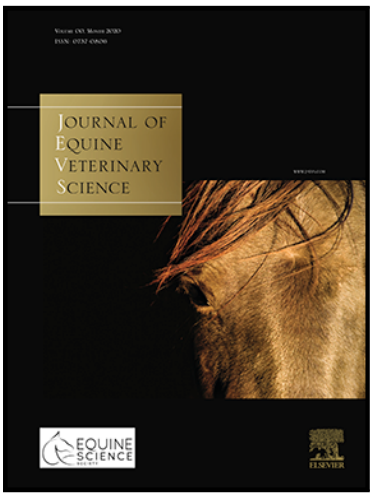

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Feasibility of echocardiographic estimation of Pulmonary Artery Stiffness in horses

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Highlights

- In humans, Pulmonary Artery Stiffness is an index of pulmonary artery elasticity
- Pulmonary Artery Stiffness can be measured by pulsed-wave Doppler
- Pulmonary Artery Stiffness is easily measurable in horses
- Measurement of Pulmonary Artery Stiffness in horses is reproducible

Summary

Pulmonary artery stiffness (PAS) is an index of pulmonary artery elasticity that permits to evaluate the pulmonary vascular bed in humans. It can early detect an increase in pulmonary artery stiffness as a consequence of remodeling of the vessel wall caused by chronic pulmonary and congenital heart diseases. This remodeling can occur also in horses with chronic respiratory diseases. Thus, PAS could be a useful echocardiographic parameter also in horses. However, in literature, there are no studies regarding PAS in horses. The aim of this study is to evaluate the feasibility of PAS in horses. Fifteen healthy horses were included in this prospective study. Maximal frequency shift (MFS) and acceleration time (AT) were measured from the pulsed-wave Doppler trace of the pulmonary artery flow, obtained from the right parasternal short-axis view at the level of the pulmonary artery, and then PAS was calculated as the ratio of MFS to AT. The low variability assessed for intra- and inter-observer variability, day-to-day variability and image acquisition variability suggests that PAS can be measured consistently in horses. Further studies could be useful to assess the clinical usefulness of PAS in unhealthy horses, such as those affected by chronic respiratory diseases.

Keywords

Pulmonary artery stiffness, pulsed-wave Doppler, horse, echocardiography

1. Introduction

The pulmonary artery stiffness (PAS) is an index of pulmonary artery elasticity that permits to evaluate the structural features and function of the pulmonary vascular bed in humans [1].

The gold standard to assess the pulmonary vascular pressure and resistance is right cardiac catheterization; however, it represents an invasive procedure and therefore, it is not useful in routine practice [2]. In humans, PAS can be calculated non-invasively by

means of computed tomography or magnetic resonance; however, the disadvantages of these two methods are radiation exposure, potential complications of contrast agents and high costs [1,3]. Görgulü and colleagues [4] proposed and validated a non-invasive method to determine PAS using echocardiographic pulsed-wave Doppler parameters in humans. PAS (KHz/sec) can be assessed from the pulsed-wave Doppler trace of the pulmonary artery flow obtained from the parasternal short-axis view, and it is calculated as the ratio of maximal frequency shift (MFS) to acceleration time (AT) [4].

In human medicine, PAS is mainly used as an early predictor of pulmonary hypertension in patients with different chronic cardiac or pulmonary diseases, which can lead to remodeling of the pulmonary vascular bed over time [1,3]. In particular, different studies reported an increase of PAS value in people with congenital heart diseases (atrial septal defect, ventricular septal defect, patent ductus arteriosus, atrioventricular septal defect) [4,5], in young and adult patients with asthma [1,6], in patients with obstructive sleep apnea syndrome [2,3] and with chronic obstructive pulmonary disease [7].

Moreover, PAS seems to be an index of subclinical right ventricular dysfunction in patients with chronic pulmonary diseases [1]. Furthermore, it has been demonstrated an increase of PAS in patients with heart failure (HF) and reduced ejection fraction without echocardiographic evidence of pulmonary hypertension, or right heart failure; in addition, in these patients, it has been demonstrated that PAS is associated with NYHA (New York Heart Association) functional class. NYHA classify the HF based on the activity that the patient can perform without the manifestation of symptoms such as dyspnea [8]. Finally, it is reported that PAS is a useful marker of early subclinical myocardial dysfunction in children at risk for obesity (children with a body mass index between the 85th and 94th percentile for age and sex); in fact, in these patients, atherosclerosis and the consequent changes in artery elasticity, begins early in childhood [9].

As in humans, chronic respiratory diseases are able to cause remodeling of the pulmonary artery wall even in horses, with a consequent reduction in pulmonary artery elasticity [10,11]. Therefore, according to the above-mentioned use of PAS in the evaluation of pulmonary vascular bed in humans, it can be assumed that PAS could be a useful parameter even in horses affected by chronic lung diseases. To the authors' knowledge, PAS was not previously measured in equines. Other authors evaluated the feasibility of right pulmonary artery fractional dimensional change (ΔRPA) measurement in healthy horses by echocardiography; ΔRPA represents an index of the right pulmonary artery elasticity [12]. There is an evident interest in the evaluation of echocardiographic parameters useful in assessing the elasticity of the pulmonary arteries in horses. However, there are no studies on the use of PAS in horses in the literature. Therefore, the aim of this study is to evaluate reproducibility of PAS measured by pulsed-wave Doppler echocardiography in healthy horses.

2. Materials and Methods

2.1. Horses

For this prospective study, approved by the Institutional Animal Care Committee (OPBA_54_2021) of the University of Milan, client-owned horses were selected according to the absence of cardiac and respiratory signs at history, clinical examination, echocardiography and ultrasonography of the thorax. A owners' informed consent was obtained.

2.2. Echocardiography

During echocardiographic examination [13], horses were held in a relaxed and standing position by means of halter and lead rope, no other physical or pharmacological containment methods were used. Echocardiographic images were obtained using an ultrasound machine (MyLabOmegaVet, Esaote, Genoa, Italy) with a 2.5 MHz phased-array transducer (P1-5, Esaote, Genoa, Italy), and an ECG was recorded simultaneously for

timing of the measurements within the cardiac cycles. Pulsed-wave Doppler trace across the pulmonary valve was performed from the right parasternal short-axis view at the level of the pulmonary artery (Figure 1) by placing the sample volume (3 mm) on the arterial side of the pulmonary valve and verifying that it remained in the center of the artery during systole [14]. No correction of the sampling angle was attempted. A satisfactory alignment between blood flow and ultrasound beam was assumed when the audible signal was clear and the envelope of the waveform was complete. Moreover, when onset of flow on the baseline was difficult to distinguish, due to low velocity signals, it was determined by extrapolation of the downstroke; to minimize the error, low velocity filters were set as low as possible [14]. Echocardiographic machine settings were adjusted to measure MFS (KHz) as the peak systolic frequency shift [4]. Acceleration time (sec) was measured from the onset of the pulmonary Doppler flow trace to the beginning of maximum velocity plateau (Figure 2) [14]. Pulmonary artery stiffness (KHz/sec) was then calculated as the ratio of MFS to AT [4]. In order to minimize the influence of respiratory and cardiac cycles, each parameter was calculated as the average of measurements taken from three consecutive Doppler flow traces.

2.3. Statistical analyses

Data distribution was tested for normality using Shapiro-Wilk test. Normally distributed data are presented as mean and standard deviation. Not normally distributed data are presented as median and interquartile range (IQR).

Inter-observer variability, intra-observer variability, variability between different echocardiographic sessions (day-to-day variability) and inter-observer variability for image acquisition were evaluated to assess reproducibility.

To assess inter-observer variability, two operators performed all the measurements independently on the same three cardiac cycles. Moreover, in order to assess intra-observer variability, one operator repeated all the measurements twice on the same three

cardiac cycles. In addition, the two operators acquired echocardiographic pulsed-wave Doppler images independently in 4 horses to assess the inter-observer variability for acquisition of the image. Finally, in 4 horses one operator repeated acquisition of the image and measurements in different days to evaluate the day-to-day variability.

A Bland-Altman test and a simple linear regression analysis were performed to evaluate inter-observer variability, intra-observer variability, variability for acquisition of image and day-to-day variability. Moreover, a Pearson correlation was used, and according to the *r* value, the correlation was classified as follows: $r = 1$ as perfect, $r > 0.8$ as very strong, $0.6 < r < 0.8$ as strong, $r < 0.6$ as weak and $r = 0$ as no correlation [15]. Finally, the within-subject variance for repeated measurements (residual mean square) was determined by a one-way ANOVA; then the within-subject standard deviation (s_w) was calculated as the square root of the residual mean square. The coefficient of variance (CV) was calculated dividing the s_w by the grand mean and expressed as percentage. According to the CV, the variability was classified as follows: CV < 15% as low variability; 15% < CV < 25% as moderate variability; CV > 25% as high variability [16].

For these statistical analyses, the operators measured each parameter from three consecutive Doppler flow traces and the median value was used.

All the statistical analyses were performed using the statistical analysis software Graphpad Prism V. 9.1.2, and statistical significance was set at $p < 0.05$.

3. Results

3.1. Horses

According to the inclusion criteria, 15 healthy horses of different breeds (6 Thoroughbreds, 4 Standardbreds, 5 other breeds) were included. Among them, there were 8 geldings, 5 females and 2 males. The age ranged between 3 years old and 16 years old (median 5 years old; IQR 8 years old). The mean body weight was 484 ± 56 kg (range 400 - 600 Kg). Nine of the racehorses were in athletic activity, according to their age and training level;

one Standardbred horse was retired from racetrack; the other five horses performed mild exercise. The heart rate ranged between 28 bpm and 44 bpm (mean 37 ± 5 bpm).

3.2. Pulsed -wave Doppler Echocardiography

Maximal frequency shift and acceleration time were measured, and pulmonary artery stiffness was calculated in all horses. All the parameters were normally distributed. The mean MFS was 1.70 ± 0.21 kHz (range 1.23-2.00 kHz). The mean AT value was 0.256 ± 0.047 seconds (range 0.184-0.360 seconds). The minimum value of PAS was 4.89 kHz/sec while the maximum value was 7.94 kHz/sec (mean value 6.76 ± 0.97 kHz/sec).

3.3. Reproducibility

The repeated measurements of MFS performed by the first operator showed a bias of <0.01 kHz (95% confidence interval, CI: -0.05 kHz to 0.05 kHz). Bias between the repeated measurements of AT was 0.003 seconds (95% CI: -0.018 sec to 0.025 sec). The calculated PAS values had a bias of 0.04 kHz/sec (95% CI: -0.41 to 0.49 kHz/sec). The correlation between the repeated measurements was very strong for all the parameters (MFS: $r = 0.99$, $p < 0.001$; AT: $r = 0.97$, $p < 0.001$; PAS: $r = 0.97$, $p < 0.001$). The intra-observer variability resulted low for all the parameters (MFS: CV = 5%; AT: CV = 8%; PAS: CV = 7%).

MFS measured by the two operators showed a bias of <-0.01 kHz (95% CI: -0.11 kHz to 0.09 kHz). Bias between the measurements of AT was <0.001 seconds (95% CI: -0.028 sec to 0.029 sec). The PAS values calculated by the two operators had a bias of 0.03 kHz/sec (95% CI: -0.70 kHz/sec to 0.76 kHz/sec). The correlation between the two operators was very strong for all the parameters (MFS: $r = 0.97$, $p < 0.001$; AT: $r = 0.96$, $p < 0.001$; PAS: $r = 0.93$, $p < 0.001$). The inter-observer variability resulted low for all the parameters (MFS: CV = 5%; AT: CV = 8%; PAS: CV = 9%).

Results of simple linear regression analysis performed for intra-observer variability and inter-observer variability are shown in Figure 3.

Regarding the inter-observer variability for acquisition of the image MFS, AT and PAS showed a bias of -0.01 kHz (95% CI: -0.18 kHz to 0.15 kHz), 0.004 sec (95% CI: -0.017 sec to 0.025 sec) and -0.01 kHz/sec (95% CI: -0.20 kHz/sec to 0.17 kHz/sec) respectively. The relationship between the two operators was good for all the parameters (MFS: $p = 0.025$, $r^2 = 0.95$; AT: $p = 0.009$, $r^2 = 0.98$; PAS: $p = 0.005$, $r^2 = 0.99$). The correlation between the two operators was very strong for all the parameters (MFS: $r = 0.97$, $p = 0.025$; AT: $r = 0.99$, $p = 0.009$; PAS: $r = 0.99$, $p = 0.005$). The inter-observer variability for image acquisition resulted low for all the parameters (MFS: CV = 5%; AT: CV = 10%; PAS: CV = 9%).

Regarding the day-to-day variability, MFS, AT and PAS had a bias of <-0.01 kHz (95% CI: -0.19 kHz to 0.18 kHz), -0.001 sec (95% CI: -0.030 sec to 0.028 sec) and 0.08 kHz/sec (95% CI: -0.13 kHz/sec to 0.30 kHz/sec) respectively. The relationship between measurements of different echocardiographic sessions was good for all the parameters (MFS: $p = 0.042$, $r^2 = 0.92$; AT: $p = 0.003$, $r^2 = 0.99$; PAS: $p = 0.007$, $r^2 = 0.98$). The correlation was very strong for all the parameters (MFS: $r = 0.96$, $p = 0.042$; AT: $r = 0.99$, $p = 0.003$; PAS: $r = 0.99$, $p = 0.007$). The day-to-day variability resulted low for all the parameters (MFS: CV = 6%; AT: CV = 12%; PAS: CV = 10%).

4. Discussion

PAS is an echocardiographic parameter introduced in human medicine; its usefulness lies in its ability to early detect an increase in pulmonary artery stiffness, as a consequence of a remodeling of the pulmonary vessel wall [1,3,4].

In human patients with chronic respiratory diseases, recurrent episodes of hypoxemia and hypercapnia, associated with inflammatory mediators and cytokines released due to chronic inflammation, cause structural modifications of the pulmonary vascular bed [3,4]. These changes consist in muscularization of pulmonary arterioles and excessive deposition of matrix proteins in the wall of large pulmonary arteries. The excess of

collagen compromises the elasticity of pulmonary arteries and this may be the reason for a high PAS value. Furthermore, stiff pulmonary artery causes elevated pulmonary vascular resistance and contributes to the raised oscillatory load that increases right ventricular systolic pressure. This stiffness itself leads to further damages to the pulmonary vascular bed over time, ultimately leading to pulmonary hypertension [1,3,4,6].

It is demonstrated that decreased pulmonary artery elasticity shortens the duration of right ventricular systolic ejection time primarily due to a reduction in the acceleration time of the pulmonary flow trace [3,4]. To maintain a constant flow within a stiff pulmonary artery where the acceleration time decreases, the velocity and MFS increase. Thus, PAS has been developed as an echocardiographic parameter calculated with a formula associated with AT [3,4].

As in humans, chronic respiratory diseases are able to cause remodeling of the pulmonary artery wall even in horses, with a consequent reduction in pulmonary artery elasticity [10,11]. Thus, PAS could be a useful echocardiographic parameter also in horses. However, to authors' knowledge no studies are available on PAS in the veterinary medicine literature, both in horses and small animals. Instead, other authors evaluated right pulmonary artery fractional dimensional change by echocardiography in dogs with pulmonary hypertension and demonstrated that ΔRPA represents an index of the right pulmonary artery distensibility, inversely related to pulmonary arterial pressure [17,18]. Recently, a study assessed the feasibility of measuring ΔRPA in healthy horses [12]. This demonstrates that there is an evident interest in the evaluation of echocardiographic parameters useful in assessing the elasticity of the pulmonary arteries.

The present study assessed the feasibility of PAS in horses. The echocardiographic evaluation of PAS can be considered easy and well tolerated by horses.

Moreover, the measurement of MFS, AT and the calculation of PAS showed a good reproducibility. The low variability (CV) for intra-observer variability, inter-observer

variability, day-to-day variability and inter-observer variability for image acquisition demonstrates how PAS can been measured consistently in horses with this technique.

No attempt to determine a reference range for PAS was done because of the limited number of subjects included in this study. The mean value of PAS in healthy horses was of 6.76 ± 0.97 kHz/sec with a minimum and a maximum value of 4.89 kHz/sec and 7.94 kHz/sec, respectively. In human medicine no agreement was found regarding the reference range of PAS measured in healthy patients by echocardiography [8]. In fact, various studies reported wide differences in PAS values in healthy people. A study investigating PAS in patients with heart failure with reduced ejection fraction, reported a PAS of 7.41 ± 1.32 kHz/sec in healthy people used as controls [8]. Another study investigated differences between recently diagnosed asthmatic patients and healthy people reporting a PAS of 22.4 ± 4.1 kHz/sec in the latter [1]. Other two studies, that evaluated PAS in people with obstructive sleep apnea syndrome, indicated for the control group a PAS of 18.0 ± 3.5 kHz/sec [3] and of 18.6 ± 6.3 kHz/sec [2].

In this preliminary study, feasibility of PAS measurement in healthy horses was assessed, while further studies on a large number of healthy horses are necessary to establish PAS reference intervals. Moreover, it would be interesting to evaluate the possible effect of age, sex, bodyweight, heart rate, training and athletic discipline on PAS value. Finally, further studies could be useful to assess the clinical utility of PAS in unhealthy horses, such as subject affected by chronic respiratory diseases; in this prospective, it would be useful to confirm the correlation between PAS and pulmonary pressure by catheterization of the right heart.

5. Conclusions

In conclusion, our results suggest that pulmonary artery stiffness can be measured consistently by pulsed-wave Doppler echocardiography across the pulmonary valve in horses. The technique is quite easy and well tolerated by subjects.

Figure capture

Figure 1. Right parasternal short-axis view at the level of pulmonary artery. This systolic view shows the pulmonary artery (a) and the aortic valve (b).

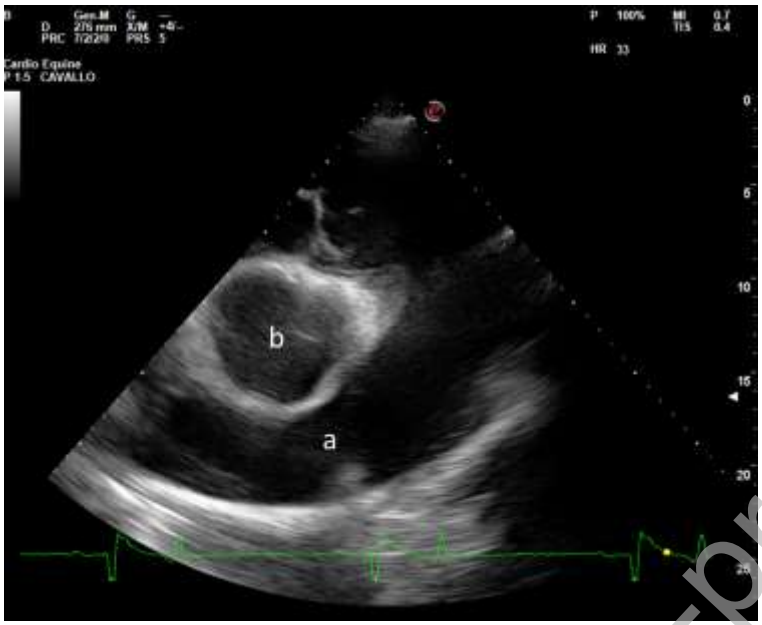


Figure 2. Pulsed wave Doppler trace across the pulmonary valve.

A) Right parasternal short-axis view at the level of the pulmonary artery demonstrating the positioning of the sample volume in proximal pulmonary artery and representative simultaneous pulsed-wave Doppler trace.

B) Zoom of the Doppler waveform from which the maximal frequency shift (MFS; blue line) and the acceleration time (AT; green lines) can be measured.

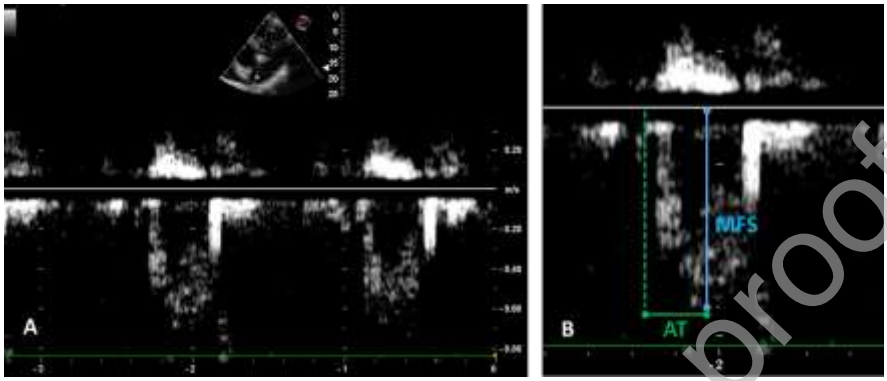
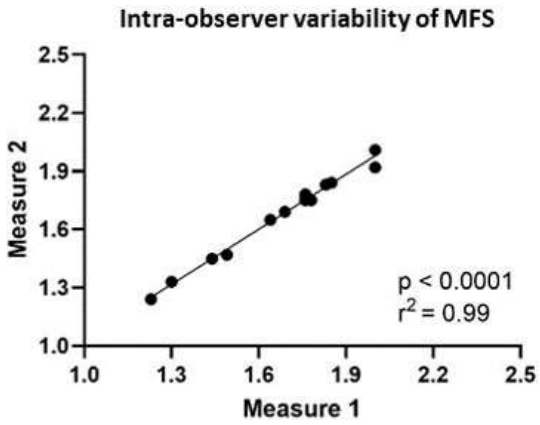
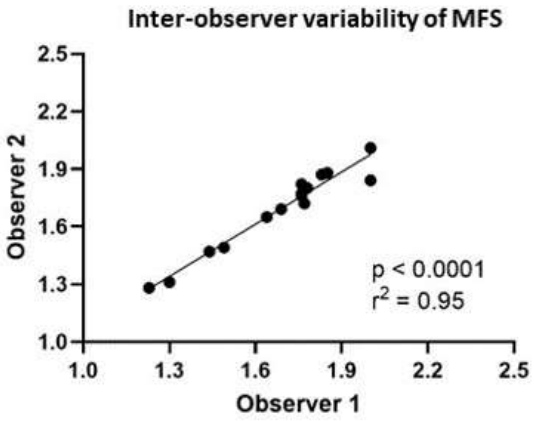
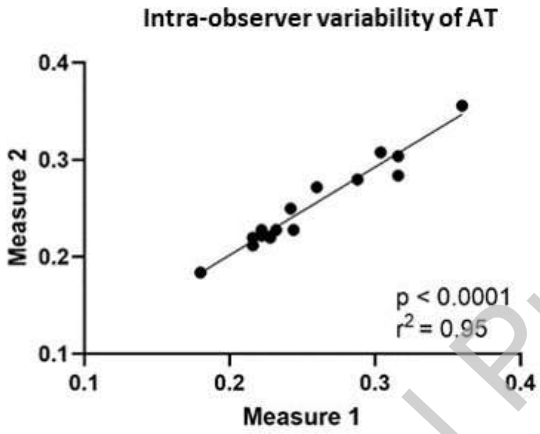
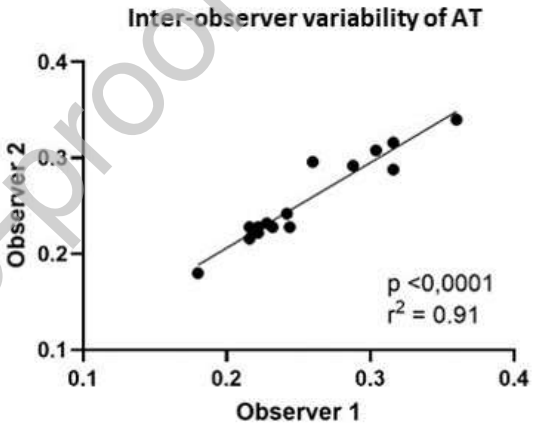


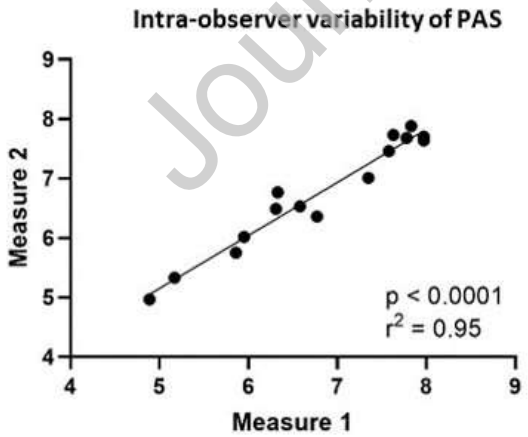
Figure 3. Results of simple linear regression analyses for the evaluation of intra-observer variability and inter-observer variability of maximal frequency shift (MFS), acceleration time (AT) and pulmonary artery stiffness (PAS).

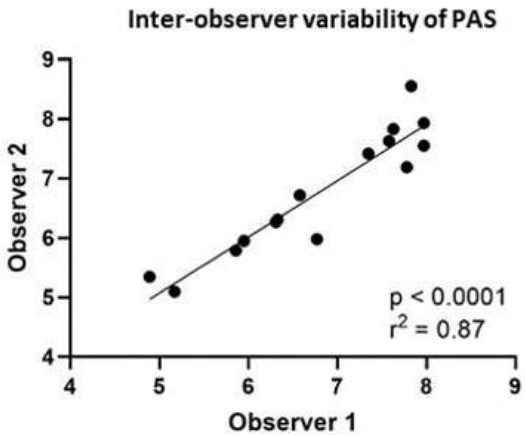












Author Contributions

Elena Alberti: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft and writing – review & editing; Luca Stucchi: investigation; Chiara Maria Lo Feudo: investigation; Francesco Ferrucci: investigation; Enrica Zucca: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization, writing – original draft and writing – review & editing

All authors approved the final version of the manuscript and agreed to be 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.

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Animal welfare statement

The study was approved by the Institutional Animal Care Committee of the University of Milan (OPBA_54_2021).

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Animal welfare statement

The study was approved by the Institutional Animal Care Committee of the University of Milan (OPBA_54_2021).

References

[1] Baysal SS, Has M. Evaluation of pulmonary artery stiffness in newly diagnosed adult patients with asthma. Echocardiography 2019, 36, 870–6. doi:10.1111/echo.14309.

[2] Ozkececi G, Ulasli SS, Akci O, Dural İE, Avsar A, Unlu M, et al. Assessment of pulmonary arterial stiffness in obstructive sleep apnea. Int J Cardiovasc Imaging 2016, 32, 799–805. doi:10.1007/s10554-016-0841-0.

[3] Altiparmak IH, Erkus ME, Polat M, Sak ZHA, Yalcin F, Gunebakmaz O, et al. Evaluation of pulmonary artery stiffness in patients with obstructive sleep apnea syndrome. Echocardiography 2016, 33, 362–71. doi:10.1111/echo.13098.

[4] Görgulü S, Eren M, Yildirim A, Özer O, Uslu N, Çelik S, et al. A new echocardiographic approach in assessing pulmonary vascular bed in patients with congenital heart disease: Pulmonary Artery Stiffness. Anadolu Kardiyol Derg 2003, 3, 92–7.

[5] Görgulü S, Eren M, Uslu N, Ozer O, Nurkalem Z. The determinants of right ventricular function in patients with atrial septal defect. Int J Cardiol 2006, 111, 127–30. doi:10.1016/j.ijcard.2005.07.037.

[6] De-Paula CR, Magalhães GS, Jentzsch NS, Botelho CF, Mota C de CC, Murça TM, et al. Echocardiographic assessment of ventricular function in young patients with asthma. Arq Bras Cardiol 2018, 110, 231–9. doi:10.5935/abc.20180052.

[7] Weir-Mccall JR, Struthers AD, Lipworth BJ, Houston JG. The role of pulmonary arterial stiffness in COPD. Respir Med 2015, 109, 1381–90. doi:10.1016/j.rmed.2015.06.005.

[8] Yildirim E, Celik M, Yukse UC, Gungor M, Bugan B, Dogan D, et al. Relationship between pulmonary artery stiffness and functional capacity in patients with heart failure with reduced ejection fraction. Korean Circ J 2017, 47, 929–38. doi:10.4070/kcj.2017.0081.

[9] Mahfouz RA, Dewedar A, Abdelmoneim A, Hossien EM. Aortic and pulmonary artery stiffness and cardiac function in children at risk for obesity. Echocardiography 2012, 29, 984–90. doi:10.1111/j.1540-8175.2012.01736.x.

[10] Ceriotti S, Bullone M, Leclere M, Ferrucci F, Lavoie JP. Severe asthma is associated with a remodeling of the pulmonary arteries in horses. PLoS One 2020, 15, 1–22. doi:10.1371/journal.pone.0239561.

[11] Decloedt A, Borowicz H, Slowikowska M, Chiers K, van Loon G, Niedzwiedz A. Right ventricular function during acute exacerbation of severe equine asthma. Equine Vet J 2017, 49, 603–8. doi:10.1111/evj.12675.

[12] Caivano D, Corda A, Rishniw M, Giorgi ME, Parpaglia MLP, Conti MB et al. Transthoracic M-mode echocardiographic assessment of pulmonary vein-to-pulmonary artery ratio in healthy horses. PLoS ONE 2019, 14(8), e0221154. doi:10.1371/journal.pone.0221154

[13] Zucca E, Ferrucci F, Croci C, Di Fabio V, Zaninelli M, Ferro E. Echocardiographic measurements of cardiac dimensions in normal Standardbred racehorses. J Vet Cardiol 2008, 10, 45–51. doi:10.1016/j.jvc.2008.04.002.

[14] Blissitt KJ, Bonagura JD. Pulsed wave Doppler echocardiography in normal horses. Equine Vet J 1995, 19, 38–46.

[15] Bunting KV, Steeds RP, Slater LT, Rogers JK, Gkoutos GV, Kotecha D. A practical guide to assess the reproducibility of echocardiographic measurements. J Am Soc Echocardiogr 2019, 32, 1505-1515. https://doi.org/10.1016/j.echo.2019.08.015

[16] Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of echocardiographic assessment of left atrial size and mechanical function in horses. Am J Vet Res 2007, 68, 735–47.

[17] Venco L, Mihaylova L, Boon JA. Right Pulmonary Artery Distensibility Index (RPAD Index). A field study of an echocardiographic method to detect early development of

pulmonary hypertension and its severity even in the absence of regurgitant jets for Doppler evaluation in heartworm infection. Vet Parasitol. 2014, 206, 60–6. doi:10.1016/j.vetpar.2014.08.016

[18] Visser LC, Im MK, Johnson LR, Stern JA. Diagnostic Value of Right Pulmonary Artery Distensibility Index in Dogs with Pulmonary Hypertension: Comparison with Doppler Echocardiographic Estimates of Pulmonary Arterial Pressure. J Vet Intern Med. 2016, 30, 543–52. doi:10.1111/jvim.13911.