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Ultrasound Elastography Detects Age-related Changes in Adult False Vocal Folds

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Abstract

Purpose To investigate the feasibility of ultrasound elastography for assessing the symmetry in stiffness values and movements of both false vocal folds (FVF).

Methods After IRB approval and written informed consent obtained, we measured ultrasound strain and shear wave velocity (SWV) of the bilateral FVF in vocal fold abduction and adduction in 30 participants using a linear array transducer (4-10 MHz). Twenty-eight participants met inclusion criteria as healthy subjects for analysis. Mean strain of FVF produced by FVF movement from abduction to adduction was analyzed using 2-D speckle tracking software offline. A SWV ratio $(SWV_{adduction} - SW_{abduction})/SW_{abduction})$ was developed to test contractibility of FVF. Statistical analysis included one-way ANOVA to test the difference in mean strain, SWV (adduction and abduction), and SWV ratio among the three age groups; linear regression to analyze correlations of stiffness and motion between the right and left FVF; and intraclass correlation coefficient (ICC) to examine intra- and inter-observer reliability in performing SWE of FVF.

Results The 28 healthy participants were divided into three age groups (10 of young 20-44y; 9 of mid-age 45-64y; and 9 of senior ≥65y). The SWV in FVF abduction was higher and SWV ratio was lower in seniors compared to young participants ($p < 0.05$). Good to excellent correlation of mean strain and SWV between both FVFs ($R^2 > 0.89$). The reliability of performing SWE of FVF was moderate to excellent.

Conclusion Ultrasound elastography is feasible to assess the stiffness, dynamic movement, and symmetry of adult FVF, and healthy seniors may exhibit increased FVF stiffness.

Keywords: Age; false vocal fold; shear wave elastography; tissue stiffness; ultrasound

Introduction

The main structural components of the larynx include the thyroid and cricoid cartilages, and thyroarytenoid, vocalis, arytenoid, and cricoarytenoid muscles. These muscles manipulate the tension and shape of the false vocal folds to influence vibration of the true vocal folds, to influence sound waves, allowing for phonation. The true vocal folds are structures that vibrate and oppose each other, which comprise of the vocal ligaments and the overlying mucosa. False vocal folds are a pair of thick folds comprised of mucous membranes that are located in the supraglottal space in the larynx. [1] The true and false vocal folds (FVF) are connected to thyroarytenoids and vocalis muscles, respectively, which are both composed of skeletal muscle fibers histologically. [2] Observations made to *in vitro* skeletal muscle tissues has led to a consensus that changes in muscle histology create dynamic responses to mechanical muscle function. Previous studies have proven that skeletal muscles undergo age-related changes over time, including increases in collagen content at the histological level as well as decreases in type 2 muscle fibers. [3,4] These changes may lead to age-related muscle tissue deterioration as one of causes to develop vocal function disorders in elderly. The term *presbyphonia* refers to various symptoms (e.g. increased vocal roughness, shortening in phonation duration, vocal instability) associated with vocal aging. [5] Histological changes in age-related human skeletal muscles have been assessed and cross-verified using non-invasive imaging modalities, including magnetic resonance imaging (MRI) [6] and ultrasound shear wave elastography (SWE). [7, 8] However, we did not find any report on the age-related change in FVF tissue properties and function using ultrasound elastography during our literature review.

B-mode ultrasonography has proven to be an effective modality to assess airway and laryngeal structures. [9] Using B-mode ultrasonography has clinical values to be an effective strategy for the assessment of functionality and overall health of FVF, and specifically through assessment of tissue morphology and symmetry of the bilateral FVF. [10] Ultrasound imaging is not only beneficial in the sense that it provides a real-time image for motion, but it also reduces the need for more invasive modalities to assess tissue functionality such as invasive transesophageal imaging of the larynx or surgical biopsy for histological assessment of FVF tissue. SWE has been used to evaluate normal mechanical properties (stiffness) of skeletal muscles, and the change in an increased passive muscle stiffness with stretched muscles. [11] There are a few reports of using ultrasound elastography (SWE and strain imaging) to assess FVF. [12] We aimed to investigate the feasibility of SWE for evaluation of mechanical properties, function, and symmetry in adult FVF with age.

Material and Methods

Participants

The Institutional Review Board at the Rocky Vista University approved the study (IRB# 2019-0088) and all participants provided written informed consent. All adult participants were volunteers recruited in the local community (Fig.1). Participants were recruited through a list of research volunteers in the community. Participants underwent a self-reported screening questionnaire to assess previous backgrounds of extensive training that may impact a normal cord function (such as professional singer or vocal performing artists).

The inclusion criteria included age older than 18 years, good health (based on an annual physical examination), and the ability to sign written informed consent, tolerate supine position, neck extension, perform the Valsalva maneuver, and undergo an ultrasound examination.

While the inclusion criteria for the study exam was broad, including healthy and subjects with potential FVF disease, the exclusion criteria for this analysis of healthy subjects were a history of hoarseness, irritant cough, and vocal fold disease of any kind, thyroid, parathyroid, and cervical spine surgery, tracheostomy, cervical trauma, or neuromuscular disorders (e.g., stroke).

Transcutaneous Ultrasound shear wave elastography of False Vocal Folds

An Acuson Sequoia ultrasound scanner (Siemens Healthineers) equipped with a 9L4 linear array transducer was used to acquire grayscale images of false vocal folds. Virtual Touch Tissue Quantification (VTIQ) software installed in the scanner was used to measure the shear wave velocity (SWV) within the bilateral false vocal folds in both abduction and adduction.

There was no specific preparation prior to the ultrasound scan. The participants were placed in the supine position. A small pillow was placed under the neck to maintain mild neck extension. The transducer was placed transversely on the anterior mid neck where the true and false vocal folds are located posterior to the thyroid cartilage (Fig. 2). Transmission gel was applied between the skin surface of the anterior neck and the transducer, a particularly relevant consideration when the angle of the thyroid cartilage was sharp, such as in the male participants examined. Machine pre-setting for thyroid ultrasound was used. We started with B-mode image to evaluate laryngeal morphologic characteristics and observe the movement of the false vocal folds to guide SWE of FVF. Then, VTIQ mode was activated to measure the SWV, representing the mechanical stiffness of bilateral false vocal folds, in both abduction (at the end of inspiration during a normal breath) and adduction (as performing maximal Valsalva maneuver) without phonation or swallowing. A shear wave quality map generated by the VTIQ software was used to ensure the quality of shear wave speed estimation before measuring quantitative SWV values. The shear wave quality was considered valid when a homogeneous green area appeared throughout the region of the false vocal folds (Fig. 3a and 3b). Images with regional absence of green in the false vocal folds within the quality map were considered poor quality and were excluded from the analysis. Quantitative SWV values were then measured within color-coded SWV maps where high quality SWE results appeared using point SWE (a circle region of interest with 0.3 cm in diameter). A total of 4 SWV measurements were obtained in each side of false vocal fold. Two investigators (L.C. and J.O., underwent 2y training in general ultrasound and 40h training in SWE) performed SWE of false vocal folds twice on each participant in FVF abduction (Fig. 3c) and again in FVF adduction (Fig. 3d), with an interval of 1 minute, in all participants. For assessing the function of false vocal folds movement, we developed an SWV ratio defined as $(SWV_{adduction} - SW_{abduction})/SW_{abduction}$ for assessing FVF contraction from abduction to adduction. The average of 4 SWV ratio measurements was used for analysis.

To test intra-observer repeatability, each investigator measured SWV of FVF (abduction and adduction) two times on each of 10 participants. To test inter-observer reproducibility, two investigators measured SWV of the FVF on the same 10 participants separately. The data were analyzed offline.

Ultrasound strain imaging of False Vocal Folds

Using the same linear ultrasound transducer, 5-second gray scale image cine loops consisting of real time ultrasound data of FVF movement from abduction to adduction were stored for offline processing. Machine settings for ultrasound strain imaging included image frame rate > 40 frames per second; off speckle reduction function; single image focus; and harmonic imaging. All recorded cine loops were recorded in Digital Imaging and Communications in Medicine (DICOM) format and transferred to a PC for offline image processing. A 2-D speckle tracking software (EchoInsight, Epsilon Imaging, Ann Arbor, Michigan, USA) was used to estimate mean strain of each FVF produced during FVF movement from abduction to adduction.[13] Mean strain of FVF is the average absolute strain produced from FVF abduction to adduction (Fig. 4).

Statistical Analyses

The variability of the mean strain, SWV, and SWV ratio within false vocal folds and the ages, body mass index (BMI) of the participants was expressed as mean ± standard deviation (SD). The difference in the SWV between the right and left false vocal folds and between false vocal folds in adduction and abduction were analyzed by a paired *t* test. The difference in SWV, SWV ratio, and mean strain among the three age groups were tested using one-way ANOVA and post-hoc. The correlation between the SWV and SWV ratio measured in the right and left false vocal folds were tested by the linear regression. Intra- and inter-observer reliability was analyzed using intraclass correlation coefficient. $P < .05$ considered statistically significant. Statistical analyses were performed with commercial software (SPSS, Version 27.0, SPSS, Intel).

Results

We initially recruited 30 participants for ultrasound elastography measurements from February to April 2021. The current study consists of the analysis of 28 healthy subjects from this population. Two participants were excluded: one participant with hoarseness and one underwent total thyroidectomy were excluded from the analysis (Fig. 1). All data from the healthy subjects were used in this analysis including mean strain, SWV, SWV ratio of FVF and demographic information of participants are listed in Table 1.

The difference in SWV in FVF abduction and SWV ratio were significant between senior and young age group ($p < .05$). SWV of FVF was higher and SWV ratio was lower in seniors compared to those in young participants. Differences in mean strain and SWV in FVF adduction were not significant among the three age groups ($p > .05$, Table 2). Strong correlations in SWV between the right and left FVF in FVF abduction ($R^2 = 0.948$, Fig. 5a), adduction ($R^2 = 0.895$, Fig. 5b), and mean strain ($R^2 = 0.984$, Fig. 5c) were observed.

Intra-observer repeatability and inter-observer reproducibility were moderate to excellent (ICC = 0.75- 0.92, Table 3).

Discussion

Yet, imaging true and false vocal folds is not well established although approximately 10% to 30% of the population are affected by problems of their voice in their lifetime, professional speakers or singers up to 50%.[14] Patient management can be improved by introducing a reliable, non-invasive method for assessment of the change in vocal folds function. Our study showed the effectiveness of SWE and ultrasound strain imaging for assessing false vocal fold stiffness and function that may change with age. This may eliminate the need for invasive procedures (such as endoscopic vocal cord biopsies or endoscopic ultrasonography) when assessing changes in vocal fold function in elderly.[15,16] To our knowledge, this is the first report of ultrasound elastography to quantify changes in the false vocal folds stiffness (in adduction and abduction) and symmetry associated with aging. Attempts were made to compare the varying muscle fiber tensions in healthy individuals across different age groups in the hope of correlating the influence of age on the FVF stiffness and function.[15,16] Our results highlight the differences in ultrasound strain and SWV in varying states of FVF movement, associated with decreased elasticity in progressively older age groups.

There was no significant difference in SWV between the right and left FVF in the same age groups. The difference in SWV between FVF abduction and adduction in each age group was significant (Table 1). SWV measured in FVF abduction, occurring with FVF associated muscle full relaxation, was lower than in FVF adduction, occurring with FVF associated muscle maximal contraction. These results are consistent with previous data showing changes in muscle stiffness with variable degrees of passive muscle tension measured by SWV.[9]

There was a statistically significant difference in the SWV of false vocal folds between the senior and young age groups in FVF abduction ($P < .05$) (Table 1). Mean SWV in seniors was higher than participants in young age group (4.06 m/s vs 3.26 m/s). This suggests that age related changes in FVF may be important in establishing references ranges for future studies or clinical applications. These data may also serve as an initial reference for applications asessing muscle stiffness changes from other disorders when applied to different age groups, since FVF stiffness increases with age. Differences in FVF stiffness measured by SWV between the young and mid-age groups, and participants between the mid-age and senior age groups were not significant (*P* > .05) (Table 2). There was no significant difference in the mean SWV representing mechanical properties of false vocal folds between all groups in FVF adduction (*P* > .05) (Table 1). Since this is the first observation of age-related changes in measured FVF stiffness by ultrasound elastography, larger studies may be needed to detect more subtle changes. There are additional factors that must be considered when assessing FVF stiffness. For example, underlying tissue stiffness changes may be compensated for by the contractibility of FVF and associated muscles in healthy seniors. Another factor is the difficulty in normalizing a "maximal contraction" when patients perform the Valsalva maneuver for measuring SWV in the FVF adducted (contraction). There are data to suggest that varying the force (strength) of voluntary muscle tension placed on the FVF may only lead to insignificant differences in evaluating the muscle stiffness in contracted muscles.[7] Given the complexity of the anatomy and mechanical changes our findings suggest that the FVF tissue properties should be evaluated in both the abducted state and adducted state to help account for changes in FVF stiffness in different stages of voluntary muscle contraction and relaxation.

The difference in the developed SWV ratio (a ratio of the difference in SWV between the FVF in abduction and adduction to FVF abduction) was also significant between the young age and senior groups (Table 1). The SWV ratio in seniors was substantially lower than young age participants (0.22 vs 0.47, *p*= .04) suggesting that the contractibility of FVF decreased with age. This may help explain clinical symptoms in the development of age-related vocal disorders.[5] These results may be helpful as an important reference when evaluating diminished function of vocal folds caused by thyroid and parathyroid surgery, as well as other neuromuscular disorders (e.g., Parkinson disease).[17-19] Our ultrasound elastography age dependent findings for SWV and SWV ratio are consistent with observations by others that age-related FVF local tissue degeneration may lead to an increase of stiffness and decrease of function of FVF.[2,3]

Having symmetry of FVF mechanical properties is essential for maintaining normal function of voice production, breathing, and swallowing.[10] In this study with healthy subjects, we found SWV and mean strain of bilateral FVF demonstrated strong correlations between sides of the false vocal folds in abduction, in adduction, and during movement from abduction to adduction (Table 3). Asymmetry may detect unilateral FVF movement abnormalities caused by either primary muscle fiber pathology or secondary to nerve damage in a neck surgery. A previous studies has shown that a poor correlation in SWV between opposite FVF sides may indicate asymmetric tissue mechanics in patients with unilateral nerve injury or unilateral vocal cord paralysis.[12,19]

An important additional observation, we successfully performed ultrasound strain and SWE of FVF in all 28 subjects, differing from previous observations that soft-tissue-air interface and calcified thyroid cartilage in adults precluded the visualization of laryngeal structures and muscles.[20] Additionally, we demonstrated good to excellent intra-observer and inter-observer reproducibility in measuring SWV of the false vocal folds performed by two junior operators (ICC > 0.75; $P < .001$) (Table 3). These two observations suggest that this technique might be more approachable for future study and clinical adoption than previously realized. There are advantages of using ultrasound strain imaging to assess FVF compared with acoustic radiation force impulse (ARFI) based SWE. Ultrasound strain (fraction) represents the relation between the altered tissue lengths (tissue shortening in contraction or deformation under compression) to its initial length for quantifying the tissue movement or deformation associated with tissue function and stiffness. [12] The locations of ultrasound speckles (kernel) in sequence of FVF grayscale real time cine loops change following FVF movement from abduction to adduction, which can be processed (tracked) using 2-D speckle tracking software frame by frame and displayed using a strain curve (Fig. 4). [12] SWE only measures the stiffness of FVF in abduction and adduction on static images. [13] Furthermore, tissue strain can be processed based on DICOM ultrasound cine loops recorded from any ultrasound scanners (portable or stationary) using an off-line 2-D speckle tracking software. To date, however, ARFI based SWE still require a dedicated high-end ultrasound scanner.

There were limitations to this study, principally related to the relatively small number of participants enrolled in the study. In addition, our study was not designed to detect pathological changes that may impact the functionality of the false vocal folds. We anticipate that the value of the SWV of false vocal folds may differ in a population with laryngeal disorders (e.g. vocal fold impairment). Nevertheless, detecting age related changes in the general population will be important in establishing references ranges for ultrasound elastography to be used in detecting FVF pathology. Disorders that lead to symmetrical FVF pathology may need age-dependent references to be used clinically. Another measurement factor that needs to be considered is that each participant had differing levels of contraction when performing the Valsalva maneuver, and thus muscle tension. These differences may lead to variation in the SWV. More work is required to understand how to interpret ultrasound elastography in FVF disorders, such as benchmarking with other imaging modalities, such as laryngeal magnetic resonance or electromyography.

Further, other patient life style and populations must be tested, such as history of smoking or smokeless tobacco use and vocal performing artists, to understand the breadth of measurement ultrasound elastography FVF results in health, different life style, and disease. The reproducibility of performing ultrasound strain of FVF over time was not conducted in the study. However, good intra-observer repeatability has been previously reported. [12]

In conclusion, our study results suggest that SWE is feasible for noninvasive assessment of stiffness, function, and symmetry of the false vocal folds through a transcutaneous approach. Additionally, we detected age-related changes in an increase of FVF stiffness and decrease of FVF contractibility in seniors compared with young age participants in this healthy population without known vocal fold disorders. Bilateral FVF symmetry tissue stiffness is demonstrated by close correlations of SWV in abduction and adduction representing SWV ratio, and mean strain representing FVF function in healthy adults. Additional study using ultrasound elastography to further assess age-related and pathologic changes in true vocal folds and false vocal folds disorders is warranted.

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Tables

Table 1. Shear wave elastography of false vocal folds among the three age groups

Parameter	Young-age	Mid-age	Old-age	ANOVA (*p*)
Number of subjects	10	9	9	
Male/Female	5/5	2/7	5/4	
Mean age (y)	25±1	54±8	69±3	< .001
Body mass index (kg/m^2)	23.3±3.21	23.3±5.02	26.7±3.9	.14
SWV: FVF-Abduction (m/s)	3.26±1.0	3.47±1.0	4.06±0.87	.04
SWV: FVF-Adduction (m/s)	4.36±1.08	4.56±0.93	4.88±1.12	.32
SWV ratio	0.47±0.37	0.44±0.34	0.22±0.19	.04
FVF mean strain	0.22±0.08	0.21±0.10	0.18±0.07	.33

Note: * p value shows the difference in shear wave velocity (SWV, m/s) in false vocal folds abduction and adduction among the three age groups tested by one-way analysis of variance (ANOVA); FVF, false vocal fold; SWV, shear wave velocity; SWV ratio is defined by SWV ratio= $(SWV_{adduction} - SW_{abduction})/SW_{abduction}$.

Table 2. Post-hoc of FVF SWV in abduction and adduction among the three age groups

Post-hoc*	Young-age vs mid-age	Young-age vs old-age	Mid-age vs old-age
SWV: FVF Abduction	§0.21, -0.54-0.96, 0.78	0.79, 0.04-1.54, 0.04	0.58, -0.19-1.35, 0.18
SWV: FVF Adduction	0.20, -0.62-1.02, 0.83	0.52, -0.30-1.34, 0.29	0.32, -0.52-1.16, 0.63
FVF SWV ratio	-0.03, -0.27-0.21, 0.95	0.25, -0.49-0.01, 0.04	-0.22, -0.47-0.03, 0.09
FVF mean strain	-0.01, -0.08-0.06, 0.93	-0.04, -0.11-0.03, 0.32	-0.03, -0.10-0.04, 0.54

Note: *Tukey HSD (Honestly Significant Difference) post-hoc test, to exam that groups significantly differ from which others and provide 95% confidence intervals around the differences between the groups. §difference, 95% confidence interval, p value based on post-hoc analysis; FVF, false vocal folds; SWV, shear wave velocity (m/s); SWV ratio is calculated by SWV ratio= $(SWV_{adduction} - SW_{abduction})/SW_{abduction}$.

Table 3. Intra- and inter-observer reliability in measuring SWV of the false vocal fold

Average Measures	Intraclass Correlation*	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	F Test with True Value 0 Value	F Test with True Value 0 Sig
Operator 1:Operator 1 -FVF adduction	.92	.85	.96	12.86	< .001
Operator 2:Operator 2 -FVF adduction	.82	.67	.91	5.64	< .001
Operator 1:Operator 2 -FVF adduction	.75	.65	.87	3.99	< .001
Operator 1:Operator 1 -FVF abduction	.89	.79	.94	9.19	< .001
Operator 2:Operator 2 -FVF abduction	.85	.72	.92	6.83	< .001
Operator 1:Operator 2 –FVF abduction	.87	.77	.93	7.66	< .001

Note: *ICC, intraclass correlation coefficient using a consistency definition; Inter-observer reliability test: measurements performed by the operator 1 to measurements performed by the operator 2 on the same patients separately; intra-observer reliability tests: measurement 1 to measurement 2 performed on the same patients by a single operator. Operator 1 and operator 2 had 2y training in performing neck and soft tissue ultrasound and 40h training for performing shear wave elastography. SWV, shear wave velocity.

Figure legends

Figure 1. Flow chart for participant enrollment.

Figure 2. Illustration shows the anatomy of true and false vocal folds.

Figure 3a-d. Virtual Touch IQ (VTIQ) was used for shear wave elastography (SWE) of false vocal folds (FVF). Shear wave quality map (quality map) is displayed side by side with B-mode image prior to measuring shear wave velocity of FVF (white arrow). The quality of SWE is considered valid once homogeneous green appearing in the entire region of FVF in abduction (a) and adduction (b). Color-coded shear wave velocity image of FVF is displayed side by side with B-mode image. Point shear wave elastography (circle region of interest with 0.3 cm in diameter) is used to measure shear wave velocity (SWV, m/s) in each side of FVF, twice in abduction (c) and twice in adduction (d). It is clear to note that SWV is low in FVF abduction (FVF relaxation) and high in FVF adduction (FVF contraction).

Figure 4a-c. Cine loops of real-time grayscale image of false vocal folds (FVF) containing FVF dynamic movement from abduction (a) to adduction (c) were recorded for offline process using 2-D speckle tracking software. Ultrasound strain represents the movement and symmetry of right (red dotted and solid lines) and left (cyan dotted and solid lines) FVF. Mean strain is the average of strain produced during one cycle of FVF movement from abduction (a and b, yellow arrow) to adduction (b and c, red arrow). The notion is that FVF tissue lengthened (b, positive strain) in FVF abduction and shortened (b, negative strain) in FVF adduction.

Figure 5a-c. Scatter plots show good correlations of shear wave velocity (SWV, m/s) between the right and left false vocal folds (FVF) stiffness in FVF abduction (a, R^2= 0.948) and in adduction (b, R^2= 0.895). A strong correlation between the right and left FVF movement assessed by the mean strain is also demonstrated by linear regression (c, R^2= 0.984).

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Date: 5/4/2022

Your Name: Jing Gao

Manuscript Title: Ultrasound Elastography Detects Age-related Changes in Adult False Vocal Folds

Manuscript Number (if known): JUM-2022-03-0305 R1

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

The author's relationships/activities/interests should be defined broadly. For example, if your manuscript pertains to the epidemiology of hypertension, you should declare all relationships with manufacturers of antihypertensive medication, even if that medication is not mentioned in the manuscript.

In item #1 below, report all support for the work reported in this manuscript without time limit. For all other items, the time frame for disclosure is the past 36 months.

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	**Specifications/Comments (e.g., if payments were made to you or to your institution)**
Time frame: Since the initial planning of the work			
1	All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.) **No time limit for this item.**	☒ None	
			Click the tab key to add additional rows.
Time frame: past 36 months			
2	Grants or contracts from any entity (if not indicated in item #1 above).	☒ **None**	
3	Royalties or licenses	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
4	Consulting fees	☒ **None**	
5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
6	Payment for expert testimony	☒ **None**	
7	Support for attending meetings and/or travel	☒ **None**	
8	Patents planned, issued or pending	☒ **None**	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ **None**	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
11	Stock or stock options	☒ **None**	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☐ **None**	
			Loaned ultrasound scanner from Siemens Healthineers to Rocky Vista University
13	Other financial or non-financial interests	☒ **None**	

Please place an "X" next to the following statement to indicate your agreement:

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ICMJE DISCLOSURE FORM

Date:	5/4/2022
Your Name:	Julian Ortiz
Manuscript Title:	Ultrasound Elastography Detects Age-related Changes in Adult False Vocal Folds
Manuscript Number (if known):	JUM-2022-03-0305 R1

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
6	Payment for expert testimony	☒ **None**	
7	Support for attending meetings and/or travel	☒ **None**	
8	Patents planned, issued or pending	☒ **None**	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ **None**	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
11	Stock or stock options	☒ **None**	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ **None**	
13	Other financial or non-financial interests	☒ **None**	

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ICMJE DISCLOSURE FORM

Date: 5/5/2022

Your Name: James Hamilton

Manuscript Title: Ultrasound Elastography Detects Age-related Changes in Adult False Vocal Folds

Manuscript Number (if known): JUM-2022-03-0305 R1

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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3	Royalties or licenses	☒ **None**	

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
6	Payment for expert testimony	☒ **None**	
7	Support for attending meetings and/or travel	☒ **None**	
8	Patents planned, issued or pending	☐ **None**	
		Epsilon Imaging Inc.	I am inventor on two issued patents assigned to Epsilon Imaging Inc., who produced SW used in analysis described in the manuscript.
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ **None**	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
11	Stock or stock options	☐ **None**	
		Epsilon Imaging Inc	I hold stock and stock options in Epsilon Imaging Inc., who produced software used in analysis described in manuscript.
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ **None**	
13	Other financial or non-financial interests	☒ **None**	

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ICMJE DISCLOSURE FORM

Date: 5/4/2022

Your Name: William Weitzel

Manuscript Title: Ultrasound Elastography Detects Age-related Changes in Adult False Vocal Folds

Manuscript Number (if known): JUM-2022-03-0305 R1

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		Time frame: past 36 months	
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3	Royalties or licenses	☒ **None**	

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ None	
6	Payment for expert testimony	☒ None	
7	Support for attending meetings and/or travel	☒ None	
8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ None	

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12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ **None**	
13	Other financial or non-financial interests	☒ **None**	

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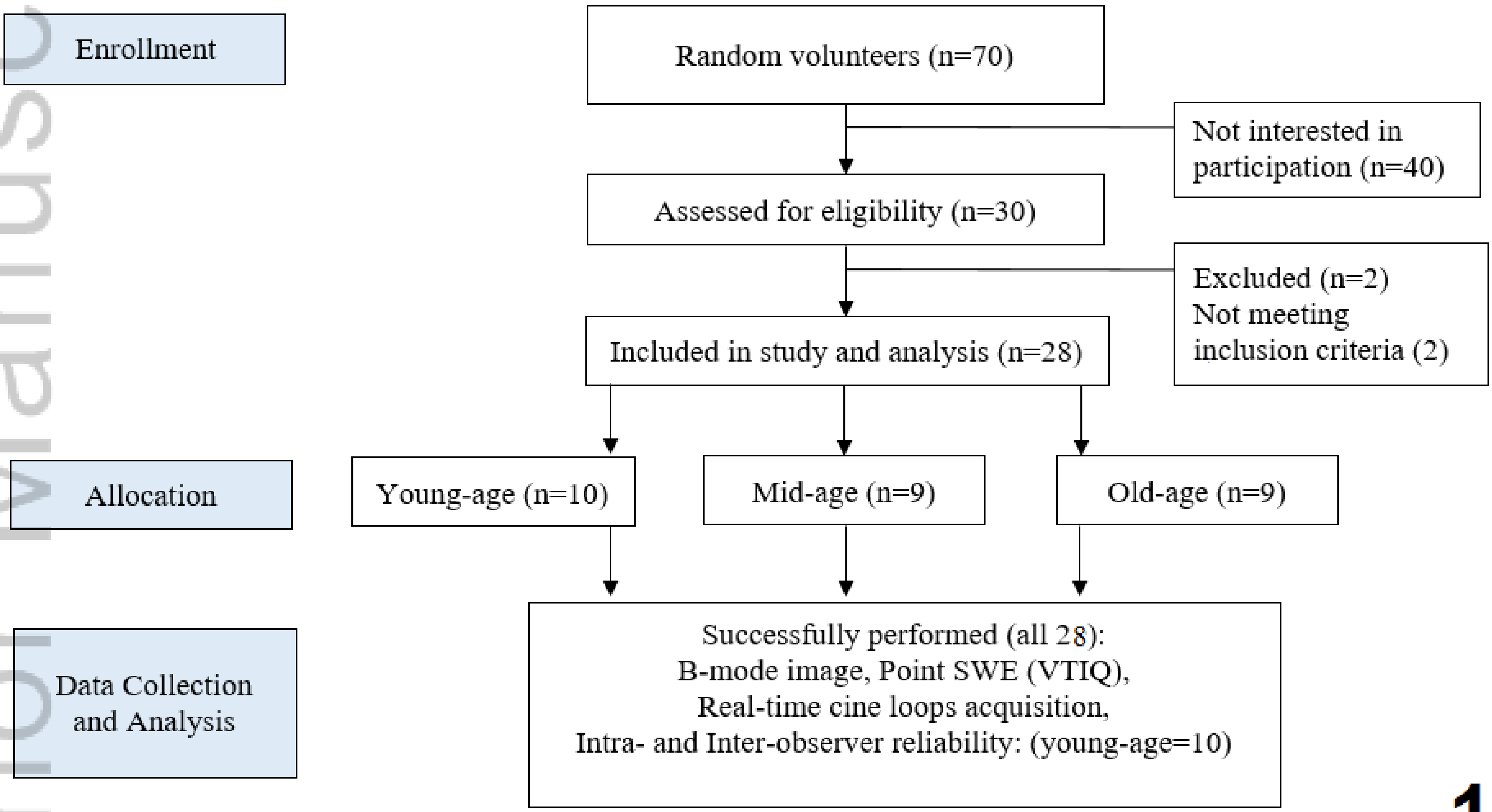

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Figure 1.tif

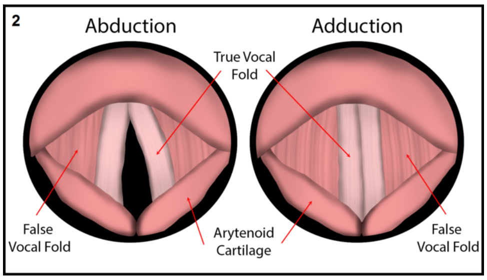
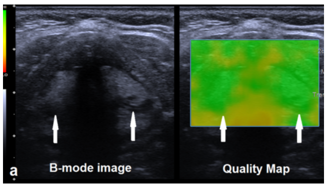


Figure 2. 600.tif



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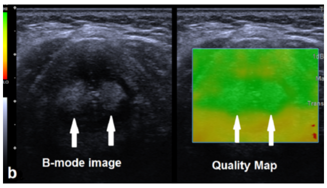
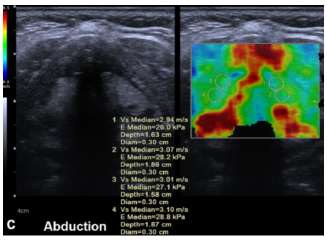


Figure 3b600.tif



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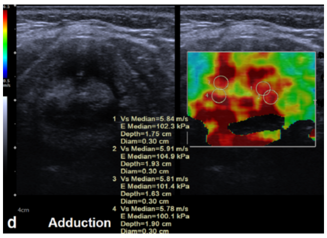

Figure 3d600.tif

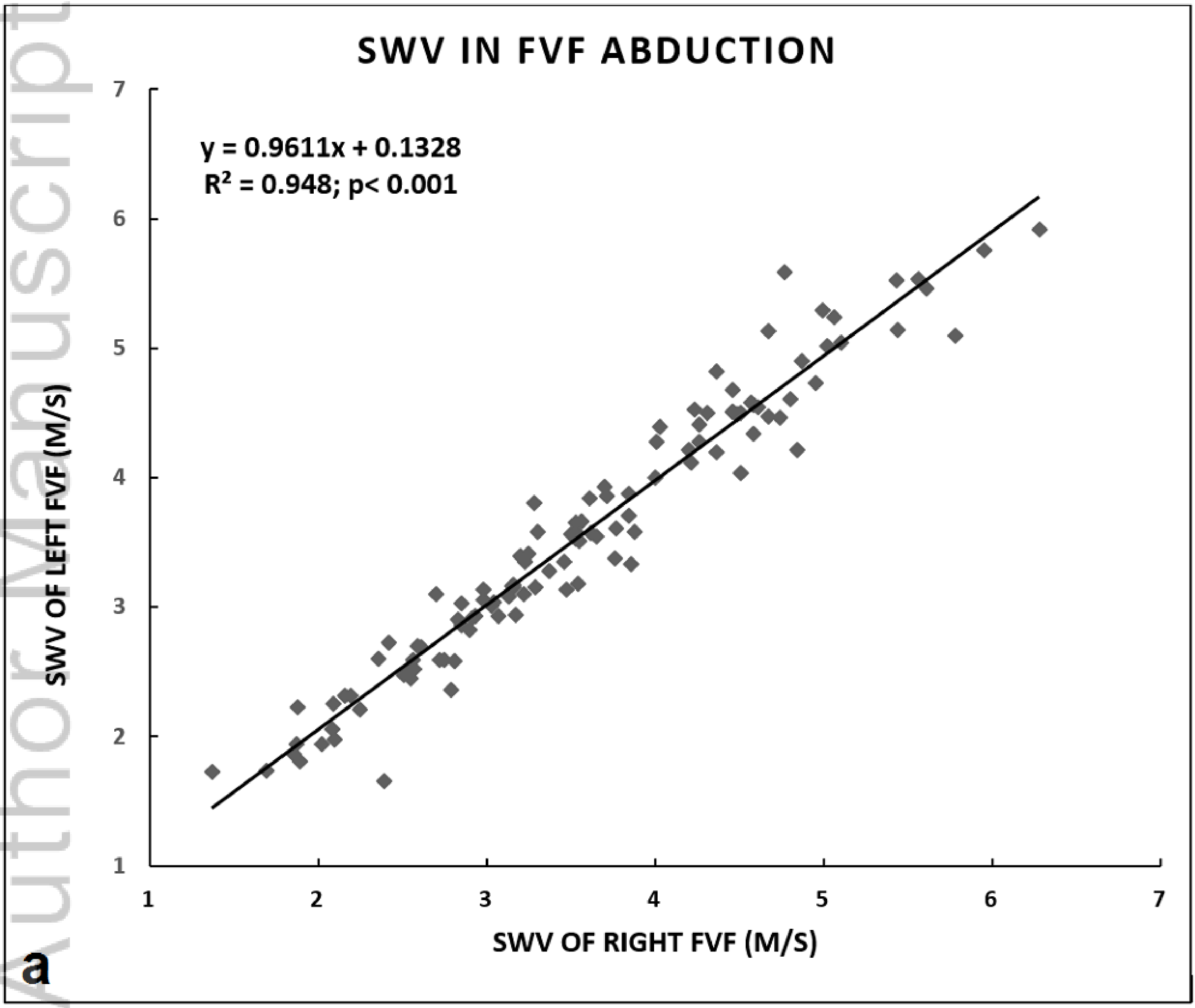

Figure 5a.tif

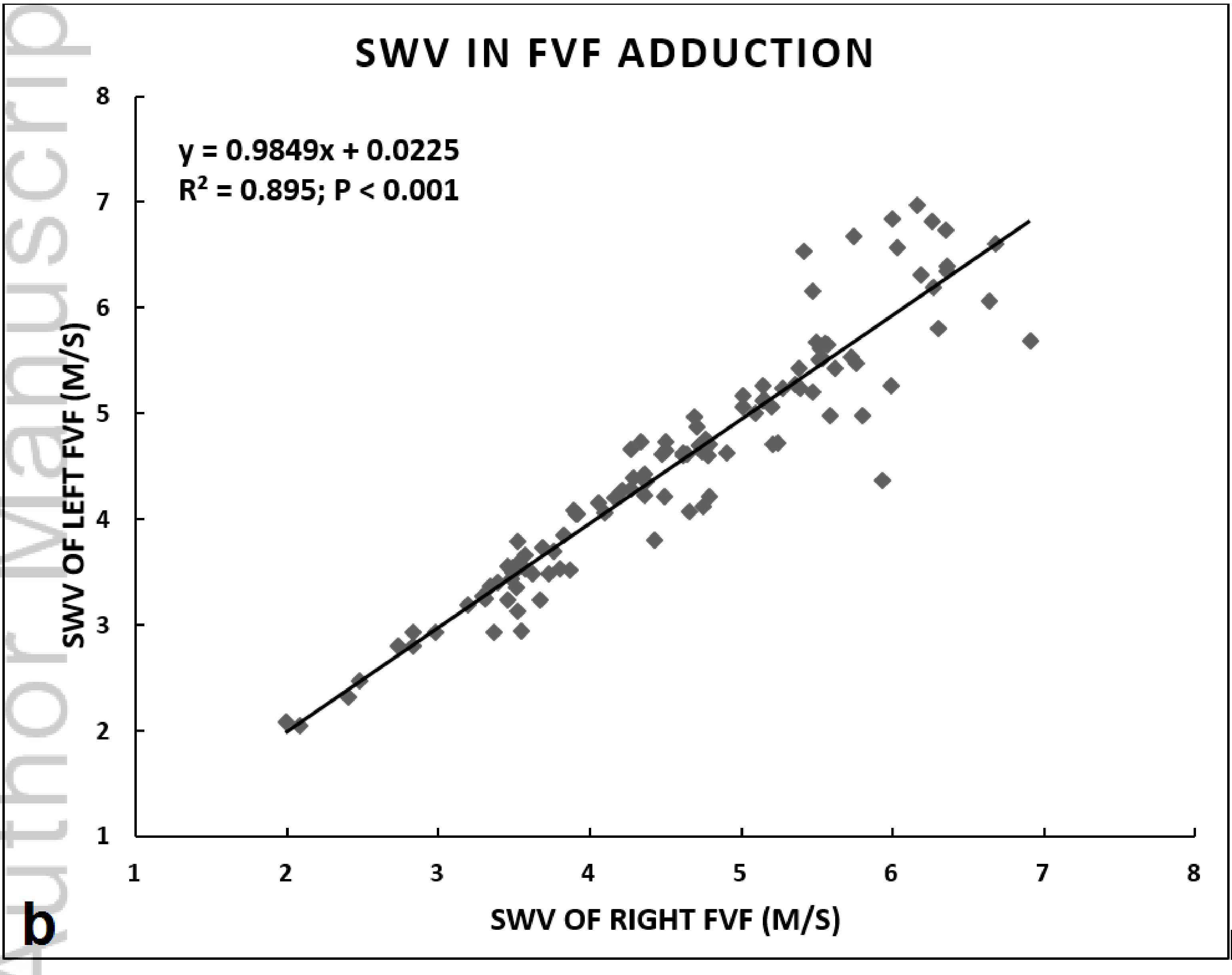

Figure 5b.tif

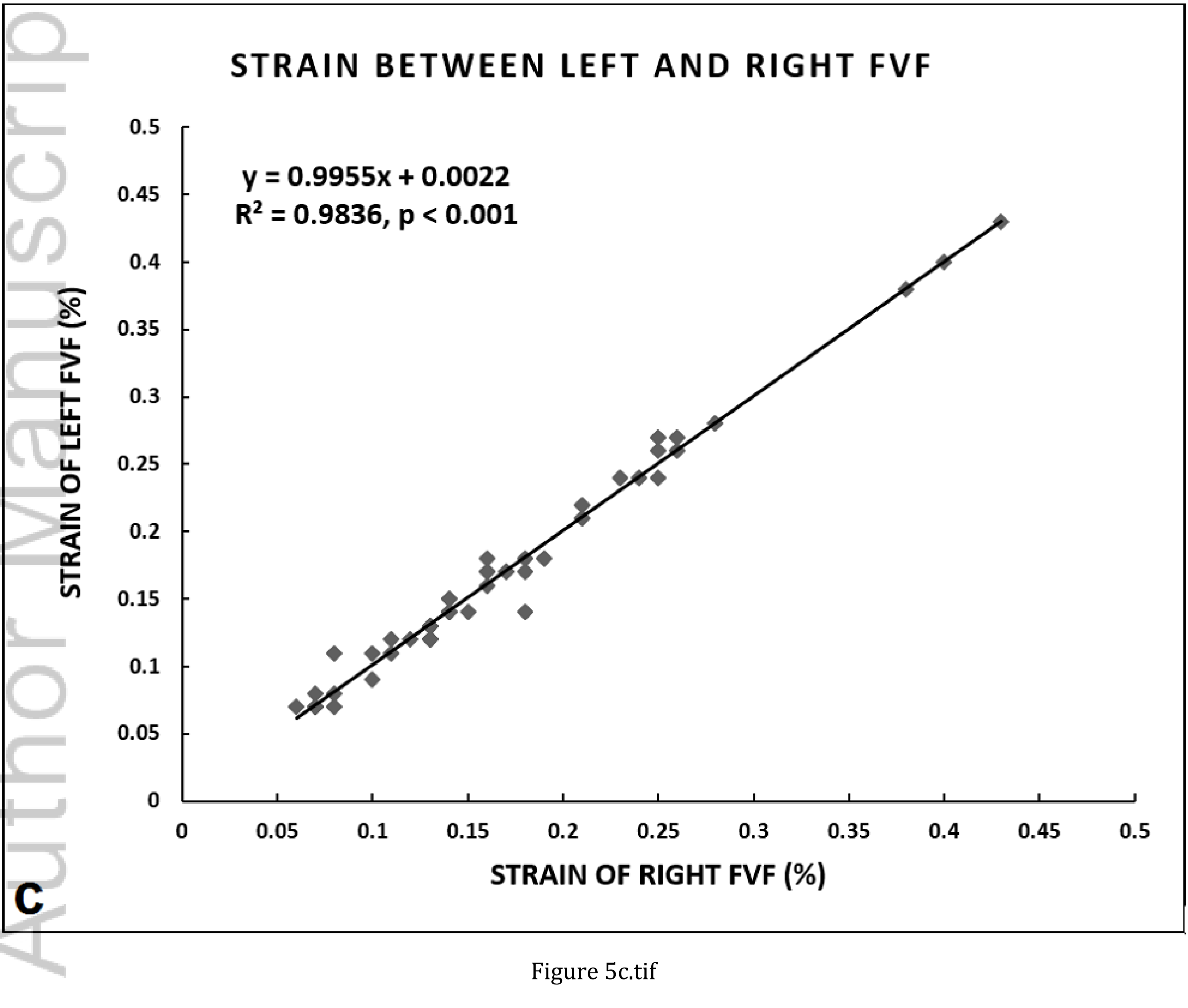

Figure 5c.tif

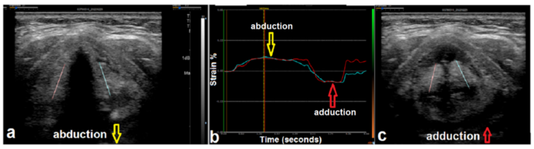

Figure 4600.tif