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Evaluation of Different Ultrasound Equipments in Detection of Power Doppler Signal in the Equine Suspensory Ligament Branches

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Abstract:
As in human medicine, the usefulness of Power Doppler (PD) ultrasonography has been suggested in the evaluation of tendinopathy in horses. However, detection of the PD flow is equipment dependent and a large variability of newer or older ultrasound machines are used in veterinary medicine. The aim of this study was to quantitatively compare the performances of different ultrasound equipments in the detection of PD signal in branches of equine suspensory ligament, especially to detect very slow flows. Our hypothesis was that there is a significant difference between ultrasound machines in their ability to detect slow flow PD signal. Thirty-six suspensory ligament branches of 5 horses were used for this study. The PD ultrasonographic (US) examination was performed for all suspensory ligament branches using three ultrasound machines. The areas of PD signal present in theUS images were measured using dedicated image analysis software. The PD performances of the three ultrasound devices were compared using quantitative image analysis and statistics tests (non-parametric, Global Linear Model and post-hoc tests with Least Square Means).A significant difference in the detection the smallest PD flow signal between one and the other two ultrasound equipments was found (P-values less than 0,05).This study indicates the importance of the ultrasound equipment for the PD US examination and the need of considering ultrasound machine performance while interpreting any PD US result on equine suspensory branches.

Keywords: *Ultrasonography, Power Doppler, Equine, Tendon, Suspensory ligament*

1. Introduction

Tendon and ligament injuries are a common disease in athletic man and in sport horse.[1,2]In both humans and horses B-mode ultrasonography is an easily available modality[2,3] and in human medicine, Colour Doppler and Power Doppler (PD) ultrasonography have proven to be useful in the assessment of tendon/ligament injuries.[4]The Doppler technique may detect and monitor hyperaemia (increased vascularisation) in musculoskeletal inflammatory disease.[5]In human's tendons, the presence of hyperaemia is interpreted as a sign of disease, since this finding is not seen in normal ligaments and tendons[4] and it is commonly present inpatients with clinical and B-mode signs of tendinopathy.[6]PD ultrasonography, in comparison to Colour Doppler, has better sensitivity to detect flow from small vessels and low velocity flow at micro vascular level.[7] In human medicine, it has been proven that ultrasound devices differ in showing the smallest detectable flow when assessing vascularity in synovitis[8]and therefore the ability of the ultrasound machine used inthe detection of Doppler signal has to be taken in account when interpreting the results. The appearance of Doppler activity seems to have the same pattern in humans and in horses in tendinopathies and desmopathies.[9,10]As in veterinary medicine older ultrasound machines coming from the human market are sometimes used to reduce cost of purchase, the purpose of this study was to quantitatively compare, the performances of three ultrasound equipments in the detection of PD signal in the suspensory ligament (SL) equine branches. We hypothesized that, while using the equine suspensory branches as target for PD signal assessment, there is a difference in detection of slow flow PD signals among ultrasound equipment and that older equipment would have significant lower detection performance compared to more recent machines.

2. Materials and Methods

Thirty-six SL equine branches (20 hindlimb SL equine branches and 16 forelimb SL equine branches) were examined for this study on 5 horses. The PD ultrasonographic (US)examination was performed for all SL equine branches using three machines (AlokaProsound SSD-3500, Mitaka, Tokyo, Japan, MyLab 25and MyLab 70, Esaote, Genova, Italy)with a linear 7,5 MHz transducer.

The PD US examination was realized at rest, on the non-weight-bearing limb in flexion, by the same operator and without using a stand-off pad. The limbs were prepared by fine clipping, washing and application of US gel. A very little pressure was applied on the transducer. Only PD US images in the transverse plane were realized. The sensitivity of the ultrasound machines was optimized for low flow with the lowest possible pulse repetition frequency and the lowest possible wall filter. The color gain was set just below the noise level. The focus was placed where the highest sensitivity was required or just below the region of interest. The color box was large and placed on the area of the branch examined.For the same SL branch, images were obtained the same day using the three machines consecutively and without any time lapse between the three US examinations. The horse was not moved between PD exams. The order in which the three machines were used was not established but depended on the availability of the machines at the beginning of the US examination.

Three areas were considered for every suspensory branch: the proximal part of the branch about 2 cm distal to the bifurcation (called middle), the sub-terminal part (about 2 cm above the proximal border of the proximal sesamoid bone) and terminal part (just proximal to the proximal sesamoid bone). In each area,a semi quantitative scale from 0 to 4 (0= absence, no PD signal, 1= a single spot of PD signal, 2= more than 1 spot of PD signal, 3= more than 1 spot of PD signal and a single vessel signal, 4= more than 1 spot of PD signal and 2 vessel signals confluent or not confluent) was used to select the images with maximal color activity. For each area of the 36 SL equine branches (middle, sub-terminal and terminal) one image was chosen for analysis. The image chosen for comparison was the image with maximal color activity with the equipment which had subjectively detected less PD signal. If no PD signal was visible at all with this equipment, the image with maximal color activity with the second subjectively less sensitive equipment was used.The image chosen based on maximal color activity was used for analysis and compared with the images obtained at the same level cased on branch with the two other machines. Three hundred and twenty-four images were analysed.

The area of PD signal present in the PD US images was measured using a dedicated image analysis software (Image J; National Institutes of Health, Bethesda, MD). The number of pixels of the area with PD signal was measured by drawing a region of interest (ROI). In order to draw the ROI, images with PD signal were zoomed four times. ROI were drawn free-hand three times by the same operator. The three measurements permitted calculation of the repeatability. The mean of the three measurements of each ROI was calculated. Subsequently, the means of pixel number of the ROI of each image were added to obtain the total PD value per image.The total PD value per image obtained with the three machines at the same level were quantitatively compared. To assess repeatability, the variance of the 3 measures of each ROI was made and the ratio of variance/variance mean of all the 3 measures was calculated. A P-value less than 0.05 meant the measurement was repeatable. The repeatability assured the precision of the measures of each ROI and make the measurement significant for the next statistical analysis. Non-parametric, Global Linear Model procedure statistics tests were used to compare the PD values. Post-hoc tests with Least Square Means were used to test the presence of a significant difference in the detection of PD signal in SL equine branches between the three machines. A P-value less than 0,05 was considered significant.

3. Results

The repeatability was considered to be very good. Only 3 out of 160 P-values were more than 0,05.

A significant difference in the detection of PD signal in each image with Doppler activity of all SL equine branches between one and the other two ultrasound equipments (**Figure 1a, b, c**) was statistically found (P-values less than 0,05). The ability in the detection of PD signal of the MyLab 70 ultrasound machine was different from the Aloka Prosound SSD-3500 ultrasound machine (P-value=0,0044) and from the MyLab 25 machine (P-value=0,001). There was no significant difference in the detection PD signal between the Aloka Prosound SSD-3500 ultrasound machine and the MyLab 25 ultrasound machine (P-value=0,2974). The MyLab70 ultrasound equipment was the most sensitive ultrasound machine for the detection of PD slow flow in the SL equine branches.

4. Discussion and Conclusion

Our hypothesis that a difference exists between ultrasound machines in their ability to detect slow flow PD signal in equine suspensory branches has been confirmed. This has to be carefully considered while interpreting PD US images in equine patients, especially as evidence based data about occurrence and evolution of PD flow signal in anatomically different regions in equine patients is lacking in veterinary literature. Standardization of Doppler exams obtained with different ultrasound equipments does not seem possible for a number of reasons. First, the many different parameters affecting Doppler performance are not present on all machines, especially if the machine differs in price range and age. When they are present, they may perform differently from model to model, from software update to software update, because of linked controls[11]and the difference in quality of equipment seems to be an insurmountable obstacle with respect to standardization.[11]

As with all US examinations, PD US results are influenced by the examiner's skill and experience.[12]In the present study all the PD exams were realized by the same examiner, thus reducing operator-related variability between exams. PD ultrasonography is extremely sensitive to tissue movement, especially at low pulse repetition frequency, which can result in ''flash'' artefacts.[13]This can be annoying in horses compared to humans, as the US exam of the non-weight bearing limb is often subjected to some degree of movement especially in the hind limbs, which are positioned with the foot toe on the ground during PD ultrasonography.

PD signal is also influenced by exercise.[14] A study has demonstrated blood flow by Doppler as a possible physiological response in tendons in healthy subjects after long distance running.[14] By examining all horses at rest at the same time with the three machines, we

believe that we have excluded any physiological Doppler response that may have persisted as a consequence of tendon stress during exercise or transfer of the horse from the stall to the ultrasound room.

In humans, PD signal has been demonstrated to occur in both acutely and chronically affected tendons/ligaments and be the result of a different mechanism depending of the age of the lesion (vasodilation in acute damage and neoangiogenesis in chronic disease).[4]Kristoffersen and collaborators[9]suggest that a similar neovascularisation process is seen in chronically injured equine and human tendons. The presence of neovascularisation in SL equine branches is of potential usefulness for monitoring disease activity and distinguishing between hyper vascular tissue from fibrous tissue and may help to better understand the pathogenic mechanisms of tendon pain in equine tendinous and ligamentous injuries. In human medicine, the highly significant correlation between PD US findings and histopathological findings supports the value of this imaging technique.[12]Further longitudinal studies on horses with SL desmopathy monitoring PD activity during time and studies comparing PD activity with histopathologic features of affected tendons will be of interest to better understand the mechanism responsible for PD signal andto correlate hypervascularity with the stage and histopathology of the lesion.

Although some difficulties exists to apply and interpret PD ultrasonography in men and in horses, we consider that in the present study we had no major technical difficulties in obtaining reliable PD images on the equine suspensory branches of the horses examined. Because of the easy applicability of the technique demonstrated in the present study, a combination of B-mode and PD ultrasonography canbe routinely used as a sensitive, non-invasive and widely available complement to standard clinical assessment for evaluating desmopathy of the SL equine branches in daily management and clinical trials. Moreover, because of their common involvement in desmopathy in sport, pleasure and race horses,[2] naturally occurring equine SL lesions can be easily used to study PD in relation to lesion B-mode appearance, age and severity and to therapeutic management. However the same ultrasound equipment should be used for all horses and its sensitivity should be taken into account in interpreting final results.

In conclusion, the results of this study remind the importance of the ultrasound equipment choice for PD US examination and the need of considering ultrasound machine performance while interpreting any PD US result. Despite difference in ultrasound machine sensitivity, the ease to obtain PD in the present study also suggests that naturally occurring SL equine branches desmopathies may be used as a research model to increase the knowledge in PD ultrasonography in tendon and ligament damage.

Figure 1a, b, c. Transverse Power Doppler ultrasonographic images demonstrating a significant difference in the detection of Power Doppler signal in the middle area of a hind limb suspensory ligament branch; images obtained at the same level with the three-ultrasound machine. **a** MyLab 70 **b** Aloka SSD-3500 **c** MyLab 25.

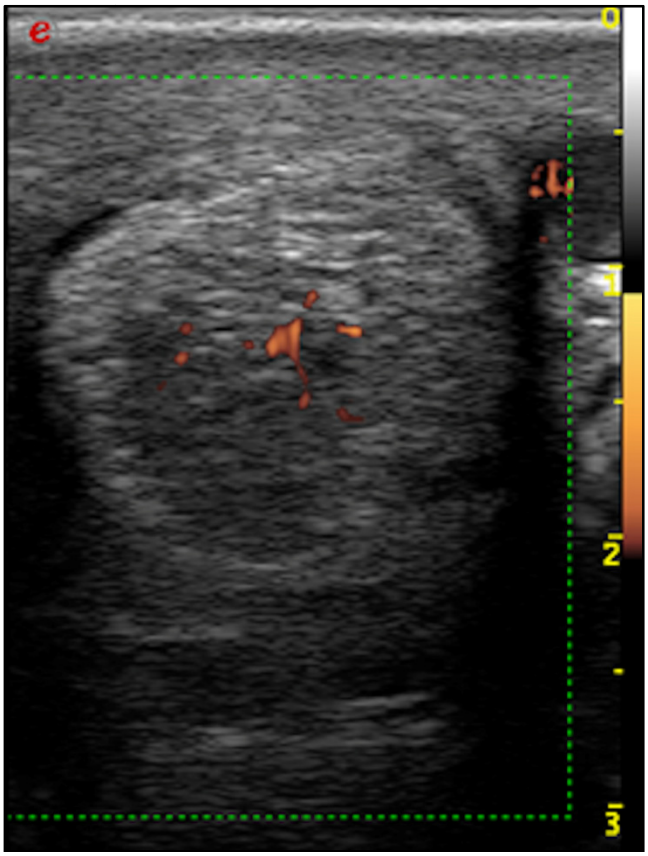

Figure 1a

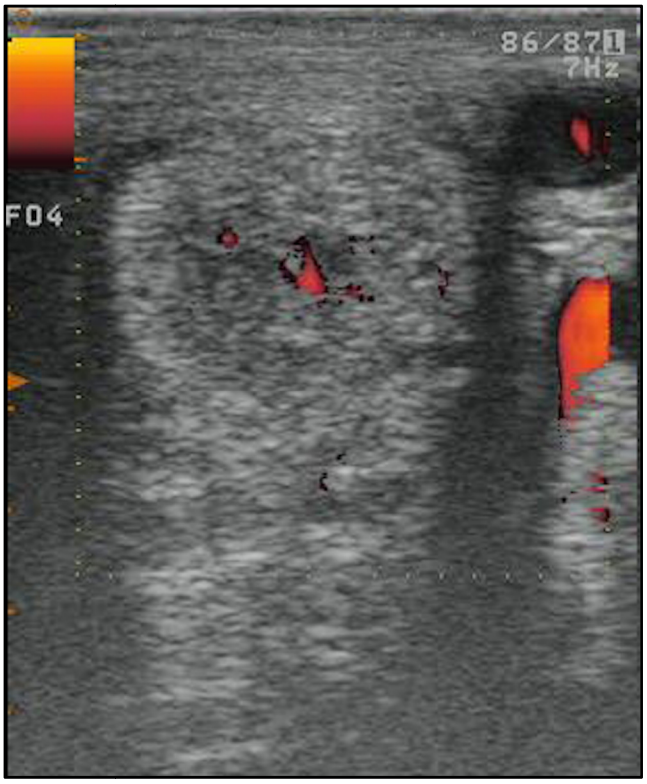

Figure 1b

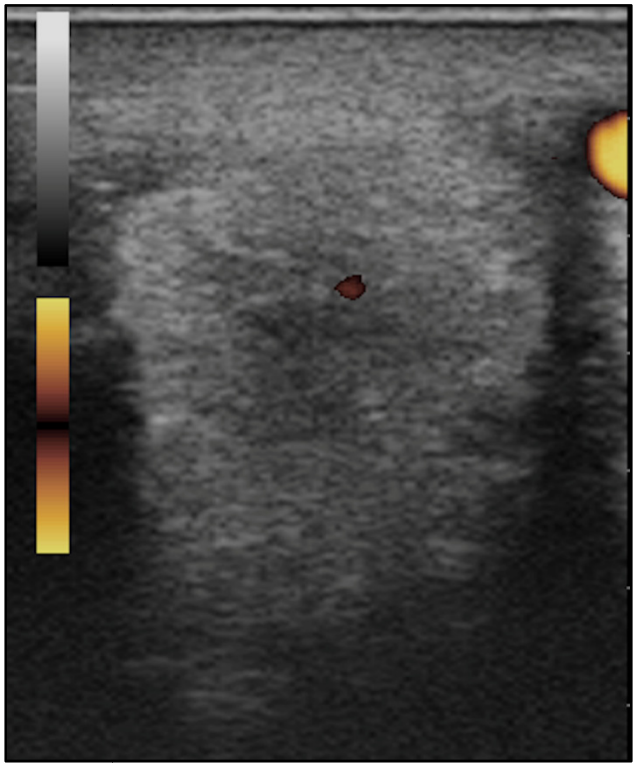

Figure 1c

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