Cancer Imaging (2010) **10,** 8–19
DOI: 10.1102/1470-7330.2010.0001

CI

e-med
www.e-med.org.uk

REVIEW

CT perfusion in oncology: how to do it

G. Petralia, L. Bonello, S. Viotti, L. Preda, G. d'Andrea and M. Bellomi

Diagnostic Radiology, European Institute of Oncology, Via Ripamonte 435, 20141 Milan, Italy

Corresponding address: Professor Massimo Bellomi, MD, Diagnostic Radiology, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy.
Email: massimo.bellomi@ieo.it

Date accepted for publication 9 December 2009

Abstract

Robust technique and accurate data analysis are required for reliable computed tomography perfusion (CTp) imaging. Multislice CT is required for high temporal resolution scanning; 16-slice (or 64-slice) scanners are preferred for adequate volume coverage. After tumour localization, the volume of CTp imaging has to be positioned to include the maximum visible area of the tumour and an adequate arterial vessel. Dynamic scans at high temporal resolution (at least 1-s gantry rotation time) are performed to visualize the first pass of contrast agent within the tumour; repeated scans with low temporal resolution can be planned for late enhancement assessment. A short bolus of conventional iodinated contrast agent, preferably with high iodine concentration, is power injected at a high flow rate (>4 ml/s) in the antecubital vein. The breath-hold technique is required for CTp imaging of the chest and upper abdomen to avoid respiratory motion; free breathing is adequate for CTp imaging of the head, neck and pelvis. Using dedicated software, a region of interest (ROI) has to be placed in an adequate artery (as arterial input) to obtain density–time curves; according to different kinetic models, colour maps of different CTp parameters are generated and generally overlaid on CT images. Additional ROIs can be positioned in the tumour, and in all other parts of the CTp volume, to obtain the values of the CTp parameters within the ROI.

Keywords: *CT perfusion; kinetic models; oncology; therapy monitoring; prediction of response to therapy; body tumours.*

Introduction

Computed tomography (CT) has become the main diagnostic tool in tumour staging and monitoring the response to therapies of various tumours due to the relatively low costs, the wide spectrum of pathologies that can be investigated, the easy standardization of protocols and wide logistic availability. The study of tumour biology is at the frontline of oncology research; in particular, neoplastic angiogenesis is considered to be an important prognostic factor[1–3] and a promising target of new antitumour therapies[4]. Recent work has led to the development of non-invasive imaging techniques, such as perfusion CT (CTp), that provide both qualitative and quantitative information regarding tumour angiogenesis. CTp is a tool which in theory can quantify the real perfusion of tissues by applying mathematical models and dedicated software to calculate the delivery of contrast agent, and therefore blood, to tissues; such a property of CTp is considered clinically useful and studies investigating the clinical application of CTp in oncology are increasingly reported in literature.

CTp has shown significant differences in perfusion values when comparing normal tissue versus tumoral tissue. Significantly higher perfusion parameters have been reported in patients with hepatic[5], rectal[6,7], lung[8], and head and neck[9,10] tumours. CTp has also been able to demonstrate different perfusion values between benign and malignant lesions[10,11]. Higher perfusion values in tumours could reflect the process of angiogenesis, with the recruitment and development of arteriovenous shunts, dilated capillary beds and hyperpermeable vessels, resulting in high values of flow, blood volume and permeability, respectively, measured with CTp. Direct correlation has been found between tumour perfusion parameters and biomarkers of angiogenesis such as microvessel density (MVD) and vascular endothelial growth factor (VEGF) in several tumours including lung[12,13], pancreatic[14] and colorectal[15,16] tumours.

This paper is available online at http://www.cancerimaging.org. In the event of a change in the URL address, please use the DOI provided to locate the paper.

1470-7330/10/000001 + 12

Another possible application of CTp is its potential role in primary tumour staging for predicting disease-free survival. Primary tumour blood flow in colorectal adenocarcinoma was significantly higher in disease-free patients, when assessed prior to curative surgery, compared with patients who eventually had metastatic disease[17]. The potential of CTp to predict outcome was also observed in head and neck tumours[18]. This predictive ability could further help clinicians in deciding on the best management plan for patients.

CTp has also shown potential in predicting response to therapy in certain tumours including head and neck, lung and rectal tumours[6,19,20]. Poorly perfused tumours were less responsive to chemotherapy possibly because there was a poor delivery of chemotherapeutic agent to the tumour. Poorly perfused tumours were also less responsive to radiotherapy because they were more likely to be hypoxic and therefore less responsive to radiotherapy.

CTp has shown a role for therapeutic assessment in tumours. It was found to be useful for monitoring conventional cytotoxic chemotherapy (CHT) in patients with tumours of the rectum[6,7], the liver[21,22], the lung[23] and the head and neck[19,24]; any conventional cytotoxic CHT is expected to have an effect on tumour angiogenesis, because it causes a loss of pro-angiogenic factors as a result of the death of tumour cells[25]. CTp was also found to be useful for monitoring anti-angiogenic therapies, as demonstrated in hepatocellular carcinoma (HCC)[21], rectal tumours[16] and other body tumours[26–28]; anti-angiogenic drugs have greater cytostatic than cytotoxic effects, determining small changes in tumour size, but early changes in tumour vasculature, that are only assessable with a functional imaging technique, such as CTp. In light of this capability of functional imaging, CTp has also been used to propose and understand possible mechanisms of action of certain drugs[29,30].

Principles of the technique

Perfusion is the transport of blood to a unit volume of tissue per unit of time[31]. It thus refers to the transport of oxygen and nutrients to tissues, which occurs at capillary level; it is very different from the concept of blood velocity, which refers to large vessels. In theory, CTp can quantify the real perfusion of tissues in an objective way, using mathematical models through dedicated software, as it only measures the difference in density produced by the delivery of contrast agent (and therefore blood) to the tissues.

CTp is based on certain fundamental requirements. One is the administration of contrast agent, in a small quantity at high flow rate, to obtain a short and sharp bolus.

Another requirement is repeated CT scanning of the same volume over time (also called dynamic, cine or perfusional scans), which has to be performed before, during and after the intravenous administration of iodinated contrast agent to allow the study of the variation in density with time. The density measured by CT in the unit of volume (voxel), which is the attenuation of X-rays expressed in Hounsfield units (HU), is directly proportional to the quantity of contrast agent present within it[32]; the contrast agent present in the volume of tissue being studied reflects the contrast agent within the blood vessels and the contrast agent that has moved to the extravascular/intracellular space (also known as interstitial space) by passive diffusion[33,34].

A third fundamental requirement is the selection of the arterial input. The placement of a region of interest (ROI) on an artery allows one to obtain a density–time curve of the artery in question, expressed in HU/s (Fig. 1). This is then compared with the density–time curve of the tissue being analysed, also obtained by placing an ROI, to distinguish between the quantity of contrast agent within blood vessels (vascular compartment) and the quantity of contrast agent present in the interstitium (extravascular/intracellular compartment). Therefore it is now possible to quantify perfusion.

Various kinetic models can be used to calculate the distribution of the contrast agent in the intravascular compartment and in the interstitial space, defining the parameters that characterize the perfusion in the tissues being analysed.

One of the kinetic models used is the double-compartmental type (described by Patlak), which describes the intravascular and extravascular compartments as separate compartments and quantifies the exchange between the two. This model provides an estimate of the volume

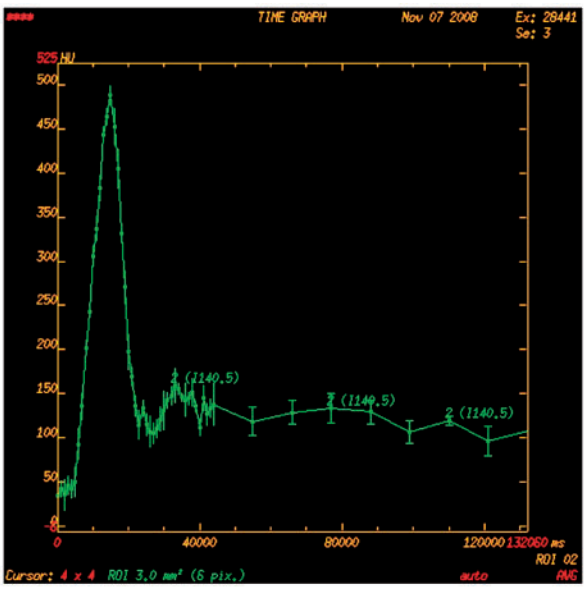

Figure 1 **Density–time curve obtained by placing an ROI over the selected arterial input, plotting density (expressed in Hounsfield units (HU)) on the *Y*-axis and time (expressed in milliseconds) on the *X*-axis.**

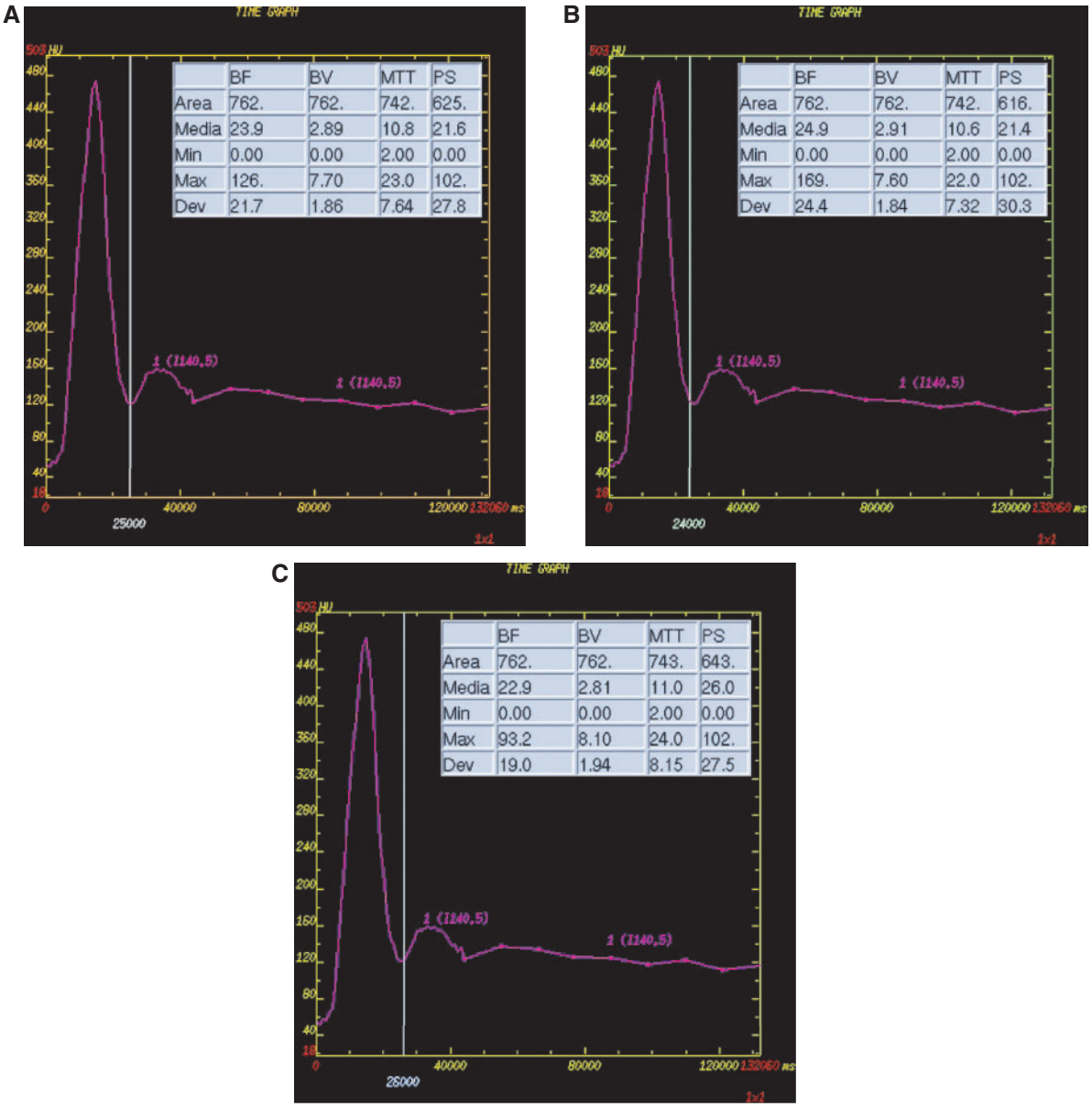

Figure 2 **Selection of the end of the first pass. (A) Correct selection of the end of the first pass by choosing the lowest point after the peak of the density–time curve. (B) Early selection of the end of the first pass (one image too early) on the density–time curve. This resulted in an erroneous calculation of the perfusion parameters (BF, BV, MTT, PS). (C) Late selection of the end of the first pass (one image too late) on the density–time curve. This resulted in an erroneous calculation of the perfusion parameters (BF, BV, MTT, PS).**

of blood within the microvessels (blood volume (BV)) and of the capillary permeability.

The single-compartmental kinetic model is based on the Fick principle and assumes that the intravascular and extravascular spaces are a single compartment, a concept that is valid for time points prior to the moment when contrast medium appears in the draining veins of the tissue of interest. Perfusion is calculated either from the maximal slope of the tissue concentration–time curve or from its peak height, normalized to the arterial input function[35–37].

The deconvolution method uses arterial and tissue time–attenuation curves to calculate the impulse residue function (IRF) for the tissue. The IRF is a theoretic tissue curve that is obtained from the direct arterial input, assuming that the concentration of contrast material in the tissue is linearly dependent on the input arterial concentration when the blood flow (BF) is constant. After accounting for the flow correction, the height of this curve reflects the tissue perfusion and the area under the curve will determine the relative blood volume; the mean transit time (MTT) can be determined from the

area under the curve divided by the height of the curve, according to the central volume (BV=BF×MTT). The deconvolution method assumes that the contrast material is non-diffusible. Although non-diffusibility is a reasonable assumption in the brain, in the testicles and in the retina, it is not the case for other organs or in the case of brain tumour disruption to the blood–brain barrier. Generally, leakage into the interstitial space is slow with respect to the transit time of the contrast material and assuming diffusion is zero only leads to small errors in most organs. For the estimation of capillary permeability, a distributed parameter model is used, which is essentially an extended deconvolution model. This approach allows the deconvolution method to provide permeability values and perfusion but requires a longer data acquisition period to determine the outflow characteristics of the extravascular contrast material[35–38].

CTp technique

Target

It is first necessary to obtain CT images without contrast agent, which basically serve as a localizer to select the appropriate tissue area to be included in the contrast-enhanced dynamic imaging range. Therefore, thin slices are not required and these baseline images can be acquired using a low dose. If a study of the first pass of the contrast agent needs to be performed at a temporal resolution of 0.5–1 s, the volume to be studied is limited along the *z*-axis by the number of detectors of the CT being used (e.g. 20 mm for 16-slice CT and 40 mm for 64-slice CT[39]) because scans with such a high temporal resolution are only possible with a stationary bed position. However, repeated spiral acquisitions have been implemented by some manufacturers with a relatively high temporal frequency, possibly as low as 1.5 s between spiral acquisitions, thus potentially allowing for the quantification of perfusion using the first pass effect. Such spiral acquisitions, with the latest scanners, allow a *z*-axis coverage on the order of 21–27 cm (depending on the scanner manufacturer)[40].

If the tumour happens to have a volume greater than that which the perfusion scans can include, it is necessary to identify the section where the maximal tumour area is visible and use that as the centre of the volume of the perfusion study.

Contrast agent

The contrast agent used is the conventional iodinated CT contrast agent; it is preferable to use a contrast agent with a high concentration of iodine (370–400 mg/l), because it gives greater tissue enhancement and can be beneficial in quantifying perfusion[35,41]. The contrast agent is administered using a short sharp bolus technique; a small quantity of contrast agent (40–50 ml) is rapidly injected at a high flow rate (4–6 ml/s), followed by 40 ml of physiologic saline at a similarly high flow rate (4–6 ml/s).

Modified bolus techniques have been used in CTp exams performed in lung tumours, with a decreasing bolus infusion rate (32 ml at 4 ml/s, 16 ml at 2 ml/s, and 60 ml at 1 ml/s) and followed by a saline flush (20 ml at 1 ml/s). The rationale for the contrast material infusion protocol was to maintain a more constant intravascular concentration of contrast material, to minimize the concentration gradient between the intravascular and extravascular spaces, to optimize conditions for Patlak analysis, and to improve the signal-to-noise ratio[42–44].

In longitudinal studies in which CTp is performed on the same patient at different stages during the therapeutic regimen, it is preferable to always use the same peripheral venous access site in order to exclude any potential sources of variability.

CT protocol

Perfusion scans performed during the first pass of contrast agent (up to 45–60 s from the start of administration of contrast agent) require a high temporal resolution (1 s for protocols defined for perfusion analysis using the deconvolution kinetic model, and 3–5 s for protocols defined for perfusion analysis using the single- or double-compartmental kinetic models)[7,35,37,38]. According to the deconvolution kinetic model, the perfusion scans performed during the interstitial phase, after analysis of the first pass of contrast agent, have to be performed with a temporal resolution of 10 s and last no longer than 2 min after the administration of contrast agent, in order to apply the adiabatic approximation of St. Lawrence and Lee. On the other hand, when using the single- or double-compartmental kinetic models, the perfusion scans performed during the interstitial phase, after analysis of the first pass of contrast agent, have to be performed with a temporal resolution between 10 and 20 s, and last for between 2 and 10 min after the administration of contrast agent[35,38].

As reported in most of the recently published studies on the use of CTp in various regions of the body, in order to reduce the x-ray dose it is advisable to use relatively low voltage (80–100 kVp) and Ampere values (120–200 mA) for the perfusion scans[45–47]. A study by Lee *et al.*[48] showed that perfusion scans performed with a voltage of 80 kV allows a 300% reduction in dose compared to those performed at a voltage of 120 kV, with only an 11% loss in the signal-to-noise ratio. A study using perfusion CT on the brain to calculate regional cerebral blood flow showed that 80 kVp acquisitions versus 120 kVp acquisitions resulted in increased contrast enhancement and thus improved regional blood flow analysis, while reducing the total radiation dose received by the patient and without any alteration in mean noise[49]. Finally it is also advisable, and almost universally used in recently published studies[50], to use a slice thickness of not less than 5 mm, which guarantees a correct

balance between the requirements for spatial resolution and the signal-to-noise ratio in perfusion scans.

For reliable analysis of perfusion using CTp it is necessary to limit movements of the volume of tissue being studied as much as possible during perfusion scanning. The anatomical regions that are most subject to movement artefacts are the lower part of the thorax and the upper part of the abdomen, due to diaphragmatic movements during breathing. Hence, efforts should be made to minimize motion by appropriate approaches, such as breath-holding instructions to patients for thoracic and upper abdominal applications. Anterior abdominal wall motion during breathing movements can be reduced by using abdominal straps[47,51–53]. Bowel peristalsis can also cause motion artefacts during perfusion scans on the bowel. These artefacts can be reduced by administering motility-inhibiting agents such as hyoscine butylbromide or glucagon, immediately before scanning, to reduces bowel peristalsis during perfusion scanning[47,51–53].

The process of deglutition can also cause significant artefacts in perfusion scans performed on the upper aerodigestive tract. Some authors described warning the patient about the sensation of warmth brought about by the administration of contrast agent in order to avoid deglutition[9,54,55]. It is possible to correct motion artefacts even after the perfusion scans have been performed using appropriate software for motion correction to increase the reliability of perfusion analysis using CT.

Post-processing

The calculation of perfusion parameters is performed using dedicated software. Some are commercially available and are therefore widely used in many centres, and in theory reliable because they are already tested and validated before their introduction into the market. Other software is developed autonomously within individual centres, and is therefore less widely available and does not undergo such an extensive process of validation. Such software is semi-automated as it requires the intervention of an operator to place an ROI and optimize the entire process; however, the mathematical analysis is performed automatically.

An arterial input is selected by placing an ROI in a blood vessel within the volume of the scans obtained. In theory, this is the ideal way to perform a reliable perfusion analysis using CT because arterial input is specific for every patient, and for every examination performed on the same patient at different times. Nonetheless, the selection of the arterial input can introduce a source of variability. It is still a debatable issue, for example, whether placing the ROI on the ipsilateral or contralateral side of the tumour, or whether placing the ROI on a blood vessel on the left side or the right side introduces a source of variability due to the anatomical variations between the left and right sides of the body. Some studies have shown a significant difference in perfusion parameters measured in squamous cell carcinomas of the upper aerodigestive tract using CTp when the arterial input is placed on the left external carotid artery or the right external carotid artery[9].

Another debatable issue is the selection of the artery; the advantage of selecting a large vessel is the reduction of artefacts caused by tortuosity in blood flow; the advantage of selecting a small calibre vessel that is a tributary of the tumour could be that, in theory, it provides a more realistic estimate of tumour blood supply. However, artefacts due to tortuosity of blood flow have been shown to be negligible for arteries with a calibre greater than 4–5 mm[6,10,56], and there were no significant differences detected in CTp parameters when the arterial input was placed on the external carotid artery or internal carotid artery, a tributary and non-tributary of the tumour, respectively, in patients with squamous cell carcinoma of the upper aerodigestive tract[10]. In addition, several studies using CTp in various regions of the body have successfully utilized the aorta as the arterial input or arteries that were not direct tributaries of the tumour[5,47].

It is therefore advisable to utilize an artery of adequate calibre (>4–5 mm) which is well visualized during perfusion scans but not necessarily a direct tributary of the tumour as the arterial input. The selection of the arterial input must also avoid artefacts due to movement and from partial volume, which could heavily influence the reliability of the analysis of perfusion using CTp. A specific comment is required for lung lesions. In most studies that report the use of CTp in lung lesions, the authors defined the arterial input by placing an ROI in the aorta[13,42–44]. However, Kiessling *et al.* showed that in some lung tumours an initial contrast enhancement was observed prior to that in the aorta, accounting for a tumour blood supply through pulmonary vessels[23]. This may have a potential role during planning of an embolization procedure and should be addressed in further CTp studies.

Accurate identification of the end of the first pass is necessary for a reliable calculation of perfusion parameters. Recirculation of blood can interfere with the calculation of perfusion parameters when using kinetic models that rely on the first pass of contrast agent, such as the single-compartmental or deconvolution models[55].

Some software automatically identifies the end of the first pass; however, for protocols where perfusion scans last longer than the end of the first pass, such an automatic identification could lead to inaccuracies and it is necessary to manually identify the first pass. In order to standardize the analysis, this can be chosen to correspond with the lowest point after the maximal peak of the density–time curve obtained from the arterial input (Fig. 2). In previous studies using CTp, some authors used mathematical formulae[57] to standardize the end of the first pass.

An ROI is manually drawn along the borders of the tumour in order for the software to quantify the perfusion parameters within it. Most authors agree that large calibre vessels, air or surrounding adipose tissue must be excluded when drawing an ROI. The ROI must be within the margins of the tumour in the images of all perfusion scans. Therefore all the images of the examination should be examined as accurately as possible, preferably in the cine-loop modality to ensure the ROI does not extend outside the margins of the tumour[9,54,55].

CTp analysis

Qualitative analysis

Qualitative analysis consists of the analysis of colour maps that are automatically generated by software for every perfusion parameter (BF, BV, MTT, permeability–surface area product (PS)). Every pixel of the images obtained is attributed a colour which represents a numeric value of the perfusion parameter calculated for that pixel. The colour scale is chosen by the operator in order to maximize the differences between areas that have different perfusion. The qualitative analysis of the colour maps yields a general view of the distribution of the perfusion within the volume of tissue being studied, with a quick identification of the areas with the highest or the lowest perfusion (Fig. 3). Most of the commercially available software packages can superimpose these colour maps on the native CT images. This can be very helpful when the margins of the tumour are not well delineated, such as in the presence of complex anatomy or in the presence of post therapy changes (chemotherapy and/or radiotherapy).

Quantitative analysis

Quantitative analysis consists of the interpretation of the numeric perfusion values calculated by dedicated software, for the area within the ROI placed on the tumour by the operator. The final numeric value represents the average of the numeric perfusion values for each voxel within the ROI. This method of quantifying perfusion has the advantage that it provides an estimate of the total perfusion of all the tumour volume selected and it is has been shown to have low interobserver variation[54,58]. However, a possible drawback to this method is that it may mask differences in perfusion within the tumour itself (i.e. tumour perfusion heterogeneity) because the final numeric value used is the average of a single voxel.

The evaluation of tumour perfusion heterogeneity can be performed either subjectively by the operator when visually analysing the spectrum of colours generated on the colour maps, or objectively using graphic representation of the distribution of perfusion values for each voxel using graphs and histogram analysis. A tumour with high perfusion heterogeneity will thus have a greater number of colours represented and a wider distribution on histogram analysis compared with a tumour with homogenous perfusion. However, to the best of our knowledge, histogram analysis is not possible for large ROIs on commercial software and this could be the reason why there are no previous studies that used graphs and histogram analysis to study tumour perfusion heterogeneity using CTp.

An alternative tool to evaluate tumour perfusion heterogeneity includes fractal analysis, which is a mathematical technique that examines underlying structural geometry. This technique may serve as a complementary measure to standard ROI analysis and potentially offers a way of investigating and quantifying the spatial heterogeneity of the tumour vasculature *in vivo*. Goh *et al.*[59] demonstrated that fractal analysis (fractal parameters analysed included fractal dimension, fractal abundance and lacunarity) for assessing the spatial pattern of colorectal tumour perfusion at dynamic contrast-enhanced CT is feasible and that colorectal tumours have fractal properties.

Clinical application

Biomarker of angiogenesis

CTp has a potential role in oncology as it could possibly indicate tumour angiogenic activity. Tumour angiogenesis is defined as the formation of new blood vessels from pre-existing ones[32]. Methods to evaluate and eventually quantify angiogenic activity are still being studied. The potential biomarkers of angiogenesis used to date including immunohistochemical markers such as the counting of microvessels (MVD) and the identification of the receptor for vascular endothelial growth factor (VEGF), and serum markers such as counting the number of circulating endothelial cells (CEC), have shown poorly concordant results.

In theory, CTp could potentially measure the volume of newly formed vessels secondary to the process of angiogenesis, by attributing a numerical value to the blood volume within blood vessels expressed in ml/100 g of tissue. It is therefore possible to hypothesize a potential correlation between BV and the standard histological technique used to date for the estimation of blood vessels, the estimation of MVD. This correlation has been shown in different body tumours, including lung tumours[12], renal cell carcinomas[60], head and neck squamous cell carcinomas[61], and colorectal tumours[62]; a similar correlation has also been found for other perfusion parameters[12,14,60–62]. There are studies, however, where this correlation was not seen. For example, Li *et al.*[63] did not observe any correlation between BF values and MVD counts in colorectal carcinomas.

Besides being associated with an increased number of vessels driven by angiogenic stimuli, these newly formed vessels during the process of angiogenesis have an abnormal wall structure making them hyperpermeable compared with normal blood vessels[64]. One of the most

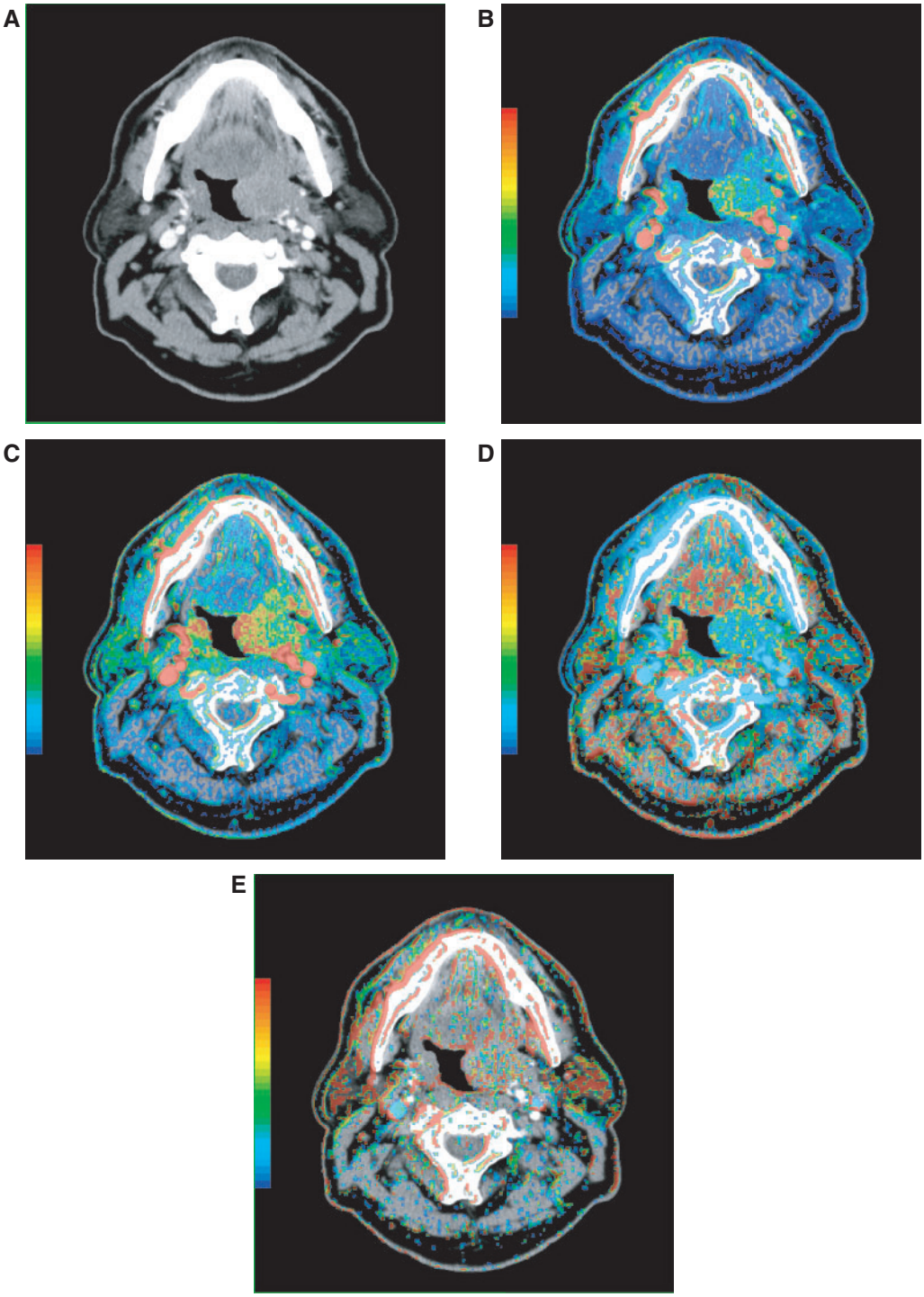

Figure 3 **A 61-year-old patient with a squamous cell carcinoma of the left oropharynx. Colour maps for BF, BV, MTT and PS automatically generated by software, in which every pixel is assigned a colour that represents a numeric value for the perfusion parameter calculated for that voxel. High numeric values are represented by colour shades of yellow and red, and low numeric values are represented by colour shades of green and blue. (A) Conventional CT image showing the extent of the tumour. (B) Colour map for BF; there is a greater presence of yellow colour shades with red traces, representing higher BF values within the tumour than in the normal tissues. (C) Colour map for BV; there is a greater presence of red colour shades representing higher BV values within the tumour than in the normal tissues. (D) Colour map for MTT; there is a greater presence of blue colour shades representing lower MTT values within the tumour than in the normal tissues. (E) Colour map for PS; there is a greater presence of red colour shades representing higher PS values within the tumour than in the normal tissues.**

prominent biomarkers of angiogenesis studied to date is VEGF, also known as vascular permeability factor, as it is thought to be directly responsible for this increased permeability of blood vessels[65]. It is possible to hypothesize that the calculation of capillary permeability using CTp could potentially reflect VEGF expression in a tumour. Ma *et al.*[13] observed a good correlation between CTp (including the estimate of capillary permeability by PS) in peripheral pulmonary nodules and VEGF expression. Other studies failed to show a correlation between perfusion parameters and VEGF values. There was no such correlation found in studies involving renal cell carcinomas[60] and colorectal carcinomas[15].

The lack of homogenous results described to date may be accounted for by the small number of patients and studies performed, and also due to the fact that CTp provides a functional assessment of tumour angiogenesis unlike the morphological assessment provided by MVD counts and the biological information provided by VEGF and CEC estimation.

Tumour assessment, characterization and staging

Perfusion parameters measured using CTp have been shown to be significantly higher in certain tumours compared with normal tissues of the same organ; this has been seen in liver[5], lung[8], rectal[6,7] and upper aerodigestive tract[9,10] tumours. Increased blood flow (BF or BE, according to the kinetic model being used for the analysis) in the tumour compared with normal tissue could be explained by the opening of arteriovenous shunts within the tumour. These shunts, offering a low resistance to flow, cause an increase in blood flow within the microvessels. As blood flow increases, the MTT of the blood flowing within these vessels decreases. A greater BV within the tumour compared with normal tissue could be explained by an increase in the number of microvessels present as a result of the production of new blood vessels[66] from pre-existing ones, due to the process of tumour angiogenesis. A greater permeability surface (PS or FE, according to the kinetic model being used for the analysis) in the tumour compared with normal tissue could be explained by the greater permeability of the endothelium of newly formed microvessels, which are structurally abnormal compared with normal microvessels[64].

Studies using CTp have also shown significantly higher perfusion values in high-grade tumours compared with low-grade tumours, as observed in lymphomas[67]. Significantly lower perfusion values have been observed in high-grade hepatocarcinomas compared with low-grade ones and this was attributed to the presence of central necrosis in high-grade hepatocarcinomas[5]. The results of the experiences to date are thus discordant and further studies are needed to draw more definite conclusions on this matter.

CTp has also been used to characterize lesions in different organs. In a study on solitary lung nodules, CTp showed significantly higher perfusion values in malignant and inflammatory solitary pulmonary nodules compared with benign solitary pulmonary nodules[11]. Rumboldt *et al.*[10] reported that CTp showed promise in differentiating between benign and malignant lesions in head and neck tumours, mostly by values of MTT, with benign lesions having longer MTT values compared with malignant lesions.

Perfusion parameters have also been correlated with tumour stage. In a series of patients with rectal carcinoma there was a significant correlation between tumour blood flow and disease stage; there was a tendency for blood flow to decrease with increasing disease stage[68]. Another potential clinical use of CTp at primary tumour staging is its potential role for predicting disease-free survival. The ability of CTp to identify those patients who are at higher risk of developing metastatic disease before the initiation of any therapy could have very useful implications, in that it would allow patients with tumours with different biological characteristics to receive the most appropriate therapy: surgery, chemotherapy, radiotherapy or a combination of these. To the best of our knowledge, there are only a few studies to date that have studied this predictive potential, mainly because such studies require long patient follow-up. Primary tumour blood flow in colorectal adenocarcinoma was significantly higher in disease-free patients, when assessed prior to curative surgery, compared with patients who eventually had metastatic disease[17]. The authors attributed this to the hypothesis that hypoxia could play an important role in the development of metastasis. Similar results were observed in another series of patients with colorectal carcinoma with high blood flow at staging associated with increased survival[68]. In another study the rate of perfusion determined using dynamic CT in head and neck tumours before radiation therapy, with or without chemotherapy, was found to be an independent predictor of local outcome in head and neck tumours[18]. Patients who had a lower median perfusion rate had a significantly higher local failure rate. The results of this study were attributed to the hypothesis that poorly perfused tumours respond poorly to radiotherapy, as tumours with low perfusion are increasingly hypoxic and therefore have a greater risk of local failure. However, greater experience is required to draw a conclusion and to demonstrate whether CTp assessment at staging may be informative on patient survival.

Predicting response to therapy

Studies have shown the potential of CTp in predicting response to therapy. Zima *et al.*[19] observed that tumours with elevated blood volume and blood flow were associated with response to induction chemotherapy in head and neck tumours. Wang *et al.*[20] observed

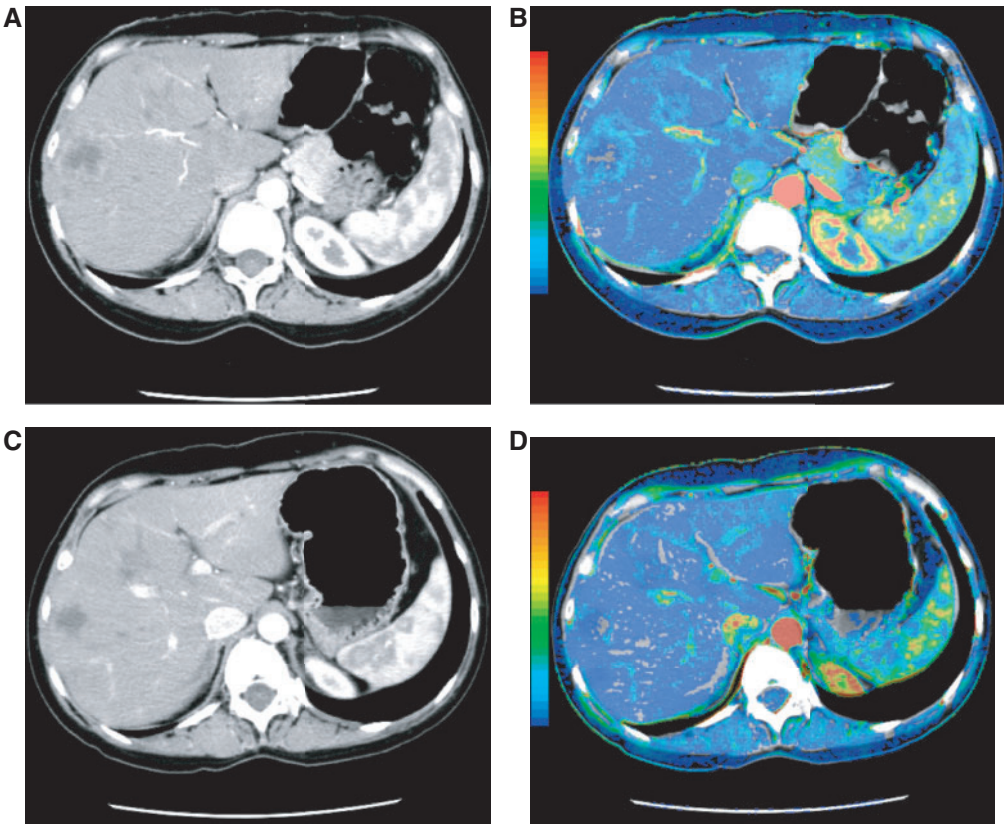

Figure 4 **A 53-year-old woman with liver metastases from breast cancer. Conventional CT images and colour maps before (A,B) and after (C,D) 4 weeks of treatment with metronomic oral vinorelbine. (A) Conventional CT image showing a hypodense metastatic lesion in the VII liver segment. (B) Colour map for BF showing a peripheral rim of yellow colour shades along the margins of the lesion, representing higher BF values than in the surrounding liver parenchyma. (C) Conventional CT performed after 4 weeks of treatment with metronomic oral vinorelbine, showing only a minimal reduction in size, not yet sufficient to be classified as response to therapy according to the RECIST criteria. (D) Colour map for BF after 4 weeks of treatment with metronomic oral vinorelbine, showing almost complete disappearance of the peripheral rim along the margins of the lesion, accounting for a decrease in BF values.**

that non-small cell lung cancers with higher blood flow were more sensitive to chemoradiation therapy than those with lower blood flow in lung tumours. Bellomi *et al.*[6] observed that patients with locally advanced rectal cancers who responded to neoadjuvant chemoradiation therapy had significantly higher blood flow and blood volume before therapy compared with non-responders.

This preliminary evidence seems to show that low tumour perfusion may determine a poor response to chemotherapy because delivery of chemotherapeutic agents to poorly perfused tumours is lower. Low tumour perfusion also seems to predict a poor response to radiotherapy because poorly perfused tumours are more likely to be hypoxic with consequently poor radiosensitivity. CTp performed before starting treatment could potentially identify those patients with poorly perfused tumours who are more likely to have a poor response to chemotherapy and radiotherapy, and offer alternative or more personalized treatment regimens, avoiding expensive and ineffective treatment with potential side effects.

Therapy monitoring and drug development

Previous studies have shown a significant reduction in perfusion parameters after chemotherapy in various types of tumours including rectum[6,7], liver[22], lung[23], and upper aerodigestive tract[18,24] tumours. These observations provide the preliminary evidence that the effects on tumour vascularization brought about by chemotherapy[69–72] can be detected by monitoring the changes in perfusion parameters measured using CTp; CTp can thus be used in the serial monitoring of such therapies.

If the effects of chemotherapy are brought about by the closing of arteriovenous shunts, this can be detected using CTp by the reduction in blood flow within the microvessels (measured using either BF or extraction-flow product (EF), according to the kinetic model used). A reduction in the blood flow within the microvessels would subsequently increase the MTT for the blood to flow within those microvessels. Similarly if the effects of chemotherapy are brought about by a reduction

in the number and volume of blood vessels, this would be detected using CTp by the reduction in BV. A reduction in the number of newly formed hyperpermeable blood vessels would also be detected using CTp by a decrease in the capillary permeability (PS or EF, according to the kinetic model used).

Anti-angiogenic drugs aim to reduce the amount of blood delivered to the tumour and thus stop its growth, therefore having a greater cytostatic effect as opposed to the more common cytotoxic effect of conventional chemotherapeutic agents. One can thus appreciate the need to monitor the effects of such therapies on tumour perfusion because the sole monitoring of tumour size as a guide to the efficacy of such treatments has little significance on its own[73], as it occurs only at a later stage (Fig. 4). In light of the increased clinical use of anti-angiogenic drugs, there is a need for a non-invasive functional tool in the clinical arena that is able to monitor changes in perfusion induced by these agents and this can be fulfilled by CTp. In fact, CTp has been able to document a reduction in perfusion parameters after administration of a single dose of an anti-angiogenic drug in rectal[16] and liver[21] tumours. Several other tumours have shown an early reduction in perfusion[26–28], even after a few hours *in vitro*[74], after therapies with anti-angiogenic or antivascular drugs.

CTp has also shown promise in helping to understand, *in vivo*, the mechanism of action of certain vascular disrupting agents. Ng *et al.*[29] proposed a possible mechanism of action for the antivascular agent, combretastin A4 phosphate, in advanced non-small cell lung cancer, when administered in conjunction with radiotherapy. Following two fractions of radiotherapy they observed an increase in tumour PS, and 4 h following administration of combretastin A4 phosphate they observed a reduction in tumour blood volume. They proposed that increase in blood vessel permeability following radiotherapy can augment the vascular disrupting effect of combrestatin A4 phosphate. CTp has the potential to show *in vivo* changes in tumour perfusion and thus help understand the mechanism of action and interaction between drugs and other associated therapies. The potential mechanism of action of a nitric oxide synthesis inhibitor has also been studied[30]. A reduction in blood volume was observed in a group of patients with various tumours (including non-small cell lung cancer, prostate, and cervical cancer) at 1 h and at 24 h after the administration of *N*-nitro-L-arginine. This could potentially indicate the effect of nitric oxide synthesis on tumour blood volume and perfusion and may help direct future drug development.

Conclusions

CTp is a tool that may serve as a non-invasive biomarker of angiogenesis. It has shown promise in tumour assessment, characterization and may play a role in tumour staging to predict disease-free survival. It has also shown good potential in predicting response to therapy in various body tumours. In light of the increasing use of anti-angiogenic and antivascular agents in oncology, besides helping us to understand the mechanism of action of these drugs, CTp may have a role in monitoring a tumour's response to these therapies. We can thus foresee a greater clinical use of CTp. However, further studies, with larger patient population and ideally multicentric, are needed to confirm the encouraging results published to date.

Acknowledgement

We would like to thank the Fondazione Umberto Veronesi for supporting this work.

References

[1] Jiao W, Xu J, Zheng J, Shen Y, Lin L, Li J. Elevation of circulating big endothelin-1: an independent prognostic factor for tumor recurrence and survival in patients with esophageal squamous cell carcinoma. BMC Cancer 2008; 8: 334. doi:10.1186/1471-2407-8-334. PMid:19014602.

[2] Szarvas T, Jäger T, Droste F, *et al.* Serum levels of angiogenic factors and their prognostic relevance in bladder cancer. Pathol Oncol Res 2009; 15: 193–201. doi:10.1007/s12253-008-9107-z.

[3] Donnem T, Al-Saad S, Al-Shibli K, *et al.* Inverse prognostic impact of angiogenic marker expression in tumor cells versus stromal cells in non small cell lung cancer. Clin Cancer Res 2007; 13(22 Pt 1): 6649–57.

[4] Rössler J, Taylor M, Geoerger B, *et al.* Angiogenesis as a target in neuroblastoma. Eur J Cancer 2008; 44: 1645–56.

[5] Sahani DV, Holalkere NS, Mueller PR, Zhu AX. Advanced hepatocellular carcinoma: CT perfusion of liver and tumor tissue – initial experience. Radiology 2007; 243: 736–43. doi:10.1148/radiol.2433052020. PMid:17517931.

[6] Bellomi M, Petralia G, Sonzogni A, Zampino MG, Rocca A. CT perfusion for the monitoring of neoadjuvant chemotherapy and radiation therapy in rectal carcinoma: initial experience. Radiology 2007; 244: 486–93. doi:10.1148/radiol.2442061189. PMid:17641369.

[7] Sahani DV, Kalva SP, Hamberg LM, *et al.* Assessing tumor perfusion and treatment response in rectal cancer with multisection CT: initial observations. Radiology 2005; 234: 785–92. doi:10.1148/radiol.2343040286. PMid:15734934.

[8] Sitartchouk I, Roberts HC, Pereira AM, Bayanati H, Waddell T, Roberts TP. Computed tomography perfusion using first pass methods for lung nodule characterization. Invest Radiol 2008; 43: 349–58. doi:10.1097/RLI.0b013e3181690148. PMid:18496039.

[9] Petralia G, Preda L, Giugliano G, *et al.* CT perfusion (CTp) for monitoring induction chemotherapy in patients with a squamous cell carcinoma of the upper aerodigestive tract: correlation between changes in tumor perfusion and tumor volume. J Comput Assist Tomogr 2009; 33: 552–9. doi:10.1097/RCT.0b013e31818d446e. PMid:19638848.

[10] Rumboldt Z, Al-Okaili R, Deveikis JP. Perfusion CT for head and neck tumors: pilot study. Am J Neuroradiol 2005; 26: 1178–85.

[11] Zhang M, Kono M. Solitary pulmonary nodules: evaluation of blood flow patterns with dynamic CT. Radiology 1997; 205: 471–8.

[12] Li Y, Yang ZG, Chen TW, Chen HJ, Sun JY, Lu YR. Peripheral lung carcinoma: correlation of angiogenesis and first-pass

perfusion parameters of 64-detector row CT. Lung Cancer 2008; 61: 44–53. doi:10.1016/j.lungcan.2007.10.021. PMid:18055062.

[13] Ma SH, Le HB, Jia BH, *et al.* Peripheral pulmonary nodules: relationship between multi-slice spiral CT perfusion imaging and tumor angiogenesis and VEGF expression. BMC Cancer 2008; 8: 186. doi:10.1186/1471-2407-8-186. PMid:18590539.

[14] d'Assignies G, Couvelard A, Bahrami S, *et al.* Pancreatic endocrine tumors: tumor blood flow assessed with perfusion CT reflects angiogenesis and correlates with prognostic factors. Radiology 2008; 250: 407–16. doi:10.1148/radiol.2501080291. PMid:19095784.

[15] Feng ST, Sun CH, Li ZP, *et al.* Evaluation of microvessel density and vascular endothelial growth factor in colorectal carcinoma with 64-multidetector-row CT perfusion imaging. Zhonghua Wei Chang Wai Ke Za Zhi. 2008; 11: 537–41.

[16] Willett CG, Boucher Y, di Tomaso E, *et al.* Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004; 10: 145–7. doi:10.1038/nm988. PMid:14745444.

[17] Goh V, Halligan S, Wellsted DM, Bartram CI. Can perfusion CT assessment of primary colorectal adenocarcinoma blood flow at staging predict for subsequent metastatic disease? A pilot study. Eur Radiol 2009; 19: 79–89. doi:10.1007/s00330-008-1128-1. PMid:18704434.

[18] Hermans R, Meijerink M, Van den Bogaert W, Rijnders A, Weltens C, Lambin P. Tumor perfusion rate determined noninvasively by dynamic computed tomography predicts outcome in head-and-neck cancer after radiotherapy. Int J Radiat Oncol Biol Phys 2003; 57: 1351–6.

[19] Zima A, Carlos R, Gandhi D, Case I, Teknos T, Mukherji SK. Can pretreatment CT perfusion predict response of advanced squamous cell carcinoma of the upper aerodigestive tract treated with induction chemotherapy? AJNR Am J Neuroradiol 2007; 28: 328–34.

[20] Wang J, Wu N, Cham MD, Song Y. Tumor response in patients with advanced non-small cell lung cancer: perfusion CT evaluation of chemotherapy and radiation therapy. AJR Am J Roentgenol 2009; 193: 1090–6. doi:10.2214/AJR.08.1367. PMid:19770333.

[21] Zhu AX, Holalkere NS, Muzikansky A, Horgan K, Sahani DV. Early antiangiogenic activity of bevacizumab evaluated by computed tomography perfusion scan in patients with advanced hepatocellular carcinoma. Oncologist 2008; 13: 120–5. doi:10.1634/theoncologist.2007-0174. PMid:18305056.

[22] Pandharipande PV, Krinsky GA, Rusinek H, Lee VS. Perfusion imaging of the liver: current challenges and future goals. Radiology 2005; 234: 661–73. doi:10.1148/radiol.2343031362. PMid:15734925.

[23] Kiessling F, Boese J, Corvinus C, *et al.* Perfusion CT in patients with advanced bronchial carcinomas: a novel chance for characterization and treatment monitoring? Eur Radiol 2004; 14: 1226–33.

[24] Gandhi D, Chepeha DB, Miller T, *et al.* Correlation between initial and early follow-up CT perfusion parameters with endoscopic tumor response in patients with advanced squamous cell carcinomas of the oropharynx treated with organ-preservation therapy. Am J Neuroradiol 2006; 27: 101–6.

[25] Ah-See ML, Makris A, Taylor NJ, *et al.* Early changes in functional dynamic magnetic resonance imaging predict for pathologic response to neoadjuvant chemotherapy in primary breast cancer. Clin Cancer Res 2008; 14: 6580–9. doi:10.1158/1078-0432.CCR-07-4310. PMid:18927299.

[26] Xiong HQ, Herbst R, Faria SC, *et al.* A phase I surrogate endpoint study of SU6668 in patients with solid tumors. Invest New Drugs 2004; 22: 459–66. doi:10.1023/B:DRUG.0000036688.96453.8d. PMid:15292716.

[27] Meijerink MR, van Cruijsen H, Hoekman K, *et al.* The use of perfusion CT for the evaluation of therapy combining AZD2171 with gefitinib in cancer patients. Eur Radiol 2007; 17: 1700–13. doi:10.1007/s00330-006-0425-9. PMid:17072618.

[28] McNeel DG, Eickhoff J, Lee FT, *et al.* Phase I trial of a monoclonal antibody specific for integrin (MEDI-522) in patients with advanced malignancies, including an assessment on tumor perfusion. Clin Cancer Res 2005; 11: 7851–60. doi:10.1158/1078-0432.CCR-05-0262. PMid:16278408.

[29] Ng QS, Goh V, Carnell D, *et al.* Tumor antivascular effects of radiotherapy combined with combretastatin a4 phosphate in human non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2007; 67: 1375–80.

[30] Ng QS, Goh V, Milner J, *et al.* Effect of nitric-oxide synthesis on tumour blood volume and vascular activity: a phase I study. Lancet Oncol 2007; 8: 111–18. doi:10.1016/S1470-2045(07)70001-3.

[31] Uusaro A, Ruokonen E, Takala J. Estimation of splanchnic blood flow by the Fick principle in man and problems in the use of indocyanine green. Cardiovasc Res 1995; 30: 106–12. doi:10.1016/0008-6363(95)00007-0.

[32] Lee TY, Purdie TG, Stewart E. CT imaging of angiogenesis. Q J Nucl Med 2003; 47: 171–87.

[33] Axel L. Cerebral blood flow determination by rapid-sequence computed tomography: theoretical analysis. Radiology 1980; 137: 679–86.

[34] Cenic A, Nabavi DG, Craen RA, Gelb AW, Lee TY. A CT method to measure hemodynamics in brain tumors: validation and application of cerebral blood flow maps. Am J Neuroradiol 2000; 21: 462–70.

[35] Miles KA. Perfusion CT for the assessment of tumour vascularity: which protocol? Br J Radiol 2003; 76(suppl 1): S36–42. doi:10.1259/bjr/18486642. PMid:15456712.

[36] Miles KA, Griffiths MR. Perfusion CT: a worthwhile enhancement? Br J Radiol 2003; 76: 220–31. doi:10.1259/bjr/13564625. PMid:12711641.

[37] Miles KA, Charnsangavej C, Lee F, Fishman E, Horton K, Lee T-Y. Application of CT in the investigation of angiogenesis in oncology. Acad Radiol 2000; 7: 840–50. doi:10.1016/S1076-6332(00)80632-7.

[38] Kambadakone AR, Sahani DV. Body perfusion CT: technique, clinical applications, and advances. Radiol Clin North Am 2009; 47: 161–78. doi:10.1016/j.rcl.2008.11.003. PMid:19195541.

[39] Platten D, Keat N, Lewis M, Edyvean S. 32 to 64 slice CT scanner: comparison report version 13. London: HMSO; 2005.

[40] Goh V, Liaw J, Bartram CI, Halligan S. Effect of temporal interval between scan acquisitions on quantitative vascular parameters in colorectal cancer: implications for helical volumetric perfusion CT techniques. AJR Am J Roentgenol 2008; 191: W288–92. doi:10.2214/AJR.07.3985. PMid:19020217.

[41] Miles KA. Functional computed tomography in oncology. Eur J Cancer 2002; 38: 2079–84. doi:10.1016/S0959-8049(02)00386-6.

[42] Ng QS, Goh V, Milner J, Padhani AR, Saunders MI, Hoskin PJ. Acute tumor vascular effects following fractionated radiotherapy in human lung cancer: *In vivo* whole tumor assessment using volumetric perfusion computed tomography. Int J Radiat Oncol Biol Phys 2007; 67: 417–24.

[43] Ng QS, Goh V, Fichte H, *et al.* Lung cancer perfusion at multidetector row CT: reproducibility of whole tumor quantitative measurements. Radiology 2006; 239: 547–53. doi:10.1148/radiol.2392050568. PMid:16543594.

[44] Ng QS, Goh V, Klotz E, *et al.* Quantitative assessment of lung cancer perfusion using MDCT: does measurement reproducibility improve with greater tumor volume coverage? AJR Am J Roentgenol 2006; 187: 1079–84. doi:10.2214/AJR.05.0889. PMid:16985160.

[45] Bisdas S, Konstantinou GN, Lee PS, *et al.* Dynamic contrast-enhanced CT of head and neck tumors: perfusion measurements using a distributed-parameter tracer kinetic model. Initial results and comparison with deconvolution-based analysis. . Phys Med

Biol 2007; 52: 6181–96. doi:10.1088/0031-9155/52/20/007. PMid:17921579.

[46] Gandhi D, Hoeffner EG, Carlos RC, *et al.* Computed tomography perfusion of squamous cell carcinoma of the upper aerodigestive tract. Initial results. J Comput Assist Tomogr 2003; 27: 687–93. doi:10.1097/00004728-200309000-00005. PMid:14501359.

[47] Goh V, Halligan S, Hugill JA, *et al.* Quantitative colorectal cancer perfusion measurement using dynamic contrast-enhanced multidetector-row computed tomography: effect of acquisition time and implications for protocols. J Comput Assist Tomogr 2005; 29: 59–63. doi:10.1097/01.rct.0000152847.00257.d7. PMid:15665684.

[48] Lee TY, Ellis RJ, Dunscombe PB, *et al.* Quantitative computed tomography of the brain with xenon enhancement: a phantom study with the GE9800 scanner. Phys Med Biol 1990; 35: 925–35. doi:10.1088/0031-9155/35/7/008. PMid:2385623.

[49] Wintermark M, Maeder P, Verdun F, *et al.* Using 80 kVp versus 120 kVp in perfusion CT measurement of regional cerebral blood flow. AJNR Am J Neuroradiol 2000; 21: 1881–4.

[50] Hirata M, Sugawara Y, Fukutomi Y, *et al.* Measurement of radiation dose in cerebral CT perfusion study. Radiat Med 2005; 23: 97–103.

[51] Goh V, Halligan S, Gartner L, Bassett P, Bartram CI. Quantitative colorectal cancer perfusion measurement by multidetector-row CT: does greater tumour coverage improve measurement reproducibility? Br J Radiol 2006; 79: 578–83. doi:10.1259/bjr/18842556. PMid:16823062.

[52] Goh V, Halligan S, Gharpuray A, Wellsted D, Sundin J, Bartram CI. Quantitative assessment of colorectal cancer tumor vascular parameters by using perfusion CT: influence of tumor region of interest. Radiology 2008; 247: 726–32. doi:10.1148/radiol.2473070414. PMid:18403621.

[53] Goh V, Halligan S, Hugill JA, Bassett P, Bartram CI. Quantitative assessment of colorectal cancer perfusion using MDCT: inter- and intraobserver agreement. AJR Am J Roentgenol 2005; 185: 225–31.

[54] Petralia G, Preda L, Raimondi S, *et al.* Intra- and interobserver agreement and impact of arterial input selection in perfusion CT measurements performed in squamous cell carcinoma of the upper aerodigestive tract. AJNR Am J Neuroradiol 2009; 30: 1107–15. doi:10.3174/ajnr.A1540. PMid:19342547.

[55] Bisdas S, Baghi M, Smolarz A, *et al.* Quantitative measurements of perfusion and permeability of oropharyngeal and oral cavity cancer, recurrent disease, and associated lymph nodes using first-pass contrast-enhanced computed tomography studies. Invest Radiol 2007; 42: 172–9. doi:10.1097/01.rli.0000252496.74242.0b. PMid:17287647.

[56] Nabavi DG, Cenic A, Craen RA, *et al.* CT assessment of cerebral perfusion: experimental validation and initial clinical experience. Radiology 1999; 213: 141–9.

[57] Hirata M, Sugawara Y, Murase K, Miki H, Mochizuki T. Evaluation of optimal scan duration end time in cerebral CT perfusion study. Radiat Med 2005; 23: 351–63.

[58] Goh V, Halligan S, Bartram CI. Quantitative tumor perfusion assessment with multidetector CT – are measurements from two commercial software packages interchangeable? Radiology 2007; 242: 777–82. doi:10.1148/radiol.2423060279. PMid:17325066.

[59] Goh V, Sanghera B, Wellsted DM, Sundin J, Halligan S. Assessment of the spatial pattern of colorectal tumour perfusion estimated at perfusion CT using two-dimensional fractal analysis. Eur Radiol 2009; 19: 1358–65. doi:10.1007/s00330-009-1304-y. PMid:19190914.

[60] Chen Y, Zhang J, Dai J, Feng X, Lu H, Zhou C. Angiogenesis of renal cell carcinoma: perfusion CT findings. Abdom Imaging 2009. doi:10.1007/s00261-009-9565-0.

[61] Ash L, Teknos TN, Gandhi D, Patel S, Mukherji SK. Head and neck squamous cell carcinoma: CT perfusion can help non invasively predict intratumoral microvessel density. Radiology 2009; 251: 422–8. doi:10.1148/radiol.2512080743. PMid:19276321.

[62] Goh V, Halligan S, Daley F, Wellsted DM, Guenther T, Bartram CI. Colorectal tumor vascularity: quantitative assessment with multidetector CT – do tumor perfusion measurements reflect angiogenesis? Radiology 2008; 249: 510–7. doi:10.1148/radiol.2492071365. PMid:18812560.

[63] Li ZP, Meng QF, Sun CH, *et al.* Tumor angiogenesis and dynamic CT in colorectal carcinoma: radiologic–pathologic correlation. World J Gastroenterol 2005; 11: 1287–91.

[64] Konerding MA, Fait E, Gaumann A. 3D microvascular architecture of pre-cancerous lesions and invasive carcinomas of the colon. Br J Cancer 2001; 84: 1354–62. doi:10.1054/bjoc.2001.1809. PMid:11355947.

[65] Senger DR, Van de Water L, Brown LF, *et al.* Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev 1993; 12: 303–24. doi:10.1007/BF00665960. PMid:8281615.

[66] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182–6.

[67] Dugdale PE, Miles KA. CT measurement of perfusion and permeability within lymphoma masses and its ability to assess grade, activity, and chemotherapeutic response. J Comput Assist Tomogr 1999; 23: 540–7. doi:10.1097/00004728-199907000-00010. PMid:10433282.

[68] Hayano K, Shuto K, Koda K, Yanagawa N, Okazumi S, Matsubara H. Quantitative measurement of blood flow using perfusion CT for assessing clinicopathologic features and prognosis in patients with rectal cancer. Dis Colon Rectum 2009; 52: 1624–9.

[69] Delille JP, Slanetz PJ, Yeh ED, Halpern EF, Kopans DB, Garrido L. Invasive ductal breast carcinoma response to neoadjuvant chemotherapy: noninvasive monitoring with functional MR imaging pilot study. Radiology 2003; 228: 63–9. doi:10.1148/radiol.2281011303. PMid:12775851.

[70] Miller KD, Sweeney CJ, Sledge Jr GW. Redefining the target? chemotherapeutics as antiangiogenics. J Clin Oncol 2001; 19: 1195–206.

[71] Kumar P, Benedict R, Urzua F, Fischbach C, Mooney D, Polverini P. Combination treatment significantly enhances the efficacy of antitumor therapy by preferentially targeting angiogenesis. Lab Invest 2005; 85: 756–67. doi:10.1038/labinvest.3700272. PMid:15864318.

[72] Lennernas B, Albertsson P, Lennernas H, Norrby K. Chemotherapy and antiangiogenesis: drug-specific, dose-related effects. Acta Oncol 2003; 42: 294–303. doi:10.1080/02841860310001835.

[73] DePrimo SE, Bello C. Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib. Ann Oncol 2007; 18(Suppl 10): 11–9. doi:10.1093/annonc/mdm409. PMid:17761718.

[74] Sabir A, Schor-Bardach R, Wilcox CJ, *et al.* Perfusion MDCT enables early detection of therapeutic response to antiangiogenic therapy. AJR Am J Roentgenol 2008; 191: 133–9. doi:10.2214/AJR.07.2848. PMid:18562736.