

Head and Neck Squamous Cell Carcinoma: CT Perfusion Can Help Noninvasively Predict Intratumoral Microvessel Density¹

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Purpose:

To determine the correlation between computed tomographic (CT) perfusion parameters and intratumoral microvessel density (MVD) in the evaluation of head and neck squamous cell carcinoma (HNSCC).

Materials and Methods:

This prospective HIPAA-compliant study was performed with institutional review board approval, and informed written consent was obtained from each patient prior to enrollment. Thirteen consecutive patients with advanced HNSCC (stage III or IV) underwent contrast material-enhanced neck CT, CT perfusion imaging, and endoscopic biopsy of the primary tumor site. The average patient age was 57.4 years (range, 41–78 years). Intratumoral MVD was determined after mouse antihuman CD31 antibody immunostaining. The mean number of stained microvessels from 10 high-power fields ($\times 400$) per specimen was recorded. CT perfusion parameters, including capillary permeability, blood volume (BV), blood flow (BF), and mean transit time (MTT), were calculated at the primary site. In the statistical analysis, Pearson and Spearman correlation coefficients were calculated.

Results:

Spearman rank correlation showed positive but not statistically significant correlation between vessel count and BF ($r = 0.30$, $P = .316$) and a positive correlation between vessel count and BV ($r = 0.59$, $P = .035$). No significant correlation was observed between vessel count and capillary permeability or between vessel count and MTT.

Conclusion:

Our findings demonstrate a positive correlation between BF and BV parameters from CT perfusion of the neck and MVD in HNSCC.

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Angiogenesis is a key element of tumor growth and metastasis. It is a complicated multistep process involving endothelial cell breakdown, proliferation and migration of endothelial cells stimulated by angiogenic factors, and formation of new capillaries (1). During angiogenesis, vessels form to supply the growing tumor with tumor endothelial cells dividing much more rapidly than normal endothelial cells. These tumoral microvessels can be detected by using antiendothelial antibodies, such as factor VIII and CD31 (2–4). Subsequently, the vessel count, or intratumoral microvessel density (MVD), can be quantified. Several studies (5–11) have shown that tumor angiogenesis has prognostic value, with an associated increased risk of local-regional recurrence, distant metastasis, and decreased survival in patients with various cancers, including breast, lung, cervix, esophagus, melanoma, and prostate. Similarly, several studies (12–16) have found that intratumoral MVD identified by using accepted immunohistochemical markers for tumor angiogenesis is predictive of outcome in a number of head and neck squamous cell carcinoma (HNSCC) disease sites and could help in the selection of patients who may require aggressive or adjuvant therapy.

Presently, intratumoral MVD determination requires endoscopic biopsy of tumors with histologic processing and microscopic evaluation. This is an invasive process with associated risks of hemorrhage, infection, and general anesthesia. Development of an alternative, efficacious, and non-invasive method for evaluating MVD could potentially spare patients the above-mentioned risks.

Advance in Knowledge

- CT perfusion parameters of the neck (blood flow and blood volume) correlate positively with microvessel density (MVD) of endoscopic biopsy specimens obtained from primary tumor sites of head and neck squamous cell carcinoma.

Deconvolution-based computed tomographic (CT) perfusion is a well-established imaging technique that can help assess physiologic parameters, such as blood volume (BV), blood flow (BF), mean transit time (MTT), and capillary permeability (CP). As a clinical tool, CT perfusion has been used in evaluating intracranial vascular disorders and to characterize intracranial masses and pathologic processes (17–19). CT perfusion in the evaluation of HNSCC is not as well established but is equally promising. Increased BF, BV, CP, and reduced MTT, compared with values for normal adjacent structures, have been shown in HNSCC (20). Hermans et al (21) further demonstrated that CT-determined tumor perfusion in HNSCC to be an independent predictor of local control after definitive radiation therapy with or without chemotherapy. More recently, Gandhi et al (22) and Zima et al (23) showed that CT-determined pretherapy BV and a reduction of BV by 20% after induction chemotherapy was significantly correlated with endoscopic response in advanced squamous cell carcinomas of the upper aerodigestive tract, a correlation that could be used in a predictive model.

As CT perfusion provides a quantitative measure of tumor perfusion, CT perfusion findings may be predictive of

intratumoral MVD. The purpose of our study was to determine the correlation between CT perfusion parameters and intratumoral MVD in the evaluation of HNSCC.

Materials and Methods

Patient Population

This Health Insurance Portability and Accountability Act–compliant prospective study was performed with institutional review board approval, and informed written consent was obtained from each patient prior to enrollment. Thirteen consecutive patients (10 men and three women) with advanced (stage III or IV) HNSCC were enrolled. The average patient age was 57.4 years (range, 41–78 years). The average age of male patients was 58.0 years (range, 50–73 years), and the average age of female patients was 55.4 years (range, 41–78 years). Primary tumor sites included base of tongue ($n = 3$), palatine tonsil ($n = 3$), epiglottis ($n = 2$), vallecula ($n = 1$), hypopharynx ($n = 1$), facial skin ($n = 1$), buccal mucosa ($n = 1$), and parotid gland ($n = 1$). All patients underwent biopsy of the primary site to confirm the diagnosis, with subsequent immunohistochemical analysis to determine the MVD count. Patients also un-

Implications for Patient Care

- Although it is unlikely that CT perfusion will replace biopsy for pretreatment assessment of MVD, CT perfusion has the potential to monitor treatment response by enabling noninvasive assessment of alterations in MVD and acting as a surrogate marker for tumor oxygenation.
- Accurate assessment of tumor response could justify dose modulation or alternative treatment options if the physiologic parameters indicate that a tumor treated with nonsurgical organ preservation therapy is not responding.

Published online before print

10.1148/radiol.2512080743

Radiology 2009; 251:422–428

Abbreviations:

BF = blood flow
 BV = blood volume
 CP = capillary permeability
 HNSCC = head and neck squamous cell carcinoma
 MTT = mean transit time
 MVD = microvessel density
 ROI = region of interest

Author contributions:

Guarantors of integrity of entire study, L.A., S.P., S.K.M.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, L.A., S.P., S.K.M.; clinical studies, T.N.T., D.G., S.P.; and manuscript editing, L.A., T.N.T., S.K.M.

Authors stated no financial relationship to disclose.

derwent standard contrast material-enhanced neck CT and CT perfusion prior to initiation of therapy.

MVD Immunohistochemistry and Determination

The biopsy specimens were taken from the tumor by using direct visualization by a head and neck surgeon (T.N.T., with 11 years of experience) to ensure that intratumoral MVD was measured from the tumor specimen. In all biopsy specimens, MVD was determined by using immunohistochemistry. Five-micrometer sections were prepared and stained by using mouse antihuman CD31 antibody at 1:250 dilution (PharMingen, San Diego, Calif). Negative and positive controls were obtained. Negative controls were slides in which no CD31 was applied, and positive controls were slides in which CD31 was used on hypervascular tumors. The total number of stained vessels was quantified by two investigators (T.N.T., S.P.) who were blinded to the patient's primary tumor site and the patient's response to therapy. Vessels were individually counted by first scanning each slide under low-power microscopy ($\times 100$) to identify areas of high vascularity in the tumor specimen. These regions were then scanned under higher-power microscopy ($\times 400$), and individual vessels were counted in 10 high-power fields per specimen. The mean number of stained microvessels was recorded for each tumor site. All tumor specimens were analyzed for MVD by consensus (T.N.T., S.P., and a predoctoral student with 1 year of experience in the field.)

CT Perfusion Imaging

CT perfusion studies were obtained with a multidetector scanner (LightSpeed Ultra; GE Medical Systems, Milwaukee, Wis). Standard CT of the neck was performed, along with CT perfusion. The acquisition of the CT perfusion studies was directly monitored by a fellowship-trained neuroradiologist (S.K.M., with particular experience in head and neck imaging and 15 years of experience). A "localizer" unenhanced CT scan was ob-

tained through the known primary site. The area of interest for measuring perfusion was centered on the largest area of gross anatomic abnormality identified on the unenhanced localizer images. Four adjacent 5-mm sections were selected at the level of the tumor. Fifty milliliters of nonionic contrast material (standard iodine concentration, 300 mg/mL, Ultravist 300; Bayer Health Care, Wayne, NJ) was injected at 4 mL/sec. At 5 seconds into the injection, a cine acquisition was initiated by using the following parameters: 120 kV, 60 mA, 4×5 -mm sections, and 1-second rotations for 50-second durations. The 1-second images were reformatted at 0.5-second intervals, and the 5-mm sections were reformatted into 10-mm-thick sections.

After completion of the perfusion acquisition, intravenous contrast material was administered at 2 mL/sec (5-second injection delay), and the routine neck study was obtained by using 2.5-mm contiguous sections (120 kV, 180 mA, 0.8-second rotation, 25-cm field of view, 512×512 matrix) from the skull base to the thoracic inlet. The perfusion data were postprocessed by using a commercially available software package (Perfusion-2; GE Medical Systems) on a workstation (Advantage Windows; GE Medical Systems). A region of interest (ROI) was placed in the internal carotid artery and internal jugular vein to generate contrast enhancement curves. The data were processed into maps that represented BV, BF, MTT, and CP.

A single observer, a neuroradiologist (S.K.M.) specializing in head and neck imaging who was aware of the primary tumor site, subsequently obtained ROIs (25 – 30 mm²) through the primary tumor. The tumor was first localized on the contrast-enhanced CT image by the radiologist at the level where the tumor cross-section was largest, and a user-defined ROI was then drawn freehand, incorporating as much of the solid portions of the tumor as possible while omitting the necrotic regions. Finally, care was taken to avoid encroaching on tumor boundaries to exclude peritumoral hyperemia. A maximal ROI was used to ob-

tain the perfusion parameters to ensure that the volume averaging effects were minimal and the perfusion parameters were representative of the tumor vascularity (Figs 1 and 2). Typically, the ROI section was at the center of the tumor and was thought to be representative of the tumor pathophysiology.

None of the patients had known symptomatic carotid disease or presence of significant-appearing stenosis

Figure 1

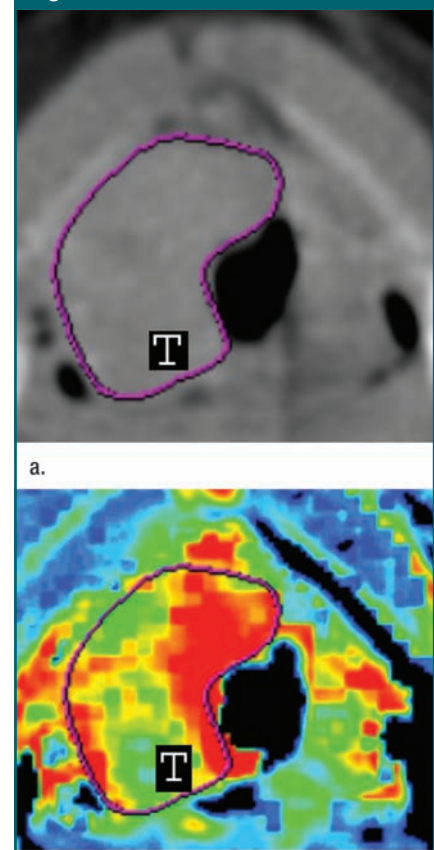


Figure 1: (a) Contrast-enhanced neck CT scan in 45-year-old man with stage IV squamous cell carcinoma in the base of the tongue. A solid mass (*T*) is localized at the right tongue base, through its largest cross-sectional area. (b) CT perfusion map shows maximal ROI used to obtain perfusion parameters such as BF in this case. This tumor (*T*) demonstrated elevated BF (190.15 mL/100 g/min), and endoscopic biopsy results revealed relative increased intratumoral MVD (47.2 vessels per square millimeter).

on the enhanced CT neck images that would have influenced the quantitative CT perfusion parameter values. Because the studies were monitored by a head and neck radiologist, perfusion maps were consistently obtained at a level not affected by substantial dental artifact. The CT perfusion studies were interpreted in consensus by two fellowship-trained neuroradiologists (S.K.M. and D.G., with extensive CT perfusion technique experience during the past 8 years).

Blinding

The surgeon performing the endoscopic biopsy was blinded to the results of CT

and CT perfusion. The investigators determining the MVD were blinded to the patient's primary tumor site and response to therapy. The radiologists evaluating the imaging studies were blinded to the results of endoscopy and biopsy.

Statistical Analysis

Pearson and Spearman correlation coefficients were computed to assess the relation between microvessel count and BF, BV, MTT, and CP. Pearson correlations were also computed after removal of one outlying value for BF and BV. Each correlation was assessed for statistical significance. A P value less than .05 was considered to indicate a significant difference. All analyses were performed by using software (SAS Institute, Cary, NC). In addition, a linear regression model was fit to vessel count, with BV as the only predictor in the model.

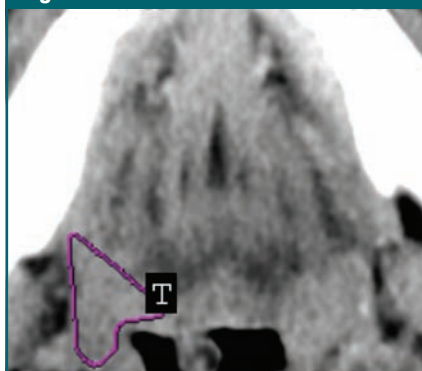
Results

Vessel count was positively correlated with BF and BV (Figs 3 and 4). Spearman rank correlation showed positive correlation between vessel count and BF ($r = 0.30$, $P = .316$) and between vessel count and BV ($r = 0.59$, $P = .035$) (Tables 1, 2). Pearson correlation was also positive although not significant. No significant correlation was observed between vessel count and per-

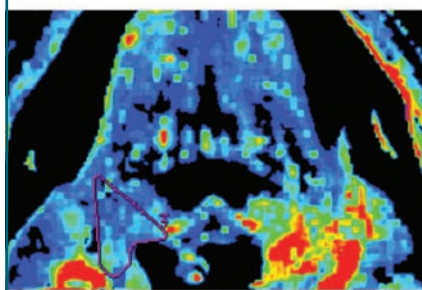
meability or between vessel count and MTT. Linear regression analysis performed on the complete data set demonstrated a slope estimate of 0.619 for BV when vessel count was used as a dependent variable. Thus, for every increase in vessel count by 1, the BV increased by 0.619.

Statistical correlation was stronger when a single outlier was removed from our study, and statistical tests were not corrected for multiple comparisons. The Spearman rank correlation coefficients for vessel count were initially 0.30 and 0.59 for BF and BV, respectively. Pearson correlation was positive although not significant. However, after the outlier was removed from the population group, the Spearman rank correlation coefficients increased to 0.48 and 0.68 for BF and BV, respectively. Pearson correlation was also significant ($P = .046$ for BF, $P = .015$ for BV). The outlier had T3N0 epiglottic cancer with a BF of 361.04 mL/100 g/min \pm 294.82 (standard deviation) and a BV of 24.15 mL/100 g \pm 15.94, as determined with CT perfusion performed through the tumor site. Endoscopic biopsy of the tumor yielded a vessel count of 24.75 vessels per square millimeter (Table 2; Figs 3, 4). In comparison, BF ranged from 39.13 to 190.80 mL/100 g/min, BV from 1.00 to 11.40 mL/100 g, and vessel count from 12.40 to

Figure 2



a.



b.

Figure 2: (a) Contrast-enhanced neck CT image in 57-year-old woman with stage III tongue base squamous cell carcinoma. A solid mass (T) is localized at the right glossotonsillar sulcus, through its largest cross-sectional area. (b) CT perfusion map shows maximal ROI used to obtain perfusion parameters such as BF in this case. This tumor (T) demonstrated decreased BF (39.13 mL/100 g/min), and endoscopic biopsy results revealed relatively decreased intratumoral MVD (19.2 vessels per square millimeter).

Figure 3

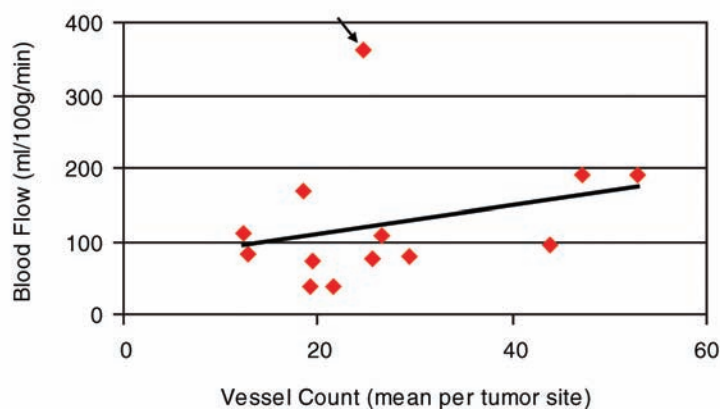


Figure 3: Scatterplot demonstrates positive correlation between CT perfusion BF and mean vessel count at HNSCC primary tumor sites. Among the 13 patients forming the study population, there was a single outlier (arrow).

52.80 vessels per square millimeter for the remainder of the patient population (Table 2).

Discussion

Tumor angiogenesis as measured by intratumor MVD has been shown in several studies to be an independent prognostic marker in several solid organ tumors (1–11). Greater tumor vascularization has also been associated with advanced tumor stage, local-regional and distant metastases, and reduced disease-free survival in patients with HNSCC (12,24–27). Similarly, several clinical studies have demonstrated MVD to be predictive of outcome in several primary sites in head HNSCC. Kupisz et al (1) found a direct correlation between tumor angiogenesis and T stage, histologic grade, and a shorter survival rate in patients with laryngeal cancer. Lentsch et al (13) demonstrated a strong correlation between MVD in primary tumor sites in HNSCC and tumor T stage. Albo et al (12) found an increase in recurrent and/or metastatic disease in patients with HNSCC with higher microvessel counts in tissues adjacent to the tumor. Other studies (14,16) have also found higher MVD to correlate with deeper levels of invasion in laryngeal squamous cell carcinoma and advanced clinical stage and tumor extension in HNSCCs. Using an increased MVD to identify patients with HNSCC with a more aggressive disease process has allowed selection of patients requiring adjuvant therapy.

Just as there is an association between intratumoral MVD and tumor angiogenesis, there is also an established relationship between tumor angiogenesis and contrast enhancement on CT scans, because the physiologic basis of contrast enhancement closely mimics the physiologic effects of tumor angiogenesis (28). Angiogenesis is associated with increased perfusion, BV, and permeability, resulting in increased contrast enhancement determined by the degree of neovascularization associated with the tumor (28). It follows that CT perfusion of

Figure 4

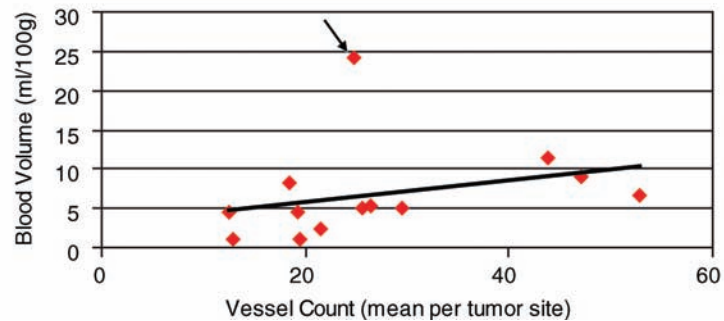


Figure 4: Scatterplot demonstrates positive correlation between CT perfusion BV and mean vessel count at HNSCC primary tumor sites. Among the 13 patients forming the study population, there was a single outlier (arrow).

the head and neck would be a valuable tool in differentiating normal tissue from malignant processes. Gandhi et al (20) demonstrated increased CP, BF, and BV in HNSCC, compared with values for normal adjacent structures. Similarly, Rumboldt et al (29) were able to differentiate malignant from nonmalignant lesions of the head and neck with CT perfusion parameters.

More recently, the use of tumor perfusion at CT has been investigated as a potential instrument to determine outcome in patients with head and neck cancers (21–23). In contrast to the above-described intratumoral MVD studies of HNSCCs that have used tumor angiogenesis in an effort to identify patients with a more aggressive disease process requiring adjuvant therapy (1,12–14,16), these latest CT perfusion studies have focused on predicting which patients will have a favorable therapeutic outcome.

The potential ability of CT perfusion to predict tumoral response to therapy is based on tumor oxygenation. Oxygen is a powerful radiosensitizer, and studies have conclusively shown that tumor hypoxia in head and neck cancers results in worse response to radiation (30–32). Tumoral oxygenation also has a significant influence on the effectiveness of chemotherapy (32). Because oxygen availability is a product of perfusion rate and arterial oxygen concentration, tumor perfusion and tumoral oxygen concentration typically have a strong association (21).

Table 1

Spearman Correlation for CT Perfusion Parameters and Intratumoral MVD in 13 Patients

Parameter	Vessel Count Correlation	P Value
BF	0.301	.316
BV	0.588	.035
MTT	−0.022	.943
CP	−0.363	.223

Hermans et al (21) have shown CT-determined perfusion rates in HNSCC to be an independent predictor of local control by definitive radiation therapy with or without chemotherapy. Patients with lower perfusion rates in this study had a significantly higher local failure rate. This corresponds to the findings of Nordmark and Overgaard (31) that local-regional tumor control in advanced HNSCC treated with radiation therapy was significantly higher among well-oxygenated tumors compared with a hypoxic subgroup.

Gandhi et al (22) have also demonstrated a significant correlation between CT perfusion-determined pretherapy BV with reduction of BV by 20% after induction chemotherapy and endoscopic response in advanced squamous cell carcinomas of the upper aerodigestive tract. Studying a similar patient population group, Zima et al (23) found that tumors with elevated BV and BF responded to trial induction chemother-

apy, and those with lower BV and BF did not. With this information, they were able to develop a predictive model to potentially eliminate the need for induction therapy when deciding on an appropriate treatment regimen.

With the use of the same principles of tumor oxygenation, similar studies have been performed with intratumoral MVD. As with CT perfusion, it is postulated that higher intratumoral MVD reflects increased oxygenation and subsequently can be used as a marker for increased sensitivity of tumors to radiation and/or chemotherapy (33,34). Intratumoral MVD has been found to be a predictor of local control of hypopharyngeal carcinoma treated with radiation therapy alone or chemotherapy and radiation therapy, and it has also been shown to correlate with local control rates in laryngeal cancer treated with radiation therapy (33,34). Whereas many studies have shown that increased intratumoral MVD correlates with more aggressive HNSCC and portends a worse prognosis, several CT perfusion studies have demonstrated improved response to radiation and/or chemotherapy in patients with increased perfusion. Thus, it seems that more aggressive tumors, characterized by increased MVD and, presumably, increased perfusion, may not only benefit from early adjuvant therapy but may also be best treated with radiation and/or chemotherapy.

Our study demonstrates a positive correlation between BF and BV in HNSCC and intratumoral MVD. These findings have potential clinical applications regarding noninvasive treatment monitoring in patients with HNSCC treated with nonsurgical organ preser-

vation therapy. It is unlikely that CT perfusion will replace biopsy for pretreatment assessment of MVD. However, CT perfusion does have the potential to monitor treatment response by enabling noninvasive assessment of alterations in MVD and serving as a surrogate marker for tumor oxygenation. Accurate assessment of tumor response could justify dose modulation or alternative treatment options if the physiologic parameters indicate that a tumor treated with nonsurgical organ preservation therapy is not responding. As Miles (28) suggested in his review, there "is much pathological data indicating a relationship between tumour angiogenesis and prognosis and thus there is potential for quantitative analysis of contrast enhancement to provide comparable information but *in vivo* rather than from a biopsy or resected specimen."

It should be noted that the statistical correlation was stronger when a single outlier was removed from our study. This patient had T3N0 epiglottic cancer. None of the other patients in the study group had epiglottic cancer, and it can only be speculated why that particular patient had such a marked increase in BF and BV compared with the others. Perhaps there was some technical error during CT perfusion, and the location of the primary tumor had no bearing. It is also feasible that certain primary sites of HNSCCs may be found to have a stronger or more predictable correlation between CT perfusion and intratumoral MVD.

Technical limitations may also have had some bearing on our results. We determined the tumor perfusion parameters by using a large ROI from a single level. It is therefore possible that our

results were not representative of the entire tumor. Recent developments allow larger coverage for CT perfusion that will permit the entire tumor volume to be imaged and prevent potential sampling error (21). We also chose to use the cervical internal carotid arteries for the arterial input rather than the branches of the external carotid artery that supply HNSCCs, because the internal carotid arteries could be consistently identified on every section in cross-section, which minimizes volume averaging artifacts. Branches of the external carotid artery can be identified, but they are small and often not seen in direct cross-section. Additionally, the external carotid artery branches that specifically supply the tumor are not always readily visualized on CT scans. Thus, it would be difficult to use the same artery reliably in the same patient at different times or even the same artery in different patients due to substantial anatomic variation.

To our knowledge, there is no published literature to support the assumption that external carotid artery output is necessarily more accurate. Furthermore, we have studied this issue at our institution and found no significant differences in BV, BF, MTT, or CP when the values were calculated by using either the internal carotid or external carotid arteries (35). Finally, we used a higher-kVp technique than is currently used. At the time of the study, 120-kVp technique was used by the majority of commercially available perfusion protocols and constituted the standard technique. It is now well known that the use of an 80-kVp technique is associated with lower radiation dose and increased contrast material conspicuity.

We also did not attempt to guide the site of the endoscopic biopsy on the basis of the findings of the perfusion study. However, the radiologist and endoscopic surgeon were guided by similar rules. The perfusion parameters were derived from the cross-section that revealed the largest cross-sectional area of the tumor. Necrotic regions were avoided, and a maximal ROI was used to obtain the perfusion parameters, ensuring that the volume averaging

Table 2

CT Perfusion and MVD Results in 13 Patients

Parameter	Mean \pm Standard Deviation	Range
BF (mL/100 g/min)	124.04 \pm 87.19	39.10–361.00
BV (mL/100 g)	6.81 \pm 6.01	1.00–24.15
MTT (sec)	5.61 \pm 2.91	1.50–10.71
CP (mL/100 g/min)	27.17 \pm 19.05	9.76–72.8
Vessel count (vessels per square millimeter)	27.23 \pm 24.75	12.40–52.80

effects were minimal and the perfusion parameters were representative of tumor vascularity. Similarly, the head and neck surgeon focused on the most bulky area of the tumor and avoided any necrotic-appearing regions. Biopsy specimens were also taken from the tumor by using direct visualization.

Our preliminary findings demonstrate a positive correlation between BV and BF parameters measured with CT perfusion of the neck and intratumoral MVD in advanced HNSCC. Although further prospective studies with larger patient populations are needed, the potential for CT perfusion to guide therapeutic decision by enabling noninvasive assessment of changes in MVD is promising and warrants further investigations.

Acknowledgment: Matthew J. Schipper, PhD, provided the statistical support.

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