Usefulness of Doppler ultrasonography to assess digital vascular dynamics in horses with systemic inflammatory response syndrome or laminitis

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Objective—To evaluate the usefulness of Doppler ultrasonography as a method to assess changes in digital vascular dynamics in horses with systemic inflammatory response syndrome (SIRS) or laminitis.

Design—Cross-sectional study.

Animals—42 adult Andalusian horses.

Procedures—Group 1 included 9 healthy horses, group 2 included 19 horses with SIRS without (n = 9) or with (10) a palpable increase in digital pulse intensity, and group 3 included 14 horses with laminitis without (8) or with (6) radiographic evidence of rotation or distal displacement (sinking) of the third phalanx. Qualitative spectrum characteristics and quantitative Doppler measurements of the lateral palmar digital artery were obtained for horses in each group.

Results—4 spectra, characterized by a positive systolic peak followed by several positive diastolic peaks, were observed in group 1 horses, group 2 horses, and group 3 horses that lacked radiographic changes. In the group 3 horses that had radiographic changes, laminar blood flow was detected. Diameter of the lateral palmar digital artery was significantly larger in the group 3 horses than in the group 2 horses; blood flow was significantly higher in the group 2 horses that had an increase in digital pulse intensity than in the group 2 horses without an increase in digital pulse intensity; velocity-time integral and acceleration time were significantly lower in group 3 horses, compared with group 2 horses.

Conclusions and Clinical Relevance—Results suggested that Doppler ultrasonography may be a useful complementary tool to detect digital blood flow changes of horses with SIRS, especially if they have a palpable increase in digital pulse intensity, or laminitis. (J Am Vet Med Assoc 2013;243:1756–1761)

Doppler ultrasonography has been established as a sensitive, noninvasive, and inexpensive method for the evaluation of digital blood flow in humans and animals.1 In horses, several diseases may disturb digital peripheral blood flow. Laminitis is one of the most severe diseases, causing important lesions and poor prognosis.2 Laminitis may be associated with endotoxemia, bearing weight overload, endocrinopathies, and excess carbohydrate consumption.3 Laminitis associated with endotoxemia is a common and acute condition occurring secondary to systemic inflammatory diseases and one of the most challenging forms because of difficulties in predicting its onset. Angiography and scintigraphy have revealed decreased blood flow in the limbs of laminitic horses with regions of hypoperfusion4 and pathological changes consistent with laminitis.5

Abbreviations

EDV  End diastolic velocity
LPDA  Lateral palmar digital artery
SIRS  Systemic inflammatory response syndrome
VTI  Velocity-time integral

Laminitis, therefore, is thought to be a consequence of altered digital blood flow. In this sense, palpation to determine digital pulse intensity is one of the most commonly used techniques to evaluate and monitor digital blood flow of the hoof and potential development of laminitis.6 However, palpation of digital pulses provides an insensitive measurement of digital blood flow and is a subjective and highly operator-dependent technique that is insufficient to quantify slight differences in blood supply to the equine digit. On the other hand, objective techniques used in fully conscious standing horses, such as laser Doppler flowmetry,7,8 gamma scintigraphy,9,10 contrast angiography,11 and use of an extracorporeal digital perfusion pump,12 provide sensitive measurements of digital blood flow in horses. However, these techniques are invasive, limiting their clinical applicability. Doppler ultrasonography has been established as the method of choice in human medicine for noninvasive objective evaluation of blood supply in peripheral vascular disorders, such as peripheral arterial occlusive disease.13 Therefore, Doppler ultrasonography was applied to assess digital vascular dynamics in horses with systemic inflammatory response syndrome or laminitis.
as obstructive arterial diseases.\textsuperscript{13–15} Doppler ultrasonography has also been established as a sensitive, noninvasive, and inexpensive procedure\textsuperscript{16,17} with repeatable measurements of vessel diameter, time-averaged mean velocity, maximum velocity, and blood flow in healthy horses.\textsuperscript{18} To our knowledge, there have been no clinical studies in horses evaluating Doppler ultrasonographic quantitative and qualitative measurements in disease states that could potentially affect digital blood flow, such as diseases that cause SIRS and laminitis. There has been one study\textsuperscript{19} in endotoxemic horses in which only quantitative variables such as vessel diameter and blood flow were measured, and another study\textsuperscript{20} in laminitic horses in which hemodynamic status, systemic blood pressure measurements, and radiographic changes were not taken into account.

The purpose of the study reported here was to compare Doppler ultrasonography of the LPDA in healthy horses, horses with SIRS, and horses with laminitis. The main hypothesis was that Doppler ultrasonography could detect changes in digital blood flow among these groups. Specific aims to address this hypothesis included identifying differences in digital blood flow between horses with SIRS with and without a palpable increase in digital pulse intensity and laminitic horses with and without radiographic changes (ie, rotation or distal displacement [sinking] of the third phalanx), compared flow characteristics among the groups, and evaluate the usefulness of Doppler ultrasonography as a method to measure digital pulse quality in horses with SIRS and laminitic horses.

**Materials and Methods**

**Animals**—Data were prospectively collected from 42 adult Andalusian horses that were referred to or were property of the Veterinary Teaching Hospital of Murcia University between 2004 to 2010. Informed consent was obtained from owners of privately owned horses. Nine healthy horses (control group) and 33 horses affected by SIRS or laminitis were included. The university committee on the ethical use of animals approved all procedures. Horses were evaluated by physical examination, hematologic tests, blood pressure measurements, and forelimb radiography. From these results, 3 groups were established: healthy horses (group 1); horses with SIRS (group 2), which included horses with enteritis, enterocolitis, acute endometritis, or pleuro-pneumonia; and horses with laminitis (group 3). Systemic inflammatory response syndrome was defined by the presence of ≥ 2 of the following abnormalities: fever or hypothermia (rectal temperature > 38.5°C [101.3°F] or < 37.2°C [98.9°F]), tachycardia (heart rate > 60 beats/min), tachypnea (respiratory rate > 30 breaths/min) or hypocapnia (PaCO\textsubscript{2} < 32 mm Hg), leukocytosis or leukopenia (leukocyte count > 12,500 or < 4,000 cells/µL), or increased numbers of immature forms of granulocytes (> 10% band neutrophils).\textsuperscript{19,21} Group 3 horses included those with a diagnosis of overt bilateral forelimb laminitis based on clinical signs (lameness, increased temperature of the hoof, percussion pain, and remarkable pulsation in the digital arteries). Group 2 horses were further characterized depending on the absence or presence of a palpable increase in digital pulse intensity, and group 3 horses were further characterized by the absence or presence of radiographic changes (rotation or distal displacement [sinking] of the third phalanx). The exclusion criteria in all groups were highly stressed behavior or having received prior treatment by the referring veterinarian.

**Study protocol**—At the time of hospital admission, medical history of each horse was obtained, and a complete physical examination was performed. Results of hematologic and serum biochemical analyses\textsuperscript{b} and arterial blood pressure measurements at the middle coccgeal artery by oscilometric method\textsuperscript{c} were also recorded. Subsequently, ultrasonography was performed within 4 hours after hospital admission in most horses. Horses were stabilized for at least 30 minutes and acclimated to a temperature-controlled environment of 21°C to 22°C (69.8° to 71.6°F). Hair was shaved from the lateral palmar aspects of the metacarpophalangeal joint of both forelimbs. Doppler ultrasonography of the right and left LPDAs was performed while the horse was bearing weight on all limbs. A commercial ultrasonographic imaging system\textsuperscript{d} equipped with a 7.5-MHz duplex linear array transducer was used, and all the examinations were performed by the same researcher who was blinded to medical information. The transducer was coupled with gel and positioned on the skin at the level of the proximal aspect of the metacarpophalangeal joint and was rotated approximately 30° to 45° abaxially until the artery appeared clear in its long axis. Anatomic landmarks used to guide transducer placement were the suspensory ligament dorsally and deep digital flexor tendon palmarly. Ultrasonographic scanner controls, including gain, grayscale, reject (allowing the operator to eliminate smaller noise signals and unimportant echoes from the image), and depth (3 cm), were adjusted to achieve optimal resolution of the LPDA walls and lumen. The transducer was orientated longitudinally and angled to allow imaging of the LPDA and to obtain at least 3 measurements in 2-D images of the diameter of the artery. The angle between the vessel axis and the ultrasound beam was adjusted to 45°, and the Doppler sample volume was set to 0.6 mm in the center of the vessel. Velocity scale was adapted to prevent the aliasing of the spectrum display as is described in the literature.\textsuperscript{1} The following variables were measured as the mean of 3 to 5 consecutive cardiac cycles: peak systolic velocity, first peak diastolic velocity, second peak diastolic velocity, EDV, mean velocity, VTI, acceleration time, peak systolic-to-diastolic velocity ratio, pulsatility index, and resistivity index. Blood flow was calculated from the following equation: blood flow = heart rate \times VTI \times \pi \times r^2, where \(r\) is the vessel radius.

As soon as the ultrasonographic measurements were obtained from all horses with SIRS, radiographic examination\textsuperscript{e} of forelimbs was performed, including lateral and dorsopalmar views of the third phalanx. The indices measured to determine the rotation or distal displacement (sinking) of the third phalanx were palmar angle, horn-lamellar zone width, extensor process—coronary band distance, and sole depth at the tip and the wing of the third phalanx.\textsuperscript{22}

**Statistical analysis**—Data analyses were performed with a commercial software package.\textsuperscript{2}

Heart
rate, PCV, serum total protein concentration, vessel diameter, systemic blood pressure values (systolic, diastolic, and mean arterial blood pressures), and Doppler ultrasonographic measurements for each horse were expressed as mean ± SD. Normality of each variable was evaluated with Shapiro-Wilk and Kolmogorov-Smirnov tests. Differences between the left and right forelimbs were obtained and evaluated by the Student t test for paired data or the Wilcoxon test, depending on the characteristics of each variable. Furthermore, Pearson or Spearman correlation tests were performed to determine correlation among all Doppler measurements. Variables with values of P ≤ 0.01 among groups were analyzed by linear regression analysis to evaluate the strength of relationship. Characteristics of blood flow in each group were compared by 2-way ANOVA. Significance was assigned for values of P ≤ 0.05.

Results

Group 1 included 9 healthy horses (6 males and 3 females) with a mean age of 7.22 ± 1.92 years and a mean body weight of 531.56 ± 35.24 kg (1,169.43 ± 77.33 lb). Group 2 included 19 horses with SIRS (9 males and 10 females) with a mean age of 7.58 ± 2.43 years and a mean body weight of 509.26 ± 58.51 kg (1,120.37 ± 128.72 lb). Group 2 was further characterized by horses without a palpable increase in digital pulse intensity (n = 9; 5 males and 4 females; mean age, 6.78 ± 2.39 years; mean body weight, 519.33 ± 79.23 kg [1,142.53 ± 174.31 lb]) and horses with a palpable increase in digital pulse intensity (10; 4 males and 6 females; mean age, 8.30 ± 2.36 years; mean body weight, 520.00 ± 32.78 kg [1,144.00 ± 72.12 lb]). Group 3 included 14 horses with overt signs of laminitis (10 males and 4 females) with a mean age of 8.14 ± 2.65 years and a mean body weight of 531.79 ± 77.77 kg (1,169.94 ± 171.09 lb). Group 3 was further characterized as laminitic horses without radiographic changes (n = 8; 6 males and 2 females; mean age, 8.50 ± 1.69 years; mean body weight, 541.50 ± 87.25 kg [1,191.3 ± 191.95 lb]) and horses with radiographic changes (6; 4 males and 2 females; mean age, 7.66 ± 3.72 years; mean body weight, 518.83 ± 68.70 kg [1,141.43 ± 151.14 lb]).
The qualitative analysis of Doppler spectrum of all horses allowed the identification of 4 Doppler spectra patterns. Nonlaminar blood flow characterized by a systolic peak followed by 2 to 4 positive diastolic waves (type 1) was found in all 9 group 1 horses and in 8 of 9 group 2 horses without a palpable increase in digital pulse intensity (Figure 1). Nonlaminar blood flow characterized by a prominent systolic peak reaching the baseline followed by 1 to 3 diastolic waves (type 2) was found in 1 of 9 group 2 horses without a palpable increase in digital pulse intensity, all 10 group 2 horses with a palpable increase in digital pulse intensity, and 6 of 8 group 3 horses without radiographic changes (Figure 2). Laminar blood flow characterized by a prominent systolic peak followed by 1 positive diastolic wave (type 3) was only found in 2 of 8 group 3 horses without radiographic changes. Laminar blood flow characterized by a prominent systolic peak, early negative diastolic wave, and late positive diastolic wave (type 4) was found in all 6 group 3 horses with radiographic changes (Figure 4).

Regarding the results obtained in the quantitative ultrasonographic study, the mean values obtained from the right and left lateral digital arteries did not differ significantly and were considered together (pooled) for further comparisons. The 2-D ultrasonographic images revealed that the diameter of the artery was significantly (P = 0.024) larger in group 3 (0.38 ± 0.07 cm) than in group 2 (0.30 ± 0.08 cm) horses and significantly (P = 0.045) larger in group 2 horses with a palpable increase in digital pulse intensity (0.32 ± 0.07 cm) than in group 2 horses without a palpable increase in digital pulse intensity (0.26 ± 0.02 cm). However, no significant difference in vessel diameter was found between subcategories of group 2 and 3 horses and control horses. The pulsed-

![Doppler waveform of the LPDA in a horse with laminitis with radiographic changes (rotation or sinking of the third phalanx) showing a laminar blood flow pattern characterized by a prominent peak systolic velocity (Pa) followed by early negative first peak diastolic velocity (Pd1) and late positive second peak diastolic velocity (Pd2). Upper insert indicates color Doppler blood flow in the LPDA.](image_url)

**Figure 4**—Doppler waveform of the LPDA in a horse with laminitis with radiographic changes (rotation or sinking of the third phalanx) showing a laminar blood flow pattern characterized by a prominent peak systolic velocity (Pa) followed by early negative first peak diastolic velocity (Pd1) and late positive second peak diastolic velocity (Pd2). Upper insert indicates color Doppler blood flow in the LPDA.

Table 1—Diameter and Doppler variables of the LPDA in healthy horses, horses with SIRS but without and with a palpable increase in digital pulse intensity (IDPI), and horses with overt signs of laminitis without and with radiographic changes (RC; ie, rotation or sinking of the third phalanx).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy horses (n = 9)</th>
<th>Horses with SIRS (group 2) Without IDPI (n = 9)</th>
<th>With IDPI (n = 10)</th>
<th>Horses with laminitis (group 3) Without RC (n = 6)</th>
<th>With RC (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (cm)</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.08</td>
<td>0.26 ± 0.02</td>
<td>0.32 ± 0.07</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>48.43 ± 15.55</td>
<td>57.04 ± 13.21</td>
<td>52.31 ± 13.99</td>
<td>61.31 ± 11.53</td>
<td>50.88 ± 11.63</td>
</tr>
<tr>
<td>FPDV (cm/s)</td>
<td>(26.29–77.43)</td>
<td>(36.06–84.81)</td>
<td>(46.46–65.81)</td>
<td>(50.88–11.63)</td>
<td>(33.96–83.71)</td>
</tr>
<tr>
<td>SPDV (cm/s)</td>
<td>40.24 ± 16.37</td>
<td>51.19 ± 18.16</td>
<td>50.89 ± 17.72</td>
<td>50.88 ± 11.63</td>
<td>17.55 ± 8.80</td>
</tr>
<tr>
<td>TPDV (cm/s)</td>
<td>37.43 ± 15.20</td>
<td>43.32–57.68</td>
<td>45.81–54.56</td>
<td>45.81–54.56</td>
<td>13.89–34.26</td>
</tr>
<tr>
<td>MV (cm/s)</td>
<td>26.85 ± 12.08</td>
<td>18.11 ± 4.72</td>
<td>17.15 ± 2.94</td>
<td>19.67 ± 3.73</td>
<td>20.50 ± 9.16</td>
</tr>
<tr>
<td>EDV (cm/s)</td>
<td>(10.43–42.91)</td>
<td>(13.76–24.84)</td>
<td>(16.93–24.94)</td>
<td>(16.93–24.84)</td>
<td>11.69 ± 5.72</td>
</tr>
<tr>
<td>AT (s)</td>
<td>22.00 ± 10.49</td>
<td>22.14 ± 11.56</td>
<td>20.72 ± 13.56</td>
<td>23.43 ± 10.00</td>
<td>15.42 ± 8.81</td>
</tr>
<tr>
<td>VT (cm)</td>
<td>31.48 ± 5.77</td>
<td>36.28 ± 14.60</td>
<td>36.72 ± 36.44</td>
<td>36.28 ± 36.44</td>
<td>15.44 ± 7.93</td>
</tr>
<tr>
<td>PSV/ratio</td>
<td>0.35 ± 0.09</td>
<td>(0.36–1.80)</td>
<td>(0.36–1.80)</td>
<td>(0.36–1.80)</td>
<td>(0.36–1.80)</td>
</tr>
<tr>
<td>PI</td>
<td>0.27 ± 0.22</td>
<td>0.41 ± 0.29</td>
<td>0.62 ± 0.27</td>
<td>0.23 ± 0.17</td>
<td>0.24 ± 0.15</td>
</tr>
<tr>
<td>BF (mL/min)</td>
<td>88.34 ± 17.10</td>
<td>119.93 ± 71.24</td>
<td>62.97 ± 30.46</td>
<td>157.92 ± 65.17</td>
<td>121.27 ± 52</td>
</tr>
</tbody>
</table>
| Data are mean ± SD (range) values. **Values with the same symbol within a row are significantly (P < 0.05) different.** \*\*AT = Acceleration time. BF = Blood flow. FPDV = First peak diastolic velocity. MV = Mean velocity. ND = Not determined. PI = Pulsatility index. PSV = Peak systolic velocity. RC = Rotation or sinking of the third phalanx. SPDV = Second peak diastolic velocity. TPDV = Third peak diastolic velocity.
wave Doppler ultrasonographic examination revealed significant differences in the blood flow volumes ($P = 0.012$) between group 2 horses with a palpable increase in digital pulse intensity (157.92 ± 65.17 mL/min) and group 2 horses without a palpable increase in digital pulse intensity (62.97 ± 30.64 mL/min) and in VTI ($P = 0.04$) and acceleration time ($P = 0.01$) between group 2 (35.28 ± 14.60 cm; 0.41 ± 0.29 seconds) and group 3 (18.44 ± 7.93 cm; 0.24 ± 0.15 seconds) horses. In contrast, peak systolic-to-diastolic velocity ratio, pulsatility index, and resistivity index variables did not differ significantly among the groups (Table 1).

**Discussion**

Detection of a palpable increase in digital pulse intensity in a systemically ill (eg, enteritis, enterocolitis, acute endometritis, and pleuropneumonia) horse is concerning and suggestive of a digital vascular disturbance with a potential for the onset of laminitis. The present study was designed to investigate and compare characteristics of digital blood flow by means of Doppler ultrasonography of the LPDA in horses predisposed to laminitis (group 2) with those in horses with overt signs of laminitis (group 3). These groups were further characterized depending on whether they had a palpable increase in digital pulse intensity or radiographic findings of rotation or sinking of the third phalanx. Sources of variability in Doppler ultrasonography measurements were minimized by consistency in procedures that included use of the following: the same ultrasonographic equipment and technique, a temperature-controlled environment, and the same operator. During each examination, horses stood and were weight bearing on all limbs. The main sources of variation in measurements, therefore, should have been intrinsic factors relating to weight bearing, vasculature disturbances, and disease.

Despite the numerous studies on improving and validating the use of Doppler ultrasonography of the LPDA in healthy horses, only 2 studies in clinically endotoxic or laminitic horses have been reported, to the author’s knowledge. In the study by Menzies-Gow et al, only quantitative variables such as vessel diameter and blood flow were measured by Doppler ultrasonography; differing from the present study, in which more quantitative variables (peak systolic velocity, first peak diastolic velocity, second peak diastolic velocity, EDV, mean velocity, VTI, acceleration time, peak systolic-to-diastolic velocity ratio, pulsatility index, resistivity index, and blood flow) and qualitative spectrum characterizations were analyzed. Regarding the study of Wongaumnuaykul et al, hematochemical and serum biochemical analyses and arterial blood pressure measurements were not performed; therefore, it is not possible to dismiss other systemic diseases in the groups established (ie, control horses, horses with pododermatitis, and laminitic horses). Also, in laminitic horses, the grade of lameness was the main criterion for the analysis of the results, whereas in the present study, besides evaluating the grade of lameness, the radiographic findings were the chosen criterion to classify horses because they provide a more objective measurement.

In our study, all healthy control horses had low-resistance spectra in accordance with previous publications. In contrast to previous studies, Wongaumnuaykul et al reported a high-resistance spectrum of the LPDA in 4 of 10 healthy control horses. Considering that hematochemical and serum biochemical analyses and systemic blood pressure measurements were not performed in that study, it is reasonable to speculate that some horses with vascular alterations could have been included in their healthy control group.

With respect to the spectra found in horses with SIRS, the results of the present investigation cannot be compared with those of an analogous study, in which only quantitative Doppler ultrasonography analysis was performed. In the present study, Doppler ultrasonography spectra in all 19 horses with SIRS were characterized by a nonlaminar blood flow. A middle-resistance pattern (types 2 and 3) was detected in all horses with SIRS that had a palpable increase in digital pulse intensity and horses with overt laminitis without radiographic changes. And a high-resistance pattern (type 4) was found in laminitic group horses with radiographic changes, similar to those found in high-caliber vessels.

In the study of Wongaumnuaykul et al, low-resistance patterns were described in laminitic horses, probably because horses with unilateral lameness were included in this group. However, in the present study, laminitic horses with bilateral forelimb lameness were selected to avoid, as much as possible, the non–weight-bearing factor. Given that the circumstances were not the same, the results obtained in the present study cannot be compared with those described in previous reports; however, a study in humans has found that in arteries with severe hypertension proximal to stenosis, a low-resistance pattern turns into a high-resistance pulsatility pattern. And in cases where stenosis becomes chronic, collateral circulation can create new capillary vessels, decreasing proximal pulsatility and providing an alternative irrigation path.

The quantitative Doppler ultrasonography analysis revealed that the variables diameter, blood flow, VTI, and acceleration time were significantly different between horses with SIRS and those with overt signs of laminitis. When comparing vessel diameter values, significantly larger size vessels were found in horses with laminitis, followed by horses with SIRS with a palpable increase in digital pulse intensity and horses with SIRS without a palpable increase in digital pulse intensity. We interpret that the increase in vessel diameter in a directly proportional way to the worsening of the horses condition is in relation to the pathophysiology of the SIRS process and the vasodilation produced due to distal stenosis. These results are in accordance with the different patterns found in the qualitative study. Both acceleration time and VTI were significantly higher in horses with SIRS than in laminitic horses, suggesting a link to ongoing hemodynamic changes in the SIRS process. This allows highlighting these variables to monitor in SIRS patients as possible risk factors for a laminitis process.

In conclusion, this study supports the usefulness of Doppler ultrasonography as a method to measure the increase of digital pulse intensity by quantitative (vessel...
diameter, blood flow, acceleration time, and VTI) and qualitative Doppler changes found in horses with SIRS and laminitis. Despite this, future studies are needed including a larger number of horses to further evaluate patient variability and monitoring of horses with SIRS and laminitis during hospitalization over time.

These findings may aid clinicians in the evaluation of changes in blood flow in horses with laminitis or in horses prone to developing laminitis. Moreover, these results could contribute to a better understanding of the digital blood supply, helping to monitor more objectively the presence of a palpable increase in digital pulse intensity that could not be evaluated by other means.

References