


# Microanatomy of the myocardial extensions of the pulmonary valve in light of modern catheter ablation methodology

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## Abstract

**Introduction:** The muscular sleeves (or myocardial extensions) derived from the right ventricle infundibulum myocardium are considered the true anatomic substrate for right ventricular outflow tract arrhythmias.

**Methods:** Pulmonary valve specimens obtained from 65 donors (24.6% females, mean age  $45.9 \pm 15.8$  years) were investigated micro-anatomically. Specimens were histologically processed, stained with Masson's Trichrome, and examined under a light microscope.

**Results:** The myocardial extensions were present in the left anterior pulmonary valve sinus in 86.2% of cases, in the right anterior sinus in 89.2% of cases and in 90.7% of cases in the posterior sinus ( $p = .699$ ). In 69.2% of examined hearts, the myocardial extensions were present in all sinuses. The mean height of the extensions was  $4.12 \pm 1.76$  (left anterior) versus  $3.69 \pm 1.47$  (right anterior) versus  $4.28 \pm 1.73$  mm (posterior) ( $p = .137$ ). The myocardial extensions occupied an average of  $28.9 \pm 10.4\%$  of the left anterior sinus,  $26.7 \pm 11.2\%$  of the right anterior sinus, and  $31.9 \pm 11.3\%$  of the posterior sinus ( $p = .044$ ). Sleeves extending beyond the fibro-arterial transition zone were present in at least one sinus in 33.8% of hearts (in 7.7% (5/65) of the left and right anterior sinuses and 21.5% (14/65) of posterior sinus,  $p = .021$ ).

**Conclusions:** The myocardial extensions of the pulmonary valve are common anatomical entities. Although the length of the myocardial sleeves is similar in all pulmonary valve sinuses, their relative extent is greatest in the posterior sinus. Long sleeves that spread beyond the fibro-arterial transition zone were observed in one-third of hearts, predominantly in the posterior sinus. Myocardial and fibrous tissue layer thicknesses varied considerably.

## KEYWORDS

myocardial extensions, pulmonary root, RVOT, supraventricular ablation, ventricular arrhythmias

## 1 | INTRODUCTION

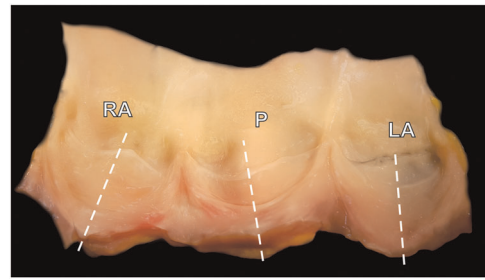
In patients with structurally normal hearts, idiopathic ventricular arrhythmias (VAs) arise predominantly from the muscular tissue of the right ventricular outflow tract (RVOT).<sup>1</sup> Such arrhythmias usually have a benign course and can be effectively managed with catheter ablation due to their focal origin. Previously, it was suggested that such arrhythmias rarely had a supra-ventricular origin, and that the prevalence of such instances was anywhere between 4.0% and 24.2%.<sup>2–4</sup> However, recent studies have challenged these results. In a study conducted by Liu et al., 46% of patients with idiopathic VAs of RVOT origin were successfully treated by ablating areas beyond the pulmonary valve, as visualized by intracardiac echocardiography.<sup>5</sup> Later, Zhang et al. showed that 90% of patients with VAs of RVOT origin were successfully treated with ablations performed within the region of the pulmonary valve sinuses.<sup>6</sup> Moreover, the authors suggested that more challenging mapping and ablation techniques (such as the “reversed U curve” technique) within the pulmonary trunk should be the preferred approach to treating idiopathic RVOT arrhythmias.

The high success rate of treating idiopathic RVOT arrhythmias with supra-ventricular ablations will lead to their increased use. In turn, clinicians will have to have a better understanding of the regional anatomy and of the potential locations that can trigger arrhythmias. Most often, the substrates responsible for the arrhythmias are the muscular sleeves (also known as myocardial extensions) derived from the myocardium of the right ventricle infundibulum.<sup>5</sup> To date, only a few studies have been published about the myocardial extensions of the pulmonary valve.<sup>5,7,8</sup> Therefore, the correlative anatomy of the pulmonary root has not been investigated adequately. Many unknowns surround the myocardial extensions—there is even a lack of consensus about their definition and prevalence.<sup>7,8</sup> Therefore, this microanatomical cadaveric study was performed to examine the myocardial extensions of the pulmonary valve and to provide morphometric descriptions of the individual parts of the pulmonary root and myocardial extensions.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Pulmonary valve specimens were obtained from routine forensic medical autopsies. We analyzed 65 donors of which 24.6% were females. The mean age of all the donors was  $45.9 \pm 15.8$  years. All studied samples were retrieved with the approval of the Jagiellonian University Bioethical Committee (No 1072.6120.31.2019 and 1072.6120.216.2019) and according to the Declaration of Helsinki guidelines. Following the heart excisions, gross inspections were done to eliminate specimens with visible cardiovascular pathologies. Afterwards, the selected samples were washed with saline solution. Cylindrical-shaped specimens were excised from each heart—these were composed of the right ventricle infundibulum, the pulmonary



**FIGURE 1** Photograph of a cadaveric heart specimen showing the pulmonary valve with marked section lines. LA, left anterior sinus; P, posterior sinus; RA, right anterior sinus

root, and part of the pulmonary trunk. These specimens were subsequently cut into three parts along the commissures separating the cusps. For each sinus, additional longitudinal sections were obtained (see Figure 1). Care was taken to ensure that the location of the cut was at the most inferior part of the cusp attachment.

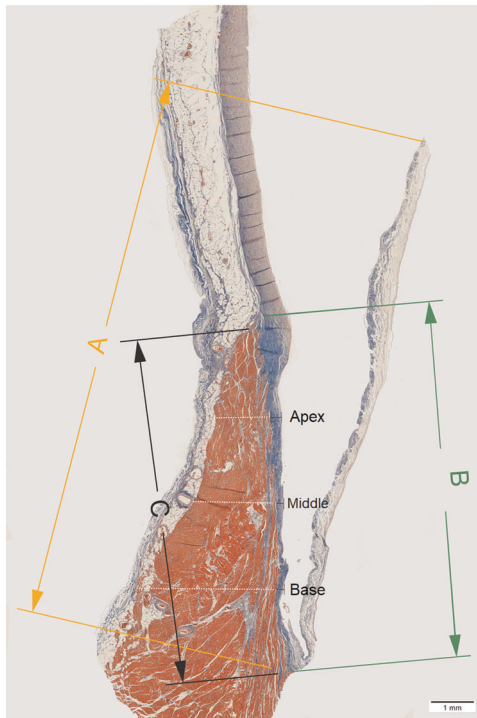
### 2.2 | Tissue processing

All tissue samples were fixed by immersion for 24 h in 10% neutral buffered formalin. After preservation, samples were dehydrated in a series of alcohols, cleared in xylene, and embedded in paraffin blocks. Samples were cut into 6- $\mu$ m serial sections (using Microtome Biocut 2035; Leica Instruments GmbH) and mounted on polylysine-coated slides (using Menzel-Glaser; Thermo Scientific). The sections were deparaffinized, rehydrated, and stained with Masson's Trichrome.

### 2.3 | Microscopic examination

The specimens were examined under a light microscope (Microscope Olympus IX83 Equipped with DP-73 digital CCD camera, Olympus). High-resolution full specimen scans (under a 10x objective lens) were used as source images. Measurements were taken using CellSens Dimension software (Olympus). For the purpose of this study, we implemented clear operating definitions of various morphometric elements. Below are their descriptions:

- Pulmonary root: part of the RVOT supporting the pulmonary valve, limited proximally by the most inferior points of cusp insertions and distally by the sino-tubular junction;
- Sinus walls: parts of the pulmonary root bounded proximally by the attachments of the semilunar cusps and distally by the sino-tubular junction;
- Fibro-arterial transition zone: part of the pulmonary root in which the dense fibrous tissue located on the arterial aspect of the sinus wall converts to a three-layered organization (comprised of an intima, media, and adventitia layer);



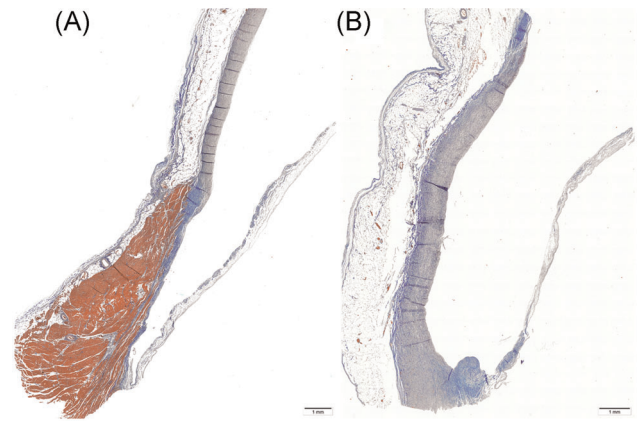
**FIGURE 2** Micrograph presenting the right anterior pulmonary valve sinus with marked measurements (Masson's Trichrome). A, cusp length; B, sinus wall height; C, myocardial tissue height

- Height of the sinus wall: the distance between the cusp insertion and the end of the fibro-arterial transition zone;
- Cusp length: the length from the cusp insertion to its free edge;
- Myocardial extension height: the length from the cusp insertion to the most distant part of the myocardial tissue;
- Myocardial tissue thickness: measured at three distinct locations below the fibro-arterial transition zone at the base, middle, and apex (see Figure 2).
- Fibrous tissue thickness: measured at three distinct locations below the fibro-arterial transition zone at the base, middle, and apex (see Figure 2).

All images were assessed by two independent researchers. To avoid bias, the mean from the two measurements was calculated and reported as the final value.

## 2.4 | Statistical analysis

The variables were expressed as a mean  $\pm$  standard deviation (SD). Statistical analyses included the *t*-Student test, the Mann-Whitney *U* test, analysis of variance test, and the Newman-Keuls post hoc analysis. The Pearson test analyzed correlations between continuous variables. Statistical analyses were performed with Statgraphics Centurion XVI (StatPoint Technologies INC) software. All tests were two-tailed and a *p*-value of  $<.05$  was considered statistically significant.



**FIGURE 3** Micrograph presenting the posterior pulmonary valve sinus with (A) and without (B) pulmonary valve myocardial extensions (Masson's Trichrome)

## 3 | RESULTS

In 69.2% of examined hearts, the myocardium extended above the cusp insertion in all pulmonary root parts. In 26% of specimens, one of the sinuses lacked extensions, whereas in 4.6% of cases, two sinuses had no myocardium extending above the valve annulus (see Figure 3). Myocardial extensions had a similar prevalence in each of the individual sinuses (left anterior: 86.2% vs. right anterior: 89.2% vs. posterior 90.7%;  $p = .699$ ). The height of the extensions was also similar between sinuses ( $4.12 \pm 1.76$  vs.  $3.69 \pm 1.47$  vs.  $4.28 \pm 1.73$  mm, respectively;  $p = .137$ , Table 1). When present, myocardial extensions covered  $28.9 \pm 10.4\%$  of the left anterior sinus,  $26.7 \pm 11.2\%$  of the right anterior sinus, and  $31.9 \pm 11.3\%$  of the posterior sinus ( $p = .044$ , Table 1). Sleeves that extended beyond the fibro-arterial transition zone were present at least in one sinus in 33.8% of hearts (in 7.7% (5/65) of the both left and right anterior sinuses, and 21.5% (14/65) of the posterior sinuses;  $p = .021$ ). Only in 13.8% of hearts, the muscular part exceeding the fibro-arterial transition zone constituted more than 10% of total sleeve length. The sinus wall height was comparable among all sinuses (left anterior:  $5.39 \pm 1.98$  vs. right anterior:  $4.96 \pm 1.51$  vs. posterior:  $5.18 \pm 1.57$  mm,  $p = .354$ ). The longest cusp was the right anterior cusp ( $13.8 \pm 2.4$  mm). The second-longest was the left anterior cusp ( $13.7 \pm 2.6$  mm) and the shortest was the posterior cusp ( $13.5 \pm 2.4$  mm). The difference between cusp lengths was insignificant. There was a strong correlation between the cusp length and the height of the myocardial extension in the left anterior cusp and in the posterior cusp ( $r = .52$ ,  $p < .001$  and  $r = .51$ ,  $p < .001$ , respectively), although this correlation was not observed in the right anterior cusp ( $r = .08$ ,  $p = .52$ ).

The examined muscular tissue was continuous with the main myocardium (see Figures 2 and 3A, Table 2,  $p < .001$ ). It had a wide base and a narrow apex. Myocardial tissue thicknesses did not differ between different sinuses at the same levels (Table 2,

	Left anterior sinus	Right anterior sinus	Posterior sinus	p-value ANOVA (sinuses comparison)
Myocardial extension height (mm)	4.12 ± 1.76 [0.99–9.05]	3.69 ± 1.47 [0.83–8.59]	4.28 ± 1.73 [1.12–8.96]	<i>p</i> = .137
Percentage of sinus height covered by extension	28.9 ± 10.4 [8.01–48.67]	26.7 ± 11.2 [6.71–57.79]	31.9 ± 11.3 [9.91–53.84]	<i>p</i> = .044

Abbreviation: ANOVA, analysis of variance.

**TABLE 1** Recorded myocardial tissue heights and sinus coverages (mean ± standard deviation with corresponding range [min.–max.])

**TABLE 2** Recorded thicknesses of the myocardial and fibrous tissue layers (mean ± standard deviation with corresponding range [min.–max.])

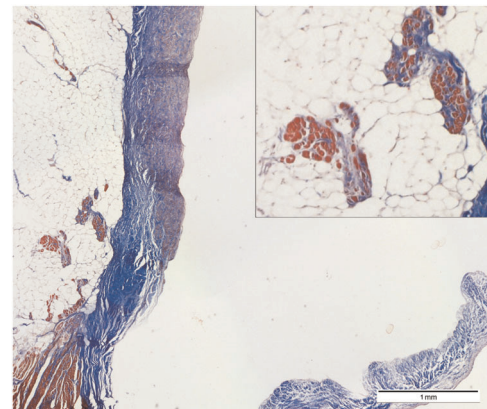
	Myocardial tissue thickness (mm)			p-value ANOVA (sinuses comparison)	Fibrous tissue thickness (mm)			p-value ANOVA (sinuses comparison)
	Left anterior sinus	Right anterior sinus	Posterior sinus		Left anterior sinus	Right anterior sinus	Posterior sinus	
Base (25% of length)	1.89 ± 0.80 [0.81–3.53]	1.94 ± 1.03 [0.55–5.59]	2.08 ± 0.77 [0.57–4.27]	<i>p</i> = .495	0.43 ± 0.21 [0.10–1.03]	0.36 ± 0.16 [0.18–0.87]	0.45 ± 0.22 [0.12–1.29]	<i>p</i> = .592
Middle (50% of length)	1.72 ± 0.74 [0.86–3.79]	1.60 ± 0.94 [0.11–5.58]	1.68 ± 0.73 [0.20–3.95]	<i>p</i> = .749	0.36 ± 0.21 [0.1–1.12]	0.47 ± 0.22 [0.17–1.22]	0.32 ± 0.20 [0.11–1.27]	<i>p</i> < .001
Apex (75% of length)	0.89 ± 0.61 [0.20–2.90]	0.96 ± 0.83 [0.10–5.45]	1.04 ± 0.55 [0.12–2.46]	<i>p</i> = .711	0.44 ± 0.20 [0.13–1.12]	0.42 ± 0.18 [0.13–0.92]	0.42 ± 0.27 [0.14–1.35]	<i>p</i> = .053
p-value ANOVA (levels comparison)	<i>p</i> < .001	<i>p</i> < .001	<i>p</i> < .001	-	<i>p</i> = .04	<i>p</i> = .007	<i>p</i> = .006	-

Abbreviation: ANOVA, analysis of variance.

*p* > .05). The myocardial tissue layer was significantly thicker than the overlying fibrous tissue (Table 2, *p* < .001). More variability was observed below the fibro-arterial transition zone. The fibrous tissue in the left anterior sinus and in the posterior sinus was significantly thinner in the middle sector than at the ends of the cross-sections. This finding was not observed for the right anterior sinus, whose fibrous layer was thinnest at the base (Table 2). Although fibrous tissue thicknesses measured at the bases and at the apexes of each sinus did not differ from one another, the middle sector was variable. It was thickest in the right anterior sinus, second thickest in the left anterior sinus and thinnest in the posterior sinus (Table 2). The classic three-layered arterial structure became noticeable caudally to the transition zone. In 33.8% of all studied hearts, in at least one of the sinuses, the “islands” of muscular tissue separated from the main myocardial extension by a substantial portion of adipose tissue were observed. Their greatest prevalence was in the right anterior sinus (14/65), followed by left anterior (5/65) and posterior (5/65; Figure 4, *p* = .021). All studied anatomical features of the myocardial extensions were not affected by the age (*p* > .05), sex (*p* > .05), or body mass index of the donor (*p* > .05).

## 4 | DISCUSSION

The current knowledge about ventricular myocardial extensions of the pulmonary valve is limited. Therefore, it is difficult to estimate their exact prevalence within the population or to thoroughly



**FIGURE 4** Micrograph presenting muscular tissue “islands” in the vicinity of fibro-arterial transition zone (Masson's Trichrome). Insert shows these muscular structures in magnification, highlighting their perpendicular orientation to the cross-section axis

describe their morphometric features. This is especially due to the major discrepancies between the few existing studies that discuss the myocardial extensions.<sup>7,8</sup> The biggest disparity in the reported myocardial extensions prevalence could be observed between current study and the research conducted by Hasdemir et al. (myocardial extensions present in 100% vs. 17% of hearts).<sup>7</sup> That variance is most likely caused by a significant difference in the adopted myocardial extension definition. In aforementioned microscopic study only the extensions that surpassed the ventriculo-arterial junction were reported. This junction is defined as the macroscopic limit of muscular tissue within the sinuses, as observed within the luminal side of the pulmonary root.<sup>9</sup> In the current study, the reported prevalence refers to myocardium that extends above the lowest point of valve cusp insertion line. Myocardial extensions above that level are naturally much more common because it lies about 4 mm inferiorly to the ventriculo-arterial junction.<sup>8</sup> Additionally, the reference to the cusp insertion line is much more clinically relevant, considering that this structure constitutes a landmark which could be easily identified during transcatheter procedures. This was demonstrated in the electrophysiological study by Liu et al., where cusp attachment line (visualized by intracardiac echocardiography) was denoted as a reference point for measurements.<sup>5</sup> On the other hand, the macro-anatomical study by Gami et al. relies on the same myocardial extensions definition to our study but reports a prevalence of 62%.<sup>8</sup> Nevertheless, in the case of Gami's research, heart specimens were fixed in 10% formaldehyde solution and analyzed 3–45 days postmortem, which would likely have caused tissue discoloration, subsequently hindering the process of myocardial extensions identification.<sup>10</sup> A microscope-based study seems to be better suited for precise myocardial extensions analysis, especially because it allows to utilize tissue stains, which are much more reliable in the matter of myocardium identification. In addition, our results are consistent with recent clinical data, that shows great effectiveness of supraventricular ablations.<sup>6</sup> Furthermore, the current study is the first to report precise height and thickness measurements of the tissues comprising sinus walls of the pulmonary root. Our reported myocardial extensions measurements are comparable to previous macroscopic cadaveric study which reported that the mean height varied from 3.7 to 4.0 mm among sinuses.<sup>8</sup> However, in the present study, the reported maximal height is significantly smaller than this reported by Gami et al. (9.05 vs. 19.0 mm).<sup>8</sup> This is possibly due to the differences in measurement acquisition methodology. In the current research, all measurements were made along the sinus midline, whereas, in Gami's study, the maximum extension height was counted with no fixed axis of measurement.

The ventricular myocardium is more extensive in the pulmonary valve than in the aortic root. In the pulmonary valve, it forms a cylindrical sleeve over its entire circumference.<sup>9</sup> Although the myocardium surpasses the cusp attachment line, it is not directly supporting them. Microscopic examination reveals that there is an intricate collagenous structure that connects the cusps to the ventricular myocardium and looks like a natural extension of the cusp's fibrosa layer (see Figure 3). It elongates distally toward the root and

constitutes the inner layer of the sinus wall. At its superior end the fibrous layer gradually gets thinner until, at some point, it becomes distinguishable as adventitia of the pulmonary trunk. At the same level, smooth muscle cells appear, constituting the media layer and giving rise to the three-layered structure present within the pulmonary artery. This transition area constitutes an identifiable landmark within the sinus, which we have designated as the fibro-arterial transition zone. This study has shown that in some cases, ventricular myocardial extensions exceeded the fibro-arterial transition area, despite it being defined as the macroscopic boundary.<sup>11</sup> Moreover, in some sections, the upper segments of the myocardial extensions appeared to form "islands" of muscular tissue separated from the main myocardium by a substantial portion of adipose tissue (Figure 4). Muscular tissue within these "islands" had a horizontal course parallel to the line of cusp attachment. Such appearance might be caused by the asymmetric superior penetration of apical muscular strands, which at some point become horizontally oriented leaving space underneath for adipose tissue infiltration. Such distinct muscular "islands" within the structure of the pulmonary valve root may cause significant anisotropy that could play a role in the arrhythmogenesis. This could be clinically relevant, although further multimodality studies are required to explain the nature and potential significance of this phenomenon.

In light of these recent scientific reports, ventricular myocardial extensions in the pulmonary valve appear much more important than previously thought. In a single-center, Zhang et al. successfully treated 90% of patients with RVOT-type origin ventricular arrhythmias by only performing ablations above the pulmonary valve. This had favorable midterm effectiveness. Since the pulmonary valve sinuses were identified as the origin of the arrhythmia, there are grounds to assume that the myocardial extensions may be the true anatomic substrates.<sup>6</sup> Moreover, Liu et al. have shown that in patients with RVOT arrhythmias, successful ablation sites were in areas where the myocardial signal ended, regardless of a supra or infra valvular localization.<sup>5</sup> Also, they reported a higher-than-expected prevalence (46%) of arrhythmias originating above the pulmonary valve. The authors hypothesized that the previously low reported prevalence could be due to the routine anterograde method of mapping the RVOT, making it unfeasible to identify the pulmonary valve in fluoroscopic views. During supraventricular ablations, the targeted myocardial tissue is hidden behind the fibroelastic parts of the sinus walls, which have variable thicknesses throughout. Their uncertain thickness may presumably influence the required time/power to achieve a successful ablation lesion.<sup>12</sup> Therefore, their presence must be remembered to prevent adverse outcomes such as valvular insufficiency/stenosis<sup>13</sup> or coronary artery damage.<sup>14</sup> Although incidents of left coronary artery damage during radiofrequency catheter ablation are rare, special precautions should be taken when ablating the region of the posterior sinus, due to the proximity of the main stem of the left coronary artery.<sup>14</sup>

There are some limitations to this study. The main one is that this was a microanatomical study based on preserved and histologically processed autopsied material. Consequently, changes to

tissue shape and size may have occurred due to fixation, dehydration, and staining. Additionally, since postmortem material was analyzed, we were not able to assess the behavior and dimensional changes of the pulmonary valve components within the cardiac cycle. Moreover, only healthy donor hearts (those without any known history of arrhythmias) were investigated and therefore our results should be interpreted with caution. Despite these limitations, we believe that they did not meaningfully disturb our morphological analyses of the myocardial extensions of the pulmonary valve.

## 5 | CONCLUSION

The myocardial extensions of the pulmonary valve are common anatomical entities present in all individual parts of the pulmonary root. While the length of the myocardial sleeves was similar in all pulmonary valve sinuses, the relative extent of myocardial coverage was greatest in the posterior sinus. Long sleeves that spread beyond the fibro-arterial transition zone were observed in one-third of hearts, predominantly in the posterior sinus. Thicknesses of the myocardial tissue and fibrous layer tissue varied considerably. Results of this study contribute to the knowledge about morphological substrates for RVOT arrhythmias. We hope they will help enhance the safety and effectiveness of ablation procedures.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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