BJR

Received:
11 December 2013

Revised:
17 December 2013

Accepted:
20 December 2013

doi: 10.1259/bjr.20130811

Cite this article as:
Goh V, Glynne-Jones R. Perfusion CT imaging of colorectal cancer. Br J Radiol 2014;87:20130811.

REVIEW ARTICLE

Perfusion CT imaging of colorectal cancer

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ABSTRACT

Imaging plays an important role in the assessment of colorectal cancer, including diagnosis, staging, selection of treatment, assessment of treatment response, surveillance and investigation of suspected disease relapse. Anatomical imaging remains the mainstay for size measurement and structural evaluation; however, functional imaging techniques may provide additional insights into the tumour microenvironment. With dynamic contrast-enhanced CT techniques, iodinated contrast agent kinetics may inform on regional tumour perfusion, shunting and microvascular function and provide a surrogate measure of tumour hypoxia and angiogenesis. In colorectal cancer, this may be relevant for clinical practice in terms of tumour phenotyping, prognostication, selection of individualized treatment and therapy response assessment.

Colorectal cancer is one of the commonest of cancers, accounting for 10% of all cancers, with approximately 1.2 million new cases each year. Colorectal cancer remains a major cause of morbidity and mortality worldwide, with approximately 609 000 deaths per annum.[1] Since a radical abdominopelvic resection approach for rectal cancer was described in 1908,[2] significant inroads have been made into its treatment, including surgery, radiotherapy and chemotherapy, which have all improved morbidity and local recurrence rates, and also had some impact on the overall survival rate. These have included the introduction of surgical techniques such as total mesorectal excision,[3,4] neoadjuvant radiotherapy prior to surgery to reduce the risk of local recurrence and an increase in the likelihood of resectability,[5–7] as well as a more aggressive treatment of oligometastatic disease. Trialling of novel targeted therapies such as bevacizumab, a recombinant humanized monoclonal antibody against the vascular endothelial growth factor (VEGF), and the selective use of epidermal growth factor receptor inhibitors, such as cetuximab and panitumumab, have also led to improvements in outcome in the metastatic setting.[8–10] These approaches have had a "knock-on" effect on imaging, requiring more accurate delineation of locoregional tumour extent and distant spread, and on the development of more sophisticated methods of tumour profiling to direct therapy and for assessing the therapy response and efficacy of the particular agent.

This article will highlight our current understanding of the molecular characterization of colorectal cancer, the architectural and physiological aspects of the vascular network in colorectal cancer, and discuss how dynamic contrast-enhanced CT (DCE-CT; perfusion CT), one of the increasing number of functional imaging techniques available in the clinic, may assist the management of colorectal cancer.

MOLECULAR CLASSIFICATION OF COLORECTAL CANCER

Traditionally, colorectal cancers have been classified by clinicopathological features, including tumour location, TNM stage, differentiation and grade. However, this may not provide sufficient information with respect to tumour profiling towards a more targeted treatment approach. Colorectal cancers are heterogeneous with respect to genetic and epigenetic mutations and may be classified by molecular characteristics.[11,12] Chromosomal instability (CIN), which reflects the tendency for chromosome breakage; microsatellite instability (MSI), which reflects defective DNA repair; and frequent CpG island hypermethylation (CIMP), which reflects gene silencing owing to methylation of the promoter gene sequence, are three common classifiers. CIMP-high colorectal tumours have a distinct clinical, pathological and molecular profile, such as associations with proximal tumour location, female sex, poor differentiation, MSI and high *BRAF* and low *TP53* mutation rates. CIN is present in the majority of sporadic cancers (85%) and may occur through different mechanisms, including whole chromosomal loss of heterozygosity, mitotic recombination and mitotic gene conversion. Loss

Table 1. Radiological–pathological correlative studies: colorectal cancer

Tumour type	Perfusion CT parameter/method	Pathological correlate/method	Findings	Study
Angiogenesis				
Colorectal, $n = 23$	*Blood volume* *Permeability surface* Whole tumour cross-sectional area Johnson–Wilson 1 s interval Limited coverage	CD34 Random field	Moderate correlations BV: $r = 0.59^a$, $p = 0.002$ PS: $r = 0.46^a$, $p = 0.03$ with CD34 expression	Goh et al[23]
Colorectal, $n = 29$	*Blood flow* *Blood volume* *Permeability Surface* Two selected areas: Luminal and invasive edge Johnson–Wilson 1 s interval Limited coverage	Factor VIII CD105 Focused region	Variable correlations, some significant for BV and PS: BF: $r = 0.05$ to 0.19; $p = 0.98$ to 0.32 BV: $r = 0.02$ to 0.55^a; $p = 0.91$ to 0.003^a PS: $r = 0.09$ to 0.43^a; $p = 0.96$ to 0.023^a	Dighe et al[24]
Colorectal, $n = 37$	*Perfusion* Whole tumour cross-sectional area Slope method 2 s interval Limited coverage	CD34 Hot spot (3)	No correlation between perfusion and CD34 $r = 0.18$, $p = 0.29$ Decrease in perfusion and CD34 expression with stage	Li et al[25]
Colorectal, $n = 32$	*Blood flow* *Blood volume* *Permeability surface* Whole tumour cross-sectional area Johnson–Wilson 1 s interval Limited coverage	CD34 Hot spot (3)	No correlations with CD34 BF: $r = -0.14$, $p > 0.45$ BV: $r = 0.11$, $p > 0.51$ PS: $r = 0.28$, $p > 0.12$	Feng et al[26]
Colorectal, $n = 27$	*Blood flow* *Blood volume* *Permeability surface* Whole tumour cross-sectional area Johnson–Wilson 1 s interval Limited coverage	CD34 Hotspot (3)	No correlations with CD34	Kim et al[27]

BF, regional blood flow; BV, regional blood volume; PS, permeability surface area product.
[a]Significant correlations.

of 18q heterozygosity is thought to reflect a worse prognosis[13] and may be a factor for selecting adjuvant therapy in Stage II cancers. MSI is present in approximately 15% of sporadic cancers. Functional loss of *MLH1* as a result of promoter methylation and gene silencing is the most common cause of MSI, particularly in sporadic MSI-high (MSI-H) cancer. MSI is typically assessed by analysing five microsatellite markers (D2S123, D5S346, D17S250, BAT25 and BAT26), referred to as the National Cancer Institute consensus panel. MSI status may also be of relevance in selecting Stage II patients to omit adjuvant therapy.[13] A systematic review of 32 studies, including 7642 colorectal cancer patients of whom 1277 had MSI-H tumours, showed that MSI-H tumours were associated with a better prognosis than MSS tumours [hazard ratio for overall survival 0.65 (95% confidence interval: 0.59 to 0.71].[14]

THE ARCHITECTURE OF THE VASCULAR NETWORK IN COLORECTAL CANCER

Angiogenesis is an important aspect of tumorigenesis. Neovascularization arises early in the adenoma–carcinoma sequence, via upregulation of VEGF, probably related to the *K-RAS* mutation, which is found in 24% of adenomas.[15] Vascular sprouting and *de novo* vascular formation from precursor endothelial cells from bone marrow are the main mechanisms by which neovascularization occurs in colorectal cancer. Tumour angiogenesis is characterized structurally by abnormal blood vessels

that are thin, fragile, tortuous and hyperpermeable because of an incomplete endothelium and a relative absence of smooth muscle and pericyte coverage. Hence, the VEGF signalling pathway represents a suitable target for anticancer agents, because it is involved in tumour angiogenesis, stimulating tumour neovascularization and promoting endothelial cell survival, migration and permeability, which in turn leads to a higher risk of relapse and a worse overall prognosis.

Architecturally unlike normal colonic mucosa, in which the capillary plexus is arranged in a hexagonal pattern around the mucosal glands, and supplied by subepithelial arteries that divide within the submucosa, the microcirculation in colorectal tumours lacks a regular pattern and vessel hierarchy.[16] The vascular architecture appears to be tumour type specific and consistent irrespective of tumour size. In colorectal carcinomas, there is a chaotic intratumoral distribution with areas of low vascular density mixed with regions of high angiogenic activity, but with a tendency for a decline in vessel density towards the tumour centre.[16] Vessel diameters in general are increased, but with an increased number of blind-ending vessels. Vessel diameters are typically $<200\,\mu m$ in diameter; capillary diameters are typically $<10\,\mu m$. Towards the centre of the tumour, where there are a higher number of elongated compressed vessels, the intervessel distance and interbranch distances are generally higher.

PHYSIOLOGICAL ASPECTS OF THE VASCULAR NETWORK IN COLORECTAL CANCER

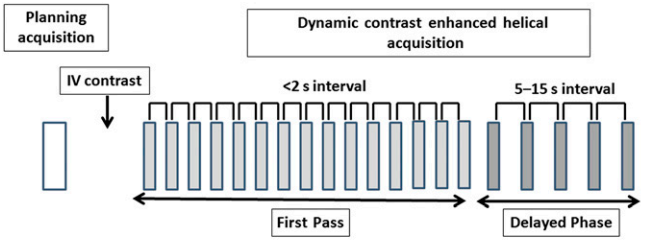
Tumours require an adequate blood supply to deliver oxygen and nutrients for growth and to remove waste products. Functionally, tumour vessels differ from normal vessels with evidence of arteriovenous shunting, intermittent flow or even reversal of flow. There may be acute vascular collapse where there are areas with raised tumour interstitial pressure, particularly towards the centre of the tumour. Higher haematocrit in cancer patients also contributes to altered flow characteristics. The normal vessel wall typically consists of a single layer of endothelial cells with supporting smooth muscle and pericytes. In tumour vessels, vascular hyperpermeability occurs as a result of looser endothelial connections, larger fenestrations and a relative lack of endothelium, smooth muscle and pericyte coverage. A secondary effect of vascular hyperpermeability is raised intratumoral interstitial pressure.

IMAGING THE VASCULAR NETWORK IN COLORECTAL CANCER

Quantitative DCE-CT (perfusion CT) based on standard low-molecular-weight, iodinated contrast agents (<1 kDa) may be incorporated easily into clinical imaging protocols.[17,18] Such an approach reflects the vascular delivery to the tumour, accumulation of contrast agent within the tumour interstitium and recirculation, and allows clinicians to combine functional assessment of the vasculature with anatomical assessment. In oncology, this is clinically relevant as it may provide an indirect measure of hypoxia[19] and angiogenesis[20–22] with data from a variety of cancers. Nevertheless, the data for colorectal cancer remain conflicted (Table 1).

A typical acquisition and contrast administration protocol is shown in Figure 1. With current state-of-the-art technology, a *z*-axis coverage of up to 28 cm may be achieved depending on the required temporal sampling rate using helical techniques or up to 16 cm with non-table-moving techniques. The dynamic acquisition allows the changes in contrast enhancement within the tumour and adjacent vessels to be plotted against time. From the tissue enhancement curve, qualitative and model-free semi-quantitative information may be derived. This includes the tissue curve shape (Type I: slow rising curve; Type II: initial rapid uptake with plateau; and Type III: initial rapid uptake with washout), time to peak enhancement, peak enhancement and area under the enhancement time curve (Figure 2). Tumours typically demonstrate an initial rapid uptake of contrast agent and washout (although some tumours also demonstrate a plateau), a shorter time to peak enhancement and a higher peak enhancement than normal tissue.

Figure 1. Typical perfusion CT acquisition protocol for cancer. IV, intravenous.



Typical acquisition parameters	Kilovoltage	mA	Contrast agent concentration and volume
Head and neck	80–100	<150	Intravenous administration: >4 ml/s Concentration: >300 ml/mg iodine Typical volumes: 30–60ml
Thorax	80–100	<150	
Abdomen and pelvis	100–120	<150	

More complex kinetic modelling may also be applied to obtain more physiologically based parameters (Table 2).

These parameters include regional blood flow (BF; blood flow per unit volume or mass of tissue); regional blood volume (BV; the proportion of tissue that comprises flowing blood); and the flow–extraction product (the rate of transfer of contrast

Figure 2. Typical enhancement time curves. PE, peak enhancement; TTP, time to peak enhancement.

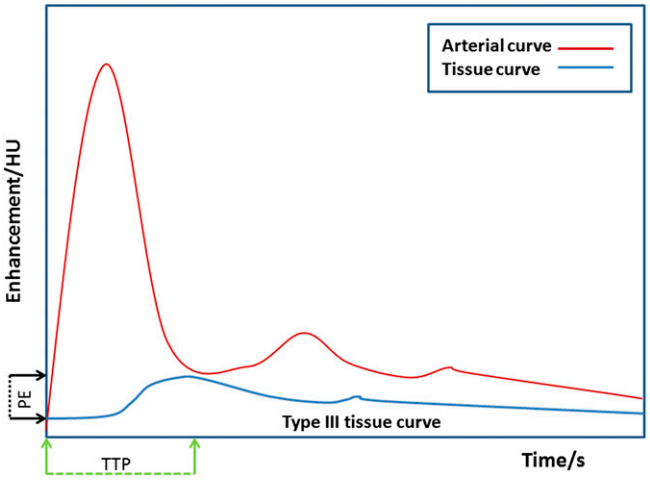


Table 2. Kinetic models used for perfusion CT analysis

Kinetic model	Compartments	Parameter measured	Assumptions
Maximum slope	Single	BF	No venous outflow
Johnson–Wilson	Dual	BF, BV, MTT, PS	Constrained IRF
Patlak	Dual	EF, BV	One way transfer Well-mixed compartments
Distributed parameter	Dual	BF, BV, PS, Ve	Constrained IRF

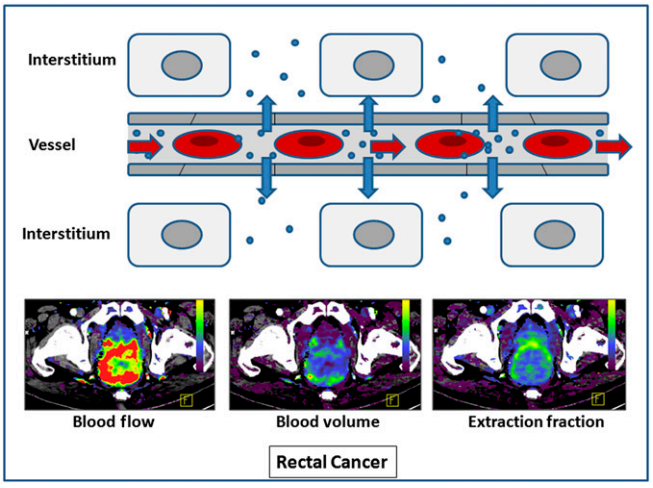
BF, regional blood flow; BV, regional blood volume; EF, extraction fraction; IRF, impulse residual function; MTT, mean transit time; PS, permeability surface area product, Ve, extravascular extracellular volume.

agent from the intravascular to extravascular space), from which the permeability–surface area product (PS; the product of permeability and total surface area of capillary endothelium in a unit mass of tissue) may be derived. BF reflects the rate of delivery of oxygen and nutrients to the tumour, BV reflects the functioning vascular volume and the flow–extraction product or PS reflects the vascular leakage rate of the microcirculation (Figure 3). Extravascular extracellular volume (Ve; %) may also be estimated.

VASCULARIZATION OF TUMOUR COMPARED WITH THAT OF NORMAL COLON

As a result of the differences in the architecture of the vascular network between normal colonic mucosa and colorectal cancer, there are differences also in the imaging characteristics. Tumour BF, BV and vascular permeability are higher than in the normal bowel wall (Figure 4). A typical range of BF values for colorectal cancer is 50–200 ml min^{-1} 100 g^{-1} tissue *vs* 10–40 ml min^{-1} 100 g^{-1} tissue for the normal bowel wall. There are regional differences in normal bowel wall perfusion, which may be related to the underlying function of the bowel, the vascular architecture and underlying supply (superior mesenteric artery, inferior mesenteric artery or other branches); BF is generally lower in the distal than in the proximal large bowel.[28] With respect to inflammation, there may be an overlap in vascular parameters between inflammation and tumour. This is to be expected given the underlying pathophysiology: an increase in vascular flow, vessel dilatation, increase in permeability, increase in vascularization (neoangiogenesis) and shunting are seen with acute inflammation. For example, a study of patients with diverticular disease, acute diverticulitis or cancer confirmed that there is a trend for higher blood flow in cancer (80 ml min^{-1} 100 g^{-1} tissue *vs* 52 ml min^{-1} 100 g^{-1} tissue for cancer and diverticulitis, respectively) but with clear overlap in parameter values for these two conditions.[29]

Figure 3. Parameters obtained from kinetic modelling. F, front.



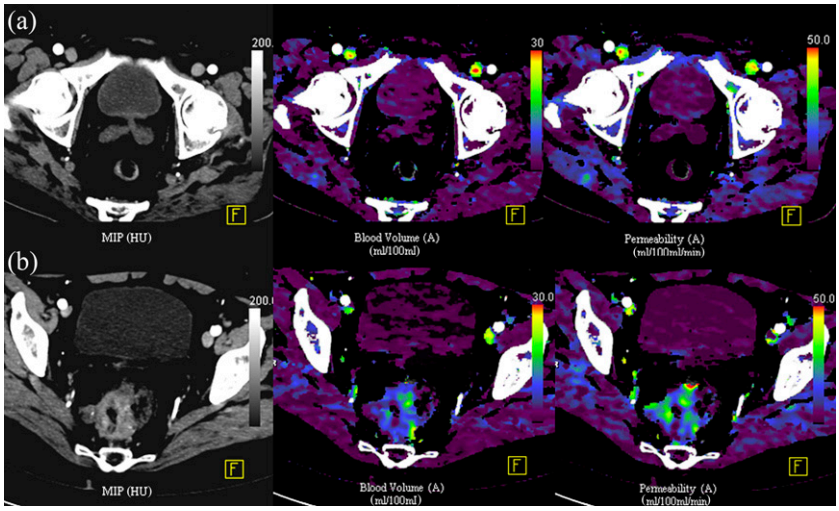
TUMOUR PHENOTYPING WITH PERFUSION CT IMAGING

The downstream physiological effects of the underlying molecular biology of tumours may be apparent with imaging. Perfusion CT techniques may provide a global overview of the degree of vascularization within the tumour as well as the associations between individual parameters, BF, BV and vascular leakage, which are inter-related. Different intratumoural patterns may be present (Figure 5). Areas of high blood flow, blood volume and leakage may reflect well-perfused areas, with the presence of shunting and areas of angiogenesis; areas of low blood flow and blood volume and low leakage areas may represent areas of poor vascularization ± necrosis; areas of low blood flow and blood volume and high leakage areas may represent poor perfusion areas with a high degree of angiogenesis. It is hypothesized that this may lead to clonal adaptation with a selection of more aggressive clones. These patterns may coexist within the tumour, reflecting the spatial and functional heterogeneity of the tumour vasculature.

In terms of clinical translation, small clinical studies have shown that more poorly perfused tumours have a poorer outcome. Hayano et al[30,31] have shown in rectal cancers ($n = 44$) and oesophageal cancers ($n = 31$) that patients with poorly perfused tumours (<40 and <50 ml min^{-1} 100 g^{-1} tissue, respectively) are more likely to have a poorer overall survival ($p \leq 0.001$). Similarly, we have shown that colorectal tumours with a lower perfusion at staging and planned for curative surgery have a greater tendency for subsequent metastatic disease.[32] Patients with these tumours may also have a poorer overall survival. In this scenario, extravascular extracellular volume may also be a relevant measure, as demonstrated by Koh et al.[33] A hypothesis for why lower extravascular extracellular volume tumours have a poorer prognosis relates to the higher grade, differentiation and larger cellular volume these tumours are likely to have.

The generalizability of these findings to more widespread clinical practice is an important issue. With respect to the prognostic value

Figure 4. Perfusion CT characteristics of the normal rectum (a) compared with a cancer (b).



of perfusion CT in colorectal cancer, this is currently undergoing evaluation as part of the National Institute for Health Research Health Technology Assessment-funded PROSPeCT study, which is in progress and aims to recruit 370 patients with primary colorectal cancer without metastatic disease at staging. To date, there have been few data from multicentre studies of perfusion CT in oncology outside of the therapy response setting, and this will provide invaluable information.

A further area of development is the integration of perfusion CT with positron emission tomography (PET) imaging, which has been facilitated by current generation integrated PET-CT scanners that allow helical volumetric perfusion CT imaging. This provides an opportunity to assess different physiological aspects, *e.g.* glucose metabolism [^{18}F-fludeoxyglucose (^{18}F-FDG)], integrin expression [^{18}F-labelled arginine–glycine–aspartic acid peptides (^{18}F-RGD-peptides)], hypoxia [^{18}F-labelled fluoromisonidazole (^{18}F-FMISO) or ^{64}Cu-labelled diacetyl-bis (N^4-methylthiosemicarbazone) (^{64}Cu-ATSM)], cellular proliferation [^{18}F-labelled fluoro-3′-deoxy-3′-L-thymidine (^{18}F-FLT)] and lipid metabolism (^{11}C-acetate) alongside perfusion, and to explore the alongside perfusion, and to explore the inter-relationships between these physiological features both at staging and in response to therapies that may produce discordant effects.

To date, most studies have focused on the relationship between tumour vascular parameters and glucose metabolism. The norm is for delivery and utilization of oxygen and nutrients to be matched, with physiological feedback mechanisms in place to promote this. However in tumours, there may be different scenarios. Vascularization and metabolism may not necessarily be matched (Figure 6), and it has been hypothesized that mismatch between vascularization and metabolism may be an indicator of a more aggressive phenotype. Tumours that are poorly

Figure 5. Different patterns of vascularization within the tumour.

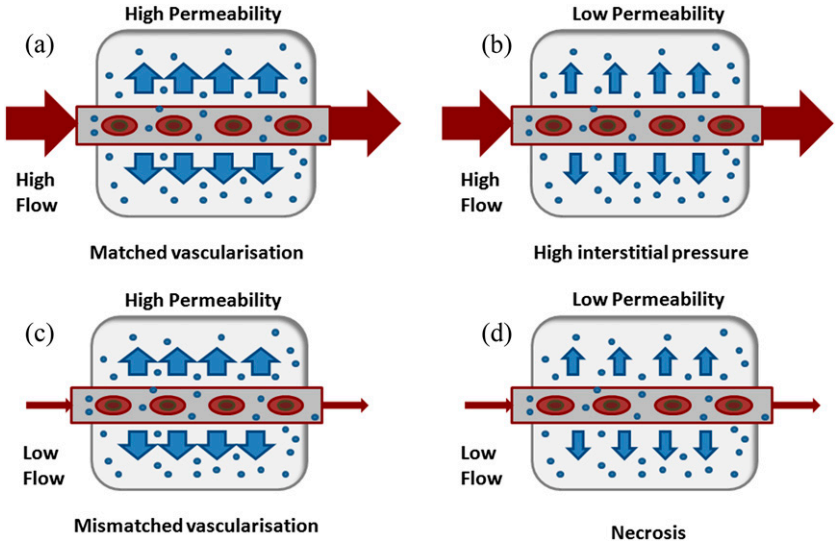
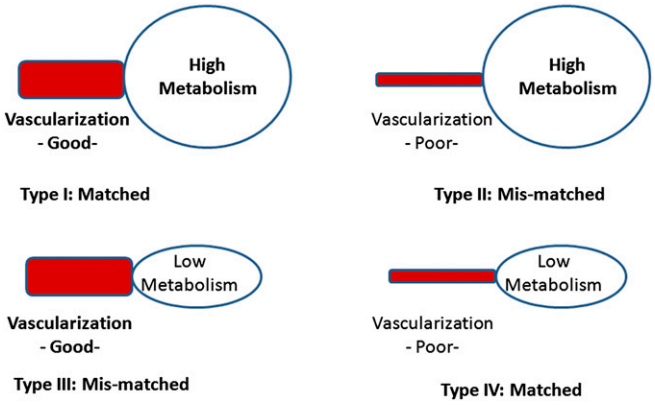


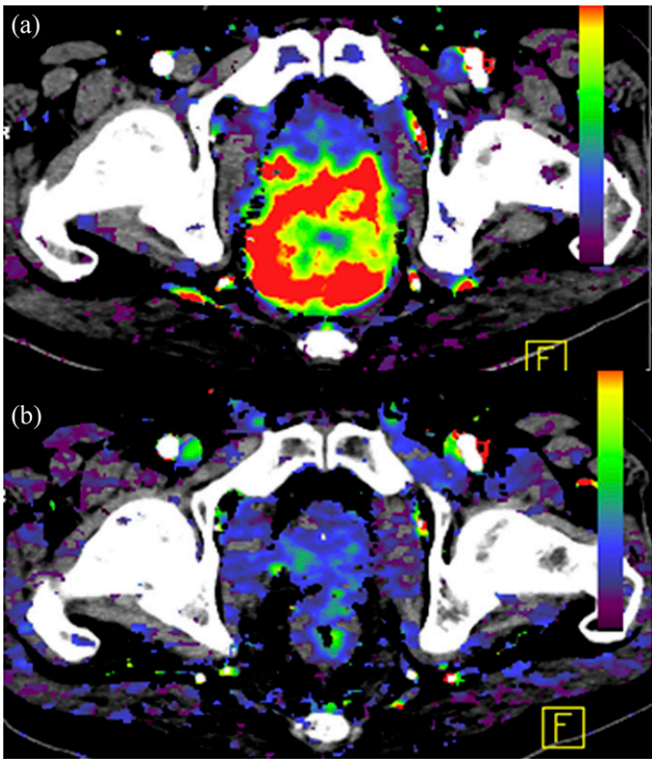
Figure 6. Different patterns of vascularization and metabolism within the tumour.



perfused but with high metabolism may reflect an adaptation to intratumoral hypoxia and may be more resistant to treatment or belie a poorer prognosis.[34]

In support of this, in colorectal cancer, a negative association between BF/maximum standardized uptake value (SUV_{max}) and higher hypoxia-inducible factor 1 (HIF-1) and VEGF expression has been shown, *i.e.* tumours with a lower BF/SUV_{max} ratio are more likely to express HIF-1 and VEGF.[35] Preliminary studies have also suggested that the relationship between vascularization and metabolism is complex depending on the tumour stage and tumour type. In colorectal cancer, vascularization and metabolism are more likely to be matched in higher than in lower stage cancers, unlike lung cancers where mismatch occurs with increasing stage.

Figure 7. Decrease in vascularization of the primary tumour before (a) and after (b) chemoradiation. F, front.



ASSESSING THERAPY RESPONSE WITH PERFUSION CT

Quantitative parameters derived from perfusion CT have a role in monitoring the effects of a variety of treatments that affect the tumour vasculature. These include chemotherapy with standard and novel agents (antiangiogenic drugs, vascular disrupting agents and immunotherapy), radiotherapy and interventional oncological procedures. These therapies typically result in a reduction in measured vascular parameters as a consequence of treatment (Figure 7). During therapy or in the immediate post-therapy period, there may be a more variable vascular effect, depending on the therapeutic mechanism of action (Table 3).

With standard chemotherapy, which affects actively replicating cells via DNA damage or interruption of DNA repair, this effect is thought to reflect the loss of angiogenic cytokine support following cell death. With antiangiogenic therapies, differing vascular effects may be seen depending on the mechanism of action of the drug under investigation and the timing of the scan. An initial effect may be a decrease in vascular permeability and a reduction in interstitial fluid pressure, with normalization of function of the vasculature resulting in a transient increase in tumour BF.[36] In the longer term, with subsequent pruning of the vasculature, a reduction in BF, BV and vascular permeability may be elicited. With vascular disrupting agents, which target the proliferating immature vasculature ± the mature vasculature, a rapid shutdown in tumour vascularization may occur that is usually transient and reversible within 24–48 h. This may be followed by a rebound revascularization.[37] With radiotherapy, the acute effects are related to an initial inflammatory effect; the permeability is related to microvascular damage, which can lead to tumour shrinkage.[38] With interventional procedures, perfusion CT parameters may provide evidence of effective treatment or the need for further procedural attempts for optimal therapeutic effect.[39]

With respect to primary colorectal cancer, there have been a few published studies. In the neoadjuvant setting, chemoradiation has been shown to decrease BF, BV and PS. The degree of reduction in blood flow has typically been >40%.[40–42] Similarly, for the antiangiogenic agent, bevacizumab, a monoclonal antibody targeted at VEGF, a reduction of up to 40% has been seen in vascularization.[43]

ASSESSMENT OF TUMOUR VASCULAR HETEROGENEITY

It is recognized that the tumour vasculature is architecturally and functionally heterogeneous. Although the vascular volume is typically <10% of the total tumour volume, changes in vascularization that occur spatially and temporally are relevant particularly with respect to quantification, where a change in quantified parameters is used to determine a vascular response/non-response. One of the limitations of current software platform methods is the reliance on a global mean value for BF, BV or vascular leakage. This clearly underestimates the extent of spatial heterogeneity. While histogram analysis can provide some information regarding the spread of data, it does not provide spatial

Table 3. Vascular effects of systemic and locoregional therapies

Therapy	Perfusion CT parameter		
	Blood flow	Blood volume	Vascular leakage
Systemic therapies			
Cytotoxic chemotherapy			
Acute effects	Unchanged	Unchanged	Unchanged
Chronic effects	Decrease	Decrease	Decrease
			Unchanged
Antiangiogenics			
Acute effects	Increase	Increase	Decrease
	Unchanged	Unchanged	
Chronic effects	Decrease	Decrease	Decrease
Locoregional therapies			
Radiotherapy			
Acute effects	Increase	Increase	Increase
Chronic effects	Decrease	Decrease	Decrease
Interventional			
Radiofrequency ablation	Decrease	Decrease	Decrease
Transarterial chemoembolization	Decrease	Decrease	Decrease
	Absent	Absent	Absent

information.[44] There has been an increasing interest in modelling methods such as fractal analysis that may better describe the spatial pattern of vascularization. Fractal dimension (FD) refers to how an object fills space. Proof of principle studies have indicated the feasibility of using two-dimensional and three-dimensional techniques for perfusion CT maps and have shown that the technique is reproducible[45] and that the FD is higher for tumours than for normal bowel.[46] To date, there have been limited data on its performance in therapy response settings. Temporal changes in vascularization may also occur related to fluctuations in vascular function. Assessment of baseline reproducibility, where two scans are performed prior to therapy and the variations in vascular parameters between the two scans are assessed, remains a way of demonstrating how much this variation is on a per patient basis.[47] This is particularly relevant in therapy response settings.

CONCLUSION

Perfusion CT is one of a number of functional imaging techniques available to us in clinics that allows us to quantify tumour vascularization. The technique is robust and, with the current state-of-the-art technology, whole tumour BF, BV and vascular leakage can be investigated. As we move towards the future, it may allow us to better phenotype the tumour and combined with PET imaging may be a more powerful tool. As technological improvements in CT continue to evolve, this will further extend clinical applications.

FUNDING

The authors hold a research grant from the National Institute for Health Research Health Technology Assessment Programme (PROSPeCT: NIHR HTA 09/22/49). The authors also acknowledge financial support from the Department of Health via the National Institute for Health Research Comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and from the King's College London/University College London Comprehensive Cancer Imaging Centre funded by Cancer Research UK and the Engineering and Physical Sciences Research Council in association with the Medical Research Council and Department of Health.

REFERENCES

1. Globocan 2008. *Colorectal cancer fact sheet*. Lyons, France: International Agency for Research on Cancer [updated 6 December 2013; cited 10 December 2013]. Available from: http://globocan.iarc.fr/factsheets/cancers/colorectal.asp
2. Miles WE. A method of performing abdominoperineal excision for carcinoma of the rectum and of the terminal portion

of the pelvic colon. *Lancet* 1908; **2**: 1812–13.
3. Heald RJ, Ryall RDH. Recurrence and survival after total mesorectal excision for rectal cancer. *Lancet* 1986; **1**: 1479–82.
4. Martling AL, Holm T, Rutqvist LE, Moran BJ, Heald RJ, Cedermark B. Effect of a surgical training programme on outcome of rectal cancer in the County of Stockholm. Stockholm Colorectal Cancer Study Group, Basingstoke Bowel Cancer Research Project. *Lancet* 2000; **356**: 93–6.
5. Improved survival with preoperative radiotherapy in respectable rectal cancer. Swedish Rectal Cancer Trial. *N Engl J Med* 1997; **336**: 980–87. doi: 10.1056/NEJM199704033361402
6. Kapiteijn E, Kranenbarg EK, Steup WH, Taat CW, Rutten HJ, Wiggers T, et al. Total mesorectal excision (TME) with or without preoperative radiotherapy in the treatment of primary rectal cancer. Prospective randomised trial with standard operative and histopathological techniques. Dutch Colo-Rectal Cancer Group. *Eur J Surg* 1999; **165**: 410–20. doi: 10.1080/110241599750006613
7. Braendengen M, Tveit KM, Berglund A, Birkemeyer E, Frykholm G, Påhlman L, et al. Randomized phase III study comparing preoperative radiotherapy with chemoradiotherapy in nonresectable rectal cancer. *J Clin Oncol* 2008; **26**: 3687–94. doi: 10.1200/JCO.2007.15.3858
8. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335–42. doi: 10.1056/NEJMoa032691
9. Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**: 2011–19. doi: 10.1200/JCO.2010.33.5091
10. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023–34. doi: 10.1056/NEJMoa1305275
11. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525–32. doi: 10.1056/NEJM198809013190901
12. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. *Proc Natl Acad Sci U S A* 2007; **104**: 18654–9. doi: 10.1073/pnas.0704652104
13. Bertagnolli MM, Redston M, Compton CC, Niedzwiecki D, Mayer RJ, Goldberg RM, et al. Microsatellite instability and loss of heterozygosity at chromosomal location 18q: prospective evaluation of biomarkers for Stage II and Stage III colon cancer—a study of CALGB 9581 and 89803. *J Clin Oncol* 2011; **29**: 3153–62. doi: 10.1200/JCO.2010.33.0092
14. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609–18. doi: 10.1200/JCO.2005.01.086
15. Ajiki T, Fujimori T, Ikehara H, Saitoh Y, Maeda S. K-ras gene mutation related to histological atypias in human colorectal adenomas. *Biotech Histochem* 1995; **70**: 90–4.
16. 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
17. Goh V, Padhani AR, Rasheed S. Functional imaging of colorectal cancer angiogenesis. *Lancet Oncol* 2007; **8**: 245–55. doi: 10.1016/S1470-2045(07)70075-X
18. Miles KA, Lee TY, Goh V, Klotz E, Cuenod C, Bisdas S, et al; Experimental Cancer Medicine Centre Imaging Network Group. Current status and guidelines for the assessment of tumour vascular support with dynamic contrast-enhanced computed tomography. *Eur Radiol* 2012; **22**: 1430–41.
19. Mandeville HC, Ng QS, Daley FM, Barber PR, Pierce G, Finch J, et al. Operable non-small cell lung cancer: correlation of volumetric helical dynamic contrast-enhanced CT parameters with immunohistochemical markers of tumor hypoxia. *Radiology* 2012; **264**: 581–9. doi: 10.1148/radiol.12111505
20. Tateishi U, Kusumoto M, Nishihara H, Nagashima K, Morikawa T, Moriyama N. Contrast-enhanced dynamic computed tomography for the evaluation of tumor angiogenesis in patients with lung carcinoma. *Cancer* 2002; **95**: 835–42. doi: 10.1002/cncr.10730
21. Sauter AW, Winterstein S, Spira D, Hetzel J, Schulze M, Mueller M, et al. Multifunctional profiling of non-small cell lung cancer using 18F-FDG PET/CT and volume perfusion CT. *J Nucl Med* 2012; **53**: 521–9. doi: 10.2967/jnumed.111.097865
22. Reiner CS, Roessle M, Thiesler T, Eberli D, Klotz E, Frauenfelder T, et al. Computed tomography perfusion imaging of renal cell carcinoma: systematic comparison with histopatholgical angiogenic and prognostic markers. *Invest Radiol* 2013; **48**: 183–91. doi: 10.1097/RLI.0b013e31827c63a3
23. 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**: 510–17. doi: 10.1148/radiol.2492071365
24. Dighe S, Blake H, Jeyadevan N, Castellano I, Koh DM, Orton M, et al. Perfusion CT vascular parameters do not correlate with immunohistochemically derived microvessel density count in colorectal tumors. *Radiology* 2013; **268**: 400–10. doi: 10.1148/radiol.13112460
25. Li ZP, Meng QF, Sun CH, Xu DS, Fan M, Yang XF, et al. Tumor angiogenesis and dynamic CT in colorectal carcinoma: radiologic-pathologic correlation. *World J Gastroenterol* 2005; **11**: 1287–91.
26. Feng ST, Sun CH, Li ZP, Mak HK, Peng ZP, Guo HY, et al. Evaluation of angiogenesis in colorectal carcinoma with multidetector-row CT multislice perfusion imaging. *Eur J Radiol* 2010; **75**: 191–6. doi: 10.1016/j.ejrad.2009.04.058
27. Kim JW, Jeong YY, Chang NK, Heo SH, Shin SS, Lee JH, et al. Perfusion CT in colorectal cancer: comparison of perfusion parameters with tumor grade and microvessel density. *Korean J Radiol* 2012; **13**(Suppl. 1): S89–97. doi: 10.3348/kjr.2012.13.S1.S89
28. Khan S, Goh V, Tam E, Wellsted D, Halligan S. Perfusion CT assessment of the colon and rectum: feasibility of quantification of bowel wall perfusion and vascularization. *Eur J Radiol* 2012; **81**: 821–4. doi: 10.1016/j.ejrad.2011.02.033
29. Goh V, Halligan S, Taylor SA, Burling D, Bassett P, Bartram CI. Differentiation between diverticulitis and colorectal cancer: quantitative CT perfusion measurements versus morphologic criteria—initial experience. *Radiology* 2007; **242**: 456–62.
30. 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. doi: 10.1007/DCR.0b013e3181afbd79
31. Hayano K, Okazumi S, Shuto K, Matsubara H, Shimada H, Nabeya Y, et al. Perfusion CT can predict the response to chemoradiation therapy and survival in esophageal squamous cell carcinoma: initial clinical results. *Oncol Rep* 2007; **18**: 901–8.
32. 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
33. Koh TS, Ng QS, Thng CH, Kwek JW, Kozarski R, Goh V. Primary colorectal cancer:

use of kinetic modeling of dynamic contrast-enhanced CT data to predict clinical outcome. *Radiology* 2013; **267**: 145–54. doi: 10.1148/radiol.12120186

34. Padhani AR, Miles KA. Multiparametric imaging of tumor response to therapy. *Radiology* 2010; **256**: 348–64. doi: 10.1148/radiol.10091760
35. Goh V, Engledow A, Rodriguez-Justo M, Shastry M, Peck J, Blackman G, et al. The flow-metabolic phenotype of primary colorectal cancer: assessment by integrated 18F-FDG PET/perfusion CT with histopathologic correlation. *J Nucl Med* 2012; **53**: 687–92. doi: 10.2967/jnumed.111.098525
36. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005; **307**: 58–62. doi: 10.1126/science.1104819
37. Hinnen P, Eskens FA. Vascular disrupting agents in clinical development. *Br J Cancer* 2007; **96**: 1159–65. doi: 10.1038/sj.bjc.6603694
38. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science* 2003; **300**: 1155–9. doi: 10.1126/science.1082504
39. Chen G, Ma DQ, He W, Zhang BF, Zhao LQ. Computed tomography perfusion in evaluating the therapeutic effect of transarterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 5738–43.
40. Curvo-Semedo L, Portilha MA, Ruivo C, Borrego M, Leite JS, Caseiro-Alves F. Usefulness of perfusion CT to assess response to neoadjuvant combined chemoradiotherapy in patients with locally advanced rectal cancer. *Acad Radiol* 2012; **19**: 203–13. doi: 10.1016/j.acra.2011.10.019
41. 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
42. Sahani DV, Kalva SP, Hamberg LM, Hahn PF, Willett CG, Saini S, 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
43. Willett CG, Boucher Y, Di Tomaso E, Duda DG, Munn LL, Tong RT, 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
44. Davnall F, Yip CS, Ljungqvist G, Selmi M, Ng F, Sanghera B, et al. Assessment of tumor heterogeneity: an emerging imaging tool for clinical practice? *Insights Imaging* 2012; **3**: 573–89. doi: 10.1007/s13244-012-0196-6
45. Sanghera B, Banerjee D, Khan A, Simcock I, Stirling JJ, Glynne-Jones R, et al. Reproducibility of 2D and 3D fractal analysis techniques for the assessment of spatial heterogeneity of regional blood flow in rectal cancer. *Radiology* 2012; **263**: 865–73. doi: 10.1148/radiol.12111316
46. 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
47. Goh V, Halligan S, Hugill JA, Bartram CI. Quantitative assessment of tissue perfusion using MDCT: comparison of colorectal cancer and skeletal muscle measurement reproducibility. *AJR Am J Roentgenol* 2006; **187**: 164–9. doi: 10.2214/AJR.05.0050