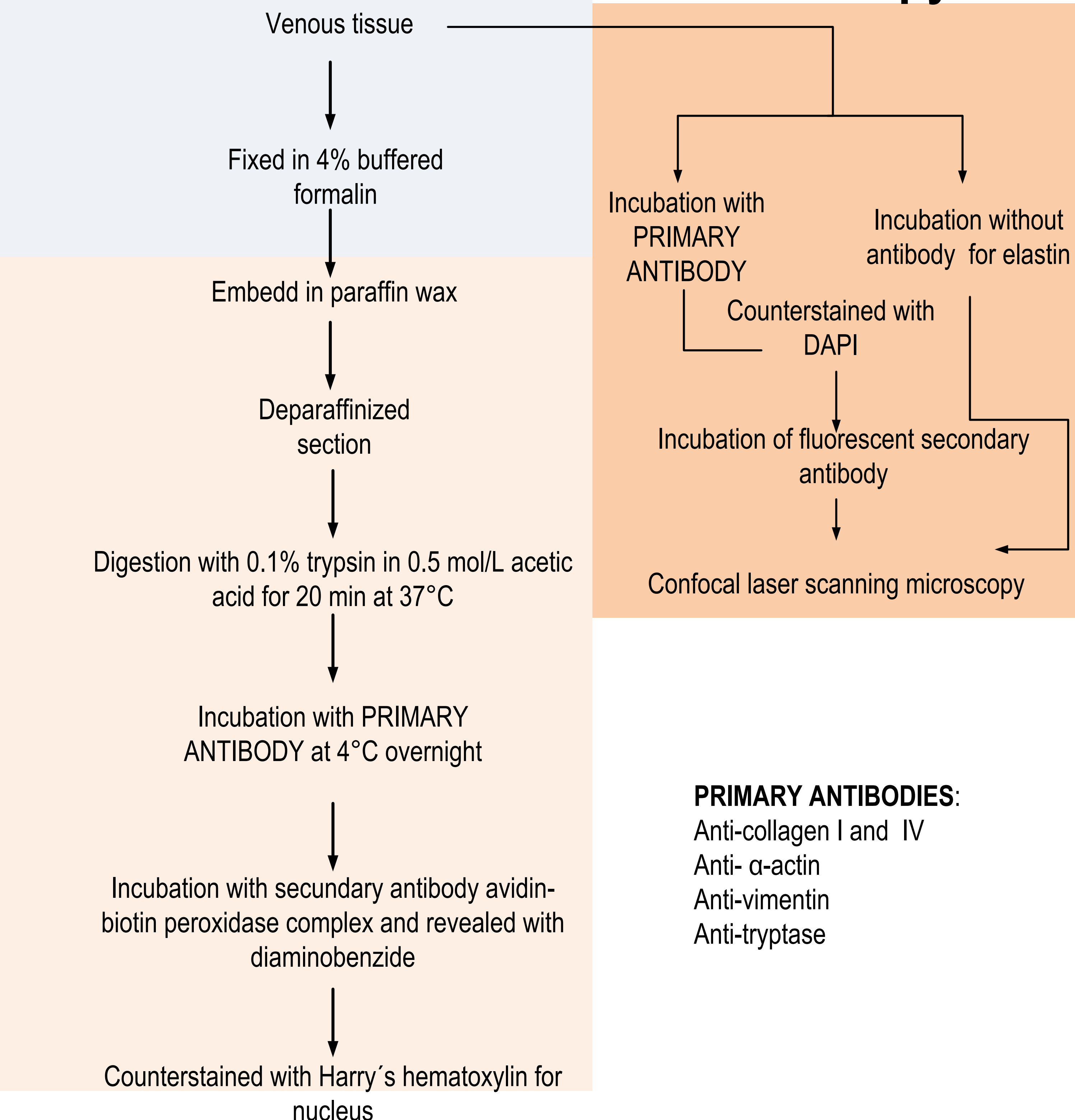
Samples

Saphenous veins from patients undergoing varicose vein surgery (CEAP-2)

Veins were divided in: **proximal** (with lesions, varicose vein, **VV**) and altered Eco-Doppler and **distal** (normal vein, **NV**) with normal Eco-Doppler.

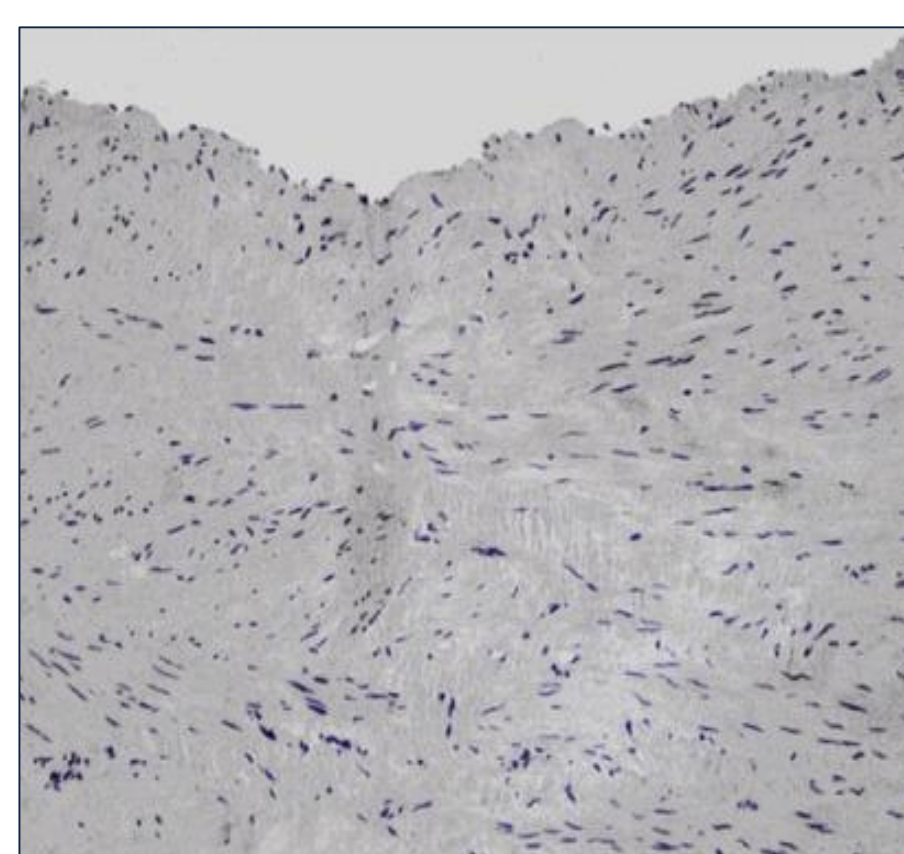
Immunohistochemistry

Microscopy

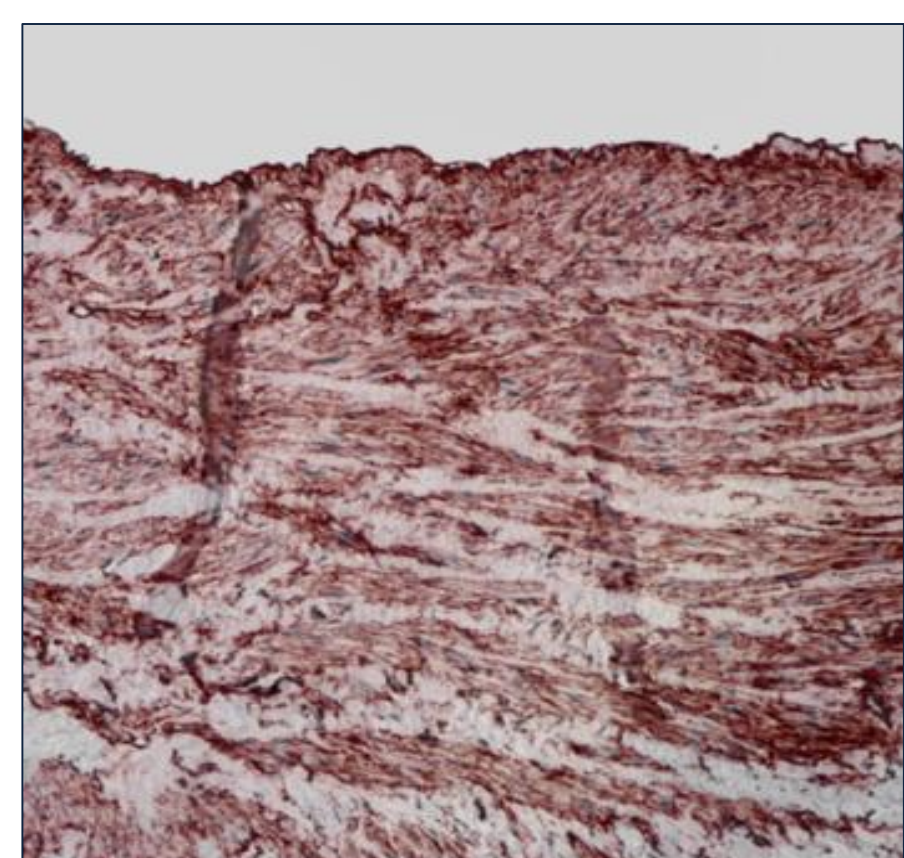


Distal Segment (normal vein)

Segment with normal histology

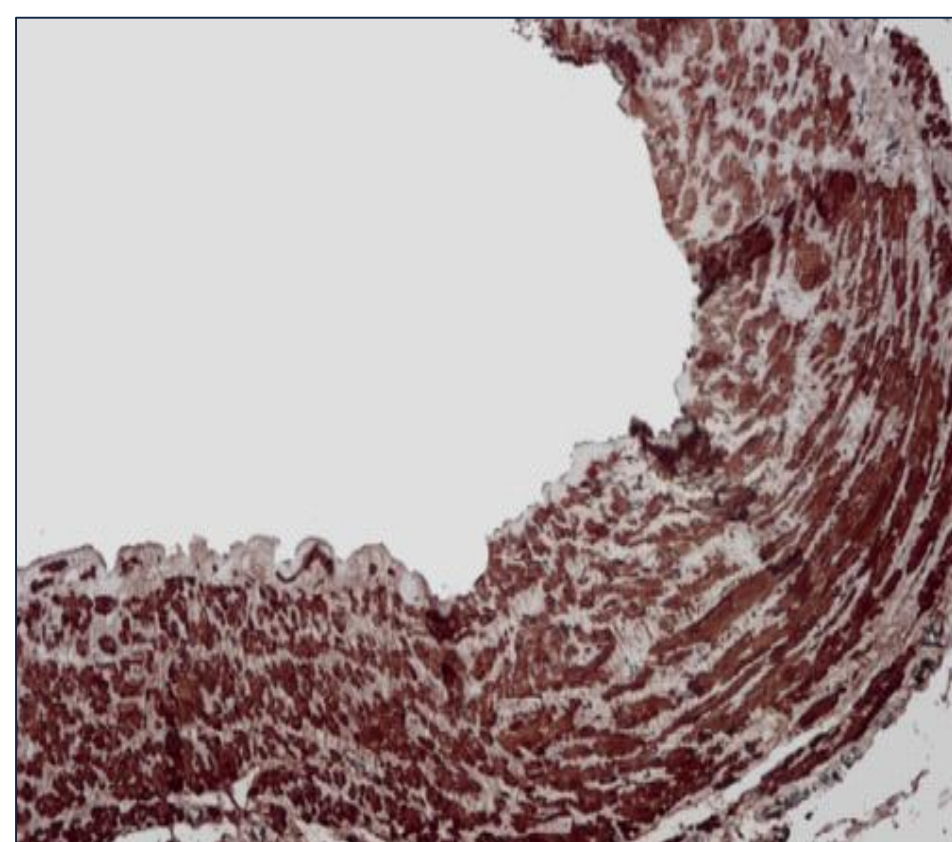


The muscular layer is thin and endothelial cells and intimal layer is normal. Primary antibody is omitted.

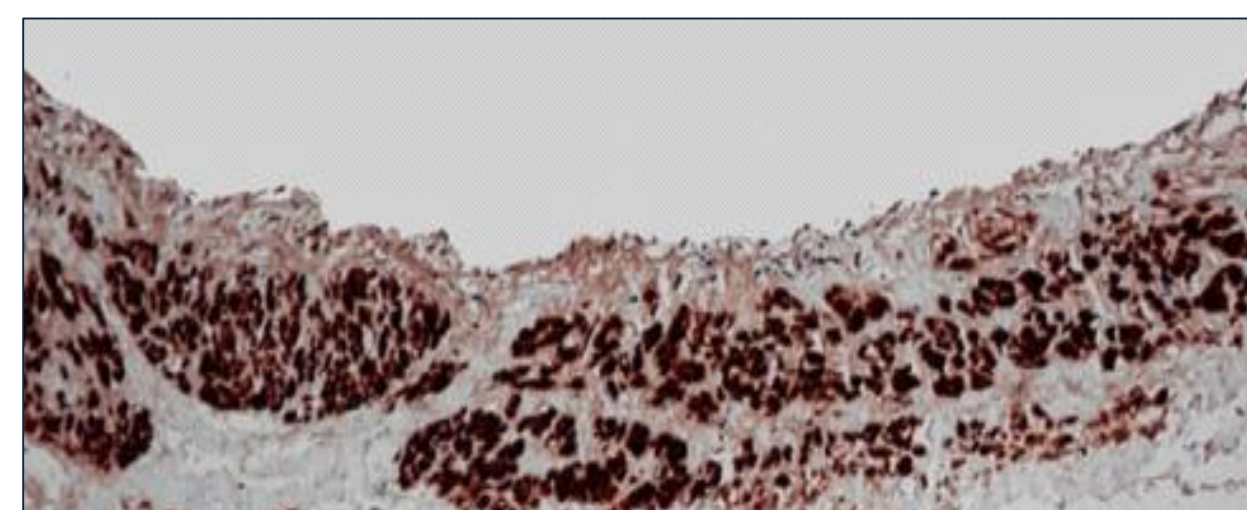


Anti-smooth muscle α -actin. Venous wall is occupied by numerous muscle fibers, which are irregularly distributed and separated by conjunctive tissue. Luminal surface is smooth, intimal layer is virtual and endothelium is directly supported on intimal conjunctive tissue. Actin expression in leiomyocytes is normal.

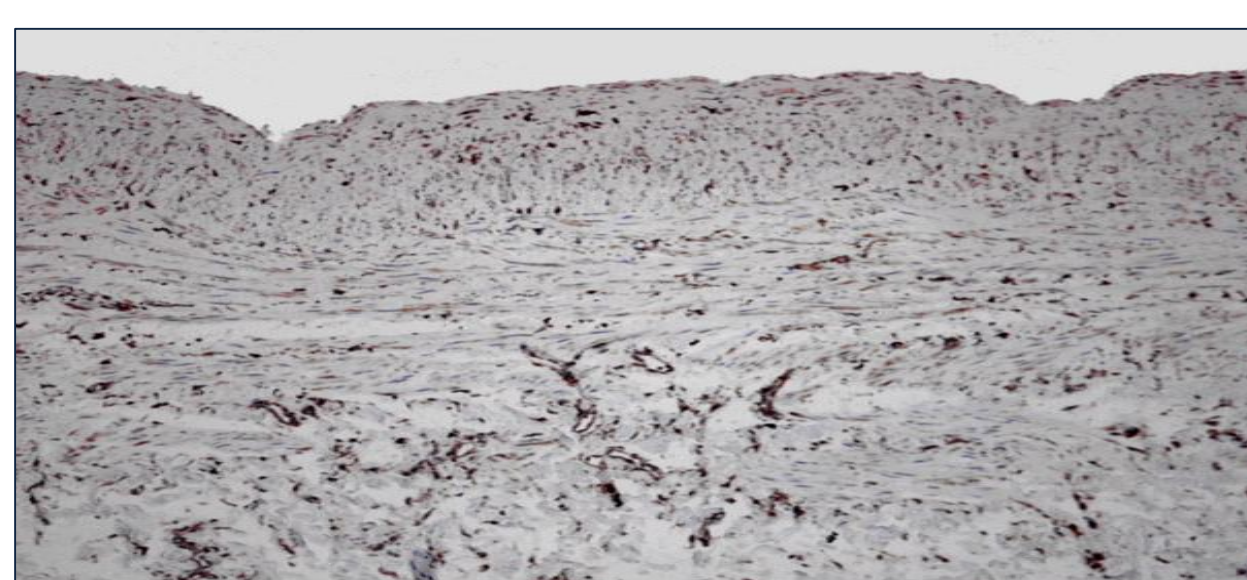
Segments with initial intimal tissue alterations



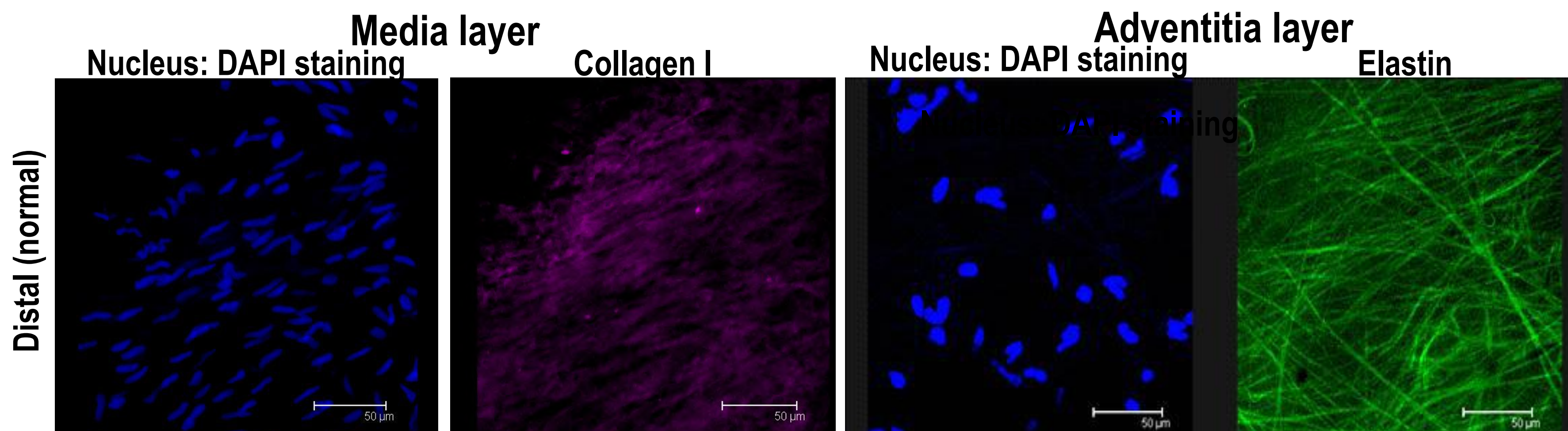
Anti-collagen IV. A focal minimal increase of intimal collagen with endothelial protrusion and gentle increase of collagenized sub-endothelial conjunctive tissue. Muscular fibers and interstitial collagen are normal. Adventitia does not show any lesion.



Anti-smooth muscle α -actin. An initial lesion can be seen in the intima of the venous segment. The muscular wall is normal.



Anti-Vimentin. Venous wall has an irregular distribution of fibroblasts into interstitial conjunctive tissue of muscular medial fascicles and in the adventitial layer. There are few cells positive to vimentin in the area where initial fibrosis was observed. Endothelial cells were also vimentin positive.



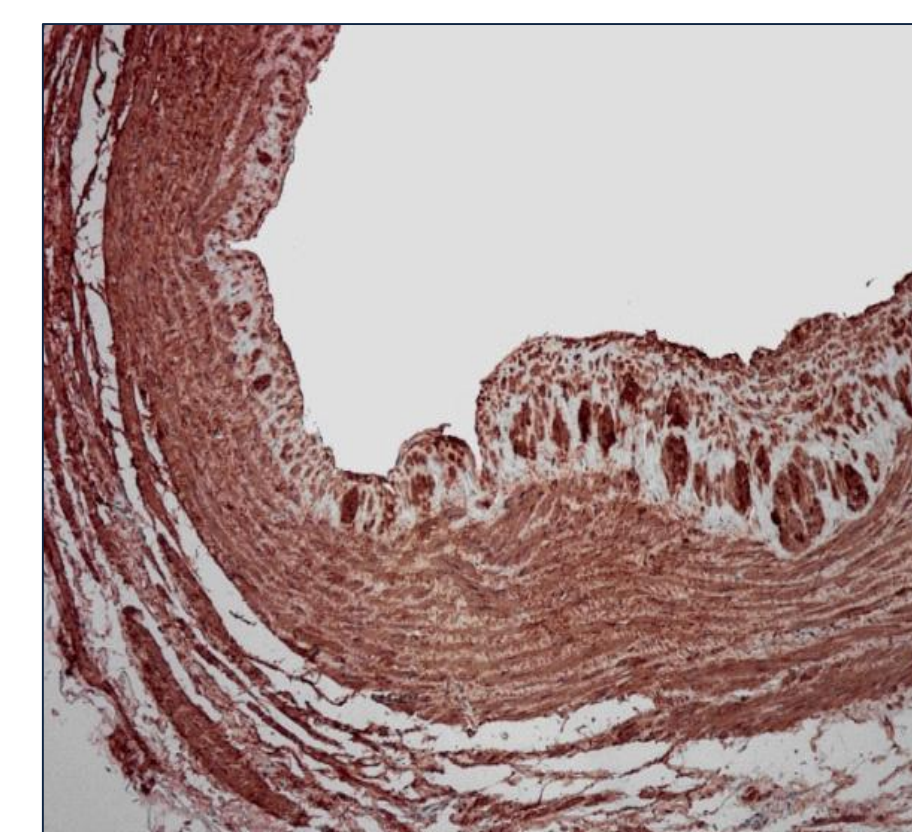
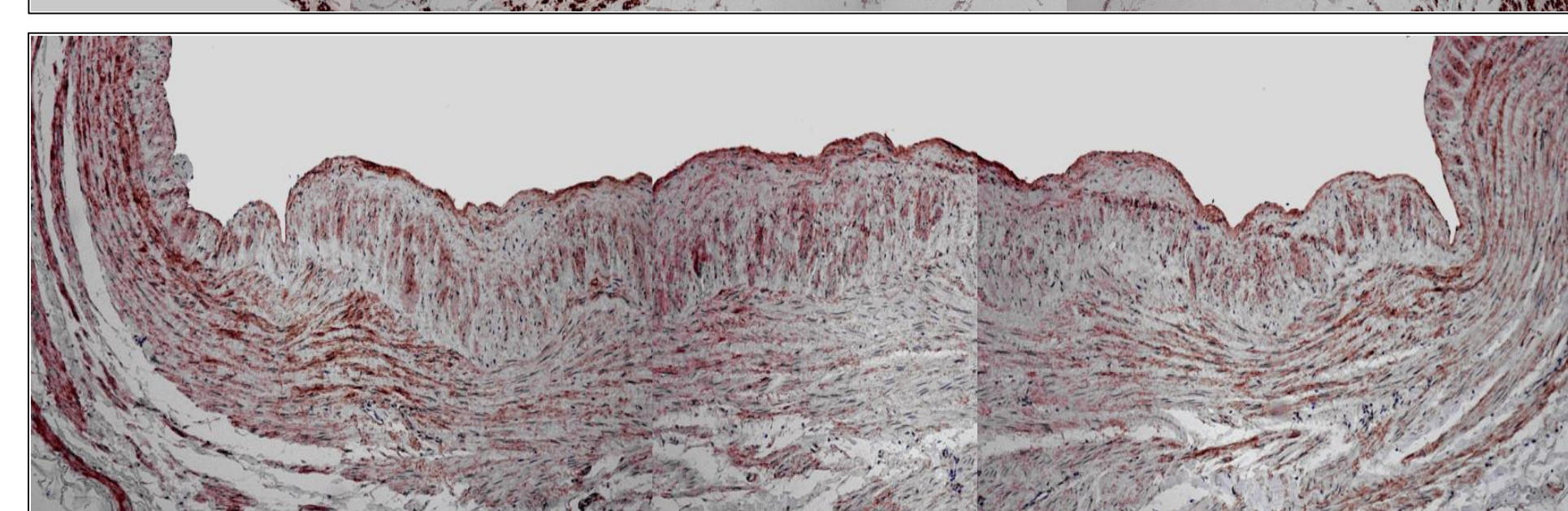
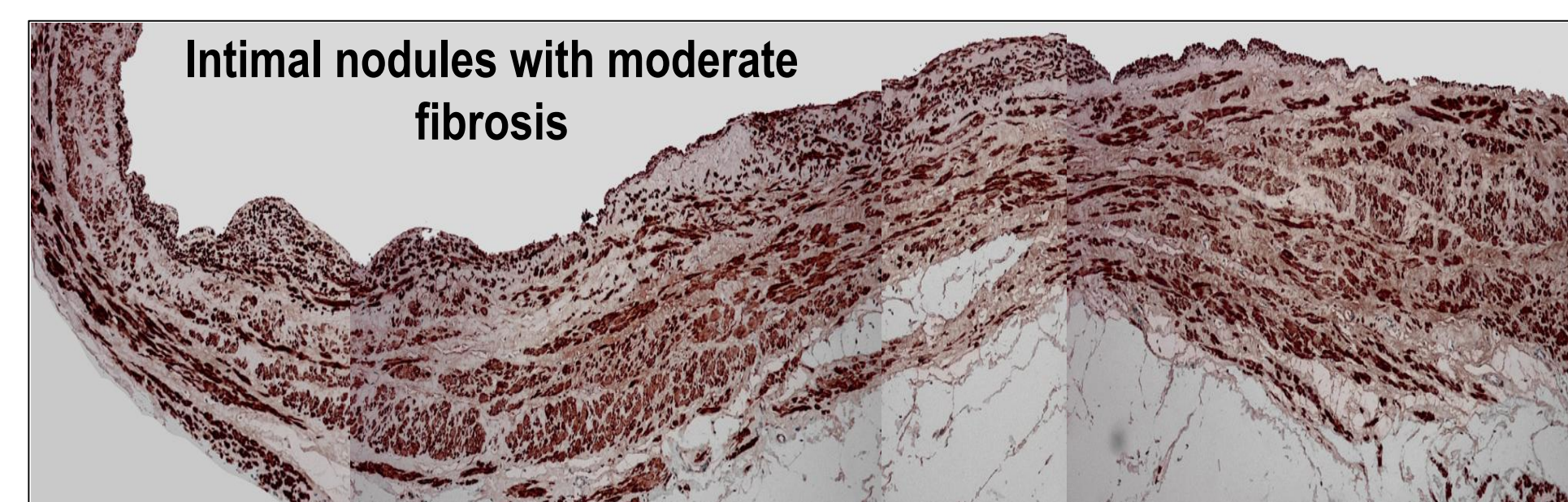
Confocal images showing the regular distribution of smooth muscle cells and small amount of collagen I in the media (left panels). Cell and elastin fibre distribution in the adventitia (right panels).

CONCLUSIONS

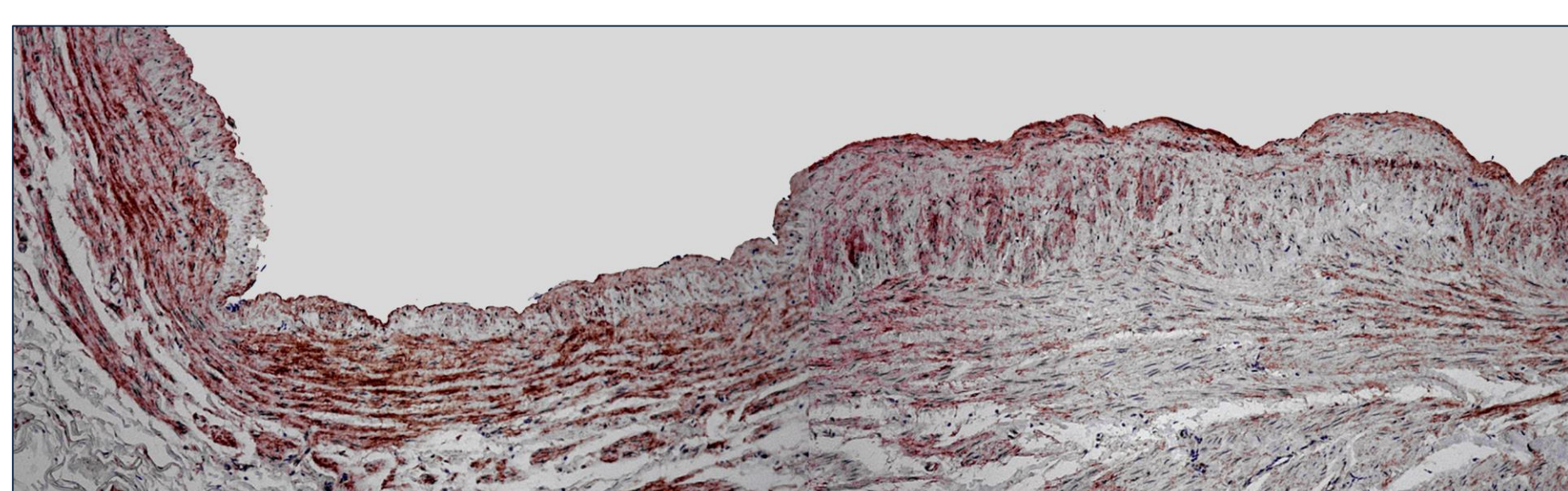
1. In the distal segment, the majority of venous wall is normal with minimal intimal conjunctive tissue in some regions.
2. In the proximal segment with normal macroscopic appearance there is moderate and severe lesions. Moderate lesions are characterized by intimal fibrosis with initial proliferation of smooth muscle cell and deposition of collagen IV, whereas medium layer shows some atrophy and presence of fibrous tissue. Severe lesion is characterized by proliferation of smooth muscle cells, deposition of collagen IV and increase of fibroblasts. Some regions show extensive loss of smooth muscle cells substituted by fibrous tissue. Adventitia do not present changes.

RESULTS

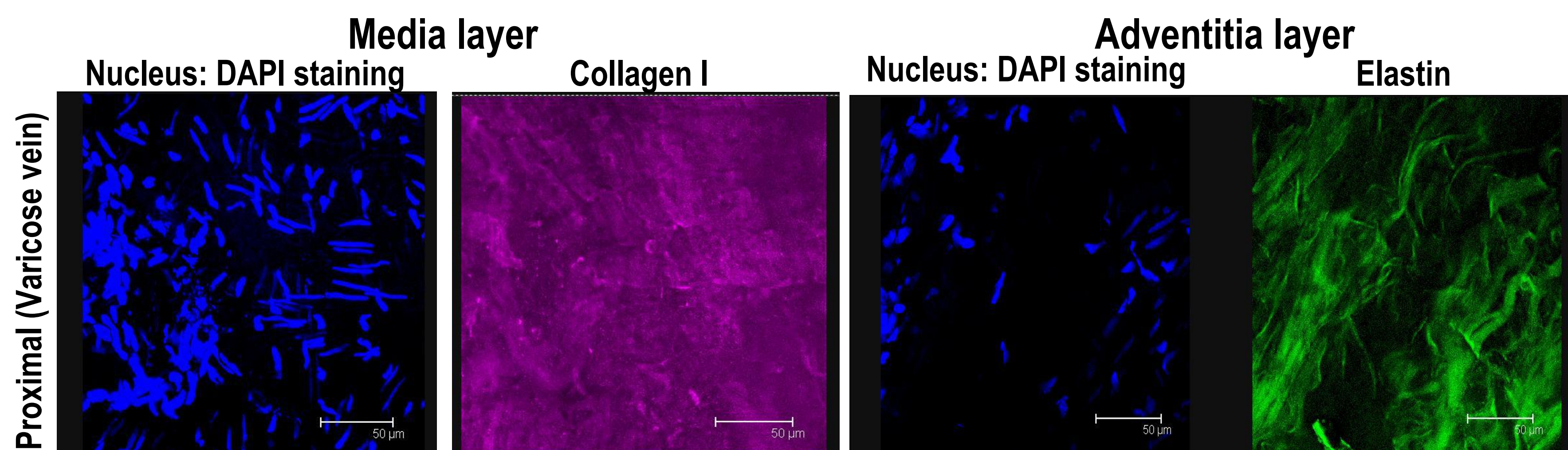
Segments with normal macroscopic appearance



Anti-smooth muscle α -actin and anti-collagen IV. Venous wall with thickness and moderate intimal fibrosis and transition of moderate to severe lesion with myointimal fibrosis. Circumferential deposition or nodules formation of collagen IV in the intimal surface.



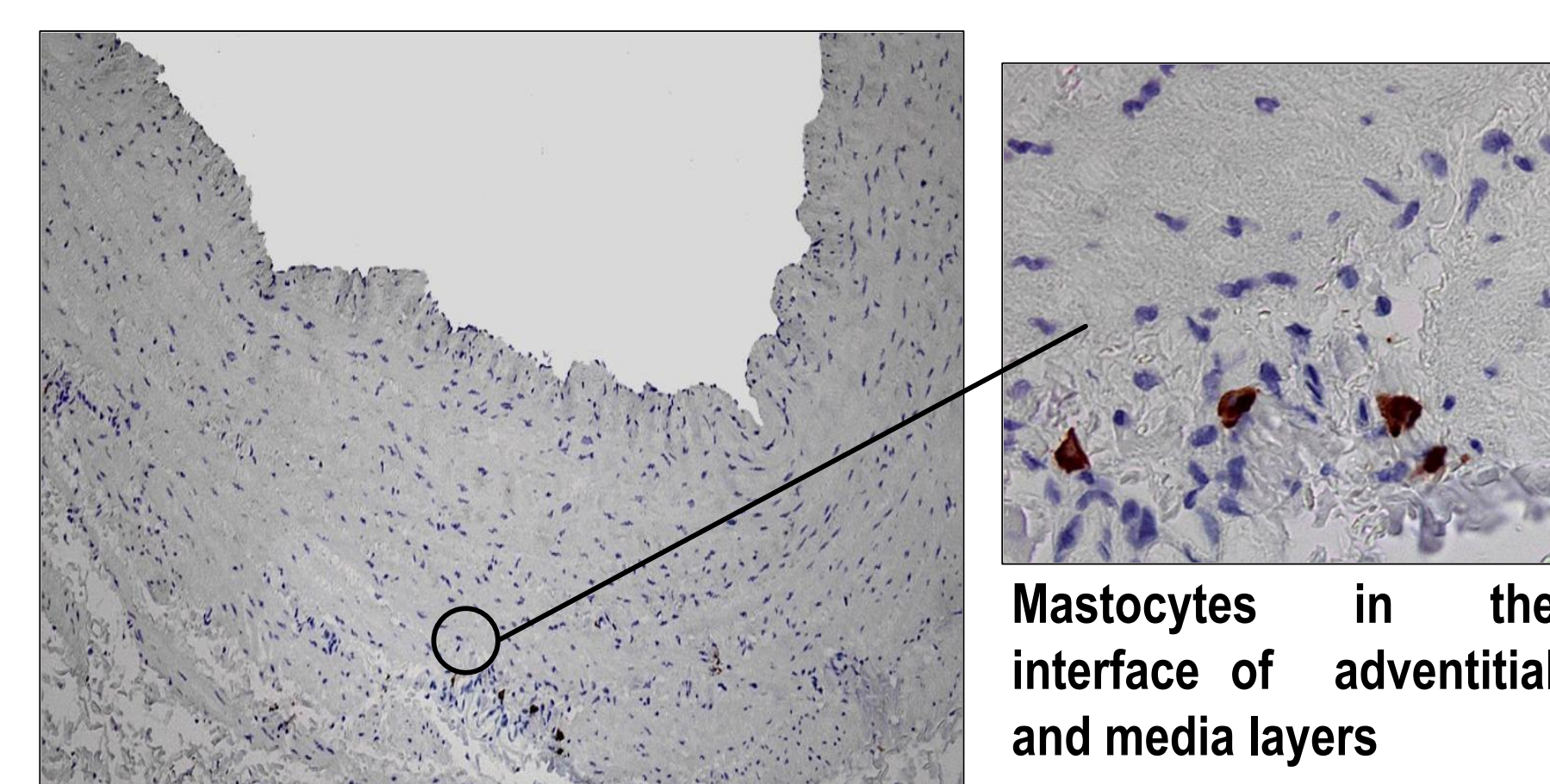
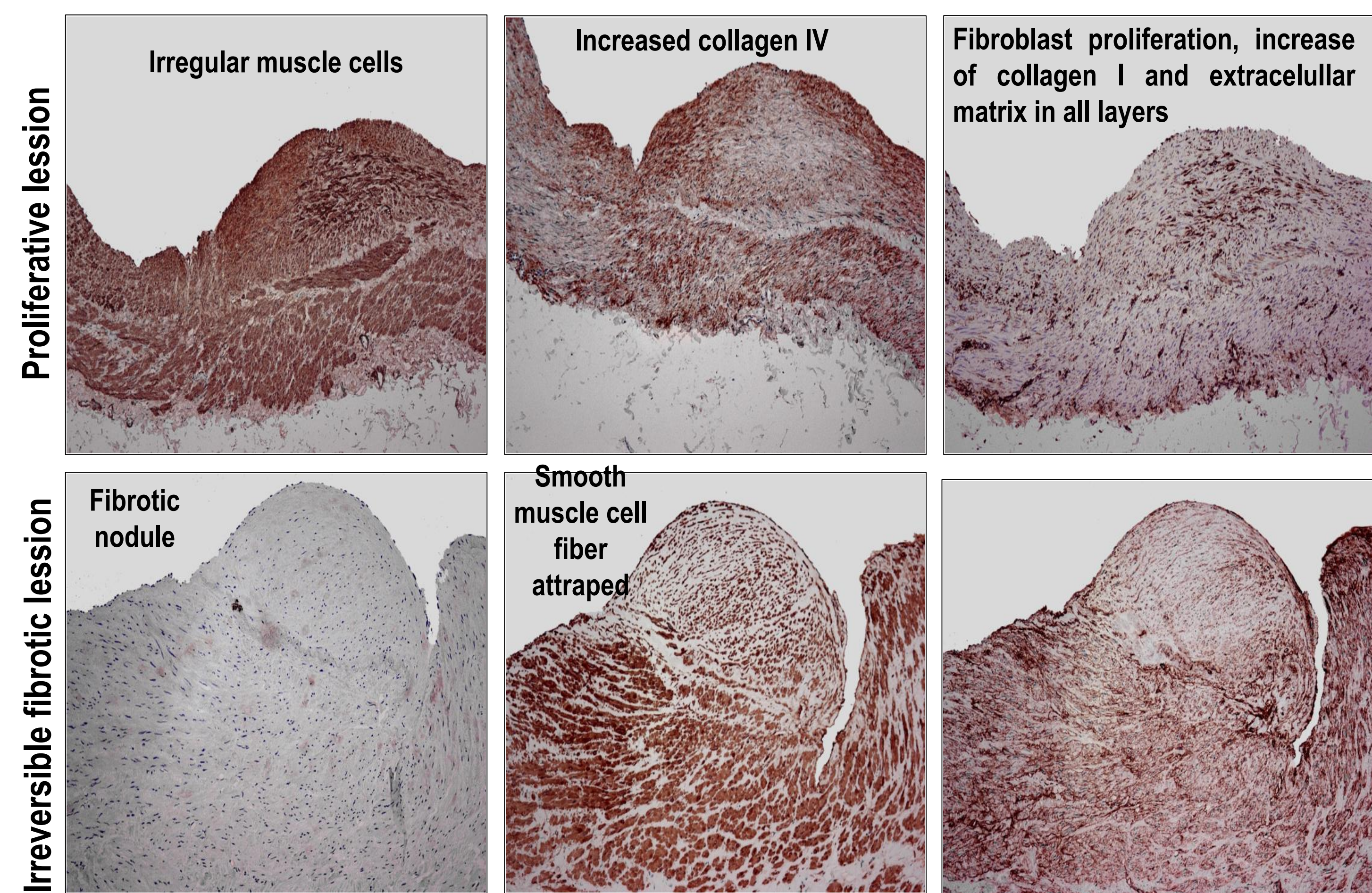
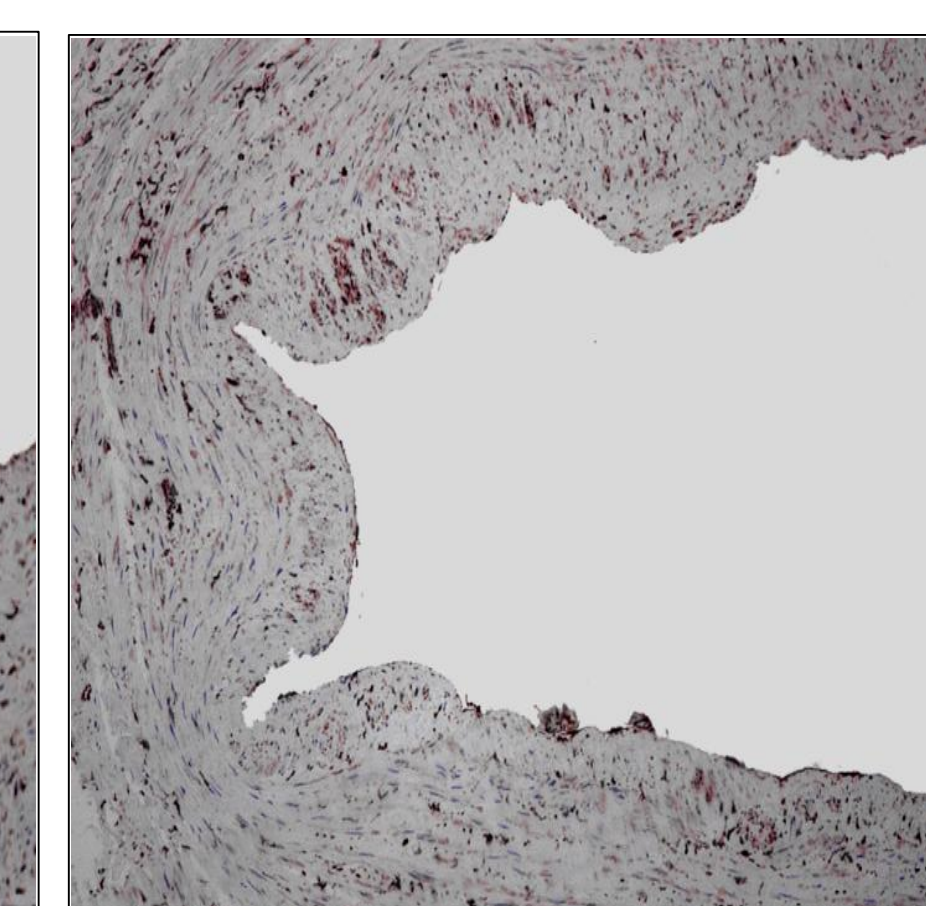
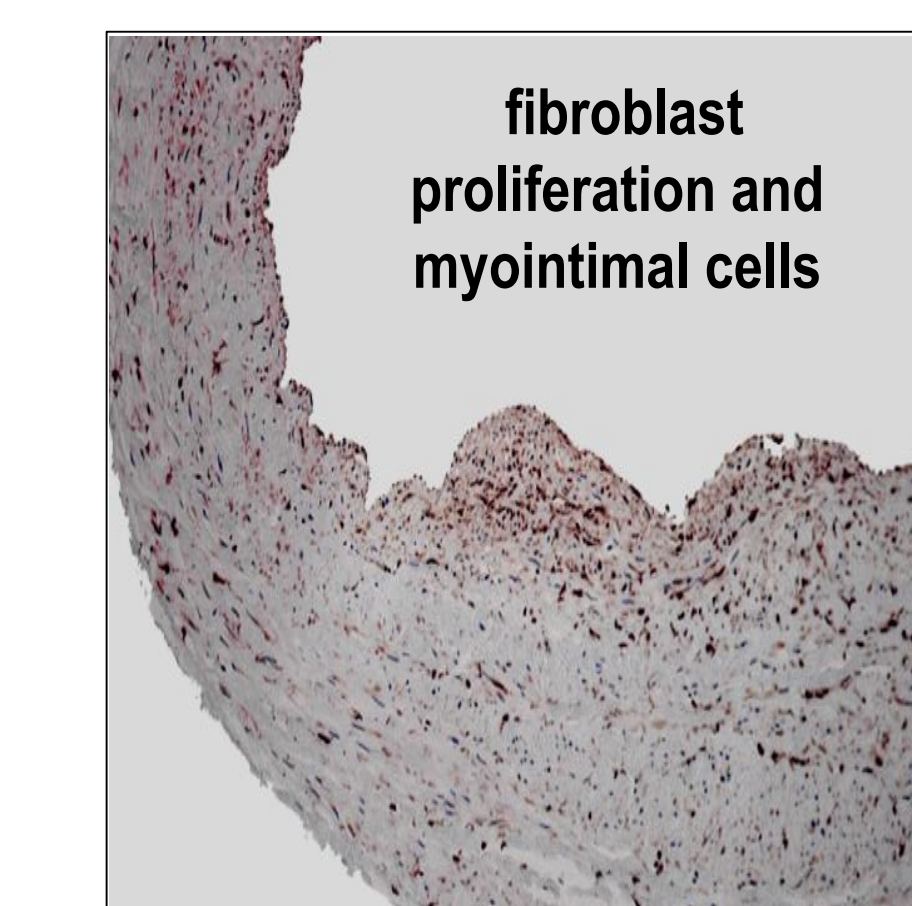
Anti-smooth muscle α -actin and anti-collagen IV. Extensive intimal fibrosis with decreased luminal venous wall. In the muscular layer, an extensive area of smooth muscle cell destruction and fibrosis is observed.



Confocal images showing the irregular distribution of smooth muscle cells and increased collagen I content in the media. In the adventitia cells and elastic fibres also are irregularly distributed.

Proximal Segment (varicose vein)

Intimal nodules



Fibrosis intimal moderated with muscular media layer normal

3. In the varicose wall wide regions of nodules of intimal fibrosclerosis are also observed. In these degenerative changes, mastocytes do not seem to play a role.

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