# Evaluation of quantified contrast-enhanced color and power Doppler ultrasonography for the assessment of vascularity and perfusion of naturally occurring tumors in dogs

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**Objective**—To investigate subjective and computerized methods of evaluation of color Doppler (CD) and power Doppler (PD) ultrasonographic images (obtained before and after administration of contrast medium) for quantitative assessment of vascularity and perfusion of various naturally occurring tumors in dogs.

**Sample Population**—34 tumors in 34 dogs.

**Procedure**—Tumors in dogs were examined via CD and PD ultrasonography before and after IV injection of a microbubble contrast agent (pre- and postcontrast examinations, respectively). Images were digitized for subjective assessment of vessel density and vascular pattern and computer-aided assessment of parameters of vascularity (fractional area [FA]) and perfusion (color-weighted FA [CWFA] and mean color level).

**Results**—With both analysis methods, more vessels were identified in precontrast PD ultrasonographic images than in precontrast CD ultrasonographic images. Moreover, compared with values for precontrast PD ultrasonography, FA, CWFA, and mean color level were higher for postcontrast PD ultrasonography. In postcontrast images, there was a significant association between vessel densities determined through subjective and computerized assessments. Although sample size was small, vascularity of squamous cell carcinomas was significantly greater than that of other tumor types. Ten of the 19 soft tissue sarcomas had low vessel density with minor contrast enhancement. With increasing gross tumor volume, FA and CWFA decreased for all Doppler ultrasonographic methods.

**Conclusions and Clinical Relevance**—Higher values of the ultrasonographic parameters representing vascularity and perfusion of tumors in dogs were determined via PD ultrasonography after administration of contrast medium than via PD or CD ultrasonography without administration of contrast medium. (*Am J Vet Res* 2005;66:21–29)

In the diagnosis of tumors and their classification as benign or malignant, the degree of vascularity and perfusion of the neoplastic tissue are of central importance. The likelihood of recurrence, metastasis, and patient survival may also depend on tumor perfusion. Ultimately, tumor vascularity and perfusion influence the design of cancer treatment protocols.[1] For example, results of a recent experimental study[2] involving radiation therapy have suggested that both tumor and endothelial cells of the tumor microvasculature are damaged by radiation. It is well documented that tumor growth beyond a certain mass requires neovasculature derived from adjacent normal vessels. The process of forming new blood vessels from preexisting vasculature is called angiogenesis. The adjacent normal cells are stimulated to produce and secrete angiogenic factors. This process is also essential in the occurrence of metastases.[3] On the basis of this knowledge, intensive research has been undertaken regarding the development of inhibitors of angiogenesis. Because these agents act directly on endothelial cells and not tumor cells, clinical monitoring of tumor regression during antiangiogenic treatment only partially reflects treatment efficacy. In studies[4,5] in tumor-bearing mice and rats, Doppler ultrasonography has provided a valuable noninvasive method for serial examinations of tumor vascularity and perfusion during antiangiogenic treatment and radiation therapy.

The most common method of quantitatively and qualitatively assessing tumor vasculature is immunohistologic analysis of intratumoral microvascular density and architecture. However, relying on serial histologic analysis to monitor response of tumors to treatment is impractical with most patients because tissue must be obtained repeatedly by invasive biopsy procedures. The tissue samples represent only a certain portion of the tumor, and histopathologic findings are not immediately available for the clinician. Doppler ultrasonography

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has been used to assess tumor vessels in vivo. During ultrasonographic examination, the entire tumor can be surveyed and its anatomic and physiologic characteristics can be evaluated without affecting the tumor itself. Ultrasonography also offers the advantage of serial examinations during a treatment regimen. The successful use of color or power Doppler ultrasonography (often in combination with a microbubble contrast medium) for assessment of the vascularity and perfusion of various tumors in human and experimentally induced tumors in animals has been described in many reports.[6-9] Power Doppler ultrasonography has been shown to be more sensitive than color Doppler ultrasonography for detecting low velocities and small parenchymal vessels.[10,11] Whereas color Doppler ultrasonography displays the change in the returning frequency of the Doppler signal to provide velocity and directional information, power Doppler ultrasonography records the amplitude (energy) of the reflected Doppler signal from moving blood cells. With the latter technique, color brightness corresponds to the number of moving cells, not their velocity. Because of the lack of angle dependence and the very low energy of random noise, higher gain settings may be used with power Doppler ultrasonography, which increases sensitivity for flow detection.[12] Because of typically high interstitial pressure in tumors and resultant low-velocity states in tumor vessels, power Doppler ultrasonography is of special value in assessing tumor vasculature.

As it is in humans, cancer is a common disease in dogs. Forty-five percent of all dogs older than 10 years are expected to die of cancer.[13] Naturally occurring tumors in dogs have previously been proposed as good model systems for cancer research.[14,15] They provide an excellent opportunity to study tumor etiology, biologic features, and response to treatment and may better represent the vascular microenvironment of human tumors than experimentally induced or transplanted tumors in animals. When dogs undergo radiation therapy, a short period of anesthesia is required during each treatment to maintain positioning. During anesthesia of dogs with tumors, it is possible to perform serial examinations of tumors via Doppler ultrasonography under standardized conditions; furthermore, those findings can be compared with data obtained via invasive procedures, such as intratumoral oxygenation measurements or microvascular density assessments. Because of this, dogs with naturally occurring tumors can be used to gain more meaningful information regarding tumor vascularity and perfusion, especially during various treatment procedures. The purpose of the study reported here was to investigate a subjective and a computerized method of evaluation of color and power Doppler ultrasonographic images (obtained before and after administration of contrast medium) for quantitative assessment of vascularity and perfusion of various naturally occurring tumors in dogs.

## Materials and Methods

**Patient selection**—The study was performed in 34 dogs with various naturally occurring tumors that were evaluated at the Section of Radio-Oncology at the Veterinary Faculty of the University of Zürich. To be included in the study, each dog's tumor had to be superficial and accessible for ultrasonographic evaluation; all tumors were either localized in the oral cavity, on the skull, or on the limbs. After owner consent was obtained, each patient underwent a physical examination, thoracic radiography, and blood analyses to determine Hct, hemoglobin concentration, and RBC count. Breed, sex, weight, and age of the dogs were recorded. If indicated, fine-needle aspiration of enlarged regional lymph nodes, abdominal ultrasonography, and computed tomography of the primary tumor were done. For tumor diagnosis and grading, routine histologic evaluation of tumor specimens was performed; tumor staging was based on the World Health Organization system. According to their histologic diagnosis, tumors were categorized as soft tissue sarcomas, bone sarcomas, squamous cell carcinomas, and miscellaneous tumors. Gross tumor volume ($cm^3$) was determined as the product of caliper measurements of width, length, and depth.

All dogs underwent either palliative or curative radiation therapy; therefore, the ultrasonographic examination of their tumors was performed under anesthesia, immediately prior to administration of the first radiation fraction. Anesthesia was initiated by use of midazolam[a] (2 mg/kg, IV) or diazepam[b] (0.2 mg/kg, IV), either with or without butorphanol[c] (0.1 to 0.3 mg/kg, IV), followed by propofol[d] given to effect; the protocol used for each dog was noted. Anesthesia was maintained with isoflurane[e] delivered in oxygen through an endotracheal tube. Heart rate and hemoglobin saturation as measured by pulse oximetry ($Spo_2$) were monitored continuously during anesthesia to assess the status of each dog. At the time of the Doppler ultrasound examination, the heart rate of each dog was recorded for the statistical analyses. For inclusion in the study and because serial episodes of anesthesia were planned, each dog had to be in good clinical condition and the results of the cardiovascular examination had to be within reference limits.

**Ultrasonographic imaging of tumors**—Whenever necessary, the hair overlying the tumor was clipped. For each ultrasonographic examination, acoustic gel was applied to the skin and imaging was performed by use of a 5- to 12-MHz linear transducer.[f] For color and power Doppler ultrasonography, settings were constant for all examinations (79% color gain, medium wall filter, and 700-Hz pulse repetition frequency for color Doppler ultrasonography and 81% color gain, medium wall filter, and 500-Hz pulse repetition frequency for power Doppler ultrasonography). The maximal image depth was 25 mm, and a frame rate of 10 to 12 Hz was used; imaging time was 5 to 10 min/dog. The ultrasound contrast agent[g] used was a first-generation microbubble suspension that was administered at a dose rate of 80 mg/kg.

Initial scanning of the entire tumor was performed with B mode to define its boundary and morphologic features ultrasonographically. A rectangular sample volume was then placed over the tumor and surrounding tissue, denoting the region in which color and power Doppler ultrasonographic data would be acquired. A sliding scan of the entire tumor was performed with color and power Doppler ultrasonography to subjectively assess overall tumor vascularity. Finally, an area that represented the tumor's typical vessel density and was also easily reproducible on the basis of anatomic landmarks was chosen. A minimum of 5 color Doppler and 5 power Doppler images (designated precontrast images) were captured on the hard disk of the ultrasound machine. The probe remained in the same location for the ultrasonographic examination after administration of contrast medium. Via an IV catheter placed in a peripheral vein, a bolus injection of the microbubble contrast agent was administered by hand. After blooming artifacts had disappeared, power Doppler and then color Doppler ultrasonographic images (designated postcontrast images) were obtained. A second injection of

contrast medium was not administered to minimize expense and keep duration of anesthesia as short as possible. This resulted in a time delay between acquisition of postcontrast power Doppler and color Doppler ultrasonographic images. A minimum of 5 color Doppler and 5 power Doppler postcontrast images were captured on the hard disk of the ultrasound machine within a maximum interval of 3 minutes. Images were transferred to a computer connected to the ultrasound unit.

**Image analysis**—Subjective image analysis included assessment of vessel density and vascular pattern in precontrast color and power Doppler and postcontrast power Doppler ultrasonographic images of tumors. Postcontrast color Doppler ultrasonographic images were not subjectively evaluated and compared because of the time delay between acquisition of postcontrast power and color Doppler ultrasonographic images. From the digitized images, vessel density was graded as low (vascularized area within a tumor, ≤ 10%), moderate (vascularized area within a tumor, 11% to 34%), or high (vascularized area within a tumor, ≥ 34%). The vascular pattern within a tumor was classified as central (if only the mid portion was vascularized), peripheral (if vessels were seen only in the peripheral zone), homogeneous (if distribution of vessels was even), or patchy (if vascularization was inhomogeneous).

Computerized image analysis of 5 images from each of the pre- and postcontrast color and power Doppler ultrasonographic examinations was performed by use of dedicated software.[h] A **region of interest (ROI)** was drawn around the tumor boundaries and, similar to the method of Fleischer et al,[16] 3 measurements were computed for each ROI: **fractional area (FA)**, **mean color level (MCL)**, and **color-weighted FA (CWFA)**. Fractional area is calculated as the number of colored pixels in the ROI divided by the total number of pixels in the ROI multiplied by 100; it represents a vascularity index and indicates the percentage area of the tumor occupied by blood vessels. In color Doppler ultrasonography, MCL is obtained by dividing the sum of red and blue color in the ROI by the total number of colored pixels in the ROI; in power Doppler ultrasonography, MCL is obtained by dividing the sum of the integrated power values in the ROI by the total number of colored pixels in the ROI. Color-weighted fractional area is the product of MCL and FA. Both MCL and CWFA are used to assess perfusion. Values of MCL indicate the mean local blood velocity in color Doppler ultrasonography or mean density of moving RBCs above a threshold velocity in power Doppler ultrasonography, whereas CWFA determines the mean blood flow through the ROI in color Doppler ultrasonography or blood volume within the tissue in power Doppler ultrasonography. Computerized parameters (FA, CWFA, and MCL) were determined for each of the 4 Doppler methods by calculating the median of each set of 5 images. The resulting 12 parameters were as follows: $FA_{precontrast\_CD}$, $FA_{precontrast\_PD}$, $CWFA_{precontrast\_CD}$, $CWFA_{precontrast\_PD}$, $MCL_{precontrast\_CD}$, $MCL_{precontrast\_PD}$, $FA_{postcontrast\_CD}$, $FA_{postcontrast\_PD}$, $CWFA_{postcontrast\_CD}$, $CWFA_{postcontrast\_PD}$, $MCL_{postcontrast\_CD}$, and $MCL_{postcontrast\_PD}$, where CD indicates color Doppler ultrasonography and PD indicates power Doppler ultrasonography.

**Statistical analyses**—Whenever data had non-Gaussian distribution, variables were transformed using the natural logarithm. To assess the association with MCL, FA, or CWFA of pre- and postcontrast color and power Doppler ultrasonography, linear regression and an ANOVA were used for continuous variables (Hct, hemoglobin concentration, RBC count, weight, age, heart rate, and gross tumor volume) and categorical variables (breed, sex, and anesthesia protocol), respectively. The relationship between histologic diagnosis of the tumor and MCL, FA, or CWFA of pre- and postcontrast color and power Doppler ultrasonography was analyzed by use of a 1-way ANOVA and the post hoc Bonferroni-Dunn test. To compare findings of post- and precontrast Doppler ultrasonography, the differences between post- and precontrast FA, CWFA, and MCL, respectively, were calculated within each Doppler method (color and power Doppler ultrasonography). The means of the differences were computed together with the 95% confidence intervals for the mean differences. Means were compared by a 1-sample *t* test, which corresponds to the paired *t* test. Similarly, to compare precontrast power and color Doppler ultrasonography for the assessment of tumor vascularity (parameter FA), the difference between $FA_{precontrast\_PD}$ and $FA_{precontrast\_CD}$ was calculated and a 1-sample *t* test was used for comparison of the mean difference with the hypothesized mean difference of 0. The variables MCL and CWFA could not be compared between color and power Doppler ultrasonography because the computerized color values differ for both methods. The association between subjectively scored vessel density and computerized vessel density (ie, $FA_{postcontrast\_PD}$) was analyzed with an ANOVA. Statistical analyses were performed with a computer software program.[i] Values of $P < 0.05$ were considered significant.

## Results

Among the 34 dogs with naturally occurring, superficially located tumors, most had soft tissue sarcomas (**Table 1**). Data obtained from 1 dog with lymphosarcoma of the oral cavity were excluded from statistical analyses because of an abnormally high heart rate during anesthesia. The age of the dogs ranged from 1 to 16 years (mean age, 8.3 years). The study group of dogs included 6 Flat-Coated Retrievers, 5 Golden Retrievers, 2 Labrador Retrievers, 8 mixed-breed dogs, and 1 dog each of 12 other small to large breeds. Twenty dogs were male, and 13 were female. In the 33 dogs, mean Hct was 43.6% (median, 44%; reference range, 42% to 55%), mean hemoglobin concentration was 15.2 g/dL (median, 15.6 g/dL; reference range, 14.4 to 19.1 g/dL), mean RBC concentration was $6.45 \times 10^6$ RBCs/μL (median, $6.59 \times 10^6$ RBCs/μL; ref-

Table 1—Location and histologic classification of 34 naturally occurring tumors in 34 dogs examined by use of color and power Doppler ultrasonography with and without administration of contrast medium.

| Histologic classification* (No. of tumors) | Location† (No. of tumors) |
|---|---|
| Soft tissue sarcoma (19) | |
| Fibrosarcoma, grade 1 (6) | Oral cavity (5), skull (1) |
| Fibrosarcoma, grade 2 (3) | Oral cavity (2), limb (1) |
| Spindle cell sarcoma, grade 2 (3) | Limb (3) |
| Histiocytic sarcoma (5) | Limb (4), oral cavity (1) |
| Myxosarcoma, grade 1 (1) | Limb |
| Hemangiopericytoma, grade 2 (1) | Limb |
| Bone sarcoma (5) | |
| Osteosarcoma, grade 2 (2) | Oral cavity (2) |
| Osteosarcoma, grade 3 (2) | Oral cavity (2) |
| Chondrosarcoma, grade 1 (1) | Vertebral column |
| Squamous cell carcinoma (4) | Rostral portion of oral cavity (4) |
| Miscellaneous (6) | |
| Melanoma (2) | Oral cavity (2) |
| Adenocarcinoma, grade 3 (1) | Nasal cavity and skull |
| Histiocytoma (1) | Skull |
| Acanthomatous epulis (1) | Oral cavity |
| Oral lymphosarcoma (1) | Oral cavity |

*For tumor diagnosis and grading, routine histologic evaluation of tumor specimens was performed; tumor staging was based on the World Health Organization system. †To be included in the study, each dog's tumor had to be superficial and accessible for ultrasonographic evaluation; all tumors were either localized in the oral cavity, on the skull, or on a limb.

erence range, 6.1 to 8.1 × $10^6$ RBCs/μL), mean heart rate during the Doppler ultrasound examination was 106 beats/min (median, 110 beats/min; reference range, 60 to 130 beats/min), and mean gross tumor volume was 90.09 $cm^3$ (median, 64.5 $cm^3$). Two dogs were anesthetized with butorphanol and midazolam, 5 with diazepam only, and 26 with midazolam only; subsequently, every dog received propofol.

**Subjective image analysis**—Of 33 dogs, 30 underwent pre- and postcontrast Doppler ultrasonography. During examination of 3 dogs, contrast medium was not available and only precontrast Doppler ultrasonography was performed.

Before the administration of contrast medium, subjective scoring of vessel density did not differ between color and power Doppler ultrasonography; via either method, low, moderate, and high vessel densities were identified in 19 of 33 (58%), 11 of 33 (33%), and 3 of 33 (9%) of the tumors, respectively. However, more small vessels were detected via power Doppler ultrasonography in 14 of 33 (42%) of the tumors, compared with color Doppler ultrasonography. After administration of contrast medium, contrast enhancement was subjectively identified via power Doppler ultrasonography in 68% of the tumors. Subjectively, vessel density in postcontrast power Doppler ultrasonographic images was higher than that assessed in precontrast color and power Doppler ultrasonographic images; overall, 15 of 30 (50%) of the tumors had low vessel density, whereas 8 of 30 (27%) tumors had moderate and 7 of 30 (23%) had high vessel densities (**Figure 1**).

Prior to administration of contrast medium, 13 of the 19 soft tissue sarcomas had low vessel density, whereas 6 had moderate vessel density. After administration of contrast medium, 10, 4, and 2 of the 19 soft tissue sarcomas had low, moderate, and high vessel densities, respectively. Of 10 tumors without apparent contrast enhancement, 7 were soft tissue sarcomas. High vessel density was detected in each of 4 squamous cell carcinomas in the oral cavity before and after

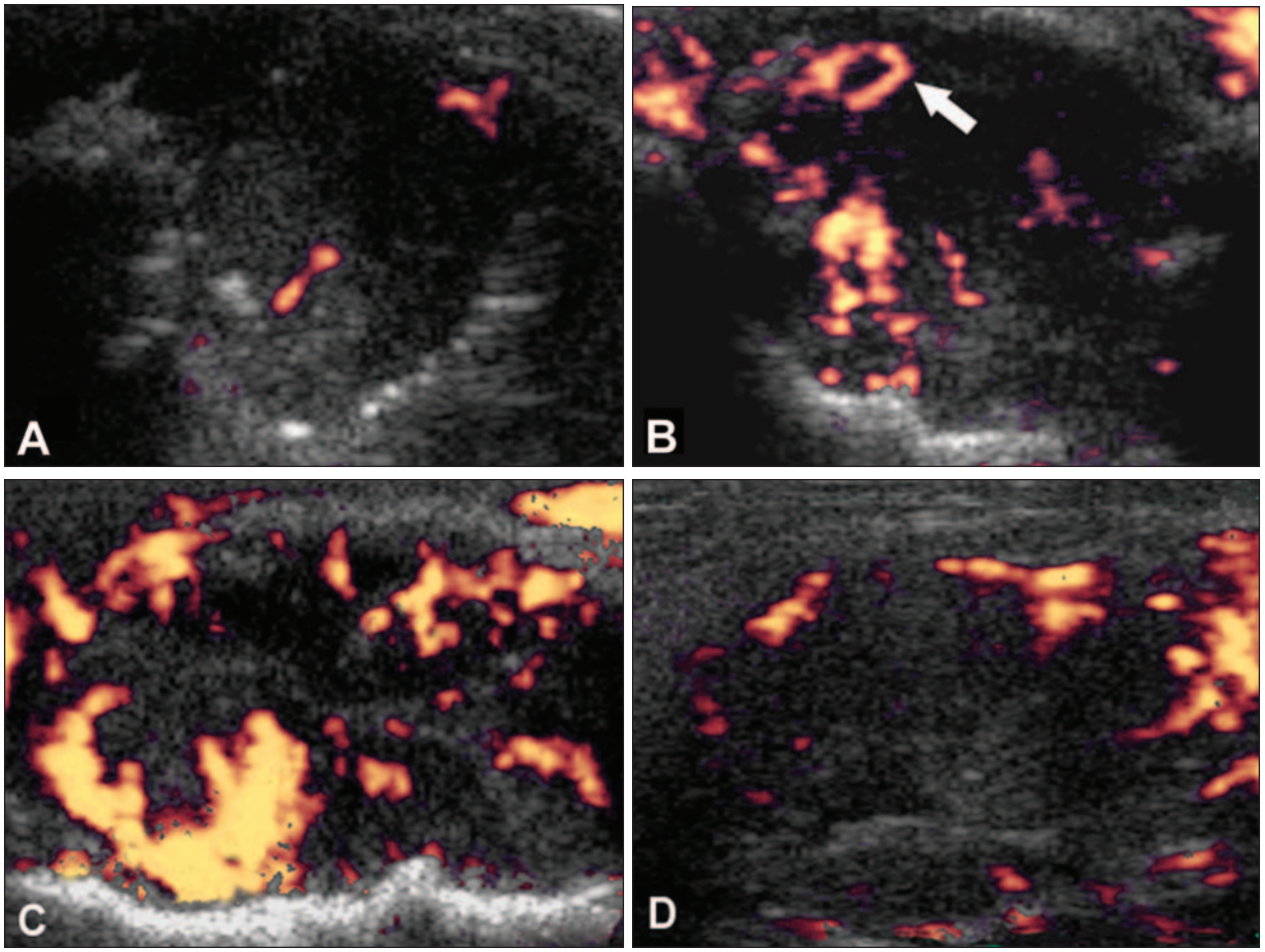


Figure 1—Power Doppler ultrasonographic images of naturally occurring tumors (various histologic types and grades) in 4 dogs obtained after IV administration of contrast medium for subjective assessment of vessel density and vascular pattern and computer-aided assessment of ultrasonographic parameters for vascularity (fractional area [FA]) and perfusion (color-weighted FA [CWFA] and mean color level [MCL]). A—Image of a low-grade fibrosarcoma located in the rostral portion of the maxilla of a dog. This tumor had low vessel density and a patchy vascular pattern (FA = 4.1%; CWFA = 1.9; and MCL = 46.3). B—Image of an acanthomatous epulis in the oral cavity of a dog. This tumor had moderate vessel density and a patchy vascular pattern (FA = 23.5%; CWFA = 12.0; and MCL = 54.0). Notice that a vessel has a self-loop formation (white arrow). C—Image of a squamous cell carcinoma of the oral cavity in a dog. This tumor had high vessel density and a homogeneous vascular pattern (FA = 31.0%; CWFA = 29.3; and MCL = 100.5). D—Image of a histiocytic sarcoma in the stifle joint of a dog. This tumor had moderate vessel density and a peripheral vascular pattern (FA = 18.8%; CWFA = 10.7; and MCL = 57.0).

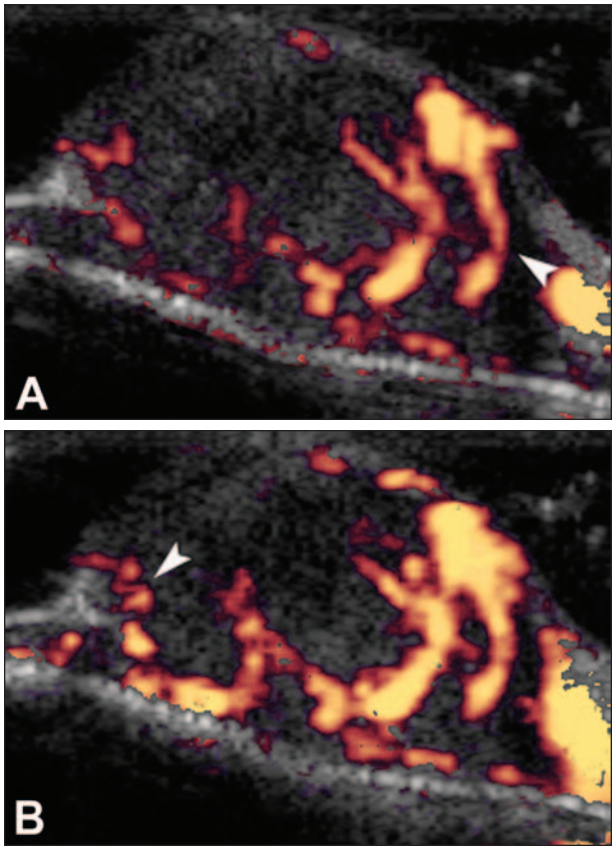


Figure 2—Power Doppler ultrasonographic images of a melanoma (with high vessel density and a patchy vascular pattern) of the oral cavity of a dog. A—Image obtained before IV administration of contrast medium (FA = 31.8%; CWFA = 21.2; and MCL = 66.8). B—Image obtained after IV administration of contrast medium (FA = 44.6%; CWFA = 39.0; and MCL = 86.8). In both panels, caliber changes are evident in multiple vessels (arrowheads). *See* figure 1 for key.

administration of contrast medium (Figure 1). The vessel densities of the remaining tumors were variable.

Three vascular patterns were identified in the tumors: peripheral, patchy, and homogeneous (Figure 1). A central vascular pattern was not detected. Precontrast vascular patterns did not differ between color and power Doppler ultrasonographic images. Before administration of contrast medium, 12 of 33 (36%) of the tumors had a peripheral vascular pattern, whereas 19 of 33 (58%) had a patchy vascular pattern. Only 6% (2/33) of the tumors had a homogeneous vascular pattern. After administration of contrast medium, a change in the vascular pattern from peripheral to patchy was detected in only 1 dog with an acanthomatous epulis. Although not a major goal of the present study, abnormal morphologic features of vessels such as stenosis, loop formation, trifurcation, and anarchic vascular pattern were commonly detected (Figures 1 and 2).

**Computerized image analysis**—Hematocrit, hemoglobin concentration, RBC count, breed, age, sex, weight, heart rate, histologic diagnosis of the tumor, and anesthesia protocol were not significantly associated with MCL, FA, or CWFA derived via pre- and postcontrast color and power Doppler ultrasonography. Gross tumor volume was significantly associated with

Table 2—Mean ± SD differences between computed power Doppler ultrasonographic parameters for vascularity (fractional area [FA]) and perfusion (color-weighted FA [CWFA] and mean color level [MCL]) assessed without and with administration of contrast medium (pre- and postcontrast values, respectively) in 34 dogs with naturally occurring tumors.

| Parameter | Difference* | 95% CI | *P* value† |
|---|---|---|---|
| FA | 3.60 ± 4.77 | 1.8–5.4 | < 0.001 |
| CWFA | 3.62 ± 5.31 | 1.6–5.6 | < 0.001 |
| MCL | 7.05 ± 9.52 | 3.5–0.6 | < 0.001 |

*Difference calculated as postcontrast value minus precontrast value for each parameter. †Statistical analysis performed by use of a 1-sample *t* test. CI = Confidence interval.

FA and CWFA of all Doppler methods ($P = 0.004$ to 0.021) but not with MCL of any of the methods. With increasing gross tumor volume, FA and CWFA significantly decreased for all Doppler methods.

Compared with values in other tumor types, FA and CWFA values of pre- and postcontrast color and power Doppler ultrasonography were significantly ($P < 0.001$) higher for squamous cell carcinomas. For precontrast color and power Doppler ultrasonography of squamous cell carcinomas of the oral cavity, median $FA_{CD}$, $FA_{PD}$, $CWFA_{CD}$, and $CWFA_{PD}$ were 16.3%, 26.0%, 27.5, and 16.8, respectively. In the other types of tumors (soft tissue sarcomas, bone sarcomas, and miscellaneous tumors), median values of precontrast $FA_{CD}$, $FA_{PD}$, $CWFA_{CD}$, and $CWFA_{PD}$ were 2.3%, 3.3%, 4.1, and 1.8, respectively. For postcontrast color and power Doppler ultrasonography of squamous cell carcinomas of the oral cavity, median $FA_{CD}$, $FA_{PD}$, $CWFA_{CD}$, and $CWFA_{PD}$ were 17.1%, 35.8%, 28.7, and 25.7, respectively. In the other types of tumors, median values of postcontrast $FA_{CD}$, $FA_{PD}$, $CWFA_{CD}$, and $CWFA_{PD}$ were 2.7%, 3.6%, 4.8, and 1.9, respectively. Mean color level values did not differ significantly among histologically classified groups of tumors. However, sample size of these tumor groups was small.

For power Doppler ultrasonography, findings of postcontrast Doppler ultrasonography were compared with those of precontrast Doppler ultrasonography (**Table 2**). Compared with values obtained prior to administration of contrast medium, significant increases of MCL, FA, and CWFA were detected after its administration (Figure 2). The same analysis was performed for color Doppler ultrasonography. Compared with values obtained prior to administration of contrast medium, FA and CWFA, but not MCL, significantly increased after administration of contrast medium; the increases in FA and CWFA were not as great as those identified for power Doppler ultrasonography. However, the actual results are not presented because they are biased as a consequence of the single injection of contrast medium.

To compare assessment of tumor vascularity (parameter FA) via power and color Doppler ultrasonography, the difference between $FA_{precontrast\_PD}$ and $FA_{precontrast\_CD}$ was calculated and was found to be highly significant ($P < 0.001$). The difference between $FA_{postcontrast\_PD}$ and $FA_{postcontrast\_CD}$ was not analyzed as a consequence of the single injection of contrast agent. Subjectively scored vessel density on postcontrast

power Doppler ultrasonographic images and computerized vessel density ($FA_{postcontrast\_PD}$) were significantly associated ($R^2 = 0.76$; $P < 0.001$).

## Discussion

In the present study, color and power Doppler ultrasonography with and without administration of contrast medium were shown to be valuable for assessment of the vascularity and perfusion of naturally occurring tumors in dogs. A computerized method was used that converted velocities (color Doppler ultrasonography) and power values (power Doppler ultrasonography) to color hue and saturation levels.[5,16] From these, blood flow parameters were derived to determine vascularity (via measurement of FA) and perfusion (via measurement of MCL and CWFA). In the tumors examined in our study, the depiction of vascularity (parameter FA) was superior in precontrast power Doppler ultrasonographic images, compared with color Doppler ultrasonographic images. This finding supports results of studies[11,17] in humans, which indicated that power Doppler ultrasonography was more sensitive than color Doppler ultrasonography for the assessment of small vessels and low intravascular velocities. This can be explained by of the lack of angle dependence and the manner in which random noise is depicted with power Doppler ultrasonography. Power Doppler ultrasonographic images are derived from the energy of the reflected ultrasound signals, and random noise has low energy; this permits higher gain settings and increased sensitivity for flow detection.[12]

A first-generation, galactose-based contrast agent containing stabilized microbubbles was administered to the dogs examined in the present study. The bubbles act as specular reflectors after IV administration, and they increase reflection of the incident fundamental frequency of the ultrasound beam. This agent also increases the Doppler signal, thereby increasing the sensitivity for low-flow situations.[18] This potential is of great interest for imaging of tumors. In mice with xenograft melanomas, contrast-enhanced power Doppler ultrasonography better depicted overall tumor vascularity and had greater correlation to histologic microvascular density than did non–contrast-enhanced power Doppler ultrasonography.[19] In breast, kidney, and liver masses and cervical lymph nodes in humans, contrast-enhanced color or power Doppler ultrasonography markedly improved diagnostic accuracy in differentiating benign from malignant lesions, compared with precontrast color or power Doppler ultrasonography.[6-9] In the study of this report, postcontrast power Doppler ultrasonographic images revealed higher estimates of vessel density, compared with densities determined via precontrast color and power Doppler ultrasonography. For power Doppler ultrasonography, the perfusion parameters MCL and CWFA also increased significantly after administration of contrast medium. Because of the study design, the postcontrast color Doppler ultrasonographic parameters could be evaluated only in a limited way. After administration of contrast medium, power Doppler ultrasonography was first performed, followed by color Doppler ultrasonography. This sequence of ultrasonographic examinations was applied because in humans and experimental animals, power Doppler ultrasonography has been proven to be more sensitive than color Doppler ultrasonography for detection of small vessels. A second injection of contrast medium was not given to minimize expense and keep duration of anesthesia as short as possible. Further, a second injection may have resulted in an accumulation of contrast medium and artificially increased postcontrast color Doppler ultrasonographic parameters. Therefore, compared with power Doppler ultrasonographic findings, a significant but lesser increase of FA and the FA-dependent variable CWFA was detected via color Doppler ultrasonography in the present study.

In our study, computerized analysis of tumor vascularity confirmed the results of the subjective assessment. Subjectively, precontrast power Doppler ultrasonography was superior to precontrast color Doppler ultrasonography for the assessment of vessel density, and postcontrast power Doppler ultrasonography appeared to be the most sensitive technique for depiction of vessel density among the 4 methods evaluated. Association between subjective score and computerized vessel density was highly significant. However, via precontrast color and power Doppler ultrasonography, the proportions of tumors determined to have low, moderate, or high vessel density were the same. This leads to the conclusion that for a single evaluation, subjective assessment may be sufficient to determine vessel density in a tumor. For serial examinations and classification of a tumor, dedicated computer software programs are required because mild differences in vascularity between or within tumors may not be recorded with a subjective semiquantitative score.[20]

The most common method for assessment of tumor vascularity is immunohistologic analysis of intratumoral microvascular density. Unfortunately, this analysis was not available for comparison with the ultrasonographic methods in the present study. However, although high correlation of Doppler ultrasonographic measurements with the quantified results of immunofluorescent staining have been reported,[20,21] results of another study[16] indicated poor correlation. It was hypothesized that the discrepancy, in part, may have been a result of differences in the techniques used in those studies. In most studies involving immunohistologic analysis of intratumoral microvascular density, the hot-spot technique is used. In this technique, an area of the tumor that appears subjectively to contain the most capillaries and small venules (ie, microvessels) is chosen; these areas are so-called neovascular hot spots. Within a 0.74-$mm^2$ region of the selected area, all microvessels are counted by use of a microscope to assess microvascular density.[22] However, the tissue samples represent only a certain area within the tumor. By use of Doppler ultrasonography, the entire tumor vasculature can be examined. Compared with histologic assessment of tumors in which vessels approximately 15 μm in diameter are detected, larger vessels (approx 100 μm in diameter) are detected via conventional power Doppler ultrasonography. In summary, measurement of tumor vascularity with histologic or Doppler techniques may provide different biolog-

ical information and consequently, methods may only partially replace each other.[23]

Depending on their stage, perfusion and vascular patterns of tumors may differ. When tumors are small, high-flow states are a prominent feature because of arteriovenous shunts. As tumors enlarge, the high-flow areas become peripheral and poor perfusion in the central areas is common.[24] The tumor architecture may become heterogeneous as a result of bulky neoplastic colonization, necrosis, edema, or desmoplastic reaction with displacement and encasement of vessels; the corresponding vascular patterns indicate patchy or peripheral distribution of vessels.[8,25] In the present study, tumors were rather large and 2 vascular patterns were most common: patchy (19/33 [58%] tumors) and peripheral (12/33 [36%] tumors). A homogeneous pattern was rare (2/33 [6%] tumors), and a central pattern was not detected in any of the tumors examined. In humans with breast cancer, liver lesions, and cervical lymph node lesions, ultrasonographic assessment of vessel pattern in combination with assessment of other neoplastic features has been successfully used to distinguish between benign and malignant lesions.[6,26-28] The vascular pattern of a tumor may also change during antiangiogenic treatment. In mice with tumors that were treated with recombinant interleukin 12, the tumors had an evenly distributed vessel pattern prior to treatment; after treatment, that vessel pattern was replaced by a more patchy pattern with a preferential loss of small vessels and overall decreased vascularity.[5]

Malignant tumor vessels are histologically characterized by lack of the muscular layer and irregular contours. They commonly form a heterogeneous network with a chaotic architecture. Instead of a normal hierarchic vascular tree (repeated divisions into 2 branches) with continuously decreasing vessel size towards the periphery, an anarchic vascular pattern consisting of caliber changes, loops (self-connective vessels), and trifurcations (divisions into 3 branches) is commonly seen. These histologic features of the microvascular architecture were first described in a mammary carcinoma of a human.[8,25] Later, they were also used for sonographic assessment. In a study[29] of musculoskeletal tumors, combined Doppler ultrasonographic techniques revealed 4 major vessel characteristics (trifurcation, anarchic vascular pattern, stenosis, and occlusion), which, if 2 criteria were combined, demonstrated high sensitivity and specificity in differentiating benign from malignant lesions. Although it was not a major goal of the present study, abnormal morphologic features of vessels, such as stenosis, loop formation, trifurcation, and anarchic vascular pattern, were commonly detected in the tumors examined.

Of the 34 tumors investigated in our study, 19 were classified as soft tissue sarcomas and 20 were located in the oral cavity. Although soft tissue sarcomas are fairly uncommon in humans, they develop commonly in dogs, and their biological behavior is similar to that of soft tissue sarcomas in humans.[15] Soft tissue sarcomas are a diverse group of cancers that accounts for 15% of all skin and subcutaneous cancers in dogs. Fibrosarcoma of the oral cavity often appears benign histologically (ie, classified as low grade) but is considered a biologically high-grade tumor with a tendency to grow to quite a large size and invade deeper structures, including bone.[30] In dogs, histiocytic sarcoma is a new classification that includes tumors of macrophage and dendritic cell origin. Such tumors are rapidly growing and locally aggressive and usually develop on the limbs or close to a joint.[31] In dogs, cancer of the oral cavity accounts for 6% of cancers and is the fourth most common cancer overall; squamous cell carcinoma, melanoma, and fibrosarcoma are the most frequently reported tumor types in dogs. Whereas melanoma has a strong predilection to metastasize to regional lymph nodes and then the lungs, metastasis of squamous cell carcinoma is very site dependent, with the rostral portion of the oral cavity having low and the caudal aspect of the tongue and tonsils having high metastatic potential.[32] Because of the site predilection for the rostral portion of the mandible and their frequency of development in large dogs, squamous cell carcinomas are easily accessible for ultrasonographic evaluation. In contrast, most ultrasonographic studies of squamous cell carcinomas of the head and neck in humans are performed on accessible lymph node metastases because of the inaccessibility of the primary tumor. It is assumed that the lymph node lesions mirror the properties of the primary tumor.[33] Therefore, squamous cell carcinoma in dogs may represent an adequate model for study of squamous cell carcinomas in humans.

In the study of this report, all squamous cell carcinomas of the oral cavity had a significantly higher vascularity than the other tumors. Vascularity of most of the soft tissue sarcomas was low with minor contrast enhancement. However, results of statistical comparison of the computerized parameters, in particular FA, for the soft tissue sarcomas versus the other tumor types were not significantly different. This is most likely because of the small number of tumors in each classification group. Nevertheless, on the basis of these preliminary results, vascularity and perfusion appear to vary between and within histologically classified tumor types. This may be partially responsible for the variation in tumor aggressiveness and treatment outcome and needs further investigation in a larger number of tumors. In humans with squamous cell carcinomas of the oral cavity, for example, the microvessel count in tumors that had metastasized to lymph nodes was significantly higher than the count in tumors that had not metastasized to lymph nodes.[34] In another study[33]of the vascularity in lymph node metastases of squamous cell carcinomas of the head and neck in humans, median survival rate and time to detection of distant metastases in patients with lymph node metastases of low vascularity were significantly higher than those values in patients with lymph node metastases of high vascularity.

In experimental studies,[35,36] an influence of Hct on blood flow velocities as detected via color or power Doppler ultrasonography has been demonstrated. For example, marked anemia may cause a decrease of blood viscosity, and as a result, changes in the velocity profile and Doppler indices can be detected.[35] A correlation between the Doppler power and Hct was deter-

mined in another study.[36] In dogs, anemia is a common paraneoplastic syndrome and may be associated with various tumors. In some dogs included in the present study, RBC count, Hct, and hemoglobin concentration were at the lower limit of the reference range or even mildly decreased, but significant associations of these variables with MCL, FA, or CWFA of pre- and postcontrast power Doppler ultrasonography were not identified. The Doppler signal is also affected by blood pressure, cardiac output, and vascular resistance to blood flow.[37,38] In the dogs in our study, systemic blood pressure was not evaluated prior to Doppler ultrasonographic examination. The authors of this report consider it unlikely that the dogs were hypo- or hypertensive prior to anesthesia. All dogs had normal mucous membrane color and capillary refill times and had no evidence of hypovolemia based on skin turgor and Hct. However, the effects of changes in Hct and systemic blood pressure should be assessed in future Doppler ultrasonographic studies.

In the present study, a protocol was used that provided rapid induction of and recovery from anesthesia, which are necessary for procedures requiring anesthesia that are repeated regularly or even performed daily, such as fractionated radiation therapy. Further, anesthetic agents that have minimal cardiovascular effects were chosen to avoid an impact on the Doppler examination.[39] Diazepam and midazolam are benzodiazepine tranquilizers and have minimal cardiovascular effects. Two dogs with joint-associated tumors were additionally given butorphanol because of signs of pain. Butorphanol is a narcotic analgesic that has minimal effects on the cardiovascular system at therapeutic doses. Every dog was given propofol IV, and anesthesia was maintained with isoflurane. Both of these agents may decrease blood pressure and cause arterial and venous vasodilation. However, because each dog was administered propofol and isoflurane, it was assumed that the cardiovascular effects were similar among dogs. Ideally, the anesthesia protocol should be more standardized for the study group. A statistical association was not found between the anesthesia protocols and the Doppler ultrasonographic parameters.

With increasing gross tumor volume, vascularity (parameter FA) and perfusion (parameter CWFA) significantly decreased for all Doppler ultrasonographic methods evaluated in our study. The explanation for this may be that as tumors enlarge, necrotic areas form and perfusion becomes poor in certain areas. Reduced function of the endothelium of the tumor vessels causes leakage of high molecular substances; the interstitial pressure increases and edema develops.[8,24]

On the basis of our data, color and power Doppler ultrasonography performed with and without administration of contrast medium appear to be valuable for the assessment of vascularity and perfusion in naturally occurring tumors in dogs. Postcontrast power Doppler ultrasonographic images depicted higher values of vessel density, compared with values determined in precontrast color and power Doppler ultrasonographic images. Although sample size was small in our study, vessel density varied with tumor type (classified histologically). A larger series of dogs with tumors should be investigated to verify the results of the present study. Further investigation of naturally occurring tumors in dogs to gain more meaningful information regarding tumor vascularity and perfusion, especially during various treatment procedures, is warranted.

a. Dormicum, Roche Pharma SA, Reinach, Switzerland.
b. Valium, Roche Pharma SA, Reinach, Switzerland.
c. Morphasol, Gräub AG, Bern, Switzerland.
d. Propofol, Fresenius Kabi AG, Stans, Switzerland.
e. Isoflo, Abbott AG, Baar, Switzerland.
f. ATL 5000, Philips AG, Zürich, Switzerland.
g. Levovist (400 mg/mL), kindly provided by Dr. A. Jäger, Schering AG, Baar, Switzerland.
h. Qwin, Leica Microsystems AG, Glattbrugg, Switzerland.
i. StatView 5.0.1, SAS Institute Inc, Cary, NC.

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