Perfusion CT to assess angiogenesis in colon cancer: technical limitations and practical challenges

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Objective: Perfusion CT may have the potential to quantify the degree of angiogenesis of solid tumours *in vivo*. This study aims to identify the practical and technical challenges inherent to the technique, and evaluate its feasibility in colorectal tumours.

Methods: 51 patients from 2 institutions prospectively underwent a single perfusion CT on 2 different multidetector scanners. The patients were advised to breath-hold as long as possible, followed by shallow breathing, and were given intravenous buscopan to reduce movement. Numerous steps were explored to identify the challenges.

Results: 43 patients successfully completed the perfusion CT as per protocol. Inability to detect the tumour ($n=3$), misplacement of dynamic sequence co-ordinates ($n=2$), failure of contrast injection ($n=2$) and displacement of tumour ($n=1$) were the reasons for failure. In 14 cases excessive respiratory motion displaced the tumour out of the scanning field along the temporal sequence, leading to erroneous data capture. In nine patients, minor displacements of the tumour were corrected by repositioning the region of interest (ROI) to its original position after reviewing each dynamic sequence slice. In 20 patients the tumour was stable, and data captured from the ROI were representative, and could have been analysed by commercially available Body Tumor Perfusion 3.0[®] software (GE Healthcare, Waukesha, WI). Hence all data were manually analysed by MATLAB[®] processing software (MathWorks, Cambridge, UK).

Conclusion: Perfusion CT in tumours susceptible to motion during acquisition makes accurate data capture challenging and requires meticulous attention to detail. Motion correction software is essential if perfusion CT is to be used routinely in colorectal cancer.

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Perfusion CT is increasingly being used to measure the vascular perfusion in tumours to gain insight into the functional nature of the tumour. The applications for this technique in solid tumours have ranged from distinguishing the presence of malignancy in suspicious lesions in the colon and the lungs [1–3] to grading the aggressiveness of lymphomas and brain tumours [4, 5]. One of the interesting aspects of perfusion CT is the potential to quantify the degree of angiogenesis in solid tumours by providing quantifiable vascular parameters [6–10]. This has gained significance owing to the development of safe neoadjuvant treatment strategies with antiangiogenesis drugs, which act by constricting tumour growth and preventing propagation of metastases [11]. There is then a possibility that perfusion CT would be able to screen patients demonstrating high angiogenic activity, who in turn would be susceptible to antiangiogenic drugs.

Despite the many promising roles perfusion CT may play in the future management of solid tumours, its

uptake in the clinical setting is restricted because of the complexity of the perfusion CT protocols [12]. The success of the scan is dependent on accurate capture of the contrast enhancement data from the area of interest for quantitative perfusion analysis. Also, the numerous steps involved in the perfusion CT protocol introduce areas of variability that affect the final analysis [13–15]. One of the main challenges in ensuring accurate data capture has been motion artefacts due to either respiration or peristalsis [16]. Perfusion CT in the lung has been affected by this, leading to loss of data in 6 of the 16 patients scanned in one study [17]. Thus perfusion studies in relatively fixed tumours such as pancreas, parotids or rectum have been easier to perform [4, 18–20]. However, in colonic tumours the potential for motion artefacts is increased owing to mobility offered by the mesentery and, being intraperitoneal, it is also susceptible to displacement during respiration.

In 2008 we undertook a prospective study to investigate the ability of perfusion CT to quantify the degree of angiogenesis in colorectal tumours. The perfusion parameters calculated were correlated with microvessel density count obtained on immunohistochemical staining of resected surgical specimen. In this paper we

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describe our experience of perfusion CT in colorectal tumours with an aim to identify the practical challenges inherent to the technique and to analyse the numerous technical steps in the methodology, in order to evaluate its practical feasibility in the treatment of colorectal cancer.

Methods and materials

Ethical approval for the principal prospective study was obtained from the Royal Marsden Hospital Ethics Committee and a written informed consent was obtained from each participant. The study was conducted at two hospitals with approval from respective research and ethics departments. It was powered to detect a correlation coefficient of >0.4 between perfusion CT and histopathology parameters with 80% power. We present our experience of 51 patients who participated in the study. Any patient with a large colorectal tumour identified on the diagnostic colonoscopy was eligible for the study. In case of rectal tumours, we excluded patients with very large rectal tumours as they would potentially receive neoadjuvant treatment in the future. We also excluded patients with contrast allergies and patients with severe comorbidities that could prevent any resectional surgery.

CT technique and practical limitations

A single perfusion CT scan was performed on each subject using either a Sensation 16 CT (Siemens Healthcare, Erlangen, Germany; $n=24$) or a LightSpeed VCT® (GE Healthcare, Waukesha, WI; $n=5$). The Sensation 16 offers a maximum of 16 slices per rotation with a total coverage of 24 mm. The LightSpeed VCT offers up to a maximum of 64 slices per rotation with a 40 mm coverage; however, for the purpose of this study only the central 20 mm of the detector was used to achieve consistency between participating sites. The scan protocol was designed on the basis of experience gained through carrying out CT perfusion scans on solid tumours at the Royal Marsden Hospital with due regard to the absorbed radiation dose within the irradiated volume [21].

Perfusion CT requires an initial baseline unenhanced scan for tumour localisation [22]. This is followed by a series of sequential images obtained over a period of time through a fixed site encompassing the tumour after injecting a bolus of intravenous iodinated contrast material. The resulting images provide a dynamic sequence of the amount of contrast medium passing through the tumour over a period of time. The enhancement data from these images can then be displayed as tissue density curves correlating with the amount of contrast present in the tissue at that point in time. These data are then analysed by commercially available perfusion software to calculate a range of vascular parameters that reflect the functional status of the vascular system. Pixel-by-pixel analysis of the data can also produce quantitative parametric images with high spatial resolution. In this study the conventional portovenous staging CT for the colorectal tumours was

performed between the dynamic sequence and the delayed phase of the perfusion CT.

Unenhanced phase for tumour localisation

The patient was instructed to inhale deeply to acquire the topogram required to set the limits for the subsequent scans. The co-ordinates for the unenhanced abdomino-pelvis (AP) locator scan were then set over a region of 20 cm so as to include the approximate region of the colonic tumour as per the colonic gas shadows seen on the topogram. The upper and lower limits for the locator scan were based on the colonoscopy report detailing the site of the tumour. The region to be scanned was restricted to an area of 20 cm, with a slice thickness of 10 mm, to reduce the radiation burden. Two gastrointestinal radiologists viewed the unenhanced images to detect the tumour and identify the slice where the tumour diameter was at its largest. This site was then used to set co-ordinates for the dynamic sequence of images so as to encapsulate a region of 20–24 mm over the tumour, depending on the scanner used.

Contrast injection and time delay for scanning

We injected 50 ml of intravenous contrast (Omnipaque™; Amersham Health, Amersham, UK) containing 300 mg of iodine per millilitre at a rate of 5 ml s^{-1} with the help of a pump injector (Percupump Touchscreen®; E-Z-Em, Westbury, NY) through an 18-gauge canula (green). We found this volume and rate of injection practical for the study as it corresponded to the volumes used for the staging aspect of the scan. A previous study comparing different rates of infusion of contrast reported that infusion rates between 4 and 10 ml s^{-1} provide effective results. Increasing the infusion rates beyond 10 ml s^{-1} has little additional benefit because of the buffering effect of the pulmonary circulation [12, 16]. The perfusion scanning in our study was started after a delay of 5 s after injection of the contrast agent and continued for 65 s to acquire information on the first pass of contrast and beyond. When performing compartmental analysis to obtain perfusion values, a bolus is needed as the validity of the method requires that peak arterial concentration occurs prior to the time of maximal increase in tissue enhancement [12]. The delay of 5 s allowed us to capture the unenhanced images of the tumour followed by the dynamic sequence visualising the passage of contrast agent through the tumour, while keeping the radiation dose under control.

Dynamic phase

The contrast material as it traverses through the tumour leaks from the intravascular space into the extravascular space to achieve equilibrium as time progresses. At this stage the rate at which the contrast material passes from intravascular to extravascular space and *vice versa* is equal in both directions. In colorectal tumours this is usually achieved by 2 min, but ultimately depends on the tumour transit time [12]. Thus we had a

dynamic sequence acquisition phase with four contiguous sections. The scanning parameters for each scanner are given in detail in Table 1. The staging CT in the porto-venous phase was performed at this point, immediately after the dynamic phase. This was followed by delayed phase, with images taken at a rate of one image every 20s for a total of 120s. The temporal sampling for the initial dynamic sequence was fixed at one image per second as decreasing the temporal sampling from one image per second to one image every 4s leads to overestimation of tumour blood flow and underestimation of blood transit in distributed parameter analysis [23]. As the changes in contrast enhancement are less at the later stages of the scan, images obtained at lower frequency are useful as they reduce the radiation burden without loss of data [12].

The volume of tumour that can be scanned temporally in the craniocaudal direction is determined by the width of the detector, which in our case was limited to 24 mm on our Siemens scanner. With the 64-slice GE scanner the region along the z-axis could have been increased to 40 mm, but in order to be consistent with the Siemens scanner the coverage was limited to 20 mm. The selected slice thickness of 5 or 6 mm, for the LightSpeed VCT or Sensation 16 CT, respectively, reduced the image noise to a reasonable level.

We tried to minimise the respiratory motion during the whole perfusion CT sequence by instructing patients to breath-hold for as long as possible, which typically lasted for 40–50s. After that period we advised them to take very shallow breaths to avoid sudden movements. We also placed an abdominal support across the abdomen to restrict abdominal motion during the shallow breaths. We avoided repeated breath-holds because of the difficulty in reproducing the same degree of inspiration each time. Colonic peristalsis was inhibited by intravenous injection of 20 mg of antiperistaltic agent Buscopan® (Boehringer Ingelheim, Ingelheim, Germany). During the delayed phase we again instructed them to take very shallow breaths to keep the tumour in the original position as much as possible.

Table 1. Site, size and phase at which the tumours experienced motion that caused problems in data capture during perfusion CT

Parameter	Successful scans (n=29)	Unsuccessful scans (n=14)
Site of tumour		
Ascending	8	3
Transverse	2	4
Descending	4	1
Sigmoid	7	4
Rectum	8	2
Size of tumour		
1–20 cm	0	3
21–30 cm	5	7
31–40 cm	11	3
>40 cm	13	1
Movement during respiratory motion	n=9 (minor motion)	n=14
Early phase	3	6
Delayed phase	4	4
Early + delayed phase	2	4

Image noise

As perfusion CT needs a large series of images at a certain anatomical location, the radiation burden is determined by the tube current–rotation time product and the time period of dynamic sequence. Similarly, a larger tube current results in less photon noise within each image, and hence greater certainty in the attenuation measurements at each point. In order to achieve a balance and keep the radiation exposure for the perfusion CT (excluding staging CT) within acceptable limits we used a tube current of 55–60 mA, which resulted in an estimated absorbed radiation dose of approximately 500 mGy within the irradiated volume of a standard-sized subject, and an effective dose of no more than 39 mSv for females and 52 mSv for males, using the ICRP 60 (International Commission on Radiological Protection 60) definition of effective dose [24]. This level of radiation dose permits repeat CT perfusion scans in quick succession without fear of causing radiation injuries to the skin or other organs in the irradiated volume. The porto-venous diagnostic study was conducted according to standard clinical practice and was in all likelihood dose modulated. The dose information provided applies only to the perfusion CT part of the examination, which was the only element of the scan that was additional to routine clinical practice. The doses quoted were submitted as part of the application for ethical approval (at the time the application was made only the additional examinations in the study required a dose assessment). As the CT perfusion scan was undertaken using manual exposure parameters, there is no benefit in quoting the total dose–length product because it will be the same for all patients.

The choice between temporal resolution and reduced image noise is determined by the analysis method. Compartmental analysis requires images with reduced noise to avoid overestimation of tissue enhancement rates and miscalculation of perfusion values, while deconvolution analysis allows for noise disturbance. We used a low tube current, which allowed us to scan for a long time, while limiting the radiation dose to the patient [12].

Image analysis

The images were then transferred to a commercial image processing workstation (Advantage Windows 4.0; GE Healthcare) and initially processed using commercially available software (Body Tumor Perfusion 3.0®; GE Healthcare) in order to capture the raw data. A processing threshold of 0–120 HU was chosen to optimise soft-tissue visualisation, and to exclude the air and bone areas.

Choosing the image sequence

The staging scan was viewed by two gastrointestinal radiologists experienced in multiplanar reconstruction to identify the axial image illustrating the most invasive site of the tumour (Figure 1). This image was then used as a template to identify the corresponding image on the dynamic sequence. Each tumour study had four series of dynamic sequences at a separation of 5–6 mm from each other (Figure 2). The four temporal slice sequences

Figure 1. The staging scan is viewed and the slice illustrating the most invasive area of the tumour is chosen. Two regions of interest are sited over the most invasive site and the luminal site by the two radiologists independently (1, invasive; 2, luminal).

performed over a 20- or 24-mm region of the tumour were reviewed and the slice sequence best corresponding to the image chosen on the staging scan was used to capture the temporal enhancement data. Only one slice sequence was used to gather data, as in many tumours the most invasive part of the tumour was not seen on all four slices along the z-axis. As colorectal tumours have vascular heterogeneity, including the data from all four sequences along the z-axis for analysis would theoretically improve reproducibility by providing a global assessment of tumour perfusion. However, this was not found to be true in a previous study, where the reproducibility was acceptable from both 5- and 20-mm tumour coverage [13]. Hence, each radiologist independently identified their corresponding 5-mm sequences to be analysed so that each tumour had data to be analysed from two separate radiologists.

Arterial region of interest

The arterial input function necessary for these calculations was obtained by placing a circular region of interest (ROI-1) over the nearest large artery (*e.g.* aorta or the iliac arteries) to provide us with an arterial tissue density curve (Figure 3). The area of this ROI-1 was kept at a minimum in order to fit into the arterial lumen (mean 25 mm²; range 19–37 mm²). The ROI-1 was placed in such a manner that it did not incorporate any surrounding soft tissue, which could lead to dilution of the data. All the temporal images in the sequence were examined to ensure that the ROI-1 was always contained in the arterial lumen. The data captured from ROI-1 measured the intra-arterial contrast concentration over the period of the perfusion scanning and were projected as a time attenuation curve with a steep curve (Figure 4).

Tumour region of interest

There are two ways that vascular parameters in a tumour can be analysed. One method is by drawing a

freehand ROI around the borders of the tumour, encompassing the whole of the visualised tumour. This has the advantage of being less operator-dependent and reduces interobserver variability. The drawback of this method is that it averages the data across the whole tumour and the intratumoral vascular heterogeneity cannot be assessed. Studies have shown that the angiogenic activity is maximal at the advancing edge of the tumour and by using this technique it is not possible to get data from that particular area [14]. Another important drawback of outlining the whole tumour is that it is difficult to adjust for motion artefact using whole-tumour ROI. If the tumour does move from its original position in any of the images in the temporal sequence, the ROI does not follow the tumour and may encompass the gas-filled bowel lumen or surrounding structures, thus capturing erroneous data. We overcame this problem by using either circular or oval ROIs of fixed areas, so that they represented the area we wanted to analyse (*i.e.* invasive edge and luminal aspect).

The tumour ROIs were drawn using the drawing tool in the software to a fixed area of about 95–100 mm². The area of the ROI was constant for each tumour in order to reduce the interobserver variability [14]. The shape of the ROIs was adjusted according to tumour orientation, however, to ensure that the boundaries of the ROIs did not lie outside the tumour. We found that setting the area at around 100 mm² enabled us to position the ROIs precisely over the region to be analysed and at the same time provided an adequate area to reduce interobserver variability.

We drew two equal-sized ROIs (the shape could be oval or circular depending on the area to be analysed in each tumour) over every tumour in order to check for intratumoral vascular heterogeneity (Figure 3). The most invasive site and the intraluminal component of the tumour were identified by scrutinising the staging scans in the multiplanar format and identifying the corresponding dynamic slice that resembled the identified region. ROI-2 corresponded to the most invasive area of the tumour, while ROI-3 was positioned over any luminal aspect of the tumour. Care was taken to ensure that the two ROIs did not overlap. We therefore had two ROIs from each radiologist, thus making four sets of data for each patient. As with the arterial ROI-1, we obtained tissue density curves for ROI-2 and ROI-3 (Figure 4).

Region of interest adjustment for motion

Even though we took utmost care to position the arterial and tumour ROI so that they did not move from the original placement, every tumour had a minor degree of movement caused by either respiration or patient shuffling on the table which caused the ROIs to displace from the original position in some of the images along the temporal sequence (Figure 5). Hence for the periods of time where the ROI was out of position the data captured were erroneous (Figure 6a). Unfortunately the version of the manufacturer's perfusion software we used would not allow us to change the position of the ROI to follow that of the tumour. As a result we could not individually move the ROI to its correct position in each of the images in the temporal sequence. As a result any analysis performed on

Figure 2. The four contiguous slices in the dynamic sequence at approximately 6-mm collimation scanned over approximately 2 cm of the tumour are illustrated in (a–d). In this case the image in (b) was chosen for analysis as it closely resembles the image in Figure 1, which demonstrates the most invasive aspect of the tumour situated in the ascending colon.

the Body Tumor Perfusion 3.0 software was invalid because of inaccurate data.

We overcame this hurdle by placing additional ROIs in the images where the ROI had been displaced to correspond to the original positions (Figure 5). We then had to examine each image to check the positioning of the ROI. The Body Perfusion 3.0 protocol provided us with the raw data of each ROI we had drawn. We then had to individually choose the correct values for ROI-1, ROI-2 and ROI-3 on each of the 71 (65 dynamic, 6 delayed) images along the temporal sequence. We then entered these values into bespoke perfusion software written in MATLAB® (MathWorks, Cambridge, UK) to

calculate the vascular parameters. We did this for all our cases to maintain homogeneity in our results (Figure 6). This process was extremely time consuming and labour intensive. In some cases it was not possible to capture data despite laborious and meticulous rearrangement of ROIs, as the motion was too violent (Figure 7).

Results

In total 51 patients were consented for perfusion CT from December 2008 to October 2009. The reasons for unsuccessful scans are detailed below.

Figure 3. Placement of three regions of interest (1, common iliac artery; 2, invasive edge; 3, luminal aspect). We ensured that the two regions of interest overlying the tumour did not overlap each other.

Unenhanced phase for tumour localisation

In some cases the colonoscopy failed to accurately site the tumour (especially if the tumour was not near an identifiable landmark such as the rectum/caecum). Thus, estimating the co-ordinates for the unenhanced scans was challenging in some cases. In three cases the tumour could not be seen on the unenhanced images, and the perfusion component of the CT had to be cancelled.

Also, as the collimation was set to about 20 mm, the detection of small tumours was not always possible. In two cases, the co-ordinates for the dynamic sequence were set over an area of normal bowel that was mistakenly presumed to be the tumour. Thus, the dynamic sequence was directed at the wrong site, making the data unsuitable for analysis. On review of the contrast-enhanced staging scan the tumour was found to be at an entirely different area.

There is a period after the AP locator scan during which a radiologist is required to identify the tumour and set the co-ordinates for the dynamic scan. This period usually lasts for about 2 min, but can be longer if the tumour is small and difficult to locate. During this period the patient is on the scanner waiting for the next phase of the scan. Any minute change of position by the patient at this stage would move the tumour out of position and the spatial co-ordinates fixed for the next phase would be inaccurate. One patient moved during this pause, causing the dynamic phase to be directed at the wrong site.

Contrast injection and time delay for scanning

The patients need a wide-bore cannula to facilitate the rapid injection of contrast. This requires a certain degree of technical expertise. In two of our patients the intravenous cannula leaked and contrast agent could not be injected at the desired rate. Thus the sequence of the scan was broken and the dynamic sequence could not be completed.

One patient experienced a flush after infusion of the contrast agent. This led to a pause in the injection of the contrast agent and loss of temporal data, again making the data unsuitable for analysis.

Dynamic phase

The total time required to complete the perfusion CT was around 5 min. Some patients found it difficult to lie perfectly still in the scanner for that amount of time. Even a small movement by the patient can displace the tumour from the 2 cm region through which the dynamic sequence and the late phase is focused. This leads to data loss due to the dynamic sequence being directed away from the region originally planned. Also, some patients are unable to hold their breath for 40–50 s of the rapid sequence. Excessive breathing during this phase can cause the tumour to move in the cranio-caudal direction out of the 2 cm region chosen for the dynamic sequence leading to loss of data. Respiratory motion in the anteroposterior plane would probably keep the tumour in the region of scanning, making data recovery possible. However, if the motion in this plane is excessive, even manual extraction of data becomes very difficult.

We managed to complete the scan in 43 patients. The median value for the contrast-to-noise ratio (CNR) for the ROI at the invasive site was 63.16 (range 42.51–108.82). The CNR for luminal sites had similar values. The CNR was calculated as peak Hounsfield units minus baseline Hounsfield units, divided by noise. After reviewing the images with the GE perfusion software (Body Tumor Perfusion 3.0), the images of 14 patients could not be analysed owing to excessive motion artefacts (Figure 7). In the 29 patients deemed suitable for analysis, the tumour was absolutely still in only 20 patients. Therefore the images from these 20 patients could be analysed with the commercially available Body Tumor Perfusion 3.0 software. In the remaining nine patients there was a slight displacement of images from the original site, which could not be adjusted using Body Tumor Perfusion 3.0 software (Table 2). In these cases we had to draw multiple ROIs for either the arterial curve or the tumour density curve in order to capture accurate data (Figure 5). We did this using the methodology described above, by manually extracting data for each slice in the sequence. This was then analysed using the perfusion software developed on MATLAB (Figure 6).

Discussion

Our experience using perfusion CT in colorectal cancer has shown that accurate data capture using the Body Tumor Perfusion 3.0 software was possible in about 39% ($n=20/51$) of patients. In this subset of patients we did not experience any motion artefacts. As a result the tumour was not displaced out of the originally placed ROIs (invasive and luminal) in any of the images along the temporal sequence. Thus, the data captured were representative of the respective regions and could be analysed by the tools available in the Body Tumor Perfusion 3.0 software.

Figure 4. Artery and tissue density curves over time (x-axis) plotted against the degree of enhancement of the tissue (y-axis) in Hounsfield units. The graph for the artery demonstrates a rapid increase in enhancement, which starts at around 6s (5s delay in starting the scan post injection) and rapidly ascends to its peak, followed by a plateau and a more gradual descent. The graphs for the invasive region of interest (a) and the luminal region of interest (b) illustrate the degree of enhancement in the tumour at their respective sites. The data points in both graphs are close to the graph lines, suggesting that motion was not an issue in this scan.

However, in 18% ($n=9/51$) of cases the data captured were inaccurate owing to tumour motion during either the dynamic sequence or the delayed phase, leading to displacement of the ROI from the original position and sometimes moving out of the tumour in some of the images along the temporal sequence. Thus, some of the data values along the time points on the sequence were no longer representative of the region originally marked. In order to capture the accurate values from such time points, we had to adjust for these errors by carefully viewing all the slices in the temporal sequence and drawing additional ROIs in the slices where the tumour

was displaced from the original position. The Body Tumor Perfusion 3.0 software did not have any function that allowed us to reposition the ROI selectively in those images where the tumour had moved out of position. We also could not choose the data set that represented the original marked area by omitting the time points with the wrong data. Thus, any further calculations performed using the tools in the software would not be representative of the original ROIs, as some time points along the enhancement curve had erroneous data.

In order to overcome this limitation of the software, we had to ensure that the data chosen for analysis were

Figure 5. Movement of tumour during the perfusion scan. The position of the tumour at 15 s (a) shifts slightly at 70 s (b) such that the regions of interest (ROIs) over the tumour and the artery are displaced from their original positions. In order to capture the information from the original areas we had to draw additional ROIs over the original position. We then collected the raw data of each ROI on each slice along the temporal sequence (75 data points) and chose the values from the ROI that overlies the original position.

representative of the originally marked ROIs in each of the time points along the time sequence. We achieved this by reviewing all the images along the temporal sequence in each of the four slides for every tumour. If on the images at certain time points the ROI did not overlie the original area owing to tumour displacement, an additional ROI was drawn over the desired area to acquire the representative data (Figure 5). Thus, each image along the temporal sequence was viewed, adjustments to ROIs were made and the data readings were recorded. These correct values for all the time points

along the tissue curve were then processed using a separate analysis tool (MATLAB; Figure 6). This was a very time-consuming and labour-intensive process, and made the whole exercise impractical for clinical use. We have to accept that in some patients with colonic tumours, accurate data capture will not be possible as they will not be able to control their breathing for the requisite period of the dynamic sequence, and the resultant tumour motion will be too severe to correct.

Previous literature on perfusion CT has raised issues regarding the difficulties associated with the technique

Figure 6. (a) Tissue density curve for a region of interest (ROI) at the invasive site when the data were not adjusted for tumour movement. In this case multiple ROIs were drawn to adjust for respiratory motion, and the accurate data were captured and analysed to provide us with a tissue density curve (b), which shows greatly reduced movement.

Figure 7. Extreme fluctuations in values for tumour enhancement in a tumour that has moved in the early dynamic phase, as well as the delayed phase. In such cases it was not possible to adjust the regions of interest to capture accurate data, and hence the studies had to be discarded.

[16]. However, none of studies evaluating perfusion CT in various tumours has discussed these challenges in the methodology or reported the effect of these on their results. Even though the commercially available analytical software packages may appear to simplify the analytical process, the technique is far from perfected and throws up numerous hurdles, as detailed in the methodology. This especially holds true in colonic tumours situated in the abdominal cavity, which are susceptible to movement during respiration. Previous studies using perfusion CT in colon cancer have shown that the technique could be applied to cancers either to quantify angiogenesis or to differentiate between benign and malignant lesions [1, 7]. They have not mentioned the number of patients in whom the technique was unsuccessful, however.

There has also been some concern regarding the mathematical modelling and the assumptions on which it is based. Sheiman and Sitek [25] highlighted the fact that the model assumes that: (a) the extravascular–extracellular space is a well-stirred compartment and the contrast agent concentration is changing slowly compared with the intravascular concentration; and (b) a

tumour ROI cannot reflect the neoplasms' heterogeneous vascular density. We tried to overcome this by using small ROIs at areas that would have similar tumour structure and uniformity. However, we still used the average value of the enhancement in the ROI, which may not represent the exact spatial heterogeneity, and this is a limitation of the software available to us.

If perfusion CT is to be clinically effective in assessing angiogenesis in the *in vivo* setting, the technique needs to be reproducible and standardised [26]. There are numerous steps involved in the technique, which need to be standardised to reduce variability in results. Until then, making any comparisons across research studies would be practically impossible. For example, different perfusion analytical software packages [e.g. Body Tumor Perfusion 3.0 or syngo Body Perfusion CT® (Siemens Healthcare)] use different mathematical models (e.g. distributed parameter analysis/Patlak analysis) to calculate the vascular indices of tissue permeability and blood volume, with varying results [27]. The technique also requires the operator to define ROIs in the identified tumours to measure the perfusion parameters. These regions can either be drawn outlining the whole of the

Table 2. Technical parameters and frequency of images for the two different scanners used in the study

Parameter	Siemens	GE Healthcare
Voltage/current	120 kV/60 mA	120 kV/55 mA
Mode	Axial mode	Cine mode
Slice collimation	6 mm	5 mm
Area of coverage	24 mm	20 mm
Rotation time	1 s	1 s
Scan field of view	50 cm	50 cm
Matrix	512 × 512 mm	512 × 512 mm
Temporal sampling in dynamic phase	1 per second	1 per second
Duration of dynamic phase	65 s	65 s
Number of images in dynamic phase	65 × 4 = 276	65 × 4 = 276
Temporal sampling in delayed phase	1 per 20 s	1 per 20 s
Duration of delayed phase	120 s	120 s
Number of images in delayed phase	6 × 4 = 24	6 × 4 = 24

Siemens, Sensation 16 CT, Siemens Healthcare, Erlangen, Germany; GE Healthcare, LightSpeed VCT®, GE Healthcare Technologies, Waukesha, WI.

Table 3. Challenges encountered in the perfusion CT of colonic cancers, with some practical tips to overcome them

Challenges	Possible solutions
Size of tumour	Tumours <3 cm in size should ideally be excluded as any movement in these small tumours leads to difficulty in accurate data capture
Co-ordinates for the unenhanced locator scan and interpretation of the unenhanced images	Accurate reporting by the endoscopist regarding the most likely positioning of the tumour in relation to the colon. An experienced gastrointestinal radiologist will need to be present during the scans to identify the tumour on the unenhanced scans and map the co-ordinates on the subsequent phases
Contrast agent injection	Appropriate technical expertise to insert large bore intravenous cannula to enable injection of the contrast at a rapid rate
Respiratory motion artefact	Patients need to be informed in detail about the steps of the procedure so that they are not anxious during the breaks between different components of the scan. They should be assessed on the ability to breath-hold and taught to maximise breath-hold with some practice prior to the scan. An abdominal band placed across would assist in reminding them to minimise respiratory motion and reduce movement. The patients should be kept well informed by constant conversation during the scan to maximise breath-hold and reduce anxiety
Area of coverage along z-axis	The development of CT scanners with wider detectors or applying one of the volumetric acquisition techniques would allow larger volumes of the tumour to be scanned, providing additional data for analysis
ROI misregistration	Analytical software chosen for analysis should have the ability to adjust for tumour displacement in the anteroposterior as well as the craniocaudal direction during the cine sequence, so that the data capture can be more accurate
ROI placement	The slice chosen for ROI placement and subsequent analysis should be matched with the slice on the staging CT that demonstrates areas of maximal tumour invasion. The arterial and tumour ROIs should be placed such that they overlie the tumour in all of the slices in the temporal sequence. Any displacement should be adjusted to enhance data capture. ROIs traced around the whole tumour have a higher probability of tumour averaging and displacement. Thus, the size of the ROIs should be limited to a smaller fixed area so that they can be placed over specific areas of interests

ROI, region of interest.

visible tumour on the slice or by identifying the most invasive part of the tumour where the maximal angiogenic activity is expected. This operator-dependent process has the potential to introduce intra- as well as interobserver variability in the analysis [26, 28]. Also, motion artefacts (especially in colonic tumours) can lead to erroneous data capture, reducing its reliability. The contrast agent had an iodine concentration of 300 mg ml^{-1} , which was routinely used clinically for staging CT in colorectal tumours in our hospitals. Iodine at higher concentrations of 340 mg (or, for that matter, 400 mg) has been found to be safe in abdominal CT; however, the dose of the iodine needs to be kept constant, which affects the volume of contrast agent that can be administered [29]. A higher concentration of iodine has been used in perfusion CT of the brain as it results in better opacification of the brain tissue in patients with acute stroke. The peak opacification was attained with a concentration of 400 mg ml^{-1} in spite of using smaller volumes of contrast agent. However, when different concentrations of contrast agents were used to assess the opacification of the abdominal aorta, the concentration of the contrast material did not influence the efficacy of contrast enhancement of the aorta [30].

The size of the tumour also plays an important part in the data capture process, and any movement in small tumours results in their displacement out of the scanning field, resulting in loss of valuable data. We observed that in tumours <3 cm in diameter the displacement caused by respiratory motion was very difficult to correct and accurate data could not be captured (Table 2).

In our study we noticed that the tumour started to enhance only after a period of around 7–8 s, and in tumours that were in the pelvis it took slightly longer (9–10 s) for the contrast to reach the tumour. This resulted in a slightly longer pre-enhancement phase than required. The radiation dose could be further reduced by increasing the time interval from 5 s to around 7–10 s, depending on the site of the tumour, without compromising the quality of the data.

There are volumetric perfusion techniques being developed to cover a z-axis region of up to 160 mm. 64-slice scanners now commercially available offer coverage of around 40 mm. Coverage of 80 and 160 mm is possible by the state-of-the-art scanners available from Philips Healthcare and Toshiba Medical Systems. Table toggling techniques (wherein the table physically toggles between two sites) can double the coverage, but at the cost of temporal sampling due to the time lost in physically moving the table. Another technique—that of helical shuttling, where the table moves back and forth during acquisition—can increase coverage to 140 mm, to overcome constraints placed by the size of the detectors. This could overcome the issue of the tumour escaping from the narrow band of 20–40 mm available in the current scanners.

Even though the tumour could be kept in the scanning field during the respiratory motion with a larger coverage area, attention would still have to be given to the duration of the dynamic sequence in order to keep the radiation exposure under control [31]. Even if we get greater coverage along the z-axis, we would still require software that allows us to track tumours if they are displaced from the original position. The software

should ideally allow us to reposition the ROI to follow the tumours all along the temporal sequence, not only in the same slice but also in the adjacent slices, if there is craniocaudal movement. This will ensure accurate data capture at all time points along the temporal sequence and ensure robust analysis. There are now CT perfusion software packages available from Toshiba, Siemens and Philips that allow us to correct for such motion artefacts by repositioning ROIs along the temporal sequence. These later versions also have the ability to reposition the ROI along the z-axis in the sequence to further improve data capture. This improvement in the data capture process would enable us to capture data from a volume of interest and may further improve observer variability by assessing the global tumour perfusion in colorectal tumours. However, we would still need to view all the images to ensure accurate positioning of the ROI and make adjustments manually wherever required.

The Experimental Cancer Medicine Network have acknowledged these issues and have published guidelines for the use of perfusion CT in the assessment of tumour vasculature to help standardise the technique [32]. For example, the recommended region of tumour scanned should be at least 4 cm in the craniocaudal direction with a gantry rotation time of 2 s or less. This would encompass a sufficient volume of tumour tissue to account for heterogeneity in angiogenic tumour vasculature. This region of scanning should ideally be achieved within an effective dose of about 20 mSv with satisfactory signal-to-noise characteristics. This can be effectively achieved by adjusting the various CT parameters (tube potential and tube current–rotation time product), reducing the frequency of temporal sampling and limiting the temporal acquisition to 45 s. Similarly, recommendations have also been provided for the contrast material concentration, dosage and injection rate, CT acquisition parameters, acquisition phases and sampling intervals. A minimum reporting data set has also been provided to standardise nomenclature and enable comparison between studies, if at all possible [32]. The numerous steps involved, along with the challenges encountered in perfusion CT of colorectal cancer and its possible solutions, are illustrated in Table 3.

Conclusion

Even though perfusion CT appears to be an attractive means of investigation to assess the functional nature of solid tumours, its applications in sites susceptible to movement during data acquisition remains challenging. If perfusion CT is to be used routinely in colorectal cancer it is essential to have motion correction software to correct for motion artefacts.

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