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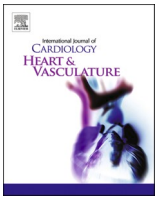

Atrial fibrillation in human patients is associated with increased collagen type V and TGFbeta1

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ABSTRACT

Background and aim: Atrial fibrosis is an important factor in initiating and maintaining atrial fibrillation (AF). Collagen V belongs to fibrillar collagens. There are, however no data on collagen V in AF. The aim of this work was to study the quantity of collagen V and its relationship with the number of fibroblasts and TGF- b 1 expression in patients in sinus rhythm (SR) and in patients with atrial fibrillation (AF).

Methods: We used quantitative immuhistochemistry to study collagen V in right and left atrial biopsies obtained from 35 patients in SR, 35 patients with paroxysmal AF (pAF) and 27 patients with chronic, long-standing persistent AF (cAF). In addition, we have quantified the number of vimentin-positive fibroblasts and expression levels of TGF-β1.

Results: Compared to patients in SR, collagen V was increased 1.8- and 3.1-fold in patients with pAF and cAF, respectively. In comparison with SR patients, the number of vimentin-positive cells increased significantly 1.46- and 1.8-fold in pAF and cAF patients, respectively.

Compared to SR patients, expression levels of TGF-ß1, expressed as fluorescence units per tissue area, was significantly increased by 77 % and 300 % in patients with pAF and cAF, respectively. Similar to intensity measurements, the number of TGFß1-positive cells per 1 mm^2 atrial tissue increased significantly from 35.5 ± 5.5 cells in SR patients to 61.9 ± 12.4 cells in pAF and 131.5 ± 23.5 cells in cAF. In both types of measurements, there was a statistically significant difference between pAF and cAF groups.

Conclusions: This is the first study to show that AF is associated with increased expression levels of collagen V and TGF-ß1indicating its role in the pathogenesis of atrial fibrosis. In addition, increases in collagen V correlate with increased number of fibroblasts and TGF-β1 and are more pronounced in cAF patients than those in pAF patients.

1. Introduction

Atrial fibrillation (AF) is the most frequent arrhythmia worldwide and is associated with considerable morbidity and mortality. The mechanisms of the initiation and maintenance of AF are certainly complex and are associated with electrical and structural remodelling (reviewed in [1–4]). Atrial structural remodelling is the key factor in AF-related mechanisms [1,2]. A hallmark of atrial structural remodelling is atrial fibrosis that is an important pathophysiological contributor to AF [5]. A recent consensus paper summarizes the available evidence and sheds more light on the term atrial fibrosis and “atrial cardiomyopathy” and its clinical implications [6].

The major heart collagens are collagen type I and type III that comprise almost 95 % of the [7]extracellular matrix [8,9]. Although these collagens are the most prevalent in cardiac tissue and their role in myocardial remodelling is increasingly studied, the role of the minor fibrillar collagens such as collagen type V is less studied.

Collagen V is a fibrillar collagen that is required for the normal

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Table 1
Baseline characteristics of patients with AF and patients in SR.

	SR	pAF	cAF	P-Value
Number	35	35	32	NS
Gender (male/female)	23/12	21/14	17/15	NS
BMI (kg/m^2)	28.4 ± 3.9	29.3 ± 3.4	29.1 ± 4.1	NS
Age (years)	68.8 ± 12.4	71.7 ± 9.3	71.2 ± 9.6	NS
NYHA class (I-IV)	2.1 ± 0.51	2.3 ± 0.47	2.3 ± 0.52	NS
Underlying cardiac disease (n/%)				
AVD	5 (14.3)	7 (20.0)	4 (12.5)	NS
CAD	9 (25.7)	8 (22.9)	7 (21.9)	NS
DCM	3 (8.6)	2 (5.7)	3 (9.4)	NS
MVD	1 (2.9)	2 (5.7)	2 (6.3)	NS
TVD	4 (11.4)	3 (8.6)	3 (9.4)	NS
CAD/AVD	8 (22.9)	8 (22.9)	6 (18.8)	NS
CAD/MVD	1 (2.9)	1 (2.9)	2 (6.3)	NS
AVD/MVD	1 (2.9)	1 (2.9)	2 (6.3)	NS
MVD/TVD	2 (5.7)	2 (5.7)	1 (3.1)	NS
CAD/MVD/TVD	1 (2.9)	1 (2.9)	2 (6.3)	NS
Type of heart surgery (n/%)				
AVR	5 (14.3)	7 (20.0)	4 (12.5)	NS
CABG	9 (25.7)	8 (22.9)	7 (21.9)	NS
CABG/AVR	8 (22.9)	8 (22.9)	6 (18.8)	NS
CABG/MVR	1 (2.9)	1 (2.9)	2 (6.3)	NS
CABG/MVR/TVR	1 (2.9)	1 (2.9)	2 (6.3)	NS
Heart transplantation	3 (8.6)	2 (5.7)	3 (9.4)	NS
MVR	1 (2.9)	2 (5.7)	2 (6.3)	NS
TVR	4 (11.4	3 (8.6)	3 (9.4)	NS
RA size (mm)	55.4 ± 8.4	56.7 ± 6.9	57.1 ± 8.7	NS
LA size	63.8 ± 9.3	64.7 ± 8.4	65.3 ± 7.9	NS
LF function				
Ejection fraction (%)	47.2 ± 7.9	45.7 ± 8.5	44.9 ± 9.8	NS
LVESD (mm)	36.3 ± 6.5	37.6 ± 8.2	35.8 ± 9.4	NS
LVEDD (mm)	55.6 ± 7.5	56.3 ± 7.9	56.4 ± 8.2	NS
Medication (n/%)				
Calcium antagonists	12 (34.3)	13 (37.1)	12 (37.5)	NS
Beta-blockers	13 (37.1)	12 (34.3)	15 (46.9)	NS
ACE inhibitors	15 (42.9)	17 (48.6)	17 (53.1)	NS
Digitalis	11 (31.4)	13 (37.1)	10 (31.3)	NS
Diuretics	7 (20.0)	11 (31.4)	12 (37.5)	NS

AVD, aortic valve disease; AVR, aortic valve replacement; CABG, coronary artery bypass graft; CAD, coronary artery disease; DCM, dilated cardiomyopathy; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; MVD, mitral valve disease; MVR, mitral valve repair/replacement; NS – not statistically significant, TVD, tricuspid valve disease; TVR, tricuspid valve repair/replacement.

Table 2
EHRAS histopathological classes of studied atrial biopsies.

	SR	pAF	cAF
Number	35	35	32
EHRAS class I (n/%)	3 (8.6)	4 (11.4)	3 (9.1)
EHRAS class II (n/%)	16 (45.7)	13 (37.1)	12 (37.5)
EHRAS class III (n/%)	13 (37.1)	15 (42.9)	14 (43.8)
EHRAS class IV (n/%)	3 (8.6)	3 (8.6)	3 (9.4)

formation of collagen I and III fibrils by initiating and determining their diameter and properties [10–15]. Recently it has been demonstrated that deletion of collagen V in mice has a protective effect after myocardial infarction by reducing fibrosis and pathological remodelling [16]. Our previous study has shown that expression levels of collagen V clearly distinguishes compensated from decompensated left ventricular hypertrophy in patients with aortic stenosis [7]. Therefore, in the present study we hypothesized that collagen V may play a role in the development of atrial fibrosis thereby contributing to the maintenance of AF in humans.

Increasing evidence indicates that collagen V is produced by fibroblasts and is regulated by transforming growth factor (TGF-β1) [11,14,15,17,18]. TGF-β1 is a known profibrotic cytokine that has been demonstrated to induce selective atrial fibrosis in a transgenic mouse model indicating the critical role of TGF-β1 in the pathogenesis of AF and provide a direct link between atrial fibrosis and AF [19–22]. Therefore, we have further hypothesized that TGF- β1 may play a role in the development of atrial fibrosis in human patients with AF.

From this background and given that fibrosis, collagen V expression and TGF-β1 are tightly interrelated [11,23], the aim of the present study was to comprehensively analyze the distribution pattern and tissue levels of collagen V and its relation with the number of fibroblasts and TGF- β1 expression in atrial tissue biopsies obtained from patients in SR and in patients with AF.

2. Material and Methods

2.1. Patients

One hundred and two patients undergoing open heart surgery were subdivided into three groups: 35 patients in sinus rhythm (SR), 35 patients with paroxysmal AF (pAF) and 32 patients with chronic, long-standing persistent AF (cAF) with a duration of AF more than 12 months. Clinical data are presented in Table 1. The study protocol was approved by the Ethics Committees of the Landesärztekammer Hessen, Frankfurt am Main (Number: FF12/2011). All patients gave written informed consent and the study followed the principles of the Declaration of Helsinki.

2.2. Tissue sampling

Tissue samples were taken intraoperatively from the right and left atrial appendages and were immediately frozen in liquid nitrogen and stored at −80 °C, or immersed in 3 % glutaraldehyde buffered with 0.1 mol/L Na cacodylate for electron microscopy. The tissue samples were mounted in Tissue-TeK® O.C.T.TM (Sakura) and 5 μm thick cryosections were prepared using a Leica CM3050S cryotome.

2.3. Immunofluorescent labelling

Prior to immunolabeling, tissue preservation, characterization and orientation were recorded by hematoxylin-eosin and Van Gieson stainings followed by histopathological analysis according to the EHRA classification [6]. Frozen sections were fixed for 10 min with 4 % paraformaldehyde. After washing in phosphate buffered saline (PBS) sections were incubated with 1 % bovine serum albumin for 30 min to block non-specific binding sites and then incubated with the primary antibodies. A monoclonal antibody against vimentin (clone V-9, Sigma) was directly conjugated with Cy3 (clone V9, Sigma) and a monoclonal antibody against TGF-β1 (MAB240, R&D) was used in dilutions as previously described [24]. Polyclonal primary antibodies against collagen V were purchased from Rockland. Anti-mouse or anti-rabbit IgG-conjugated with Cy3 or Cy2 (Biotrend) served as detection systems in single or double immunolabelings. The nuclei were stained with 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI, Molecular Probes). F-actin was fluorescently stained using TRITC-conjugated (Sigma) or Alexa633-conjugated phalloidin (Molecular Probes). Negative controls were obtained by omitting the primary antibody, in an otherwise similar protocol. Sections were embedded in Mowiol and coverslipped.

2.4. Confocal microscopy

Tissue sections were examined by laser scanning confocal microscopy (Leica TCS SP2 and Leica SP5). Series of confocal optical sections were taken using a Leica Planapo ×40/1.00 or ×63/1.32 objective lens. Each recorded image was taken using four channel scanning and consisted of 1024 × 1024 pixels. To improve image quality and to obtain a high signal to noise ratio, each image from the series was signal-averaged. After data acquisition, the images were further processed for restoration, quantification and three-dimensional reconstruction using an Imaris multichannel image processing software (Bitplane,

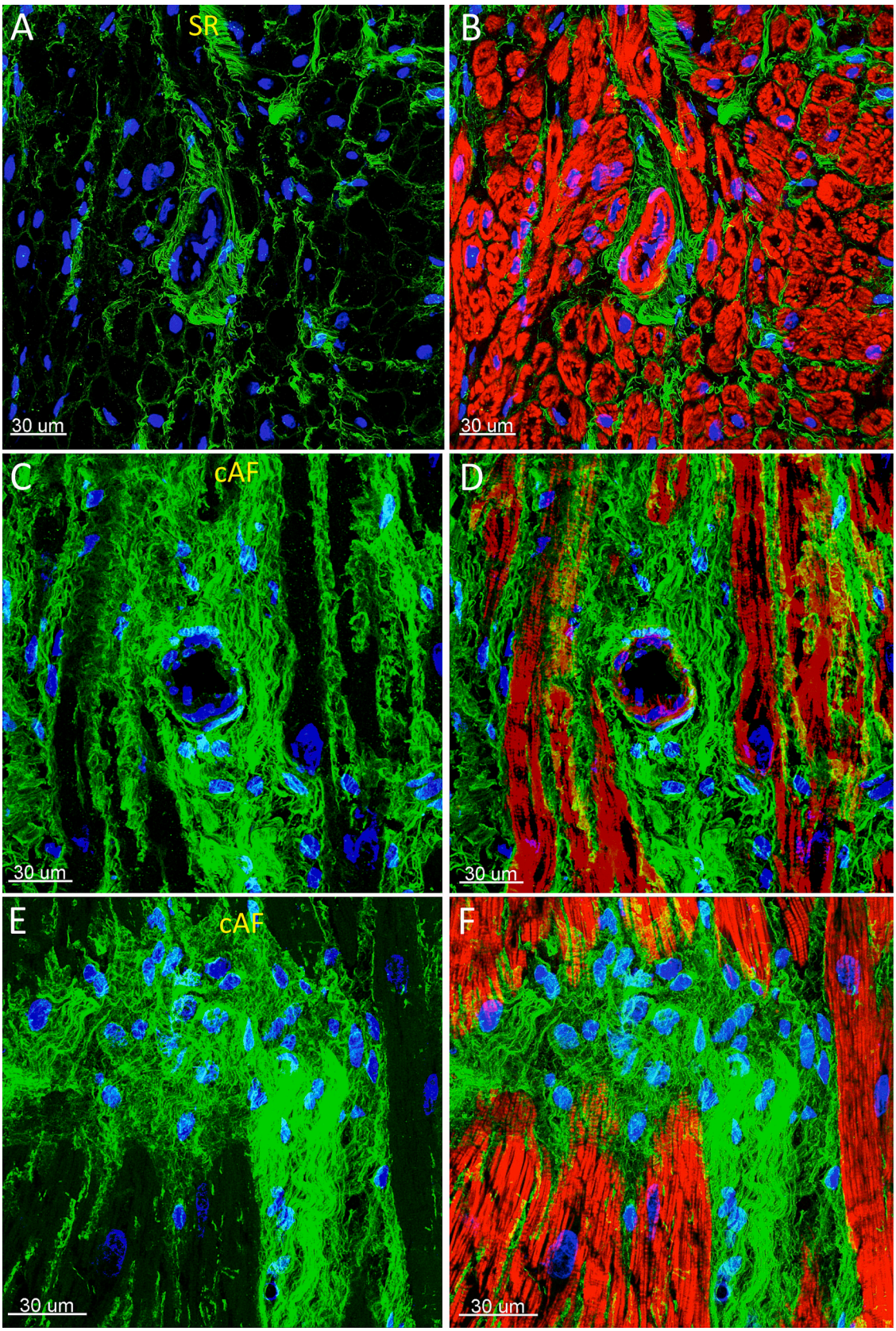

Fig. 1. Representative confocal micrographs of the perivascular and replacement fibrosis. Panels **A** and **B** are merged images showing the amount of collagen V in the perivascular area in a patient in SR. Panels **C** and **D** shows the amount of collagen V in the perivascular area in a patient in pAF. Notice a significant increase in perivascular fibrosis in pAF than in SR. A typical example of replacement fibrosis in a patient with cAF is shown in merged images (panels **E** and **F**). Collagen V is shown in green, nuclei are shown in blue after staining with DAPI and F-actin is shown in red after labelling with Alexa633-phalloidin.

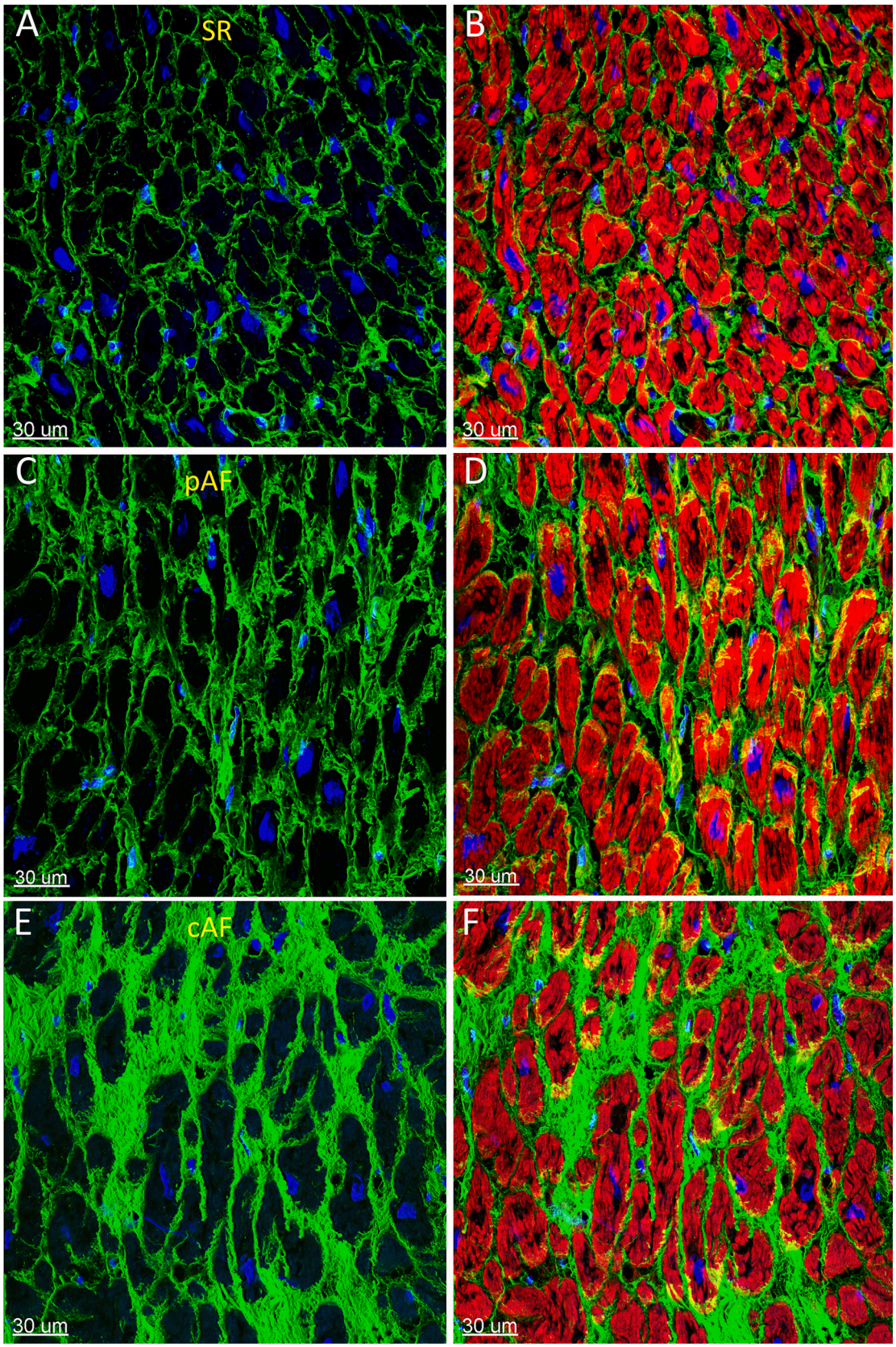

Fig. 2. Representative confocal images of the interstitial atrial fibrosis. In comparison with patients in SR (panels **A** and **B**), the interstitial collagen V is already increased in patients with pAF (panels **C** and **D**) and is drastically augmented in patients with cAF (panels **E** and **F**). Collagen V is shown in green, nuclei are shown in blue after staining with DAPI and F-actin is shown in red after labelling with Alexa633-phalloidin.

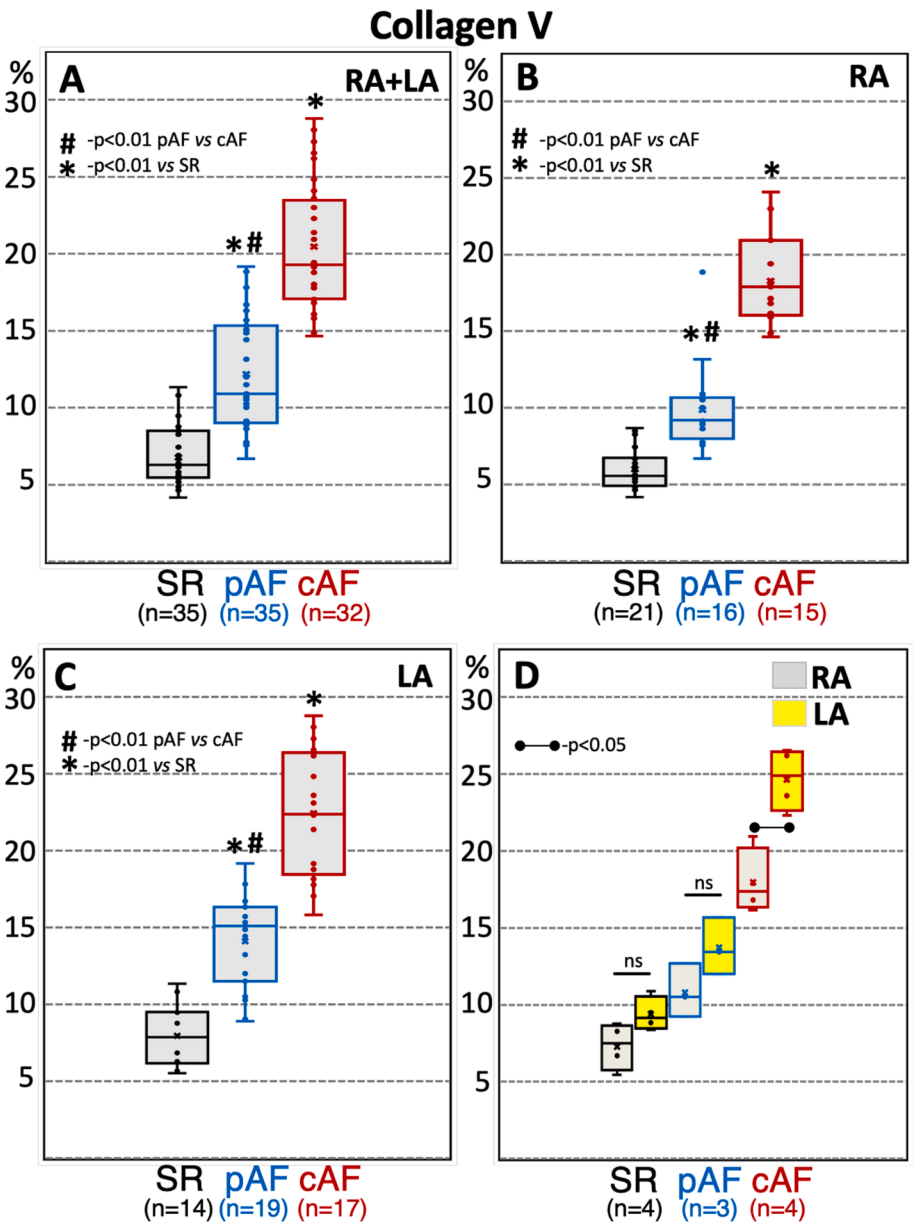

Fig. 3. Quantitative analysis of collagen V in patients with pAF (n = 35), cAF (n = 32) and patients in SR (n = 35). Collagen V was expressed as percent per tissue area in both RA and LA from different patients (panel **A**), or in RA (panel **B**), or in LA (panel **C**) or in RA and LA obtained from the same patient (panel **D**). Note that when comparing collagen V in RA and LA obtained from the same patient, only LA from cAF patients differed significantly from RA in terms of collagen V. ns – not statistically significant.

Zürich, Switzerland).

2.5. Quantitative immunofluorescent microscopy

Measurements of immunofluorescence were done using using a ×40 Planapo objective (Leica) and a Leica (Leitz DMRB) fluorescent microscope equipped with a Leica DC380 digital camera. Cryosections from at least two different tissue blocks in each case were used. For quantitative analysis all sections were immunolabeled simultaneously using identical dilutions of primary and secondary antibodies and other reagents. Immunofluorescent images were obtained under identical parameters of imaging, zoom, pinholes, objectives, and fluorescence power. Sections exposed to PBS instead of primary antibodies served as negative controls. The image acquisition settings were standardized for all groups to ensure that the image collected demonstrated a full range of fluorescence intensity from 0 to 255 pixel intensity level and were kept constant during all measurements. Quantification of each protein was performed blindly, having on the screen only one channel showing F-actin labelling. For each patient at least 10 random fields of vision were analyzed using image analysis software (Leica) and Image J program as described [25]. The area occupied by collagen V was calculated as percentage of collagen V per tissue area.

Quantification of TGF-β1 was performed by measurements of fluorescence intensity by using a range of 0 to 255 values. Arbitrary units were calculated per unit surface area (AU/mm^2) as previously described [24]. Another quantification of TGF-β1 was performed by counting the number TGF-β1-positive cells per 1 mm^2 tissue area as previously described [26].

2.6. Quantitative real time PCR

RNA was isolated from 30 mg of atrial tissue with a RNeasy mini

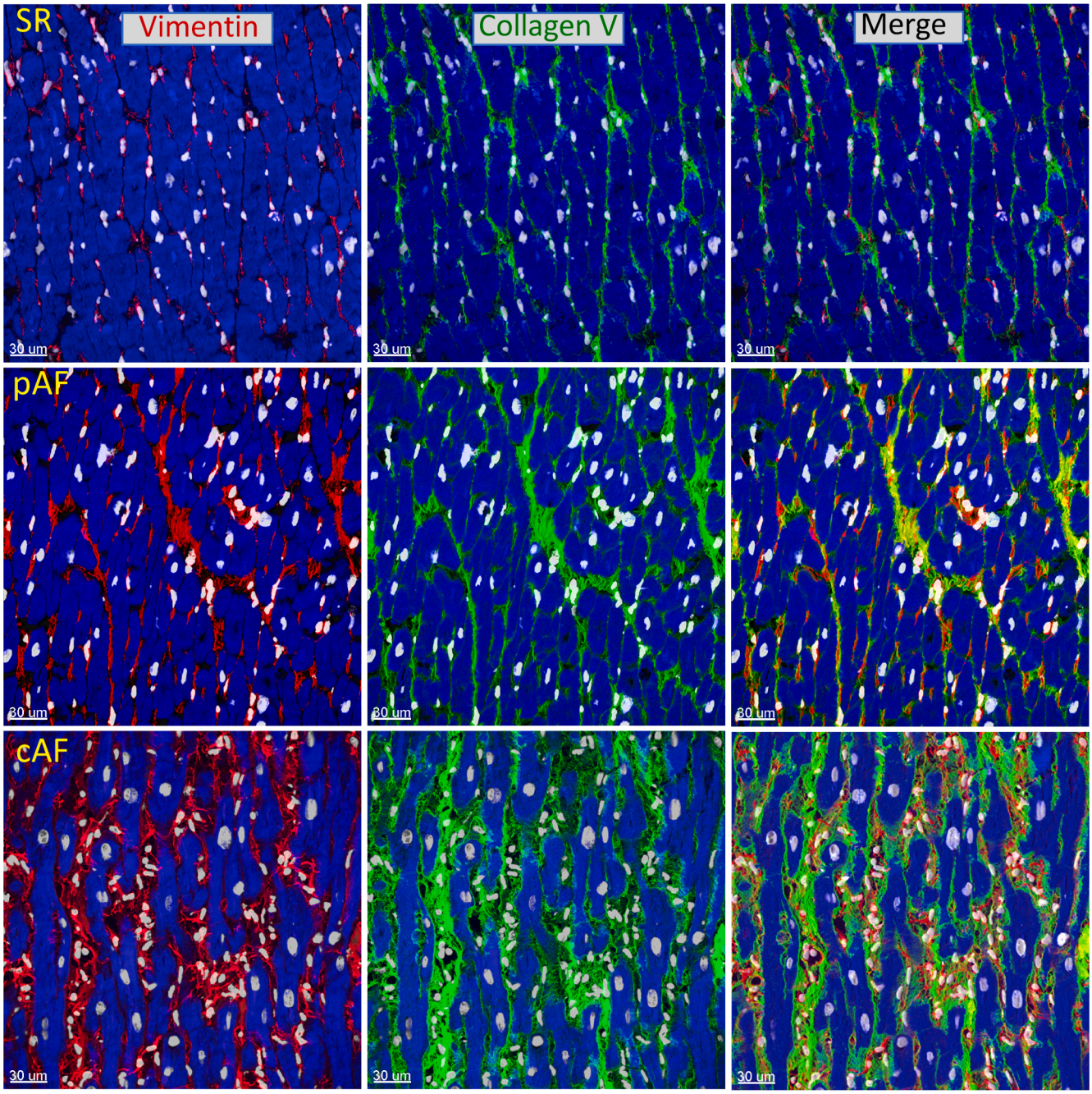

Fig. 4. Confocal images of vimentin-positive cells (red) and collagen V (green) in SR, pAF and cAF groups. Nuclei are shown in white after after staining with DAPI and F-actin is shown in blue after labeling with Alexa633-phalloidin. Note that in comparison with patients in SR (upper panels), the number of vimentin-positive cells and the amount of collagen V is steadily increased from patients with pAF (middle panels) to patients with cAF (lower panels).

tissue kit (Qiagen). cDNA libraries were synthetized on the total RNA using High Capacity cDNA reverse transcriptase kit (Applied Biosystems). TGFbeta1 primers (catalog number HP200609, OriGene), forward sequence: TACCTGAACCCGTGTTGCTCTC, reverse sequence: GTTGCTGAGGTATCGCCAGGAA were used. For quantitative PCRs, the cDNA pool was diluted 1 in 10 with miliQ water and used as a template. To assess expression level of TGFbeta1 gene, the quantitative PCR was performed with 25 cycles using a standard PCR thermocycler (BioRad laboratories). GAPDH was served as a reference gene. Both transcripts were detected using on the 1 % agarose gel developed at 5 V/cm^2.

2.7. Transmission electron microscopy

After overnight fixation in 3 % glutaraldehyde, atriall tissue samples were embedded in Epon following routine procedures. Ultrathin sections were stained with uranyl acetate and lead citrate, and viewed and photographed in a Philips CM 10 electron microscope. For quantitative ultrastructural analysis of fibroblasts, at least 10 fields (200 × 200 μm) per each patient were analyzed.

2.8. Statistical analysis

All data are presented as means ± SD. For multiple comparisons we used ANOVA, followed by analysis with the Bonferroni *t*-test.

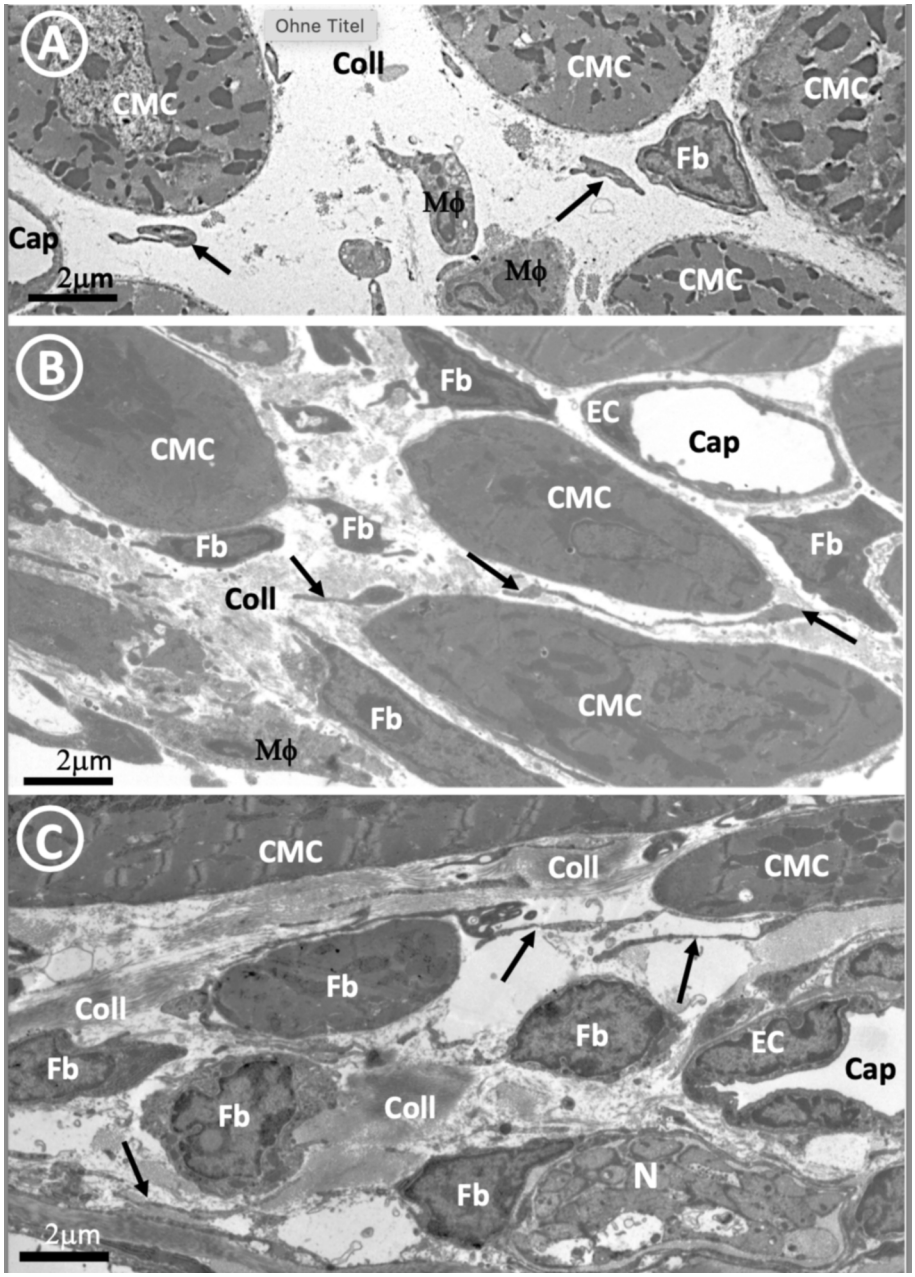

Fig. 5. Representative electron microscopic images of atrial tissue in SR (panel **A**), pAF (panel **B**) and cAF patients (panel **C**). Note that in comparison with patients in SR, the number of fibroblasts and collagen fibrils are densely compacted and obviously increased in patients with pAF and in patients with cAF. Arrows indicates filopodia of the fibroblasts. CMC – cardiomyocytes, Coll – collagen fibrils, EC – endothelial cells, Mf - macrophage, N – nonmyelinated nerve.

Differences between two groups were analyzed using the two-way unpaired *t*-test or the Mann-Whitney *U* test for two comparisons. Associations between variables were assessed using Spearman's rank correlation. Differences between groups were considered significant at $p < 0.05$.

3. Results

3.1. Demographic characteristics of study participants

The clinical data of the patients are presented in Table 1. The clinical characteristics did not differ among the groups ($p > 0.05$). It is noteworthy that only very few patients (1–2 pro group) were admitted for a repair or replacement of the mitral valve, which is often associated with the occurrence of AF and atrial fibrosis.

3.2. Histopathology of atrial tissue biopsies

Histopathologic characteristics of the atrial tissue were classified according to the EHRAS scheme [6] and the results are summarized in Table 2. Histologically, fibrosis alone (class II) or in combination with myocyte changes (class III) was observed in more than 80 % of patients, irrespective whether AF was present or not. Similarly, accumulations of interstitial mononuclear cells (class IV) were observed in about 5.5 % of patients and there was no statistical difference between the groups.

3.3. Collagen V and atrial fibrosis

Representative confocal images of collagen V localization and distribution are shown in Figs. 1 and 2 and depict that fibers formed by collagen V surrounds individual cardiomyocytes and vessels. Using

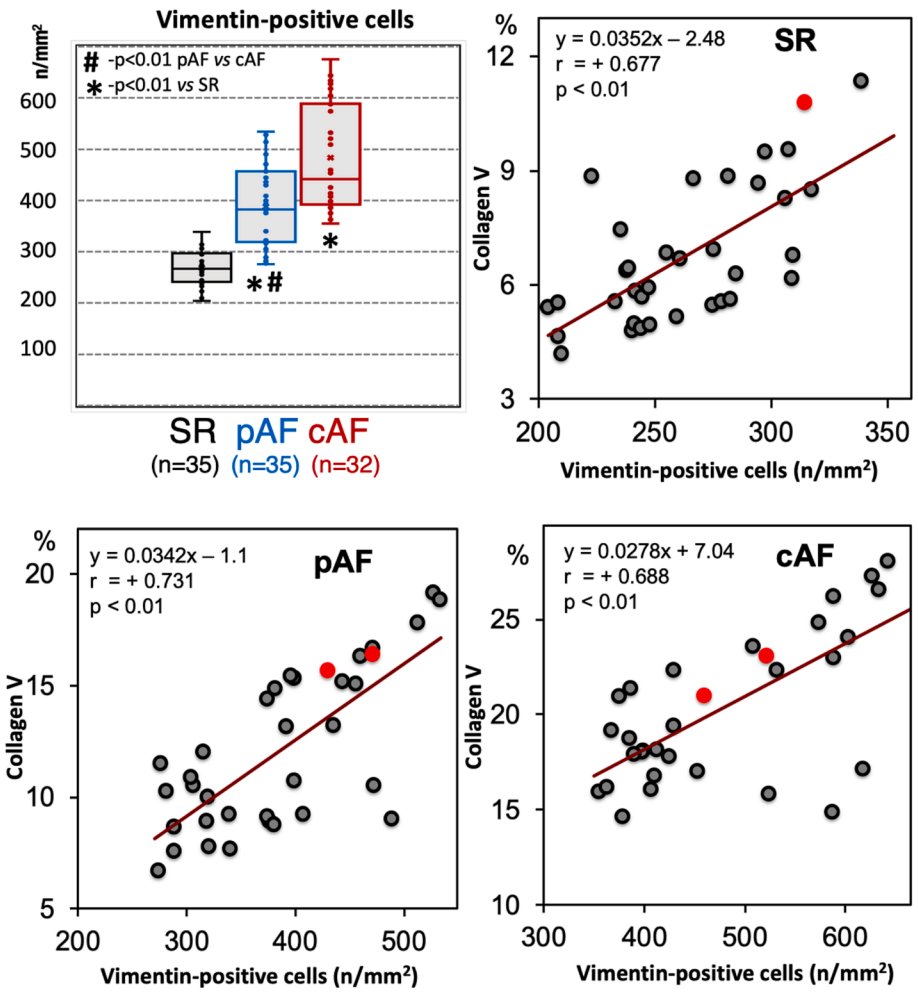

Fig. 6. Quantification of vimentin-positive cells per 1 mm^2 tissue area in SR, pAF and cAF groups. Note that in comparison with patients in SR, the number of vimentin cells is 1.38 and 1.81-fold higher respectively in patients with pAF and in patients with cAF. Shown in diagrams is a statistically significant correlation between collagen V and vimentin-positive cells in all groups. Red dots are values obtained from MVD patients.

immunolabeling for collagen V, we found in our patients 3 forms of atrial fibrosis, namely: perivascular, focal replacement (reparative) and diffuse interstitial (endomysial). Fig. 1A though 1D shows typical examples of arteries with and without perivascular fibrosis. Perivascular fibrosis was found in 9 (25.7 %) patients in SR, in 20 (57.1 %) patients with pAF and in 23 (85.2 %) with cAF.

Fig. 1E and Fig. 1D are representative images of replacement fibrosis. This type of fibrosis was observed in 4 (11.4 %) patients in SR, in 13 (37.1 %) patients with pAF and in 22 (81.5 %) with cAF.

Fig. 2 show examples of collagen V deposition in form of diffuse interstitial fibrosis. Interstitial collagen V was determined quantitatively and the results are shown in Fig. 3. Collagen V occupied 6.8 % of the atrial tissue in patients in SR and it was 1.8-fold augmented in patients with pAF and 3.1 times elevated in cAF patients. Similar changes in collagen V were observed in both, RA and LA tissues obtained from the different patients (Fig. 3B and 3C). However, comparing the amount of collagen V in RA and LA tissues obtained from the same patient, only patients with cAF showed a statistically significant difference between RA and LA (Fig. 3D).

3.4. Cellular and molecular regulators of collagen V

Several studies have shown that the fibroblasts are the major cell type involved in production of different types of collagen, including V production [14,15,17,18,27]. Therefore, in the present study, vimentin was used as a fibroblast marker and used in double immunolabeling procedures with collagen V. As shown in Fig. 4, compared with patients in SR, the number of vimentin-positive cells were obviously increased and were associated with augmented deposition of collagen V in both, pAF and cAF groups.

Our observations of vimentin-positive number of fibroblasts were confirmed by electron microscopy. The results are shown in Fig. 5. It was found that 1 mm^2 of the atrial tissue in SR patients ($n = 12$) comprises an average of 32.4 ± 6.1 fibroblasts. The same tissue surface comprises 76.6 ± 7.4 fibroblasts in pAF ($n = 15$) and 98.8 ± 10.2 in cAF ($n = 21$). It should be emphasized that the values of the latter group differ significantly ($p < 0.001$) from those found in SR and pAF groups. These findings were further confirmed by the quantitative determination of the number of vimentin-positive cells. Compared to SR patients (263.5 ± 35.4 cells per 1 mm^2 atrial tissue), the number of vimentin-positive cells increased significantly 1.46- and 1.8-fold in patients with pAF and cAF, respectively. Moreover, the number of vimentin-positive cells significantly correlated with collagen V in all groups of patients (Fig. 6B through 6D).

Given the evidence that fibrillar collagens, including collagen V are tightly regulated by TGF-ß1 [11,28], we have performed double labeling for TGF-ß1 with collagen V. The results are shown in Fig. 7 and illustrate that TGFß1 was mainly confined to interstitial cells. TGFß1 was obviously increased in both pAF and cAF groups and paralleled the increased deposition of collagen V. Compared to SR patients, expression levels of TGF-ß1, expressed as fluorescence units per tissue area, was significantly increased by 77 % and 300 % in patients with pAF and cAF, respectively.

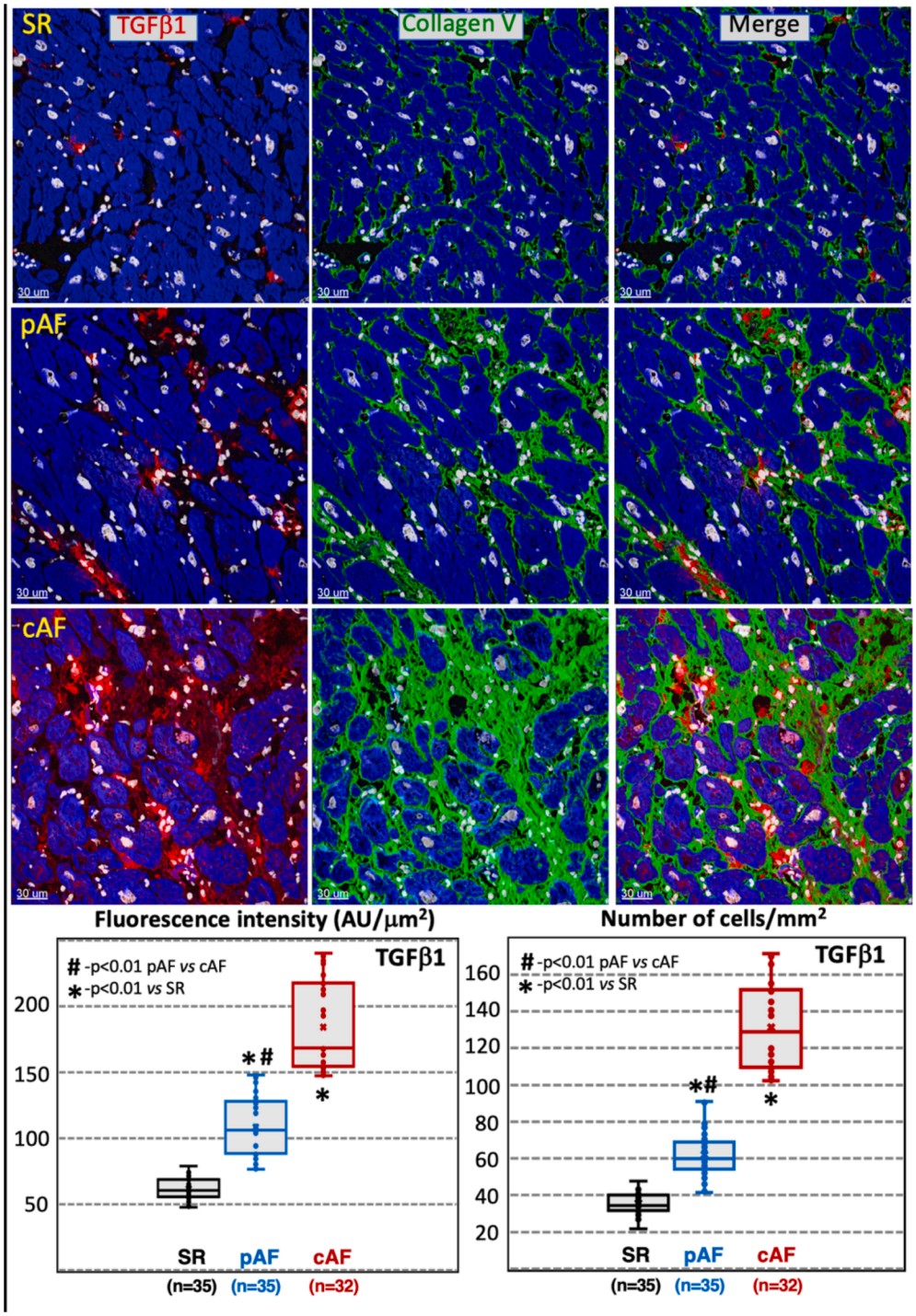

Fig. 7. Confocal images of TGF-ß1 (red) and collagen V (green) in SR, pAF and cAF groups. Cardiomyocytes are stained blue with Alexa633-phalloidin and nuclei are stained white with DAPI. Note that TGFß1 is mainly confined to interstitial cells and, compared with SR patients, is obviously increased in the pAF and cAF groups. Note also that increases in TGF-ß1 are associated with increased abundance of collagen V. The lower left and right graphs are quantifications of TGFß1 expressed as arbitrary units of fluorescence per 1 mm^2 surface area or the number of TGF-β1-positive cells per 1 mm^2 of atrial tissue area.

Similar to intensity measurements, the number of TGFß1-positive cells per 1 mm^2 atrial tissue increased significantly from 35.5 ± 5.5 cells in SR patients to 61.9 ± 12.4 cells in pAF and 131.5 ± 23.5 cells in cAF. In both types of measurements, there was a statistically significant difference between pAF and cAF groups.

The quantitative data obtained by immunohistochemistry were confirmed by quantitative PCR analysis. It is evident that compared to SR, TGFβ1 in pAF and cAF is significantly 3-fod elevated at the transcript level (Fig. 8). There was not statistically significant difference between pAF and cAF groups ($p = 0.11$).

4. Discussion

In our previous studies we have established that upregulation of collagen V and TGF-β1 clearly distinguishes compensated from decompensated left ventricular hypertrophy due to aortic stenosis [24,29]. In the present study we further analyzed the expression levels of collagen V and TGF-β1 in patients with AF. Here we demonstrate that collagen V

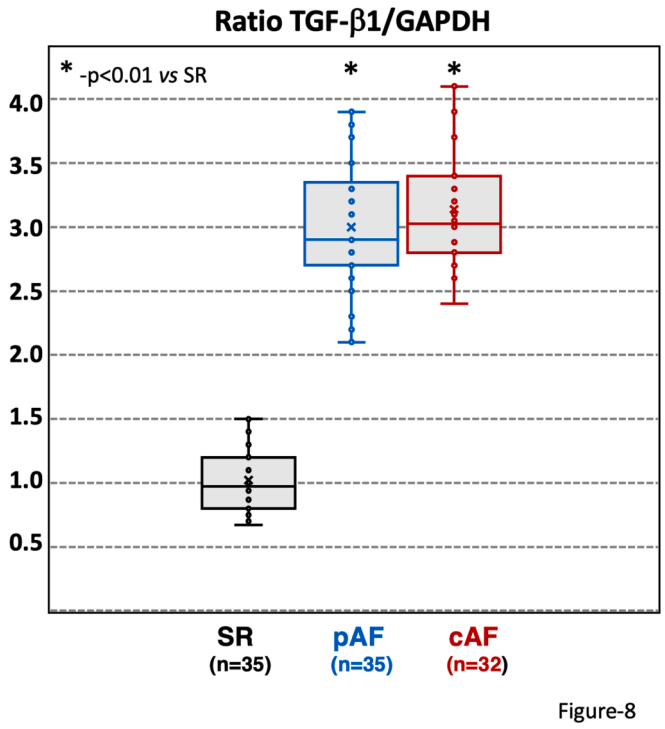

Fig. 8. Quantification of TGF-ß1 and its ratio to GAPDH in SR, pAF and cAF groups. Note that TGFß1 is drastically increased in pAF and cAF, while there is no difference between pAF and cAF in the TGF-ß1/GAPDH ratio.

and TGF-β1 expression levels in cAF patients are significantly higher than those in SR patients and, importantly, than those found in pAF patients. These data suggest that collagen V and TGF-β1 are important pathophysiological contributor to AF progression from its pAF to cAF forms. Moreover, our data showing a gradual increase of collagen V, fibroblasts and TGF-β1 from SR toward cAF corroborate the view that AF is a "clinicopathologic continuum from paroxysmal to permanent AF" [30] and that "atrial fibrillation begets atrial fibrillation" [31]. Moreover, our data indicate that collagen V and TGF-β1 are useful tissue markers of different stages of AF. In this context, early detection of atrial fibrosis is crucial in initiating an early rhythm-control therapy of AF [32].

Several experimental models demonstrated that atrial fibrosis is a substrate that promotes and maintains AF [20,21,31,33–35]. Increased amount of fibrotic tissue consisting of fibrillar collagen types I and III has been previously shown in the atria of patients with AF [36,37]. The present study has documented also an abundant collagen V deposition in fibrotic areas in patients with AF. The increase of fibrosis may cause an abnormal conduction through the atria by disturbing anisotropic conduction and generating re-entry circuits [34,38]. Importantly, atrial tissue fibrosis detected by delayed enhancement magnetic resonance imaging technique was independently associated with likelihood of recurrent arrhythmia [32].

Type I collagen represents 50–80 % of total cardiac collagen followed by collagen III (10–45 %) [8]. Collagen type V is a fibrillar collagen that is required for the normal formation of collagen types I and III fibrils [12,13]. In line with such a function of collagen V, the upregulation of collagen V in our AF patients, especially those with cAF, would predict its role in the abundant deposition of other fibrillar collagens that has been consistently found in patients with AF [29,36].

Numerous and convincing studies have demonstrated that TGF-β1 and its signalling pathways are important regulators of collagen-producing fibroblasts in the heart ventricles and atria [1,28,29,39,40]. However, Nakajima et al. found that mice with constitutive expression of TGF-β1 developed selective fibrosis in the atria and rather than the ventricles, suggesting that atrial fibroblasts may be especially sensitive to the actions of TGF-β1 [19]. Another study has demonstrated that goats with transgenic overexpression of TGF-β1 are prone to atrial fibrillation development as a result of raised levels of atrial fibrosis [41]. Taken together, studies in transgenic TGF-β1-overexpressing mice and goats have clearly demonstrated that atria are more sensitive to TGF-β1-induced fibrosis than ventricles [19,22,41]. Our data showing increased levels of TGF-β1 in pAF and cAF patients concur with the hypothesis that TGF-β1 is an important contributor of AF.

From a clinical perspective, knowing that the extent of atrial fibrosis appears to be the strongest independent predictor of AF recurrence after ablation [32], our findings may further explain the lower success rates of ablation in patients with cAF compared to pAF likely due to the presence of collagen V. In that context special magnetic resonance image acquisition techniques were developed [42,43] and clinical trials confirmed the correlation between atrial fibrosis content and AF recurrence after ablation [32].

5. Study limitations

The findings of the present study should be interpreted in light of certain limitations. Because the study groups were characterized by different underlying heart diseases and different severities of heart failure, our results should be interpreted with some caution in view of the high interindividual variability of the parameters studied. Nonetheless, our study appropriately matched patient groups for most clinical parameters, minimizing potential sources of bias.

6. Conclusions

This is the first study demonstrating that AF is associated with increased expression levels of collagen V and TGF-β1 indicating their role in the pathogenesis of atrial fibrosis. In addition, increases in collagen V correlate with increased number of fibroblasts and TGF-β1 and are more pronounced in cAF patients than those in pAF patients indicating that collagen V and TGF-β1 are useful tissue markers of different stages of AF.

Ethical statement

One hundred and two patients undergoing open heart surgery were subdivided into three groups: 35 patients in sinus rhythm (SR), 35 patients with paroxysmal AF (pAF) and 32 patients with chronic, long-standing persistent AF (cAF) with a duration of AF more than 12 months. Clinical data are presented in Table 1. The study protocol was approved by the Ethics Committees of the Landesärztekammer Hessen, Frankfurt am Main (Number: FF12/2011). All patients gave written informed consent and the study followed the principles of the Declaration of Helsinki.

CRediT authorship contribution statement

Sawa Kostin: . **Manfred Richter:** Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Data curation. **Natalia Ganceva:** Supervision, Investigation, Data curation. **Benjamin Sasko:** Writing – review & editing, Writing – original draft, Formal analysis. **Theodoros Giannakopoulos:** Writing – review & editing, Methodology, Formal analysis. **Oliver Ritter:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Zoltan Szalay:** Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Nikolaos Pagonas:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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