



# Right ventricular longitudinal shortening and right atrial volumes are not associated in healthy adults – detailed analysis from the three-dimensional speckle-tracking echocardiographic MAGYAR-Healthy Study

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**Background:** There is a close relationship between volumes of the right atrium (RA) and dimensions and derived functional sphincter-like features of the tricuspid annulus (TA). However, its relation to longitudinal TA motion is not clear, which can even be considered to be a characteristic of the longitudinal shortening of the right ventricle (RV) and represented by TA plane systolic excursion (TAPSE). Therefore, the aim of this cohort study was to perform a detailed analysis of the relationship of three-dimensional speckle-tracking echocardiography (3DSTE)-derived RA volumes and RV longitudinal shortening in healthy individuals. These parameters were also examined in case of average values and larger/smaller than mean values.

**Methods:** The present study comprised 93 healthy adults (mean age: 27.7±6.3 years, 46 men), who participated in a complete medical investigation including two-dimensional, TAPSE, Doppler and 3DSTE-derived RA volumetric echocardiographic assessments.

**Results:** RA volumes, stroke volumes and emptying fractions were not related to TAPSE. In case of low, mean and high TAPSE, maximum [50.4±22.4 vs. 49.5±15.5 vs. 49.0±15.8 mL, P= not significant (ns)], preatrial contraction (36.9±16.8 vs. 34.5±10.4 vs. 35.6±10.5 mL, P= ns) and minimum (28.7±13.6 vs. 27.2±9.4 vs. 26.6±9.3 mL, P= ns) RA volumes did not differ. Higher RA volumes showed no associations with TAPSE either.

**Conclusions:** 3DSTE-derived RA volumes and M-mode echocardiography-derived TAPSE representing RV longitudinal shortening are not associated in healthy adults. None of the RA volumes showed correlations with TAPSE.

**Keywords:** Right atrium (RA); volume; tricuspid annular plane systolic excursion (TAPSE); three-dimensional (3D); echocardiography

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## Introduction

In the past decades, echocardiography has undergone enormous technical development. It is now possible to perform a detailed volumetric analysis of various heart chambers including the right atrium (RA), this latter measurement takes into account the heart cycle as well. Three-dimensional (3D) speckle-tracking echocardiography (3DSTE), which measures volumetric data using virtual 3D models, is ideally suited for this task (1-4). In recent studies, capability of 3DSTE was demonstrated in accurate volumetric assessment of RA with defined normal reference values (5-7). Moreover, the exact relationship of RA volumes, volume-based functional properties and strains was examined in healthy circumstances (7). The RA forms an organic unit with the right ventricle (RV), and the tricuspid annular (TA) (8,9). The TA has a special 3D hyperbolic paraboloid shape with a dynamic 3D motion including longitudinal and sphincter-like movements (10). In a recent study, close relationship between RA volumes and TA dimensions and derived functional sphincter-like features could be detected (5). However, its relation to TA longitudinal motion is not clear, which can even be considered to be a characteristic of RV longitudinal shortening. M-mode echocardiography-derived TA plane systolic excursion (TAPSE) is a long-used parameter, which is suitable for characterising RV longitudinal function with a well-documented strong prognostic impact (8,11-13). Therefore, the aim of this cohort study was to perform a detailed analysis of the relationship of 3DSTE-derived RA volumes and RV longitudinal shortening in healthy individuals. These parameters were examined in the case of average values and larger/smaller values as well. We present this article in accordance with the STROBE reporting checklist (available at <https://qims.amegroups.com/article/view/10.21037/qims-24-223/rc>).

## Methods

### Subject population

The present study comprised 93 healthy adults (mean age: 27.7±6.3 years, 46 men) being in sinus rhythm, who participated in a complete medical investigation including physical examination, laboratory test, standard 12-lead electrocardiography and two-dimensional (2D) Doppler echocardiography with results within the normal range. 3DSTE data acquisition was obtained immediately after a

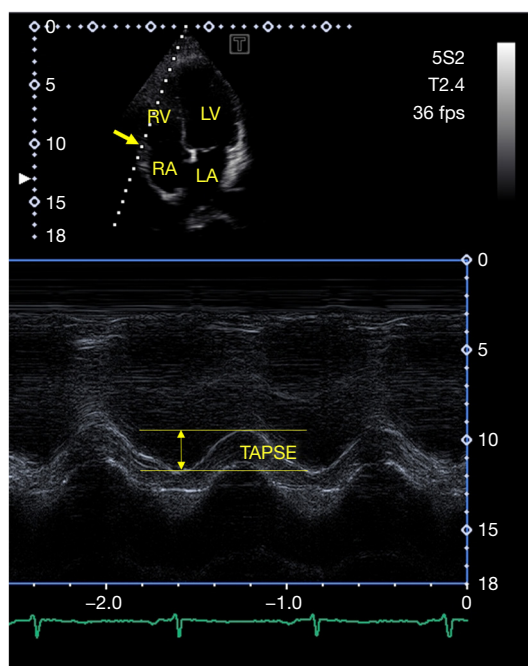
routine echocardiographic examination and datasets were analysed at a later date. No healthy participant took any medication or drug regularly, none of them were obese or were smoking regularly, and none of them had any known pathological state or disease. The present retrospective cohort study is part of the 'Motion Analysis of the heart and Great vessels by three-dimensional speckle-tracking echocardiography in Healthy subjects' (MAGYAR-Healthy) Study, which was organized in part to investigate physiological relationships in healthy adults ('Magyar' means 'Hungarian' in Hungarian language). All individuals who participated in the study signed informed consent. The study was in line with the Helsinki Declaration (as revised in 2013). The study was approved by the Institutional and Regional Human Biomedical Research Committee of University of Szeged, Hungary (registration number: 71/2011, latest approval for prolongation: February 20, 2023).

### 2D Doppler echocardiography

During 2D Doppler echocardiography, professional guidelines were followed. Measurements were made using a Toshiba Artida™ echocardiography machine (Toshiba Medical Systems, Tokyo, Japan), which was attached to a PST-30BT (1–5 MHz) phased-array transducer. All healthy individuals were in the left lateral decubitus position, and after placing the transducer on their chest, examinations were made from the apical and parasternal views. The left atrium (LA) and left ventricle (LV) were quantified according to guidelines, and LV ejection fraction (EF) values were determined using the Simpson's method (8,14). Continuous Doppler echocardiography was used to exclude valvular stenosis or regurgitation on any valves. Pulsed Doppler echocardiography was used to determine transmitral E and A flow velocities and E/A ratio featuring LV diastolic function. To represent longitudinal shortening of the RV, TAPSE was measured in apical long-axis view by measuring TA lateral edge movement toward the apex in systole in longitudinal direction (*Figure 1*) (8,14).

### 3DSTE-derived data acquisition

In accordance with the established practice, the 3DSTE examination took place in two phases (1-7). For the first time, digital 3D datasets were acquired by the same observer (Á.K.) using the same Toshiba Artida™ cardiac ultrasound with a PST-25SX matrix transducer attached.



**Figure 1** Measurement of tricuspid annular plane systolic excursion by M-mode echocardiography. Yellow arrow represents the plane of measurement. RV, right ventricle; RA, right atrium; LV, left ventricle; LA, left atrium; TAPSE, tricuspid annular plane systolic excursion.

An important condition during data acquisition was that the individual had to be in sinus rhythm, and the volunteer was asked to lie on his left side. During data acquisition, 6 wedge-shaped datasets (subvolumes) were collected within a single breath-hold within 6 heart cycles from the apical window. The automatically merged subvolumes helped to create a complete dataset called full volume. At a second stage, datasets were analysed offline with a 3D Wall Motion Tracking software version 2.7 (Ultra Extend, Toshiba Medical Systems, Tokyo, Japan).

### RA volumetric evaluation by 3DSTE

During the analysis of the RA, virtual 3D models were created in the images focused on RA using the methodology already described. Shortly, datasets were presented in apical two- (AP2CH) and four-chamber (AP4CH) views and in short-axis views at basal, midatrial and superior RA levels at end-diastole. After defining the septal and lateral RA-TA edges and the RA apex in AP2CH and AP4CH views at end-diastole, the RA endocardial surface was reconstructed

and a sequential analysis was performed. Respecting the cardiac cycle, the following volumes of the RA were measured (Figure 2) (5-7):

- ❖ Maximum RA volume, measured at end-systole, just before tricuspid valve opening ( $V_{\max}$ ).
- ❖ RA volume before atrial contraction, measured at early-diastole at the time of the P wave on the electrocardiogram ( $V_{\text{preA}}$ ).
- ❖ Minimum RA volume measured at end-diastole, just before tricuspid valve closure ( $V_{\min}$ ).

Using RA volumes several RA stroke volumes and emptying fractions were calculated respecting the cardiac cycle as presented in Table 1.

### Statistical analysis

Depending on the distribution of parameters, for continuous variables, mean  $\pm$  standard deviation (SD) format and for categorical variables, number/percentage format were used. During the statistical analyses, P less than 0.05 was considered to be significant. Independent sample *t*-test and analysis of variance (ANOVA) tests were applied for group comparisons. Pearson's coefficients were determined for featuring correlations between variables. The Bland-Altman method was used for interobserver and intraobserver agreements and intraclass correlation coefficients (ICCs) were also calculated. SPSS software (version 22, SPSS Inc., Chicago, IL, USA) was applied for statistical analyses.

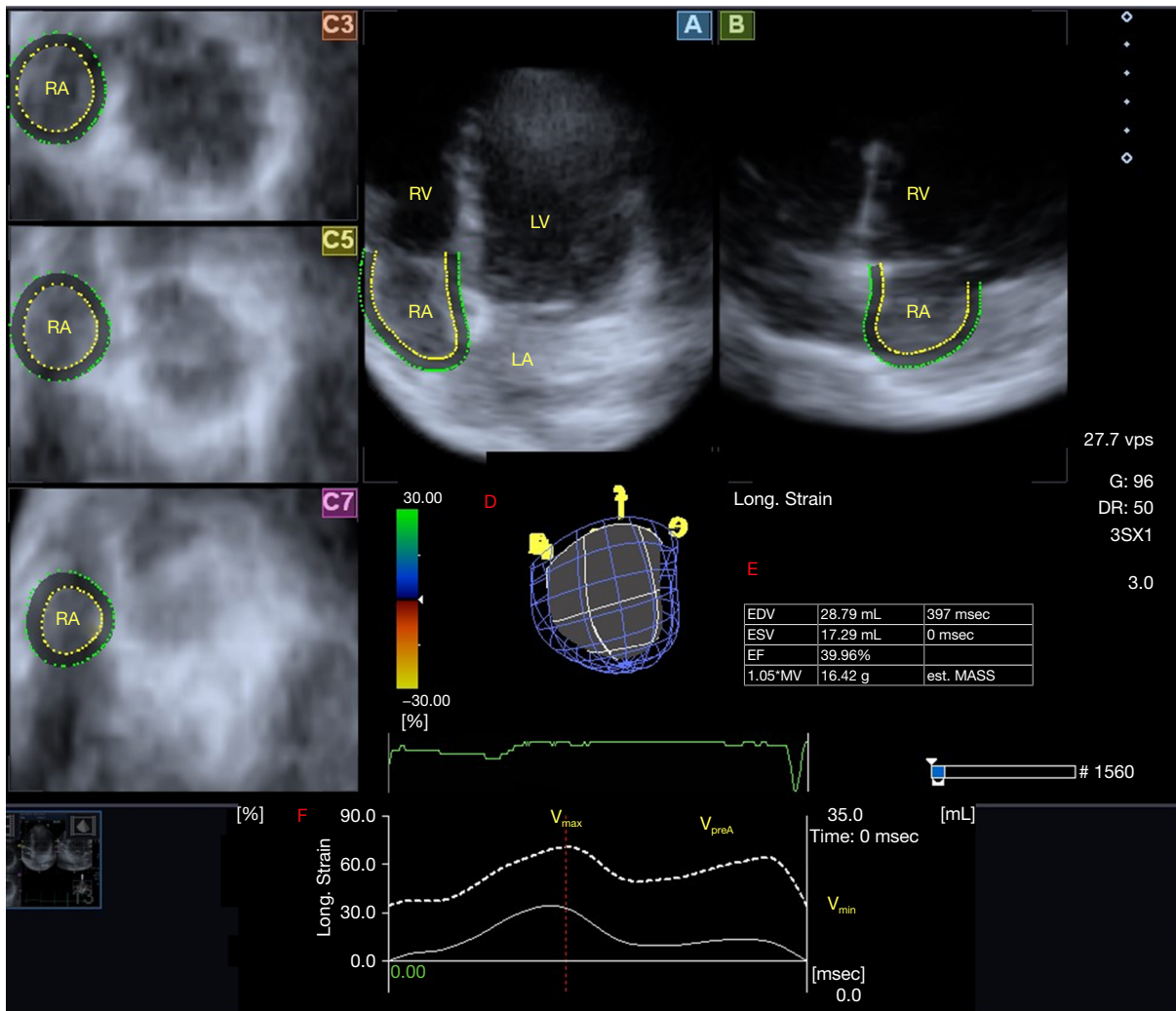
## Results

### Clinical and two-dimensional Doppler echocardiographic data

All routine two-dimensional echocardiographic data are demonstrated in Table 2. Significant valvular disease were not present in any of subjects.

### Classification of subjects

The study population was divided into several groups of healthy individuals considering their mean  $\pm$  SD of 3DSTE-derived RA- $V_{\max}$ , RA- $V_{\text{preA}}$  and RA- $V_{\min}$  and TAPSE as assessed by M-mode echocardiography, which proved to be  $49.5 \pm 16.3$ ,  $35.0 \pm 11.2$ ,  $27.2 \pm 9.9$  mL and  $23.8 \pm 2.9$  mm, respectively. According to these values, the subgroups were classified based on the lower (33.2, 23.8, 17.3 mL and 20.9 mm, respectively) and upper (65.8, 46.2, 37.1 mL and 26.7 mm, respectively) values of these parameters.



**Figure 2** Creation and analysis of 3D model of the RA by 3D speckle-tracking echocardiography. By using this 3D model, several two-dimensional views including apical longitudinal four-chamber (A) and two-chamber views (B) and basal (C3), midatrial (C5) and superior short-axis views (C7) are created together with a 3D cast of the RA (D), measured RA volumes respecting the cardiac cycle (E) and time-RA volume changes curve (white dotted curve) and time-global RA longitudinal strain curve (white solid curve) (F). RV, right ventricle; RA, right atrium; LV, left ventricle; LA, left atrium; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction;  $V_{max}$ , end-systolic maximum RA volume;  $V_{preA}$ , early diastolic preatrial contraction RA volume;  $V_{min}$ , end-diastolic minimum RA volume; 3D, three-dimensional.

**Table 1** Assessment of right atrial stroke volumes and emptying fractions respecting the cardiac cycle

Parameters	Systolic reservoir RA function	Early diastolic conduit RA function	Late diastolic booster pump RA function
RA stroke volumes	$TASV = V_{max} - V_{min}$	$PASV = V_{max} - V_{preA}$	$AASV = V_{preA} - V_{min}$
RA emptying fractions	$TAEF = TASV/V_{max} \times 100$	$PAEF = PASV/V_{max} \times 100$	$AAEF = AASV/V_{preA} \times 100$

RA, right atrial; TASV, total atrial stroke volume;  $V_{max}$ , end-systolic maximum RA volume;  $V_{min}$ , end-diastolic minimum RA volume; PASV, passive atrial stroke volume;  $V_{preA}$ , early diastolic preatrial contraction RA volume; AASV, active atrial stroke volume; TAEF, total atrial emptying fraction; PAEF, passive atrial emptying fraction; AAEF, active atrial emptying fraction.



**Table 2** Two-dimensional and three-dimensional speckle-tracking echocardiographic data

Data	Measurements
Two-dimensional echocardiographic data	
Left atrial diameter (mm)	36.9±3.4
LV end-diastolic diameter (mm)	48.1±3.6
LV end-systolic diameter (mm)	32.3±3.2
LV end-diastolic volume (mL)	105.8±24.5
LV end-systolic volume (mL)	38.2±10.1
Interventricular septum (mm)	9.0±1.2
LV posterior wall (mm)	9.3±1.5
LV ejection fraction (%)	64.6±4.1
Early diastolic mitral inflow velocity – E (cm/s)	83.5±15.2
Late diastolic mitral inflow velocity – A (cm/s)	56.9±11.1
Tricuspid annular plane systolic excursion (mm)	23.8±2.9
Three-dimensional speckle-tracking echocardiographic data	
End-systolic maximum RA volume (mL)	49.5±16.3
Early diastolic pre-atrial contraction RA volume (mL)	35.0±11.2
Late diastolic minimum RA volume (mL)	27.2±9.9
Total RA stroke volume (mL)	22.3±10.2
Passive RA stroke volume (mL)	14.4±8.3
Active RA stroke volume (mL)	7.8±4.9
Total RA emptying fraction (%)	44.3±11.6
Passive RA emptying fraction (%)	28.0±10.6
Active RA emptying fraction (%)	22.5±12.1

Data are presented as mean ± standard deviation. LV, left ventricular; RA, right atrial.

### *Degree of TAPSE and RA properties*

RA volumes, stroke volumes and emptying fractions were not related to TAPSE (Table 3).

### *Degree of RA volumes and TAPSE*

Higher RA volumes showed no associations with TAPSE (Table 4).

### *Correlations*

None of the RA volumes showed correlations with TAPSE.

### *Interobserver and intraobserver agreement*

The mean difference in measured parameters by

2 examiners for RA- $V_{max}$ , RA- $V_{preA}$ , RA- $V_{min}$  and TAPSE was  $0.9 \pm 5.0$ ,  $-1.3 \pm 7.9$ ,  $0.9 \pm 4.1$  mL and  $1.2 \pm 0.4$  mm, respectively. ICC proved to be 0.95 ( $P < 0.001$ ), 0.90 ( $P < 0.001$ ), 0.93 ( $P < 0.001$ ) and 0.98 ( $P < 0.001$ ) (interobserver agreement). The mean difference in measured parameters by examiner 1 two times for RA- $V_{max}$ , RA- $V_{preA}$ , RA- $V_{min}$  and TAPSE was  $1.0 \pm 6.1$ ,  $-1.5 \pm 8.9$ ,  $0.7 \pm 5.1$  mL, and  $1.2 \pm 0.4$  mm, respectively. ICC proved to be 0.96 ( $P < 0.001$ ), 0.88 ( $P < 0.001$ ), 0.97 ( $P < 0.001$ ) and 0.98 ( $P < 0.001$ ) (intraobserver agreement).

## **Discussion**

In order to get to know the coordinated functioning of the RV, TA and RA, easy-to-perform clinical tests, even on healthy subjects, can help. 3DSTE is suitable for the volumetric examination of the RA by taking into account its changes according to the cardiac cycle. The RA has different phases of function: it behaves like a reservoir during systole, a conduit in early diastole allowing blood flow from the veins to the RV and acting like an actively contracting cavity in late diastole like a booster pump. While the LV looks like a bullet or egg, the RV is fundamentally different from the LV: it is located around the LV and has a triangular shape from the sides, its cross-sectional view it resembles a crescent, widening from the apex of the heart to its base. The deep RV wall muscle fibers are responsible for the base-to-apex longitudinal movement, as a result of which the RV longitudinal axis shortens and the tricuspid valve (TV) moves towards the apex. Superficially located circumferential fibers parallel to the TV are responsible for the movements towards the cavity of the RV (“bellows” effect) (8,15,16). TAPSE characterizes the longitudinal displacement of the TA, which is related to RV contraction with a prognostic power that has long been known and used to characterize RV longitudinal shortening (8,11-13).

According to the presented findings, RA volumes, SVs and EFs were not related to TAPSE, and RA volumes showed no associations and correlations with TAPSE under healthy circumstances. These results suggest independency of RA volumes from RV longitudinal shortening and vice versa. These findings have to be considered in the context of previous results. Lower TAPSE was associated with lower 3DSTE-derived TA fractional area change, which was related to more dilated end-systolic TA area. However, direct correlations between TA longitudinal and sphincter-like features could not be detected (17). Moreover, strong associations between TA dimensions and RA volumes could

**Table 3** Tricuspid annular plane systolic excursion and right atrial volumes in different tricuspid annular plane systolic excursion groups

Data	TAPSE $\leq$ 20.9 mm (n=11)	20.9 mm < TAPSE <26.7 mm (n=61)	26.7 mm $\leq$ TAPSE (n=21)
RA- $V_{\max}$ (mL)	50.4 $\pm$ 22.4	49.5 $\pm$ 15.5	49.0 $\pm$ 15.8
RA- $V_{\text{preA}}$ (mL)	36.9 $\pm$ 16.8	34.5 $\pm$ 10.4	35.6 $\pm$ 10.5
RA- $V_{\min}$ (mL)	28.7 $\pm$ 13.6	27.2 $\pm$ 9.4	26.6 $\pm$ 9.3
TASV (mL)	21.7 $\pm$ 12.6	22.3 $\pm$ 9.9	22.4 $\pm$ 10.5
PASV (mL)	13.5 $\pm$ 9.1	15.0 $\pm$ 8.3	13.4 $\pm$ 8.4
AASV (mL)	8.2 $\pm$ 8.4	7.3 $\pm$ 4.0	9.0 $\pm$ 5.0
TAEF (%)	42.3 $\pm$ 14.8	44.6 $\pm$ 11.0	44.7 $\pm$ 11.9
PAEF (%)	26.7 $\pm$ 9.3	29.1 $\pm$ 10.4	25.5 $\pm$ 11.6
AAEF (%)	20.7 $\pm$ 19.3	21.8 $\pm$ 10.4	25.7 $\pm$ 12.0
TAPSE (mm)	19.6 $\pm$ 0.5	23.1 $\pm$ 1.6*	28.1 $\pm$ 1.2*†

Data are presented as mean  $\pm$  standard deviation. \*,  $P < 0.05$  vs. TAPSE  $\leq$ 20.9 mm; †,  $P < 0.05$  vs. 20.9 mm < TAPSE <26.7 mm. TAPSE, tricuspid annular plane systolic excursion; RA, right atrial;  $V_{\max}$ , end-systolic maximum RA volume;  $V_{\text{preA}}$ , early diastolic preatrial contraction RA volume;  $V_{\min}$ , end-diastolic minimum RA volume; TASV, total atrial stroke volume; PASV, passive atrial stroke volume; AASV, active atrial stroke volume; TAEF, total atrial emptying fraction; PAEF, passive atrial emptying fraction; AAEF, active atrial emptying fraction.

be demonstrated even under healthy circumstances (5). In summary, these results suggest that although TAPSE-represented RV longitudinal shortening does not correlate with RA volumes, it does associate with TA sphincter-like features, with certain relationship between the two types of TA functions. It is important to emphasize that these associations were found in healthy subjects who did not have functional tricuspid regurgitation (FTR). With other words, while our previous results suggested the role of TA sphincter-like features in the development of FTR, the same cannot be said about RV longitudinal shortening. However, further studies are warranted to confirm our findings, even in certain disorders with concomitant FTR.

### Limitation section

Here the most important limitations are listed:

- ❖ The lower image quality associated with 3DSTE compared to two-dimensional echocardiography is a well-known fact, which could significantly influence our results (1-4).
- ❖ Only limited number of healthy subjects being in sinus rhythm were involved into this study. The findings would have been stronger, if larger number of healthy cases were included.
- ❖ Although not only volumetric, but strain data characterizing RA wall contractility can be measured as well using the same virtual RA cast

during its assessment, we did not aim to analyze them in this study.

- ❖ Moreover, neither volumetric nor strain and other functional features of other cardiac chambers were performed either.
- ❖ 3DSTE-derived atrial volumetric assessments and TAPSE measurement are validated procedures, therefore it was not aimed to validate them again (13,18).
- ❖ Six wedge-shaped subvolumes during 6 cardiac cycles were acquired during the acquisition phase of 3DSTE analysis, which increases the risk of stitching and motion artifacts. However, considering all benefits of 3DSTE-based RA volumetric analysis, its importance and accuracy are unquestionable.
- ❖ STE-derived TA functionality analysis was not purposed either.
- ❖ It would have been more convincing, if more advanced methods were used for FTR quantification.
- ❖ Although healthy individuals were involved, the possibility that individuals with latent disease were included in the clinical trial cannot be completely excluded. Further special laboratory or imaging tests would have helped with these exclusions.

### Conclusions

3DSTE-derived RA volumes and M-mode echocardiography-

**Table 4** Tricuspid annular plane systolic excursion and right atrial volumes in different right atrial volumes groups

Data	RA-V <sub>max</sub>				RA-V <sub>preA</sub>				RA-V <sub>min</sub>		
	≤33.2 mL (n=15)	>33.2 to <65.8 mL (n=62)	≥65.8 mL (n=16)	≤23.8 mL (n=15)	>23.8 to <46.2 mL (n=64)	≥46.2 mL (n=14)	≤17.3 mL (n=13)	>17.3 to <37.1 mL (n=62)	≥37.1 mL (n=18)		
RA-V <sub>max</sub> (mL)	28.4±3.3	47.7±8.9*	76.1±10.0*†	28.9±3.8	49.3±11.1 <sup>§</sup>	72.4±15.0 <sup>§†</sup>	32.5±10.2	47.5±11.7 <sup>#</sup>	68.5±16.1 <sup>#§</sup>		
RA-V <sub>preA</sub> (mL)	21.6±3.4	34.3±6.9*	50.6±11.4*†	21.1±3.0	34.1±6.0 <sup>§</sup>	54.3±9.1 <sup>§†</sup>	21.5±3.6	33.2±6.3 <sup>#</sup>	51.1±10.3 <sup>#§</sup>		
RA-V <sub>min</sub> (mL)	16.6±2.8	26.5±7.6*	39.9±8.3*†	15.7±2.5	26.2±6.2 <sup>§</sup>	44.3±4.9 <sup>§†</sup>	14.8±1.7	25.2±5.3 <sup>#</sup>	43.2±4.8 <sup>#§</sup>		
TASV (mL)	11.8±2.8	21.2±7.9*	36.2±8.1*†	13.2±4.0	23.1±9.3 <sup>§</sup>	28.0±13.2 <sup>§</sup>	17.8±9.5	22.3±9.0	25.4±13.7		
PASV (mL)	6.8±3.4	13.4±6.1*	25.5±8.4*†	7.7±3.7	15.3±7.8 <sup>§</sup>	18.0±10.7 <sup>§</sup>	11.0±8.6	14.3±7.4	17.4±10.4		
AASV (mL)	5.1±2.3	7.7±4.8*	10.7±5.7*†	5.5±2.7	7.9±4.8 <sup>§</sup>	10.0±6.3 <sup>§</sup>	6.7±2.7	8.0±4.5	8.0±7.1		
TAEF (%)	41.6±8.0	44.2±12.8	47.6±8.5*	45.2±9.4	45.7±11.7	36.9±10.8 <sup>§†</sup>	51.7±11.9	45.6±9.5 <sup>#</sup>	34.5±12.4 <sup>#§</sup>		
PAEF (%)	23.5±11.1	27.7±10.0	33.8±10.6*†	26.2±11.4	29.4±10.3	23.5±10.6 <sup>†</sup>	30.6±15.5	28.6±9.3	24.0±10.2		
AAEF (%)	23.0±9.3	22.9±13.4	20.6±8.6	25.2±10.9	23.0±12.7	17.5±8.8 <sup>§</sup>	30.2±9.7	23.5±11.2 <sup>#</sup>	13.8±12.0 <sup>#§</sup>		
TAPSE (mm)	23.0±3.0	23.9±3.0	24.2±2.4	23.1±3.0	23.9±3.0	24.1±2.7	23.5±3.1	23.9±3.0	23.9±2.7		

Data are presented as mean ± standard deviation. \*, P<0.05 vs. RA-V<sub>max</sub> ≤33.2 mL; †, P<0.05 vs. 33.2 mL < RA-V<sub>max</sub> <65.8 mL; ‡, P<0.05 vs. RA-V<sub>preA</sub> ≤23.8 mL; §, P<0.05 vs. RA-V<sub>preA</sub> >23.8 mL < RA-V<sub>preA</sub> <46.2 mL; #P<0.05 vs. RA-V<sub>min</sub> ≤17.3 mL; §P<0.05 vs. 17.3 mL < RA-V<sub>min</sub> <37.1 mL. RA, right atrial; V<sub>max</sub>, end-systolic maximum RA volume; V<sub>preA</sub>, early diastolic preatrial contraction RA volume; V<sub>min</sub>, end-diastolic minimum RA volume; TASV, total atrial stroke volume; PASV, passive atrial stroke volume; AASV, active atrial stroke volume; TAEF, total atrial emptying fraction; PAEF, passive atrial emptying fraction; AAEF, active atrial emptying fraction; TAPSE, tricuspid annular plane systolic excursion.

derived TAPSE representing RV longitudinal shortening are not associated in healthy adults.

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## Footnote

*Reporting Checklist:* The authors have completed the STROBE reporting checklist. Available at <https://qims.amegroupp.com/article/view/10.21037/qims-24-223/rc>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://qims.amegroupp.com/article/view/10.21037/qims-24-223/coif>). A.N. serves as an unpaid editorial board member of *Quantitative Imaging in Medicine and Surgery*. The other authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Institutional and Regional Human Biomedical Research Committee of University of Szeged, Hungary (registration number: 71/2011, latest approval for prolongation: February 20, 2023). All individuals who participated in the study signed informed consent. The study was in line with the Helsinki Declaration (as revised in 2013).

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