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Can perfusion CT assessment of primary colorectal adenocarcinoma blood flow at staging predict for subsequent metastatic disease? A pilot study

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Abstract We aimed to determine whether perfusion CT measurements at colorectal cancer staging may predict for subsequent metastatic relapse. Fifty two prospective patients underwent perfusion CT at staging to estimate tumour blood flow, blood volume, mean transit time, and permeability surface area product. Patients considered metastasis free and suitable for surgery underwent curative resection subsequently. At final analysis, a median of 48.6 months post-surgery, patients were divided into those who remained disease free, and those with subsequent metastases. Vascular parameters for these two groups were compared using t-testing, and receiver operator curve analysis was performed to determine the sensitivity and specificity of these vascular parameters for predicting metastases. Thirty seven (71%) patients underwent curative surgery; data were available for 35: 26 (74%) remained disease free; 9 (26%) recurred (8 metastatic, 1 local). Tumour blood flow differed significantly between disease-free and metastatic patients (76.0 versus 45.7 ml/min/100 g tissue; $p=0.008$). With blood flow <64 ml/min/100 g tissue, sensitivity and specificity (95% CI) for development of metastases were 100% (60–100%) and 73% (53–87%), respectively. Our preliminary findings suggest that primary tumour blood flow might potentially be a useful predictor warranting further study.

Keywords Perfusion CT · Dynamic CT · Cancer, colon · Cancer, rectum

Introduction

Approximately 70% of cases of colorectal cancer are treated initially with curative intent but up to 30% of these patients will relapse subsequently, usually within 3 years [1–3]. Only a small percentage of this group will be suitable for further surgical resection, thus prognosis is generally poor once metastases are evident. Currently a median overall survival of 21 months might be achieved for patients with advanced disease treated with palliative chemotherapy [4]. In theory it would be beneficial to be able to predict for metastases in advance of their development, in order to improve patient selection for adjuvant treatment, yet in practice this is difficult to achieve reliably. Conventional staging (e.g. TNM or Dukes' staging) is used widely to decide on patients for further treatment. However it has limitations, and may not be sufficiently predictive with the trend towards chemotherapy that is individualised increasingly to the biology of the tumour in question [5].

With the licensing of anti-angiogenic drugs for colorectal cancer e.g. Bevacizumab, techniques that assess tumour angiogenesis on an individual basis have become more

relevant to clinical practice. Angiogenesis can be evaluated by immunohistological techniques such as microvessel density (MVD) or vascular endothelial growth factor (VEGF) expression but these are time-consuming, may be subject to substantial variability, and are not performed routinely in clinical practice. Imaging techniques are an attractive alternative given their already widespread use for tumour staging. Ultrasound (US) Doppler techniques have shown promise in the past, even though vessels <1 mm in diameter are not assessed, thus excluding direct examination of microvessel functionality. Colour Doppler ultrasound has been correlated positively with microvessel density, and shown to be predictive of metastatic disease [6, 7]. The Doppler US hepatic perfusion index has also been shown to be predictive of metastatic disease due to alterations in hepatic arterial blood flow with the presence of micro-metastases [8, 9]. However these techniques have not found widespread acceptance in this clinical setting as they are technically challenging to perform and are highly operator-dependent, potentially limiting generalisability [10].

To date few studies have assessed the prognostic potential of quantitative dynamic contrast-enhanced CT techniques, known as 'perfusion CT'. Perfusion CT has been performed increasingly in clinical practice to assess therapeutic response [11–14], as it may reflect tumour angiogenesis [15, 16], and can provide robust assessment of the functional tumour microvasculature [17–20]. To our knowledge no studies have been published of its potential at primary tumour staging for predicting subsequent metastatic relapse. We aimed to determine if quantitative perfusion CT vascular measurements at staging can predict for subsequent metastatic relapse in primary colorectal cancer.

Material and methods

Patients

Ethical approval was obtained for this prospective study. Adult patients attending for whole body CT staging of a proven primary colorectal adenocarcinoma were eligible, and consecutive consenting adults were recruited over a 33-month period between 2001 and 2004. Written informed consent was obtained from each patient; each received an information sheet detailing the purpose of the study and included information on the additional radiation exposure conferred by the entire perfusion CT study (estimated effective dose 12 mSv). Patients were excluded at CT if they had a previous reaction to iodinated CT contrast, if there was renal impairment, or if intravenous access with an 18-gauge venous cannula was not possible. Patients were also excluded if their tumour could not be identified on the initial planning acquisition. Thus 52 patients (mean age 69 years, range 33.5–90.4 years; 27 male, 25 female) were recruited into the study.

Computed tomography

No additional patient preparation over and above that of a standard abdominal-pelvic study was performed. An 18G venous cannula was sited in the antecubital fossa and 20 mg of the spasmolytic hyoscine *N*-butylbromide (Buscopan; Boehringer Ingelheim, Ingelheim am Rheim, Germany) administered intravenously to diminish bowel peristalsis. Abdominal wall movement was minimised by placing a restraining band around the abdomen. Patients were examined using a four-detector row CT system (Lightspeed Plus; GE Healthcare Technologies, Waukesha, WI, USA). An abdominal-pelvic study (120 kV, 180 mAs, slice collimation 10 mm, field of view 50 cm, matrix 512×512 mm) was performed initially, without intravenous contrast medium, in order to locate the known colorectal tumour. The images were then inspected by the supervising radiologist, the coordinates of the tumour noted, and used to plan the subsequent dynamic study.

A pump injector (Percupump Touchscreen; EZ-EM, Westbury, NY, USA) was used to inject 100 ml iopamidol 340 (Niopam 340; Bracco, Milan, Italy) intravenously at a rate of 5 ml/s. Four contiguous slices of 5-mm slice thickness were obtained through the midpoint of the tumour using a 'cine mode' acquisition (120 kV, 60 mA, field of view 50 cm, matrix 512×512 mm; temporal resolution 1 acquisition per second) thus encompassing a 2-cm tumour section. CT data acquisition commenced 5 s following the start of intravenous injection to allow acquisition of baseline unenhanced images and continued for a total duration of 65 s. The dynamic study was followed immediately by a diagnostic portal venous phase abdominal-pelvic study that started 75 s following commencement of intravenous injection. Chest CT was also performed to complete staging either on the same occasion or on a separate occasion.

Image analysis

All measurements were made by a radiologist experienced in perfusion CT analysis, using previously validated commercially available software based on modified distributed parameter analysis (Body Tumor, Perfusion 3.0; GE Healthcare Technologies, Waukesha, WI, USA; [16,19]). The 65-s dynamic study was loaded initially into the software. The first of the four available 5-mm tumour sections was selected. A processing threshold of 0 to 120 Hounsfield units was selected so that the subsequent analysis appropriately included both unenhanced and enhanced soft tissue. The arterial input was determined by placing a circular region of interest (ROI), 10 mm² in size, within the artery best visualised on the image, either the aorta, iliac or femoral arteries. An arterial enhancement–time curve for the 65-s acquisition was displayed automatically, along with parametric maps of blood flow,

blood volume, mean transit time, and permeability surface area product for all tissues lying within the selected processing threshold, each pixel representing a parameter value (Fig. 1).

A region of interest was then drawn freehand (using an electronic cursor and mouse) around the peripheral boundary of the tumour visible. Care was taken to exclude pericolonic fat and intraluminal gas, which was facilitated by viewing a cine-loop of the acquisition in order to gauge the degree of patient movement, and that of the tumour margins. A tumour enhancement–time curve and vascular parameter values (blood flow, blood volume, mean transit time, and permeability surface area product) were displayed by the software for the selected tumour region of interest. Mean values for each parameter were recorded for each patient. The entire process was repeated for each of
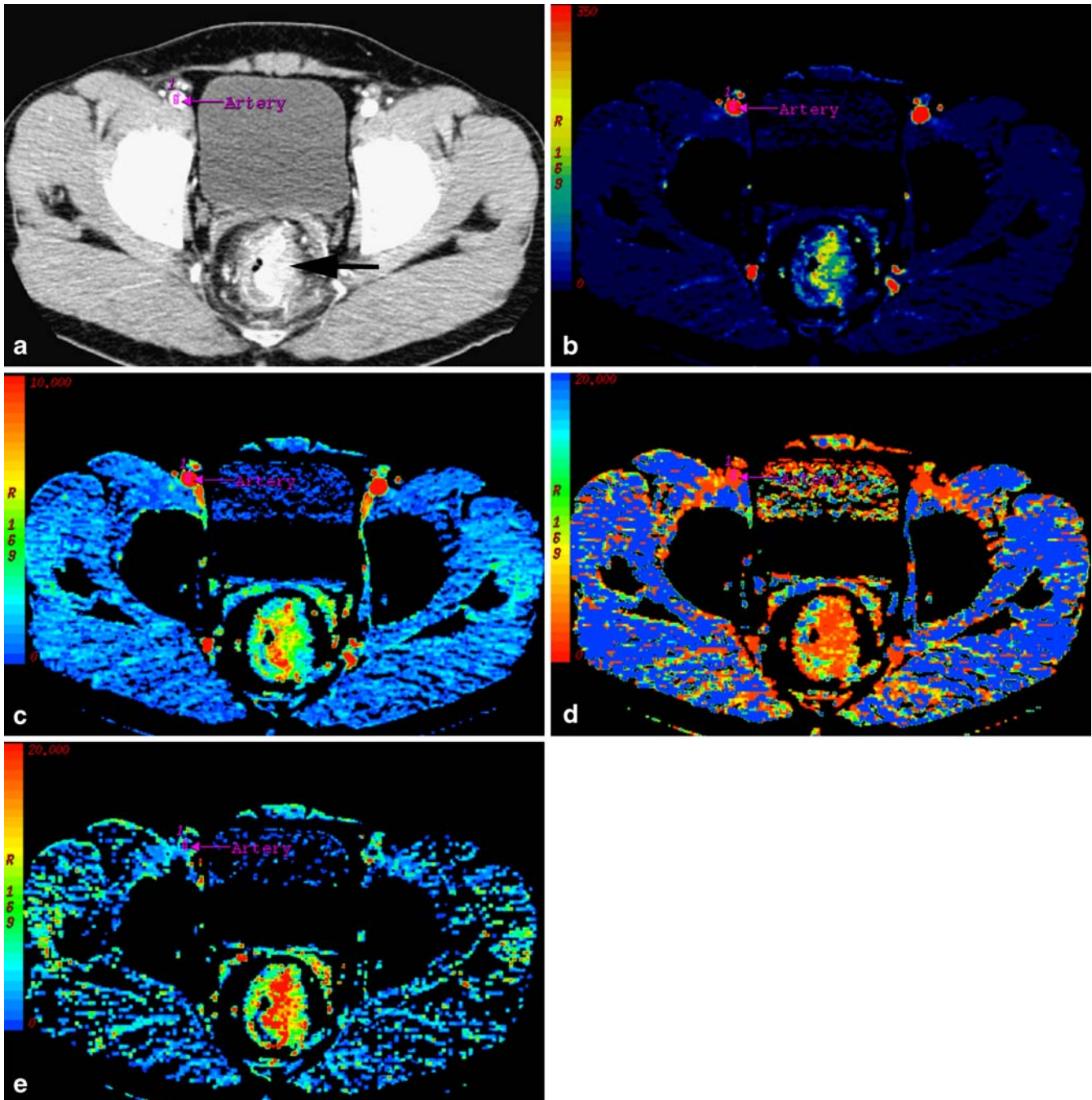
Fig. 1 Contrast-enhanced image and corresponding vascular parametric maps of a rectal tumour (**a**; *black arrow*) showing heterogeneous tumour blood flow (**b**), blood volume (**c**), mean transit time (**d**) and permeability surface area product (**e**) measurements

the remaining three adjacent 5-mm tumour sections until the entire 2-cm tumour section had been evaluated. An overall mean value for the four perfusion CT parameters for the entire 2-cm tumour section was then calculated for each patient.

Follow-up

Of the 52 patients recruited, nine did not proceed to surgery because of significant comorbidity (5 patients), or the burden of metastatic disease (4 patients). Thirty seven of the 43 patients who underwent surgery had a resection performed with curative intent, while six had a palliative resection only as there were concomitant metastases. Patients with metastases were offered standard chemotherapy consisting of 5-fluorouracil and oxaliplatin or irinotecan where performance status permitted this. Of the 37 cancers resected with curative intent, cancers were located in the caecum (7), ascending colon (2), descending colon (1), sigmoid colon (13), and rectum (14). Tumour stage is summarised in Table 1. All tumours were moderately differentiated adenocarcinomas apart from four poorly differentiated adenocarcinomas. Mean tumour length at pathological evaluation was 5.9 cm (range 3–11.5 cm). Four patients with rectal cancers threatening the resection margin received preoperative chemoradiation following CT. All patients with evidence of nodal metastases at surgery were offered standard adjuvant chemotherapy consisting of intravenous 5-fluorouracil or oral capecitabine as per usual practice in our institution. No patients underwent adjuvant radiotherapy.

Following surgery, patients were followed up as per usual clinical practice with clinical examination, blood tests including liver function and CEA levels, and colonoscopy. All patients also underwent imaging surveillance consisting of liver ultrasound at 6-monthly intervals. Whole body CT was performed also in the majority of patients at yearly intervals, or sooner if clinically indicated by any symptom suggesting relapse. Final disease status at final analysis was confirmed by case note and imaging review. Any patient lost to follow up was excluded at final analysis.

Statistical analysis

Statistical analysis was performed by a statistician using Stata (Version 7.0, Stata Corporation, College Station, Texas, USA). Mean and standard deviation (SD) of blood flow, blood volume, mean transit time, and permeability surface area product were determined for patients who remained free of disease, and patients with evidence of metastatic relapse at final analysis. Vascular parameters of each group were compared using independent samples t-testing. Additionally the mean values (SD) for all four vascular parameters were determined for the group of patients with evidence of metastases at presentation, and comparison made subsequently between these patients and patients who developed subsequent metastatic relapse.

Receiver operator curve (ROC) analysis was performed to identify the parameter threshold at which patients did or did not develop metastatic disease. The area under the ROC curve (AUC) was assessed to determine if this was significantly greater than 0.5 (= no discrimination). The sensitivity and specificity (and 95% confidence intervals) of each vascular parameter for the prediction of metastatic disease were established. The extent to which these parameters were independent predictors of metastatic status was determined by multivariate logistic regression using a stepwise approach. The relative performance of histological stage and vascular measures in predicting metastasis-free survival were examined in a contingency table. Two-tailed statistical significance was assigned at the 5% level.

Results

Patients were followed up for a median of 48.6 months; range 37.2–69.9 months. Of the 37 patients who underwent 'curative' surgery, no follow-up at our institution was available for two patients who were therefore excluded, leaving 35 patients at analysis. All of these 35 patients underwent US imaging surveillance; 26 of these 35 patients (74%) also underwent whole body CT surveillance; nine (26%) patients did not undergo CT due to (1) their exclusion at the discretion of their physician; (2) their inclusion into a clinical trial of surveillance methods following surgery.

At final analysis nine of these 35 patients (26%) had relapsed (8 of 35 patients (23%) had developed metastatic disease, 1 of 35 patients (3%) had developed local recurrence without evidence of distant metastasis), and 26 of 35 patients (74%) remained disease free. The stage and original tumour site of the nine patients with disease relapse are shown in Table 2. All relapsed tumours were

Table 1 TNM and Dukes' stage of patients who underwent curative surgery (n=35)

TMN stage	Dukes' stage	Number of patients
T2N0M0	A	7
T3N0M0	B	14
T2N1M0	C1	11
T3N1M0		
T4N1M0		
T2N2M0	C2	3
T3N2M0		
T4N2M0		

Table 2 Pathological TNM and Dukes' stage and original tumour site of patients with subsequent local and metastatic relapse (*n*=9)

Type of relapse	TMN stage	Dukes' stage	Number of patients	Original tumour site
Distant metastases (*n*=8)	T2N0M0	A	1	Sigmoid
	T3N0M0	B	2	Sigmoid
	T3N1M0 (1)	C1	3	Rectum
	T4N1M0 (2)			Sigmoid & caecum
	T3N2M0 (1)	C2	2	Rectum
	T4N2M0 (1)			Sigmoid
Local recurrence (*n*=1)	T4N1M0	C1	1	Sigmoid

moderately differentiated apart from two poorly differentiated adenocarcinomas. The distant metastatic sites in the eight patients were as follows: liver only (5); nonregional nodes (1); multiple sites including liver (2). These were confirmed by histology (5), or subsequent disease progression on follow-up imaging (3). The patient with local tumour recurrence had a positive resection margin at pathological assessment.

The mean values (standard deviation, SD) of blood flow, blood volume, mean transit time, and permeability surface area product for patients who remained disease free following surgery and for patients who developed metastases following surgery are summarised in Table 3. Patients who developed metastatic disease following curative surgery had a significantly lower baseline mean blood flow (t=2.81, df=32, p=0.008), longer mean transit time (t=2.51, df=32, p=0.02), and lower permeability surface area product (t=2.06, df=32, p=0.05) in comparison to patients who remained disease free (Fig. 2). Blood volume was not significantly different between patients who developed metastatic disease and patients who remained disease free following curative surgery (t=0.82, df=32, p=0.42, respectively; Fig. 2). There were no demographic biases between patients with and without disease relapse.

Mean blood flow, blood volume and transit time of patients with metastatic disease at presentation lay intermediate between measurements of patients with subsequent metastatic relapse and measurements of patients remaining disease free: mean (SD) of 63.8 (25.7) ml/min/100 g tissue; 5.67 (1.19) ml/100 g tissue; and 10.09 (2.91) s for blood flow, blood volume, and mean transit time, respectively. These measurements were not significantly different from those of patients with subsequent metastatic relapse: p>0.05. In comparison mean permeability surface area product of patients with metastatic disease at presentation was significantly elevated compared with patients who developed subsequent metastatic relapse (t=2.72, df=15, p=0.01).

At ROC analysis, the AUC of both blood flow and mean transit time were found to be significantly greater than 0.5 (p=0.006 and 0.007, respectively; Table 4; Fig. 3) in contrast to blood volume and permeability surface area product measurements (p=0.32 and 0.07, respectively) indicating these measurements were capable of producing a diagnostically useful threshold value. A threshold value of 64 ml/100 g tissue/min or lower for blood flow was found to have a sensitivity and specificity (95% confidence interval, CI) of 100% (60–100%) and 73% (53–87%), respectively, for metastases. A mean transit time value of 8.2 s or higher had a sensitivity and specificity (95% CI) of 100% (60–100%) and 56% (37–74%), respectively, for metastases. Logistic regression indicated that both blood flow and mean transit time were independent predictors of metastases (p=0.02 and 0.03, respectively). Pathological stage appeared to be worse than perfusion CT parameters at predicting subsequent metastatic disease (χ^2<2.4, p>0.05; Table 5)

Discussion

Adjuvant chemotherapy has a well-established role in colorectal cancer patients found to have lymph node

Table 3 Mean (standard deviation, SD) of perfusion CT measurements for patients remaining disease free (*n*=26) and patients with distant metastatic disease (*n*=8) subsequent to curative surgery

Perfusion CT measurement	Disease free following surgery (*n*=26)	Metastases following surgery (*n*=8)	*p* value
Blood flow (ml/min/100 g tissue)	76.0 (29.7)	45.7 (11.2)	0.008*
Blood volume (ml/100 g tissue)	5.89 (1.24)	5.48 (1.12)	0.42
Mean transit time (s)	9.14 (4.02)	13.03 (3.05)	0.02*
Permeability surface area product (ml/min/100 g tissue)	13.5 (3.19)	10.9 (2.91)	0.05*

*Significant at the 5% level. Two-tailed *p* values of independent *t*-testing are also shown
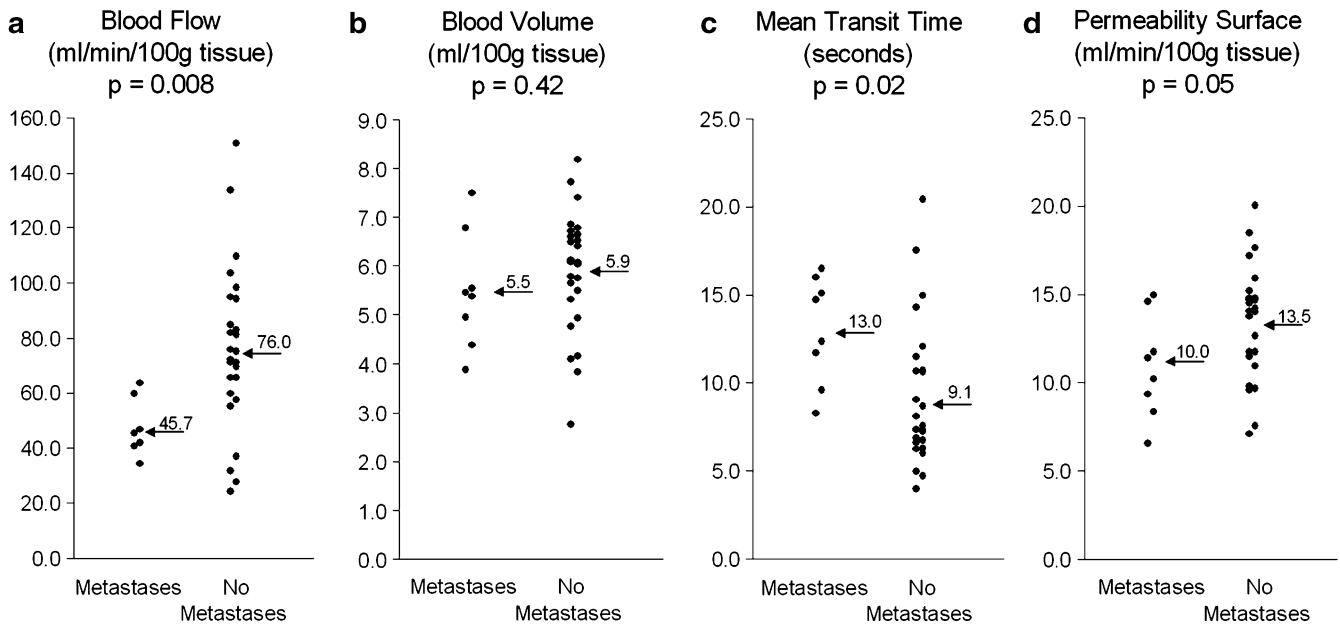
Fig. 2 Distribution of values for blood flow (**a**), blood volume (**b**), mean transit time (**c**) and permeability surface area product (**d**) for patients with metastases and for patients remaining disease free with involvement at surgery [21, 22]; mean and *p* values indicated. Mean blood flow, mean transit time, and permeability surface area product values were significantly different between the two groups

involvement at surgery [21, 22]; however, adjuvant treatment for stage II (T3N0M0, Dukes' B) tumours remains controversial. The majority of patients will remain disease free [23–25] but up to 40% of this group may potentially relapse. In our study eight (23%) patients who underwent surgery with the intention of curative resection subsequently relapsed with distant metastases. This proportion likely reflects the initial TNM staging: the majority of patients (80%) were stage T3N0M0 or greater. Three of these eight patients were stage II or less and would not have been offered adjuvant treatment as standard care.

Adjuvant chemotherapy would be the sensible course if these particular individuals could be identified in advance. Adjuvant drug treatment has also become increasingly tailored to the individual biological behaviour of the tumour [5], thus reliable markers are needed to improve individual patient selection for such adjuvant treatment. Although microvessel density and vascular endothelial growth factor

expression have prognostic value [26–31] and can be used to select patients for anti-angiogenic therapy [32], these are not routinely performed in practice and there are limitations to such analysis e.g. variation in suggested threshold values.

Since the introduction of helical CT, development of computing methods to display data as parametric maps [33, 34], and more recently introduction of commercial software, perfusion CT has been used increasingly to assess tumour vascularity [11–14]. The vascular parameters measured by CT reflect both the regional and microvasculature of the tumour. For example, blood flow reflects vascular supply to the tumour, mean transit time reflects transit through the tumour bed (and is thus influenced by vascular density, vascular morphology and functionality such as shunting, as well as interstitial pressure), blood volume reflects functional vascular volume, while permeability surface area product reflects the leakiness of the microvasculature, and thus may provide some insight into

Table 4 ROC analysis of CT measurements

Perfusion CT measurement	AUC	SE	*p* value	Asymptotic 95% CI for AUC	
				Lower bound	Upper bound
Blood flow	0.83	0.07	0.006*	0.69	0.97
Blood volume	0.72	0.12	0.32	0.38	0.85
Mean transit time	0.81	0.07	0.007*	0.67	0.96
Permeability surface area product	0.62	0.10	0.07	0.52	0.92

Area under the ROC curve (AUC) testing has been performed, with standard error (SE) calculated using the nonparametric assumption, and the null hypothesis that the true AUC is 0.5. The AUC for blood flow and transit time measurements is significantly greater than 0.5 indicating that these variables are capable of producing a diagnostically useful threshold value
*Significant at the 5% level
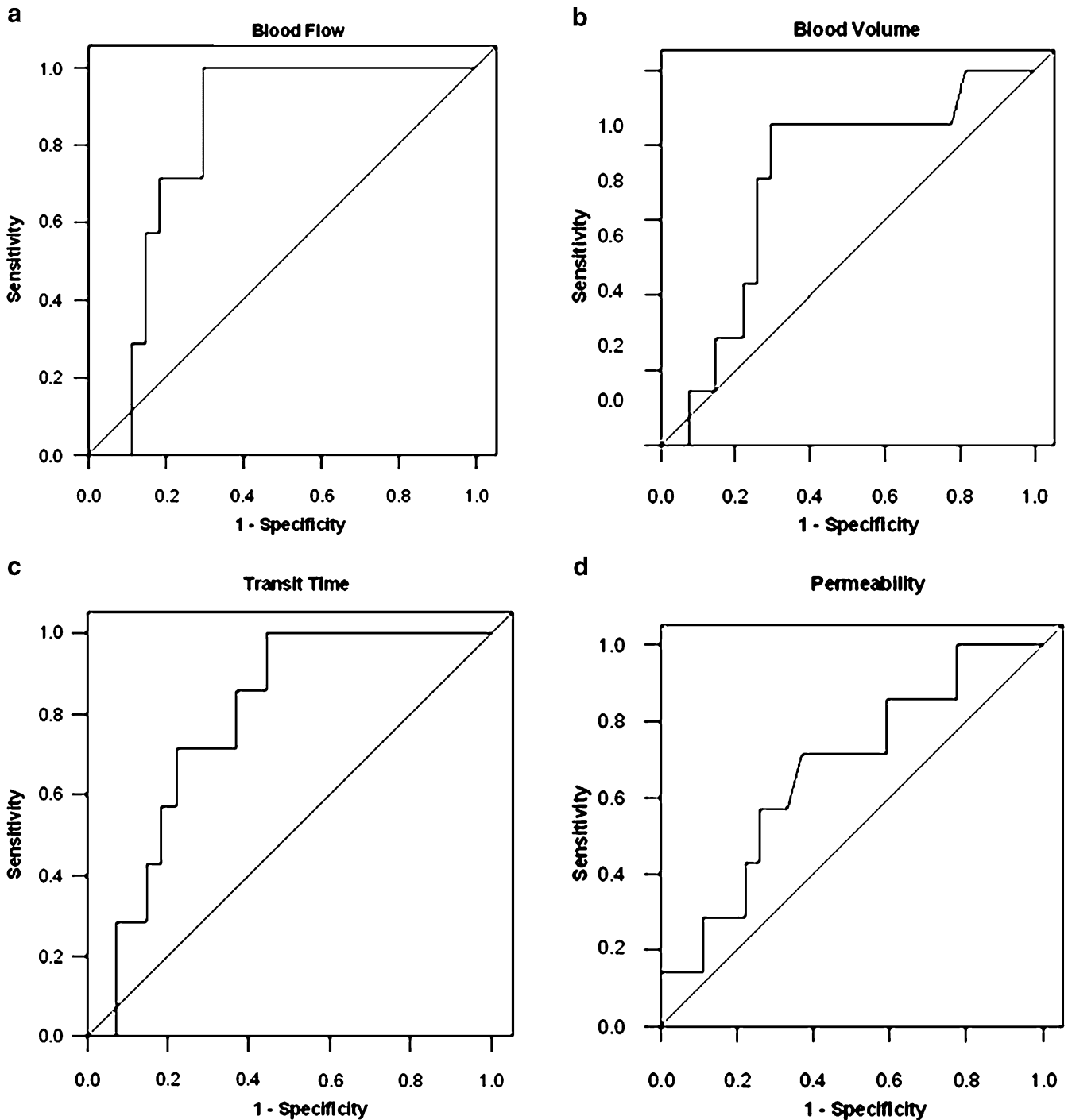
Fig. 3 Receiver operator curves (ROC) for blood flow (**a**), blood volume (**b**), mean transit time (**c**) and permeability surface area product (**d**). Blood flow and transit time curves are removed from the diagonal, with the area under the curve (AUC) significantly different from 0.5 indicating that these variables are capable of producing a useable cutoff value for predicting metastatic disease

angiogenesis. In our study the vascularity of colorectal tumours at initial staging of patients who subsequently developed metastases, despite potentially curative surgery, appeared different to that of patients with tumours that did not metastasize. These measurements could potentially provide evidential support for patients who would not normally be considered for adjuvant therapy following surgery from pathological stage alone or vice versa.

Measured tumour blood flow was significantly lower, mean transit time significantly higher, and permeability

Table 5 Contingency tables demonstrating the prognostic value of pathological stage, and perfusion CT measurements in predicting metastatic disease

	Metastatic	Disease free	Total
Dukes' stage			
A	1	6	7
B	2	12	14
C	5	8	13
Total	8	26	34
TNM nodal stage			
N0	3	17	20
N1 & N2	5	9	14
Total	8	26	34
Blood flow			
≥64 ml/min/100 g tissue	0	19	19
<64 ml/min/100 g tissue	8	7	15
Total	8	26	34
Mean transit time			
<8.2 s	0	15	15
≥8.2 s	8	11	19
Total	8	26	34

surface area product was lower in patients who ultimately developed metastatic disease. A previous study of cervical cancer has shown that tumour blood flow at perfusion CT correlates positively with tumour oxygenation: the higher the blood flow, the better the in vivo tumour oxygenation status [35]. Thus our findings appear to support a previously proposed hypothesis that tumour hypoxia plays an important part in the development of metastatic disease. Preclinical and clinical studies have indicated that hypoxia is an important selective variable in the clonal evolution of tumours, promoting oncogenic mutations, cell survival and more aggressive behaviour in tumours including cervical cancer, and soft tissue sarcoma [36–40]. Hypoxic or anoxic tumour areas arise as a result of an imbalance between supply and consumption of oxygen [41]. Both regions of acute and chronic hypoxia have been demonstrated in human colorectal tumours using extrinsic hypoxic markers such as iododeoxyuridine and pimonidazole [42]. Hypoxia also appears to be an important factor for the angiogenic phenotype, upregulating the angiogenic pathway via hypoxia inducible factor (HIF-1)-induced expression of VEGF [43]. In vitro studies of colon cancer cells have demonstrated that chronic intermittent exposure to low oxygen levels induces adaptive changes in apoptotic susceptibility and angiogenic profile such that hypoxia-conditioned cell lines grow more rapidly in vivo than their parental cell lines, and show enhanced vascular proliferation, when implanted as xenografts in immunodeficient mice [44].

Whether this enhanced vascular proliferation is reflected by an increase in measured permeability surface area product at CT will be dependent on whether these vessels are functional. It is notable that permeability surface area product measurements were significantly higher in patients who already had evidence of metastatic disease at presentation when compared with patients who subsequently developed metastatic disease giving some credence to the theory of enhanced angiogenesis in tumours that have metastasized.

To our knowledge no perfusion CT studies have been published on the prognostic potential of primary colorectal tumour measurements at staging. Although perfusion CT studies investigating colorectal cancer exist, these have concentrated on therapeutic assessment, for example, following bevacizumab [11] and radiotherapy [45, 46]. While these studies have attempted to predict treatment response from perfusion characteristics, they have not investigated the ability of perfusion CT parameters to predict subsequent metastasis. Patient numbers undergoing imaging before and after radiotherapy have been small, ranging from 9 to 19 [45, 46]. However, the changes in functional tumour vascularity that have been recorded following radiotherapy are inconsistent, with some patients showing a reduction [46] and others an increase in blood flow [45].

Limited studies have assessed prognostic potential in other tumour types. These have concentrated on the relationship between vascular parameters and eventual local relapse at the primary tumour site following radical radiotherapy, rather than the metastatic potential of the tumours in question. As an example, in head and neck cancers treated with curative intent, blood flow at perfusion CT was found to be an independent prognostic indicator, with a lower blood flow associated with a significantly higher local failure rate following radiotherapy [47]. The authors hypothesized that reduced blood flow reflected tumour hypoxia reducing the efficacy of radiotherapy.

Some ultrasound (US) studies have assessed the prognostic potential of in vivo functional vascular measurements of colorectal cancer: a transrectal colour Doppler US study of primary rectal cancer found no difference in peak systolic velocity and time-averaged maximal velocity in patients with and without metastases [6]. Other Doppler US studies have concentrated on liver vascularity and found alterations in Doppler flow to be predictive of both locally recurrent and metastatic disease [9]. In particular, the hepatic perfusion index (ratio of arterial to total liver perfusion) was found to be elevated in patients with metastases [8], and a sensitivity of 95% has been claimed for prediction of disease relapse [9]. However, no distinction was made between patients with metastatic disease and patients with locally recurrent disease: elevated hepatic perfusion index in patients with local recurrence was attributed to the presence of occult hepatic metastases but this was not proven [9]. Furthermore histological studies have shown that the vascularity of colorectal hepatic metastases differs from that of the primary tumour, with the former having lower vascular counts [48] and VEGF expression [49]. Other investigators have also found the hepatic perfusion index difficult to measure reliably [10]. To date although dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has been performed in primary colorectal cancer for example to assess the effects of radiotherapy [50–52], no DCE-MRI studies have assessed the prognostic potential of primary colorectal cancer vascular measurements in predicting metastatic disease.

Our study does have limitations. Firstly, while our results suggest that blood flow and mean transit time may potentially select patients for adjuvant treatment, this is based on thresholds derived from single centre data. These thresholds should be validated by further prospective studies that examine whether informed prognosis on an individual basis can be generalised outside of our original study setting [53]. The numbers involved, for example in the metastatic group, are also relatively small, but this reflects the demographics of colorectal cancer. Again our findings need to be confirmed via multicentre investigation. This presents its own problems since consensus and standardisation of data acquisition and analysis methods have yet to be established for perfusion CT.

Although these vascular measurements can be obtained relatively easily using current CT scanners and validated commercial software, and have been shown to be robust [17–19], it remains unclear which of the commonly used mathematical analysis methods (e.g. distributed parameter analysis, uni- or two-compartment analysis) is optimal for colorectal cancer, and whether data obtained by one type of analysis method is directly comparable to others [54]. The definition of the tumour ROI is subject to similar consideration, since the method by which the ROI is delineated clearly influences ultimate vascular parameter values. Our rationale for choosing a region of interest of the tumour outline rather than any other, e.g. an area of highest vascularity, was simply to ensure that results would be more reproducible, even if this averaged out parameter value.

Secondly, measurements were only obtained from part of the colorectal tumour and not the entire tumour, as tumours were at least 3 cm, due to the limited z-axis coverage at a single scan level of our four detector-row CT scanner. While the CT technique described here has not altered substantially since the introduction of helical CT scanners in the 1990s, the z-axis coverage has increased with newer generation scanners such that 4 cm is achievable with 64-MDCT. How such technical improvements impact on our findings is unknown at the time of writing; the increase in volume coverage potentially improves the spatial representation of the tumour vasculature in lung cancer [55, 56] but there are no data to suggest that this will benefit colorectal cancer assessment.

Thirdly, colorectal tumours were analysed as a single group rather than separately according to tumour site i.e. rectum versus other colonic sites. This was for pragmatic reasons since the small numbers at each site preclude meaningful statistical analysis. Fourthly, the length of follow-up did not reach 5 years in some patients. However all patients achieved 3 years, which has been shown to be a predictor for 5-year survival [2]. Criticisms could be levied at our use of US and CT instead of potentially more sensitive integrated 18-fluorodeoxyglucose PET/CT for surveillance of metastatic disease. However FDG PET/CT is not used for surveillance in our institution. Finally while our data appear supportive of the hypothesis that tumour hypoxia is an important factor in the development of metastatic disease, no immunohistological correlation with intrinsic or extrinsic markers of hypoxia was performed as this is not a routine procedure in our institution. This will require further investigation.

In conclusion, primary colorectal cancer blood flow at staging appears to be significantly lower in patients who

subsequently present with metastases despite 'curative' surgery. If substantiated by further larger scale studies this could potentially be used in clinical practice to provide evidential support for adjuvant treatment in patients who would not normally be offered such treatment based on pathological stage alone.

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