Assessment of microcirculatory changes by use of sidestream dark field microscopy during hemorrhagic shock in dogs

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Objective—To directly assess microcirculatory changes associated with induced hemorrhagic shock by use of sidestream dark field microscopy (SDM) and correlate those values with concurrently measured macrovascular and blood gas variables in healthy anesthetized dogs.

Animals—12 adult dogs.

Procedures—Dogs were anesthetized and splenectomized. Instrumentation and catheterization were performed for determination of macrohemodynamic and blood gas variables. Hemorrhagic shock was induced via controlled hemorrhage to a mean arterial blood pressure (MAP) of 40 mm Hg. Dogs were maintained in the shock state (MAP, 35 to 45 mm Hg) for 60 minutes. An SDM device was used to image microcirculation of buccal mucosa, and vascular analysis software was used to determine microcirculatory variables. These values were compared with other cardiovascular and blood gas variables to determine correlations.

Results—Following hemorrhage, there was a significant decrease in microvascular variables (mean ± SD), including proportion of perfused vessels (82.77 ± 8.32% vs 57.21 ± 28.83%), perfused vessel density (14.86 ± 2.64 mm/mm^2 vs 6.66 ± 4.75 mm/mm^2), and microvascular flow index (2.54 ± 0.52 vs 1.59 ± 0.85). Perfused vessel density individually correlated well with macrovascular variables, with heart rate (zero order, partial correlation, and part correlation coefficients = –0.762, –0.884, and –0.793, respectively) and oxygen extraction ratio (–0.734, –0.832, and –0.746, respectively) being the most important predictors.

Conclusions and Clinical Relevance—SDM allowed real-time imaging of the microvasculature and has potential as an effective tool in experimental and clinical applications for monitoring microcirculatory changes associated with hemorrhagic shock and resuscitation in dogs. (*Am J Vet Res* 2011;72:438–445)

Traditionally, macrohemodynamic variables (eg, arterial blood pressure, heart rate, cardiac output, and oxygen delivery) have been used to determine the severity of hemorrhagic shock and aid in determining fluid therapy for resuscitation. However, there is increasing evidence to suggest that these global indices may not be reflective of changes in the microcirculation with regard to capillary perfusion and tissue oxygenation. Several studies[1–5] have evaluated response to various treatments for shock, and results indicate that microvascular flow remains deranged despite return of blood pressure, cardiac output, and other macrovascular variables to reference limits. Microhemodynamic variables might therefore serve as a better therapeutic guide during resuscitation. Indirect (or downstream) global measurements of cellular perfusion and microvascular hemodynamics are widely available and include such variables as lactate concentration, base excess, and central venous oxygen saturation.[6–8] An additional modality for indirectly evaluating the regional microcirculation includes the use of fiber-optic sensors for the direct measurement of tissue oxygenation in skeletal muscle[9] or intestinal mucosa.[10] Gastric tonometry and sublingual capnometry have also been used as indicators of perfusion and oxygen supply to the gastrointestinal tract.[8,11] The indirect nature of these monitoring techniques may be associated with limitations that reduce their ability to truly reflect changes in the microcirculation, especially given its unique structure and local regulation.

Abbreviations

FCD	Functional capillary density
MAP	Mean arterial blood pressure
MFI	Microvascular flow index
O_2ER	Systemic oxygen extraction ratio
PPV	Proportion of perfused vessels
PVD	Perfused vessel density
SDM	Sidestream dark field microscopy
SVRI	Systemic vascular resistance index
TVD	Total vessel density

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The microcirculation is composed of networks of small vessels, including arterioles, capillaries, and venules, all measuring < 200 µm in diameter. The capillaries are the smallest of all vessels, having a diameter of < 20 µm. The metabolic demand of the local tissue contributes to capillary resistance, with increased oxygen demand or low oxygen tension causing capillary dilation and decreased resistance secondary to release of nitric oxide.[12] Blood viscosity in the capillaries is also important because shear stress exerted by blood flow on the endothelium leads to production of nitric oxide and preservation of capillary perfusion.[13] During hemorrhage, the loss of RBC mass can result in reduction of blood viscosity, decreased shear force, and resultant vasoconstriction.[14] With close proximity to the terminal arterioles, oxygen tension and other metabolites such as lactate and carbon dioxide in the venules act upon the precapillary arterioles to further regulate flow into the capillary bed. There are also systemic regulators of the microcirculation, such as catecholamines and angiotensin II, which constrict the precapillary sphincter of terminal arterioles and restrict the amount of blood entering a capillary bed. This becomes important in shock states and during resuscitation as blood is preferentially shunted to vital tissues such as the brain and heart. Normalization of macrohemodymamic variables such as heart rate and MAP might therefore not necessarily reflect what is happening at the level of the microcirculation, and in fact, some studies[14–16] reveal that ongoing tissue hypoxia and oxygen debt can occur despite return of macrovascular variables to reference limits. Perhaps a more effective way to assess microcirculatory perfusion is by direct imaging of the microvasculature.

Previously, direct assessment of the microcirculation was limited to experimental use of intravital microscopy, especially in the hamster cheek pouch model.[13,14,17,18] Through determination of FCD, defined as the number of capillaries with passage of RBCs per unit of surface area in microscopically observed tissues, it has been determined that there is a strong correlation between maintenance of FCD and tissue survival.[19] This technology works well in the laboratory setting, but is impractical for use in clinical patients. More recently, handheld videographic microscopy for microvascular assessment and potential clinical use has become available. One of these devices[a] uses SDM whereby emitted green light (wavelength, 530 nm) is absorbed by the hemoglobin of erthrocytes.[20] With the 5X objective lens, the erythrocytes are illuminated as they flow through the microcirculation, resulting in a real-time videographic image with 326X magnification.[20] These videographs can be analyzed later to determine several microcirculatory variables including PVD, which is a value similar to the previously described FCD, PPV, and MFI. That handheld microscope has been used in a number of experimental animal models as well as human clinical patients.[20–26] One study[20] in human sepsis patients revealed that collapse of the microcirculation was associated with decreased tissue oxygenation and organ dysfunction. Another study[24] verified that the device can provide repeatable measurements on both sublingual tissue and abdominal stomata. Sidestream dark field microscopy has been explored to a limited extent in canine patients in a study[26] that described the technique, established baseline values, and assessed reproducibility in anesthetized dogs, but has not been used to monitor microvascular changes associated with hemorrhagic shock in dogs.

The purpose of the study reported here was to directly assess microcirculatory changes associated with induced hemorrhagic shock by use of SDM and correlate those values with concurrently measured macrovascular and blood gas variables in healthy anesthetized dogs. In addition, macrohemodynamic and blood gas variables were simultaneously assessed for comparison with PVD. We hypothesized that hemorrhagic shock would lead to a significant reduction in values for microcirculatory variables and that macrohemodynamic and blood gas variables would not correlate well with PVD.

Materials and Methods

Animals—All procedures were performed with approval from The Ohio State University Institutional Animal Care and Use Committee. Twelve purpose-bred male Foxhounds, ranging from 1 to 6 years of age and with a mean ± SD body weight of 24.9 ± 2.56 kg (range, 19 to 29 kg) had food withheld for 12 hours prior to procedures but were allowed free access to water.

Anesthesia and instrumentation—In preparation for the procedure, the dogs were administered general anesthesia. Briefly, an 18-gauge catheter was placed in a cephalic vein, and the dog was administered hydromorphone[b] (0.1 mg/kg, IV) as a premedication. Anesthetic induction was performed by administration of diazepam[c] (0.5 mg/kg, IV) and ketamine[d] (5 to 10 mg/kg, IV). An orotracheal tube was placed, and the dogs were anesthetized by use of sevoflurane[e] in 100% oxygen. Mechanical ventilation was provided through the anesthetic circuit and was used to maintain end-tidal CO_2 partial pressure between 35 and 45 mm Hg. Fentanyl[f] (6 to 10 µg/kg/h) was administered as a constant rate infusion to provide continued analgesia. Lactated Ringer's solution[g] (5 mL/kg/h) was administered IV for the duration of the procedure. Continuous monitoring of heart rate and end-tidal CO_2[h] partial pressure, pulse oximetry,[i] and electrocardiography[h] were performed. A 20-gauge, 3-cm catheter was placed in the dorsal metatarsal artery for continuous direct arterial pressure monitoring and lithium dilution cardiac output determination.[j] A 20-cm double-lumen central venous catheter was placed in a jugular vein for venous blood sampling and lithium administration. The left carotid artery was surgically exposed for IV placement of a 10F, 5-cm polyethylene catheter connected to a 3-way stopcock. This catheter was used to obtain arterial blood samples and induce controlled hemorrhage. The dog was then placed in dorsal recumbency, and a ventral midline celiotomy was performed. Hemostasis was maintained by use of electrocautery. A splenectomy was performed to limit the variable hemodynamic effects of splenic contraction that can occur during hemorrhagic shock.[27,28] The dogs were allowed a 30-minute equilibration period prior to baseline measurements and induction of shock.

Induction and maintenance of hemorrhagic shock—Following the equilibration period, baseline measurements were taken and shock was induced. The carotid artery catheter was connected to a closed collection system with a stopcock, and blood was withdrawn until an MAP of 40 mm Hg was reached. The dogs were maintained in this shock state (MAP, 35 to 45 mm Hg) by removal of additional blood or administration of lactated Ringer's solution as needed for a period of 60 minutes, at which time measurements of all variables were again made. The dogs received interventions as part of another protocol, which involved resuscitation from hemorrhagic shock with a novel hyperviscous blood substitute. At the conclusion of the experiment, the dogs were euthanized with pentobarbital[k] (100 mg/kg, IV) while still anesthetized.

Measurement of macrohemodynamic and blood gas variables—Macrovascular variables included heart rate, systolic arterial pressure, diastolic arterial pressure, and MAP. Cardiac output was determined by use of the lithium dilution technique, as described.[29,30] Data obtained from arterial and central venous blood gas analysis[l] included pH, oxygen tension, carbon dioxide tension, oxygen saturation, lactate concentration, base excess, bicarbonate concentration, total hemoglobin concentration, and sodium concentration. Sodium concentration values were obtained for use in lithium dilution cardiac output determination.[29] Packed cell volume was determined via centrifugation and total plasma protein concentration via refractometry. Calculated values included cardiac index, SVRI, systemic oxygen delivery, systemic oxygen consumption, and O_2ER, by use of standard equations.

Measurement of microvascular variables—At both time periods, videographic recordings of the buccal mucosal microcirculation were obtained by use of the videomicroscope.[a] According to established consensus criteria, videographic recordings of 20-second duration are recommended.[31] However, because of technical and logistic limitations of the study design, videographic recordings of only 5 seconds' duration were obtained. For each dog at each time point, 3 videographic recordings were obtained by the same operator from adjacent areas of the buccal mucosa. In dogs with little or no oral pigmentation, videographic recordings were obtained from the mucogingival junction dorsal to a carnassial tooth. In dogs with extensive oral pigmentation, videographic recordings were obtained wherever nonpigmented mucosa or gingiva could be found. Care was taken to minimize application pressure and risk of vessel compression. The videographic recordings were exported offline and analyzed by use of specialized vascular analysis software[m] by a single investigator who was unaware of the study time point at which the videographic recordings were obtained.

Videographic recordings were assessed for overall quality. Videographic recordings of insufficient quality (eg, poor image resolution, excessive motion, pressure artifact) were withdrawn from analysis. The analysis software was used to determine microcirculatory variables established by consensus criteria.[31] One of these values is TVD, which was derived by superimposing a grid of 3 equidistant horizontal and 3 vertical lines over the image. The TVD was then calculated on the basis of the number of vessels crossing the lines divided by the length of the lines.[31] The PPV is a value based on the assignment of a flow category to each vessel (continuous, intermittent, or no-flow). The PPV was calculated as follows:

$$\text{PPV} = 100 \times (\text{total number of vessels} - [\text{no flow} + \text{intermittent flow}])/\text{total number of vessels}$$

The PVD was calculated by multiplying TVD and PPV. The analysis software was also used to determine the MFI. This value is obtained by dividing the visual field into quadrants and characterizing the flow as absent (0), intermittent (1), sluggish (2), or normal (3).[31] The mean value from each quadrant is then calculated to determine the MFI. Mean measurements from videographic recordings of acceptable quality were used for determination of microcirculatory variables for each subject at each time point.

To assess measurement reliability, intraobserver variability was determined for all microvascular variables. Videographic recordings from 8 dogs were randomly selected and analyzed in triplicate by the same investigator who performed the study analysis. The investigator was unaware of the previous videographic measurements as well as the hemodynamic state of the dog being analyzed.

Statistical analysis—All data were tested for normality by use of the Kolmogorov-Smirnov test. General linear relationships between predictive macrovascular variables and PVD were evaluated by inspection of scatterplots prior to linear regression. Descriptive statistics (mean, median, SEM, and SD) were calculated for all independent variables across the treatment periods. Differences between pre- and postshock hemodynamic variables were evaluated by use of a paired *t* test. Intraobserver variability for microvascular variables was determined with the coefficient of variation, which is calculated by use of this equation: (SD of the measurements/mean of measurements) × 100.[32]

Linear correlations between PVD and independent macrovascular variables were evaluated by use of repeated-measures linear regression, a form of multiple linear regression, as described by Glantz and Slinker.[33] A general linear model was used to predict PVD from the independent variables. To account for repeated measurements in dogs, each subject was coded by use of dummy variables. The model used was the following:

$$\text{PVD} = B_o + B_{IV} \cdot \text{IV} + B_1 \cdot D_1 + B_2 * D_{2+} \ldots, B_{11} \cdot D_{11}$$

where B_o is a constant (Y intercept), B is a coefficient, IV is the independent predictive variable of interest, and D_1 through D_{11} are dummy variables coding for the 12 dogs.[33] The dummy variables accounted for the repeated measurements obtained from each dog and adjusted the model for those influences. To isolate the effects of the independent variable of interest, zero-order, partial, and semipartial (part) correlation coefficients were calculated. The partial correlation coefficient reveals the correlation that remains between 2 variables after

removal of the correlation attributable to their mutual association with the other variables; that is, the partial correlation coefficient reveals correlation between the dependent variable and an independent variable when the linear effects of the other independent (dummy) variables have been removed from both. The semipartial (part) correlation coefficient is the correlation between the dependent variable and an independent variable when the linear effects of the other independent variables in the model have been removed from the independent variable. This relates to the change in R^2 when a variable is added to the equation.[34] Backward stepwise linear regression was used to determine whether a combination of the independent variables could be used to predict PVD. For all analyses, $P < 0.05$ was considered significant. Statistical analyses were performed by use of commercial statistical software packages.[n,o] Data are presented as mean ± SD because the data were normally distributed.

Results

During videographic analysis, 3 dogs at each time point had 1 videographic recording of unacceptable diagnostic quality. Therefore, 66 of 72 (92%) videographic recordings were included in the microvascular analysis. Determination of intraobserver variability revealed coefficients of variation that were within the acceptable limit of < 10%.[32]

Perfused vessel density was significantly lower during hemorrhagic shock, compared with baseline (**Table 1**). This was evident on gross inspection of the images as well as with determination of PVD (**Figure 1**). Microvascular flow index, TVD, and PPV were similarly affected. Significant differences between time periods were also detected for several other variables and were considered for further statistical analysis, including heart rate, PCV, systolic and diastolic arterial blood pressures, MAP, cardiac index, oxygen delivery, oxygen consumption, O_2ER, central venous pH, central venous oxygen saturation, central venous base excess, arterial lactate concentration, and central venous lactate concentration. Systemic vascular resistance index was not different among time points.

Table 1—Mean ± SD values of micro- and macrohemodynamic variables measured at baseline and during hemorrhagic shock in a group of 12 dogs.

Variable	Baseline	Shock
TVD (mm/mm^2)	17.53 ± 1.78	11.72 ± 4.60*
PPV (%)	82.77. ± 8.32	57.21 ± 28.83*
PVD (mm/mm^2)	14.86 ± 2.64	6.66 ± 4.75*
MFI	2.54 ± 0.52	1.59 ± 0.85*
HR (beats/min)	63 ± 27	205 ± 19*
SAP (mm Hg)	142 ± 22	45 ± 5*
MAP (mm Hg)	94 ± 19	38 ± 2*
DAP (mm Hg)	80 ± 17	33 ± 2*
CI (L/min/m^2)	3.24 ± 0.82	1.46 ± 0.62*
SVRI (dynes • s • cm^5)	2,375 ± 921	2,486 ± 984
$Lactate_A$ (mmol/L)	1.77 ± 0.34	4.41 ± 1.16*
$Lactate_{CV}$ (mmol/L)	1.60 ± 0.31	3.84 ± 1.01*
pH_{CV}	7.33 ± 0.03	7.11 ± 0.04*
BE_{CV} (mmol/L)	–1.78 ± 1.51	–6.86 ± 2.19*
$S_{CV}O_2$ (%)	95.3 ± 3.66	34.5 ± 6.9*
DO_2 (mL/min)	65.4 ± 20.1	22.7 ± 10.7*
VO_2 (mL/min)	38.8 ± 33.4	151.2 ± 69.5*
O_2ER (%)	6.15 ± 4.4	65.9 ± 6.7*

*Significant ($P < 0.05$) difference from baseline values.

A = Arterial blood gas value. BE = Base excess. CI = Cardiac index. CV = Central venous blood gas value. DAP = Diastolic arterial pressure. DO_2 = Systemic oxygen delivery. HR = Heart rate. SAP = Systolic arterial pressure. $S_{CV}O_2$ = Central venous oxygen saturation. VO_2 = Systemic oxygen consumption.

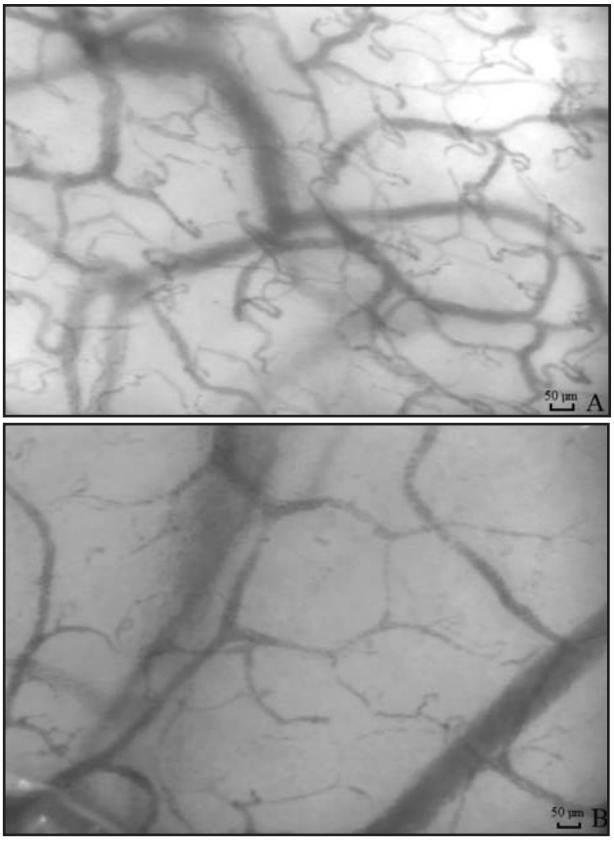

Figure 1—Sidestream dark field microscopic images obtained from the buccal mucosa of an anesthetized dog at baseline (A) and during hemorrhagic shock (B). Notice the decrease in the number of visible vessels, consistent with a decrease in TVD and PVD, in B. Bar = 50 µm.

Table 2—Correlation coefficients between PVD and various variables determined via repeated-measures linear regression during hemorrhagic shock in a group of 12 dogs.

Variable	*P* value	Zero-order	Partial	Part
HR	< 0.001	–0.762	–0.884	–0.793
SAP	0.002	0.669	0.777	0.697
MAP	0.006	0.600	0.714	0.641
DAP	0.008	0.588	0.701	0.629
CI	0.002	0.596	0.773	0.694
$Lactate_A$	0.007	–0.654	–0.703	–0.630
$Lactate_{CV}$	0.018	–0.585	–0.643	–0.577
pH_{CV}	0.001	0.736	0.822	0.738
BE_{CV}	0.002	0.629	0.766	0.687
$S_{CV}O_2$	0.001	0.732	0.810	0.727
DO_2	0.001	0.612	0.798	0.716
VO_2	0.023	–0.584	–0.646	–0.567
O_2ER	< 0.001	–0.734	–0.832	–0.746

Part = Part (semipartial) correlation coefficient. Partial = Partial correlation coefficient. Zero-order = Zero-order correlation coefficient.

See Table 1 for remainder of key.

Analysis of the variance inflation factors obtained from the stepwise linear regression indicated severe multicollinearity among all predictors. Accordingly, each independent variable was isolated by use of a repeated-measures linear regression model for PVD.[33] All the independent variables studied were significant univariate predictors of PVD (**Table 2**). Heart rate and O_2ER had the greatest part correlation coefficients, indicating the strongest predictive value for PVD after accounting for the influence of each individual dog on the data.

Discussion

A significant reduction in all microvascular variables (TVD, PPV, PVD, and MFI) during hemorrhagic shock was detected in this study by use of SDM imaging, likely reflecting hypovolemia-induced hypoperfusion. The decrease in TVD reflects a reduction in the number of vessels in the visual field, potentially indicating vasoconstriction, microvascular shunting, or failure of those vessels to fill because of diminished vascular volume. Reduction in PPV reflects an increase in heterogeneity of blood flow during hemorrhagic shock because a smaller proportion of visible vessels had continuous flow. Intermittent or stagnant flow could indicate a loss of hydraulic or perfusion pressure associated with severe hypovolemia and hypotension. Markedly decreased PVD, as a product of TVD and PPV, represents an alteration in both vascular density and heterogeneity of flow and reflects substantial impairment in tissue perfusion. The reduction in MFI suggested a decrease in flow velocity, also likely indicating a reduction in blood volume and perfusion pressure, because its determination allows for continuous flow to be further characterized as sluggish or normal. The macrohemodynamic and blood gas variables in this study changed predictably with hemorrhagic shock. In addition, those variables correlated well with changes in PVD. Heart rate and O_2ER appeared to have the strongest predictive value for PVD.

The baseline values for PVD and PPV determined in this study (mean PVD, 15 vessels/mm [range, 11 to 19 vessels/mm]; mean PPV, 82.77% [range, 70.1% to 96.1%]) were considerably less than those in another study that evaluated values in healthy dogs (mean PVD, 24 vessels/mm [range, 17 to 30 vessels/mm]; mean PPV, 100% [94% to 100%]).[26] There are several potential reasons for this variation. In the present study, the subjects underwent a laparotomy and splenectomy prior to determination of baseline values. Surgical manipulation and resultant sympathetic response could have had an effect on microcirculation by causing a reduction in PVD. This notion is supported by a study[35] that found significant changes in microvascular flow after major abdominal surgery in human patients. Differences in anesthetic protocol could have also played a role. The present study also had a uniform canine population (Foxhounds), whereas the other study had representation from several breeds. Therefore, breed differences in PVD could have resulted in some of the disparity. Several dogs in the present study had extensive oral pigmentation at the desired site for videographic acquisition (at the mucogingival junction above a carnassial tooth). As a result, not all videographic recordings could be recorded from the same anatomic site, and regional changes in the buccal mucosal microcirculation may have had an effect as well. Finally, the operator-dependent nature of videographic acquisition and analysis could have been a contributing factor. Unlike PVD and PPV, baseline values for MFI in this study (mean, 2.5; range, 2.2 to 3) were similar to those reported in the other study (mean, 2.7; range, 2 to 3).

Although this study revealed a significant reduction in all microvascular variables during hemorrhagic shock, there was not a significant change in SVRI. Given the expected compensatory response to hemorrhagic shock, the decrease in microvascular variables might have occurred secondary to vasoconstriction. However, this should have resulted in an increase in SVRI, which was not observed. The use of inhalant anesthetic, which can cause vasodilation secondary to direct effects on vascular smooth muscle, may have blunted this response and prevented an increase in SVRI.[36,37] In addition, SVRI is a macrovascular variable representing global vascular tone and therefore might poorly reflect changes in the microcirculation, especially if there is substantial microvascular shunting and regional vasoconstriction. Finally, severe hypovolemia could result in failure to completely fill (and thereby perfuse) vessels, resulting in reduction in microvascular variables independent of vascular tone.

Perfused vessel density was the microcirculatory variable used in correlation analysis to determine which macrovascular variables might be the best predictors of microcirculatory changes associated with hemorrhagic shock. The authors chose PVD because it provides a composite representation of the microcirculation that most closely approximates the FCD, a value that correlates with tissue survival.[19] In the present study, a good correlation between macrovascular variables and changes in PVD was found. This was in contrast to studies[1–5] that found substantial discordance between macro- and microcirculation variables. Some of the discrepancy might be related to the type of shock being assessed. Many of the previous studies[3–5] that used this technology involved septic shock, a disease process known to be associated with local release of vasoactive mediators and microcirculatory disregulation. Without these differences in local mediators, it is possible that, with hemorrhagic shock, macro- and microcirculatory changes are more like to occur in concert. This notion is supported by a study[38] comparing microcirculatory changes in hemorrhagic versus septic shock. The investigators found that PVD closely paralleled changes in macrovascular variables in hemorrhagic shock but not in septic shock. In addition, the severity of hemorrhagic shock may potentially play a role, with more severe shock states resulting in loss of microcirculatory autoregulation and a more parallel relationship between these variables. Perhaps a greater discrepancy would be found in less severe hemorrhagic shock (such as compensated hemorrhagic shock) in which macrohemodynamic variables are preserved at the expense of microcirculation. In addition, there is potential for the discrepancy to become more apparent after resus-

citation, in which macrohemodynamic variables are improved but microcirculatory variables are still abnormal. Both of these scenarios constitute further avenues of exploration with this technology.

Heart rate and O_2ER were significant predictors of PVD. The O_2ER is determined by the balance between oxygen delivery to the tissues and cellular utilization. During hemorrhagic shock, as microvascular perfusion worsens, there will be an attempt, through changes in local blood flow and cellular oxygen uptake, to increase oxygen extraction from the blood.[39] Therefore, it makes sense that O_2ER could be a good postcapillary indicator of the microcirculation because it will be affected by changes in tissue perfusion, oxygen-carrying capacity, and tissue oxygen debt. Heart rate is a determinant of cardiac output and therefore the amount of blood that can potentially feed into the microcirculation. In hemorrhagic shock, increase of heart rate is reflective of a sympathetically driven compensatory response attempting to maintain cardiac output in the face of diminished stroke volume (from hypovolemia). The increased sympathetic tone driving the heart rate will also promote vasoconstriction and impaired microvascular blood flow. It stands to reason that increased heart rate was also predictive of decreased PVD during shock in the present study.

There were several limitations to this study. One major limitation was the acquisition of videographic recordings that were only 5 seconds in duration, as opposed to the 20 seconds recommended by established consensus criteria.[31] This recommendation is based on the notion that vessels should be assessed for 20 seconds to determine adequacy and consistency of flow and thereby perfusion. In the available literature, however, there is no clear indication as to exactly how this optimal time frame was derived (as opposed to a shorter or longer time frame). In the present study, the authors encountered technical and logistic constraints that limited the videographic capture time to 5 seconds for each videographic recording. The shorter observational period could therefore have resulted in overestimation of PVD if flow proved to be intermittent rather than continuous when assessed for a longer period of time. Additionally, while videographic quality appeared adequate at the time of acquisition, some videographic recordings were deemed to be nondiagnostic at the time of analysis. Three dogs at each time point only had 2 videographic recordings for analysis instead of the recommended 3.[31] This is a relatively small proportion of the videographic recordings, but their removal could have influenced the final results. The design of the experimental model used in this study could have resulted in further limitations. Induction and maintenance of anesthesia was performed with sevoflurane gas, which can have a substantial effect on vasomotor tone. The vasodilatory effects of inhalation anesthesia could therefore have increased values for microcirculatory variables at baseline and blunted their reduction in hemorrhagic shock. As such, values for PVD and the other variables may be even lower in conscious dogs with naturally occurring hemorrhage. The subjects in the present study also received ketamine as an induction agent. Ketamine increases heart rate, myocardial work, cardiac output, and myocardial oxygen demand. It has direct negative inotropic effects on the heart and causes increased sympathetic outflow from the CNS.[40] The use of ketamine could have led to changes in microvascular perfusion. However, there was no substantial effect on heart rate at baseline associated with its administration, and the expected decrease in PVD during hemorrhagic shock was detected. Additionally, hemorrhagic shock is a common sequel of severe trauma or underlying systemic disease in clinical patients. The controlled hemorrhage, fixed-pressure shock protocol used in this experiment did not account for the multifactorial effects of tissue trauma and systemic inflammation that result from naturally occurring hemorrhagic shock and that could therefore affect microcirculatory flow.

Finally, there are several potential limitations to application of the SDF technology in dogs. One of the major concerns regarding the use of buccal mucosa for monitoring the microcirculation is that it only provides information about one tissue bed and might not be reflective of changes in microcirculation of other tissues. However, results of a recent study[41] indicate a strong correlation between microcirculatory changes in the oral and intestinal mucosa in a porcine sepsis model. This suggests that the variable PVD of the buccal mucosa could provide information regarding a major organ of concern in shock states, the gastrointestinal tract, especially with regard to risk of bacterial translocation and septic complications.[42,43] During the present study, we discovered that the device worked best on nonpigmented tissue, and excessive pigmentation or salivation was detrimental to the videographic quality. Acquisition of quality videographic recordings also depends on the skill level of the device operator, and there is a substantial learning curve.[26] To obtain quality images, some pressure must be applied to keep the device in apposition to the tissues. If too much pressure is applied, however, the small and thin-walled capillaries can be compressed and lead to falsely decreased microcirculatory variables. One way to circumvent this problem is to watch for absence of flow in large veins as the videographic recordings are being obtained. This finding represents evidence of pressure artifact, and pressure should be reduced to obtain quality images.[31] General anesthesia made videographic acquisition in our study dogs simple, but patient compliance and restraint may also be a factor in conscious dogs because motion can affect videographic quality. Image analysis and determination of microvascular variables are performed after acquiring and storing the videos and are fairly time-consuming (approx 1 h/video). These factors could substantially limit the clinical application of this monitoring tool.

Intravital microscopy performed by use of SDF technology allows real-time imaging of the microvasculature and has potential as an effective tool for monitoring microcirculatory changes associated with hemorrhagic shock and resuscitation in dogs. The present study revealed a significant reduction in values for microcirculatory variables associated with a state of severe hemorrhagic shock that has not been previously reported in dogs. A strong correlation between changes in PVD and macrohemodynamic and blood gas vari-

ables was also detected. On the basis of the results of this investigation, further experimental and clinical research is warranted to determine the value of PVD and other microcirculatory variables for assessing severity of shock and effectiveness of resuscitation in dogs.

a. Microscan, MicroVision Medical, Amsterdam, The Netherlands.
b. Hydromorphone, Baxter Healthcare, Deerfield, Ill.
c. Diazepam, Hospira Inc, Lake Forest, Ill.
d. Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa.
e. SevoFlo, Abbott Animal Health, Abbott Park, Ill.
f. Fentanyl citrate, Hospira Inc, Lake Forest, Ill.
g. Baxter Healthcare, Deerfield, Ill.
h. Passport 2, Datascope Corp, Fairfield, NJ.
i. Datex, Instrumentarium Corp, Helsinki, Finland.
j. LiDCO, LiDCO Ltd, London, England.
k. Euthasol euthanasia solution, Virbac Animal Health, Fort Worth, Tex.
l. Blood gas, ABL 725, Radiometer America, Westlake, Ohio.
m. AVA MicroScan Analysis Software, version 3.0, MicroVision Medical, Amsterdam, The Netherlands.
n. PASW Statistics, version 18, SPSS Inc, Chicago, Ill.
o. SigmaStat, version 3.5, Systat, San Jose, Calif.

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