Comparison of transverse facial venous sinus and jugular blood values in healthy and critically ill horses

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Summary

Reasons for performing study: The transverse facial venous sinus (TFVS) can be used for blood collection in horses, but information on the validity of blood values from this site is limited.

Objectives: To determine if packed cell volume (PCV), total solids (TS) and blood lactate concentrations in blood drawn simultaneously from a TFVS and jugular vein of critically ill horses are correlated and determine the effect of serial TFVS sampling on the same parameters in healthy horses.

Study design: Prospective observational study.

Methods: Critically ill horses had simultaneous blood samples drawn from a TFVS and jugular vein. Blood was also drawn from the left TFVS and jugular vein from 6 healthy, adult horses q. 6 h for 24 h, then q. 24 h for 72 h. Blood was drawn from the right TFVS and jugular vein q. 24 h for 96 h. All samples were analysed for PCV, TS and blood lactate concentration. Data were analysed with 2-way repeated measures ANOVA. Significance was set at P≤0.05.

Results: There were no significant differences in PCV, TS or blood lactate concentrations of TFVS samples compared with jugular blood in critically ill horses. Serial TFVS sampling in healthy horses had no significant effect on TS or blood lactate concentrations. Although PCV in the TFVS was significantly lower than in jugular blood on serial sampling, the difference was not considered clinically relevant.

Conclusions: Packed cell volume, TS and blood lactate concentrations were comparable between the TFVS and jugular vein.

Keywords: horse; transverse facial venous sinus (TFVS); serial blood samples; critically ill

Introduction

Serial blood samples are used to monitor clinicopathological parameters in critically ill horses and the jugular vein is the most common site for venipuncture [1]. However, damage to the vascular endothelium occurs with venipuncture and in critically ill horses this may increase the risk for thrombophlebitis [2]. Consequences of jugular thrombophlebitis include vessel occlusion, head swelling with subsequent respiratory distress, sepsis and release of emboli leading to organ infarction [2]. The jugular vein is the preferred site for i.v. catheter placement in the horse and clinicians typically make every effort to preserve its integrity for this purpose. Thrombophlebitis is among the most common complications that occur in critically ill horses secondary to gastrointestinal disease with incidences of 7.5% reported in post operative colics [3]. Although thrombophlebitis typically occurs in catheterised veins, this is not always the case [4]. Thus, there is clinical value in identifying alternative sites for blood sample collection.

The TFVS, the most dorsal branch of the facial vein, lies ventral to the facial crest deep to the masseter [5]. As it courses caudally, the transverse facial vein penetrates the masseter to lie superficially just ventral to the zygomatic bone before anastomosing with the superficial temporal vein. While the caudal segment is accompanied by the transverse facial artery, the rostral segment is not. Thus, inserting a 20 gauge 3.8 cm needle ventral to the facial crest at the level of the medial canthus [6] is guaranteed to yield venous blood.

To spare the jugular vein, the transverse facial venous sinus (TFVS) has been proposed as an alternative blood sampling site in the horse [1,6,7], but there is limited information on the accuracy with which the systemic circulation is represented by blood from this site. Packed cell volume (PCV) and total solids (TS) in samples from the TFVS are reportedly comparable to those in jugular blood, but the health status of horses in that study was not reported [7]. Blood gas parameters from TFVS blood reportedly reflect jugular blood in healthy horses, but similar information in critically ill horses are unavailable [8]. Use of the TFVS for multiple blood collections in one day has been recommended [7], but haematomas may form over the site with multiple sample collections in the experience of these authors. The effect of serial sampling and haematoma formation on blood parameters from the TFVS has not been reported in healthy or critically ill horses. In critically ill horses, blood collected from jugular catheters has been shown to be equivalent to blood obtained by direct jugular venipuncture [9]. Some have suggested that sampling from catheters may decrease catheter longevity and increase risk of thrombophlebitis [1]. Studies evaluating this in horses are lacking. Transverse facial venous sinus venipuncture is minimally challenging, safe for the handler and well tolerated by most horses. Sampling from this site is a good alternative to sampling from jugular veins if blood values from both sites are equivalent.

This study had 2 objectives. One was to compare serially collected TFVS blood samples with simultaneously collected jugular samples from clinically healthy horses through the evaluation of PCV, TS and blood lactate concentration. The other was to compare TFVS with jugular blood in critically ill horses through evaluation of the same parameters. We hypothesised that there would be no significant differences between TFVS and jugular blood values in healthy or critically ill horses at any collection time.

Materials and methods

Serial TFVS sampling in healthy horses

To determine the effects of repeated sampling on TFVS blood analysis, 6 adult horses (4 Quarter Horses, one Thoroughbred, one Warmblood cross, age 10–24 years, weight 490–631 kg) were used. They were judged to be healthy based on physical examination findings and housed in box stalls with a diet of grass hay and free access to water. Indwelling 14 gauge, 14 cm catheters (Abbocath-T®) were placed in the left jugular vein using an aseptic technique and remained in place for the duration of the study. Jugular blood samples of 2 ml were collected into glass vials containing 7.5% EDTA liquid (Monoject®) from the catheters after discarding the first 12 ml of blood withdrawn. Transverse facial venous sinus samples (1.5–2 ml) were collected with 20 gauge 3.8 cm needles into glass vials containing 7.5% EDTA liquid (Monoject®) using previously described landmarks and technique [5]. Samples were drawn from the right TFVS every 24 h for 96 h and from the left TFVS every 6 h for 24 h, then every 24 h for another 72 h. Jugular vein samples were obtained each time a TFVS sample was taken. Blood lactate concentrations were determined for all samples immediately after collection prior to placement of blood in EDTA tubes. Packed cell volume and TS were determined within 60 min of collection. All samples were collected and processed by one author (B.H.).
Blood lactate concentrations were determined using a handheld point of care lactate meter (Lactate Scout® previously validated for use in horses [10]. The lactate meter had a reporting range of 0.5–25 mmol/l. Samples with blood lactate concentrations below the reporting range were assigned a value of 0.5 mmol/l for analysis. Packed cell volume and TS were determined from samples of EDTA anticoagulated whole blood centrifuged at 10,062 g for 5 min in nonheparinised micro-haematocrit tubes. Packed cell volume was calculated using a micro-haematocrit capillary tube reader and TS determined using refractometry. At the time of each TFVS venipuncture, haematoma presence over the sampling site was recorded if visibly and palpably apparent. All procedures were performed with the approval of the Institutional Animal Care and Use Committee.

Clinical study in hospitalised patients

Critically ill horses presenting to a referral hospital for treatment had blood samples drawn from a TFVS at the same time jugular blood samples were being drawn for patient assessment. Blood lactate concentrations of paired samples were determined using the handheld point of care lactate meter (Lactate Scout®). The PCV and TS were determined as above. Horses were deemed critically ill if they had clinical abnormalities that could alter systemic perfusion. All samples were subcategorised into one of 2 groups. Group 1 were designated as ‘post operative colics’; samples from these horses were collected within 10 min of recovery from general anaesthesia for abdominal surgery. Group 2 were critically ill horses that had either not undergone general anaesthesia, or were post operative colics ≥6 h after recovery from general anaesthesia. These horses had one or more of the following clinical signs at the time of sample collection: heart rate >60 beats/min, signs of severe abdominal pain, profuse watery diarrhoea, mucous membranes that were cyanotic, pale or brick red in colour, or prolonged jugular refill. Jugular refill was graded as normal, prolonged or severely prolonged, with severely prolonged defined as veins that did not visibly or palpably fill after being occluded for ≥10 s. Horses were monitored for clinical signs of thrombophlebitis in catheterised jugular veins during hospitalisation. Transverse facial venous sinus samples were collected with the approval of the Institutional Animal Care and Use and Committee and client consent.

Data analysis

For serial sampling, blood parameter data were compared for differences in sampling site or repeated sampling by 2-way repeated measures ANOVA. Clinical patient blood data were compared for differences in sampling site and group by 2-way repeated measures ANOVA. Results are reported as mean ± s.d. Blood parameter data from both sample sites in all horses were correlated using a Pearson product moment correlation and R values are reported. For all comparisons, significance was set at P≤0.05. The power for each data set was calculated during 2-way repeated measures ANOVA analysis with a computerised statistical programme (SigmaStat®). Alpha was set at 0.05 and observed standard deviations and differences in sample means were used for calculations.

Data for both healthy and clinically ill horses were combined and Bland Altman plots created for each parameter. These plots were created by plotting the difference in parameter values (e.g.: TFVS PCV - JV PCV) over the mean parameter value (ITFVS PCV + JV PCV)/2 at each sample collection to create a visual representation of the variation in parameter values between sites independent of time.

Results

In serial samples from healthy horses, significant differences in blood lactate concentrations between the right TFVS, left TFVS and jugular vein on samples taken every 24 h were not found (P = 0.18, Power = 0.18) or between the left TFVS and jugular vein on samples taken every 6 h (P = 0.58, Power = 0.65). The PCV of blood taken from left TFVS was significantly lower than blood taken from the right TFVS or jugular vein in samples taken every 24 h (P = 0.05, Power = 0.47). The PCV of blood taken every 6 h was significantly lower in left TFVS as compared with the jugular vein (P = 0.027, Power = 0.65). Inspection of the data set revealed that, while most jugular vein samples had a PCV within 3% of its TFVS pair, one jugular vein sample at the 72 h collection was 7% higher than both TFVS samples. When this sample was treated as an outlier and removed from the data set, PCV of blood taken from the left TFVS every 24 h was no longer significantly different from jugular blood (P = 0.07, Power = 0.40). Significant differences in total solid concentrations between sampling sites on samples drawn every 24 h (P = 0.09, Power = 0.34) or every 6 h (P = 0.06, Power = 0.43) were not found. Blood lactate, PCV and TS concentrations from all collection sites were within normal reference range (blood lactate ≤2.0 mmol/l, PCV = 28–42%, TS = 58–76 g/l) with the exception of a single outlier for PCV (45%). Individual values are presented in Fig 1. Forty-five of 49 samples from the left TFVS and 26 of 30 samples from the right TFVS were within 3% of their paired jugular blood sample.

Twenty-eight samples were drawn from 21 horses that met the inclusion criteria for critical illness. Horses ranged in age from 4 to 25 years (median: 15 years) and in weight from 412 to 652 kg (median: 523 kg). Genders represented were mares (9), geldings (10) and stallions (2). Breeds included American Quarter Horses (6), Warmbloods (3), American Paint Horses (4), Thoroughbred/Thoroughbred cross (4), Appaloosa (1), Friesian (1), Clydesdale cross (1) and a Fjord. Eleven samples were drawn from horses within 10 min of recovery from general anaesthesia for exploratory laparotomy. Seventeen samples were drawn from horses that had not undergone general anaesthesia or that were ≥6 h post recovery from general anaesthesia for abdominal surgery.

Blood lactate, PCV and TS values from critically ill horses are presented in Fig 2. Hyperlactataemia was present in 25/28 samples. Powers of analyses in critically ill horses were 0.05 and, given this low power, results...
Healthy horses that underwent serial sampling did not display clinical signs of thrombophlebitis associated with a jugular catheter during venipuncture was not displayed by any horse. No horses resisted haltering or palpation of their face during hospitalisation. Two horses displayed clinical signs of thrombophlebitis associated with a jugular catheter during hospitalisation.

**Discussion**

Data comparing clinical values in blood from the TFVS with blood collected from the jugular vein in critically ill horses have not been previously reported. The findings of this study support the hypothesis that blood samples drawn from the TFVS are comparable with jugular blood samples in critically ill horses. Variations in parameter values secondary to serial sampling from the TFVS have not been reported, but information on disparities is important as serial blood samples are routinely drawn from critically ill horses and samples may be drawn as often as every 6 h [11]. Haematoma formation is one of the most common complications associated with TFVS sampling [6], but our results revealed no apparent effect of haematoma formation on these parameters.

Packed cell volume, TS and blood lactate concentrations were assessed in critically ill horses and samples may be drawn as often as every 6 h [11]. Haematoma formation is one of the most common complications associated with TFVS sampling [6], but our results revealed no apparent effect of haematoma formation on these parameters.

The strong correlations between sampling sites in this study suggest that collection from the TFVS in healthy and critically ill horses, particularly for repeated small samples. The strong correlations between sampling sites in this study suggest that collection from the TFVS in healthy and critically ill horses, particularly for repeated small samples.

In this study as they are commonly monitored in critical care patients. Use of critically ill horses allowed assessment of these parameters over a broader range of values than those occurring in healthy horses. For example, 19 of the 21 horses in this clinical study had hyperlactataemia in 25/28 samples. Even over extended ranges, there were minimal differences between collection sites and there was a linear relationship in parameters between collection sites.

In addition to the strong correlation in parameter values between collection sites, minimal variation in parameter values is apparent between sites as is evident in the Bland Altman plots (Fig 3). These plots depict the distribution of the difference between sites for each parameter around the mean value of that parameter at each sampling time. The minimal variation in parameter values between sites suggests that collection from the TFVS yields values clinically equivalent to values obtained from jugular blood. The strong correlations between sampling sites in this study suggest that the TFVS is a good alternative to the jugular vein for blood sampling in healthy and critically ill horses, particularly for repeated small samples.

Collecting blood from sites other than the jugular vein is necessary if both jugular veins have overlying haematomas, are surrounded by cellulitis, or are thrombosed. In horses with only one patent jugular vein, it is prudent to avoid use of that vein for simple blood collection. In some cases, avoidance of the jugular veins altogether is considered prudent if they

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**TABLE 1: R values for Pearson product moment correlations between sampling sites.**

<table>
<thead>
<tr>
<th>Sites correlated</th>
<th>Parameter</th>
<th>R value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy horses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JV vs. left TFVS</td>
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</tr>
<tr>
<td>JV vs. right TFVS</td>
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</tr>
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</tr>
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<td>TS</td>
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</tr>
<tr>
<td>Left vs. right TFVS</td>
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<td>0.95</td>
</tr>
<tr>
<td>Critically ill horses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JV vs. TFVS</td>
<td>BL</td>
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<tr>
<td>JV vs. TFVS</td>
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<tr>
<td>JV vs. TFVS</td>
<td>TS</td>
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</tbody>
</table>

BL = Blood lactate; PCV = Packed cell volume; TS = Total solids.

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**Fig 2:** Packed cell volume, total solids and blood lactate concentrations for critically ill horses. JV = jugular vein; TFVS = transverse facial venous sinus.
Facial sinus vs. jugular blood in critically ill horses

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PCV Bland Altman plot

Total solids Bland Altman plot

Blood lactate Bland Altman plot

Fig 3: Bland Altman plots for PCV, total solids and blood lactate concentrations in healthy and critically ill horses. JV = jugular vein; TFVS = transverse facial venous sinus.

have been used frequently in the past or if the horse has evidence of a hypercoagulable state. The TFVS has been equated in reliability to the coccygeal vein in cattle [6]. Additional peripheral sites for blood collection have been evaluated in other species. In cats, marginal ear vein samples have proven to be reliable for evaluation of blood glucose [12], while PCV of blood from the dorsal fin of sharks is consistently 8% lower than blood from the caudal tail artery [13]. Limb vessels have been used in horses, but lactate concentrations in blood samples from those vessels have been shown to be greater than in jugular venous samples in healthy horses [14] and one would expect this effect to be magnified in critically ill horses or after general anaesthesia.

The PCV and TS of blood drawn from the right TFVS was equivalent to jugular blood, but the PCV of samples drawn from the left TFVS were significantly different from the jugular venous samples. Inspection of the raw data revealed that most samples from the left and right TFVS were within 3% of the values obtained using the jugular venous blood. Disparities of 4–5% occurred between 4 left and 4 right TFVS samples and their jugular vein pairs. Two of these disparities between the left TFVS and jugular vein occurred during baseline sampling while the disparities between the right TFVS and jugular vein were more evenly distributed over time. Jugular samples were taken after TFVS sample collection in all cases. One horse in the ‘healthy’ group became excited after TFVS sample collection and the PCV in jugular blood was subsequently 8% higher than both TFVS samples. Assuming that this disparity was secondary to splenic contraction, data were re-analysed with these samples omitted and there was no significant difference between PCV of left TFVS and jugular venous blood. The differences in distributions of disparities ≥4% over time led us to believe that the PCV obtained from TFVS blood can occasionally be aberrant in a clinically relevant manner, but these aberrations are unlikely to be associated with serial sampling as they do not occur in a predictable fashion.

The TFVS is the safest and easiest alternative site for collection of blood samples from most horses. The only complications noted in this study were haematoma formation over the venipuncture site following repeated venipuncture and temporary head shyness in some horses. Although thrombosis of the TFVS is possible, the clinical implications of thrombosis are reportedly minimal [1]. In contrast, the clinical ramifications of thrombosis or thrombophlebitis of the jugular vein are important and critically ill horses are at an increased risk for development of thrombophlebitis [15]. Jugular thrombophlebitis is one of the 6 most common complications in horses after abdominal surgery [16] with reported incidences of 7.5% [3] and 25% [17]. Thrombophlebitis is typically associated with catheterised veins in critically ill horses and several patient-derived factors are known to increase the risk of occurrence [4,15]. Blood collected from i.v. catheters is equivalent to that drawn by jugular venipuncture in horses [9], but it is unknown if collection from catheters increases the risk of phlebitis.

If the presence of haematoma was to affect TFVS samples, changes in blood lactate and PCV would be expected findings. Packed cell volume would be expected to increase with erythrocyte stagnation and the small amounts of lactate normally produced during red blood cell metabolism would be expected to accumulate when production exceeds clearance [18]. Neither of these changes was evident in TFVS samples obtained after haematoma formation in the overlying soft tissues.

The number of clinical patients evaluated in this study was small, but the data obtained provide useful information for future studies. These data suggest that the TFVS supplies reliable values for blood lactate concentration and TS in both critically ill and healthy horses. Additionally, haematoma formation over the TFVS has no apparent effect on the parameters measured. While PCV of TFVS blood was not significantly different from jugular venous blood in critically ill horses, the power of the study was low and a larger sample size may reveal disparities between sites. Serial sampling in healthy horses revealed that while most TFVS samples have a PCV within 3% of jugular blood, occasionally the disparity between the 2 sites is greater. These disparities, while uncommon, do not appear to occur in a predictable fashion. Studies with greater sample size and evaluating additional haematological and biochemical parameters are warranted to validate TFVS blood as equivalent to jugular blood, but this study provides initial data that is clinically relevant and a useful basis for future studies.

Authors’ declaration of interests

No competing interests have been declared.

Ethical animal research

This study was approved by the Institutional Animal Care and Use Committee and performed with client consent.

Source of funding

College of Veterinary Medicine, Oregon State University, USA.

Authorship

The study was designed and executed by Dr Barbara Hunter and Dr John Schlipf with assistance from Dr Chris Cebra. Data was analysed by Dr Chris...
Cebra with assistance from Dr Barbara Hunter and Dr John Schlipf. All authors contributed equally to interpretation of data. The manuscript was prepared by Dr Barbara Hunter with assistance from Dr John Schlipf and Dr Chris Cebra. All authors approved the final version of the manuscript.

Manufacturers’ addresses

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dSigmaStat 2.0, SPSS Inc, Chicago, Illinois, USA.

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