research article

Quantifying the changes in the tumour vascular micro-environment in spinal metastases treated with stereotactic body radiotherapy - a single arm prospective study

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Background. The primary objective was to quantify changes in vascular micro-environment in spinal metastases (SM) patients treated with stereotactic body radiotherapy (SBRT) with multi-parametric dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI). The secondary objective was to study plasma biomarkers related to endothelial apoptosis.

Patients and methods. Patients were imaged with DCE-MRI at baseline/1-week/12-weeks post-SBRT. Metrics including normalised time-dependent leakage (Ktrans), permeability surface product (PS), fractional plasma volume (Vp), extracellular volume (Ve) and perfusion (F) were estimated using distributed parameter model. Serum acid sphingomyelinase (ASM) and sphingosine-1-phosphate (S1P) were quantified using ELISA. Clinical outcomes including physician-scored and patient-reported toxicity were collected.

Results. Twelve patients (with varying primary histology) were recruited, of whom 10 underwent SBRT. Nine patients (with 10 lesions) completed all 3 imaging assessment timepoints. One patient died due to pneumonia (unrelated) before follow-up scans were performed. Median SBRT dose was 27 Gy (range: 24–27) over 3 fractions (range: 2–3). Median follow-up for alive patients was 42-months (range: 22.3–54.3), with local control rate of 90% and one grade 2 or higher toxicity (vertebral compression fracture). In general, we found an overall trend of reduction at 12-weeks in all parameters (Ktrans/PS/Vp/Ve/F). Ktrans and PS showed a reduction as early as 1-week. Ve/Vp/F exhibited a slight rise 1-week post-SBRT before reducing below the baseline value. There were no significant changes, post-SBRT, in plasma biomarkers (ASM/S1P).

Conclusions. Tumour vascular micro-environment (measured by various metrics) showed a general trend towards downregulation post-SBRT. It is likely that vascular-mediated cell killing contributes to excellent local control rates seen with SBRT. Future studies should evaluate the effect of SBRT on primary-specific spinal metastases (e.g., renal cell carcinoma).

Key words: spine metastases; stereotactic body radiotherapy; DCE-MRI; endothelial apoptosis

Introduction

Approximately 40% of patients with cancer, will develop spinal metastases (SM) in their cancer journey.[1] Symptomatic SM is usually treated with a combination of analgesia, radiotherapy and/or surgery.[2] Stereotactic body radiotherapy (SBRT) is an emerging treatment technique which is indicated for patients with oligo-metastatic disease, symptomatic SM from radio-resistant histological subtypes (e.g., renal cell carcinoma, colon adenocarcinoma), or in selected patients with expected long survival where durable tumour control becomes a priority.

SMs from some primaries such as renal cell carcinoma are known to be vascular, as evidenced by large intra-operative blood losses, in patients undergoing surgical resection (e.g., decompression, separation surgery, corpectomy). Data from our research group has estimated the mean intra-operative blood loss to be 870 + 720 ml, with an average blood transfusion requirement of 1.5 + 1.9 units.[3] This has prompted surgeons to utilise pre-operative angio-embolisation prior to resection, however, the effectiveness of this is highly variable.[4]

SBRT uses highly focused ablative radiotherapy, with the key feature being large fraction sizes (ranging 6–24 Gy), given over 1–5 sessions. This contrasts with palliative conventional external beam radiotherapy, with smaller fraction sizes (2.5–4 Gy) given over 5 – 15 sessions. Large fraction sizes have been mechanistic linked to a novel way of vascular-mediated cell killing, through the ceramide pathway.[5] The key players in the ceramide pathway include ASM and S1P.[6] The use of large fraction sizes has been shown in pre-clinical studies to significantly reduce the vascular volume.[7] However, this is poorly understood in the clinical setting.

The effect of SBRT on tumour vasculature has been explored by other research groups, particularly with the use of Dynamic Contrast enhanced MRI imaging (DCE-MRI).[8-11] DCE-MRI is an advanced non-invasive modality which provides functional information on vascular micro-environment and hemo-dynamics, where quantitative assessment of vascular parameters can be obtained through a pharmacokinetic model of contrast uptake to determine the signal intensity changes over time.[12] There are multiple models available to obtain quantitative information, and our group prefers the use of the distributed parameter (DP) model, over compartment models (e.g. Toft's model), especially in the post-treatment setting.[13] In contrast to Toft's model, the DP model does not assume well-mixed compartments (between the plasma and extracellular, extravascular spaces) and accounts for concentration changes with both time and distance along the capillary length. A schematic representation of the DP model can be seen in Figure 1. The variables of the DP model include time-dependent leakage (Ktrans), perfusion (F), permeability surface area product (PS), fractional plasma volume (Vp) and fractional extracellular volume (Ve). As such, the DP model offers the possibility of estimating flow and permeability separately, as well as estimating fractional vascular and interstitial volumes.

DCE parameters such as Vp and Ktrans were found to be reduced post-SBRT, and Vp has been suggested to be an early response biomarker for tumour control. Notably, most of these studies were not done in a prospective manner, where the time points of assessment were highly variable between patients. Moreover, only two parameters have been reported (Vp and Ktrans) in these studies, and this may not provide a comprehensive assessment of tumour vascular compartment.

We hypothesize that SBRT to SM will reduce the vascular micro-environment and perfusion parameters, in keeping with previous studies. Our aim is to prospectively quantify the effects of SBRT on SM using DCE-MRI metrics, and to describe changes in correlative plasma biomarkers of the ceramide pathway.

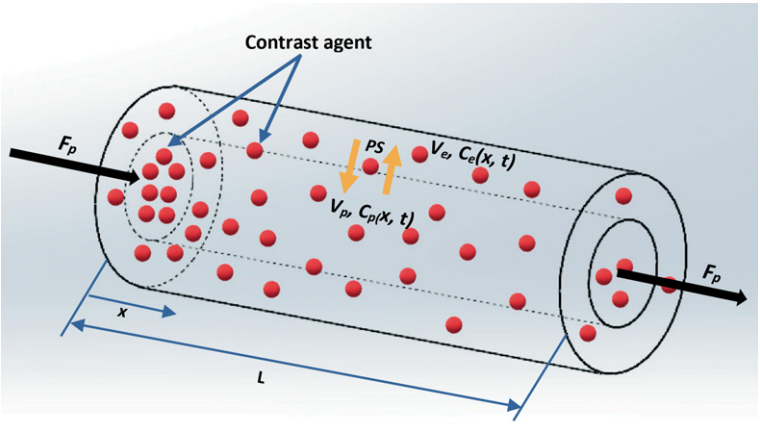

FIGURE 1. Schematic illustration of the DP model. Contrast agent (CA) concentration within the vessel decreases with position (x) along the vessel length (L), producing concentration gradients between the arterial (x = 0) and venous (x = L) capillary ends. During the CA passage, a portion of the CA molecules diffuses between the plasma and extracellular, extravascular space (EES) at a controlled permeability surface area product (PS) rate, so that the plasma, Cp(x, t), and EES, Ce(x, t), concentrations show both spatial and temporal dependence.

TABLE 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Age ≥ 21 years	1. Metastatic haematological and germ cell neoplasms
2. Proven metastatic disease	2. Inability to undergo MRI or receive gadolinium contrast
3. Life expectancy > 3 months	3. Prior radiotherapy to region of interest
4. Eastern Cooperative Oncology group (ECOG) 0–2	4. Recent surgery to affected spinal levels, or patients requiring immediate surgical intervention
5. ≤ 3 contiguous vertebral body segments, including para-spinal disease	5. Spinal instability score (SINS) > 12
6. Able to lie supine for ≥ 60 minutes	6. Symptomatic cord compression (Bilksy grade 2 or 3), or worsening neurological deficits

Patients and methods

This prospective study was approved by Institutional Review Board (NHG, 2016/1179), and registered on clinicaltrials.gov (NCT03072979). This was designed as a single-arm cohort study, conducted from May 2017 to December 2018, and patients were recruited from National University Hospital, Singapore. Written consent was obtained from all patients prior to any study related procedures. This study was conducted in accordance with the declaration of Helsinki.

Participants

Only adult patients who in whom SBRT for SM was clinically indicated were eligible for recruitment. Inclusion and exclusion criteria are described in Table 1. Patients who were undergoing systemic chemotherapy at recruitment, had to observe a one-week wash-out period between their last chemotherapy and first SBRT treatment.

SBRT planning and treatment details

SBRT for SM were carried out as per our department protocol. In brief, patients with lesions at T3 and above were immobilized using a rigid 5-point thermoplastic mask, and patients with lesions below T3 were immobilized using a rigid body bag (Elekta AB, Stockholm, Sweden). CT simulation imaging was performed and reconstructed at 2 mm for RT planning. A dedicated MRI in the region of interest was obtained within a few days of CT simulation. Axial T1 and T2 volumetric MRI sequences were co-registered with CT simulation images to define the target volumes and organs-at-risk. The gross tumour volume (GTV) included bony metastatic disease within the vertebral level, including any para-vertebral extension. Clinical target volume (CTV) included the adjacent compartment, as per contouring guidelines.[14] A 2-5 mm margin was applied in patients with para-vertebral extension to form the final CTV. The CTV was expanded by 2 mm isotropically to create the planning target volume (PTV). The spinal cord was defined using the axial T2 imaging, and a 2 mm planning organ at risk volume (PRV) margin was applied. For lesions at L2 and below, the thecal sac was contoured and a PRV margin was not given. The portion of the PTV that overlapped with the PRV Cord was carved out. Recommended SBRT doses are 24 – 27 Gy in 3 fractions, or 24 Gy in 2 fractions (delivered on alternate days). SBRT treatment was planned using Monaco (Elekta AB, Stockholm, Sweden) using volumetric modulated arc therapy (1 or 2 arcs). We aimed for a CTV coverage of D90/90. Priority was given to the avoidance of critical organs at risk (such as spinal cord PRV, thecal sac, brachial and sacral plexuses), and published dose limits were adhered to.[15] Image guided radiotherapy was performed on Elekta Infinity, with cone-beam CT (CBCT) guidance pre- and post-treatment. For long treatment sessions lasting more than 20 minutes, a mid-treatment CBCT was performed.

Clinical follow-up protocol

Patients were assessed clinically at time of CT simulation, 1-week and 3-months post RT as part of the study. Patients were followed up 3–6 months thereafter (as part of shared care between medical oncology and radiation oncology). Patients were recommended to have a MR imaging every 3–6 months as part of follow-up. Information collected at baseline and 3 months include: primary histology, baseline pain score (VAS), analgesia requirement and spinal instability score (SINS). Pain score was assessed at time of CT simulation (approximately 2 weeks prior to SBRT) and 12-week post-SBRT, using VAS 0-10.

Local tumour response was evaluated according to the MD Anderson criteria[16] at 3 months

TABLE 2. Patient characteristics

Patient ID	Age	Gender	Primary histology	Level of spinal metastases	Extraspinal disease site	Baseline analgesia requirement: opioid/ non-opioid/nil	Prior chemotherapy	Prior anti-VEGF therapy	Prior immunotherapy	ECOG	Pre-treatment VAS	SINS	Indication	SBRT	
														Dose (Gy)	Fractions
1	69	M	RCC	C1	Lung	Non-opioid	No	No	Yes	1	8	6	Radioresistant histology, pain control	24	3
2	60	M	RCC	S1	Nil	Nil	No	No	No	0	3	3	Oligometatasis	27	3
3	60	M	NSCLC-EGFR -	T5	Brain, bone. lung	Nil	Yes	No	No	1	5	6	Oligometastasis	24	3
4	62	F	NSCLC-EGFR -	C5	Brain, nodal, bone	Opioid	No	No	No	1	8	7	Oligometastasis, pain control	24	2
5	75	M	Prostate adenocarcinoma	T1	Bone	Nil	No	No	No	1	6	5	Oligometastasis	24	3
6	62	M	Colon adenocarcinoma	L1	LN, lung	Nil	Yes	No	No	1	3	9	Oligometastasis	27	3
7a	52	M	RCC	L1	Bone	Opioid	No	No	Yes	1	7	7	Radioresistant histology, pain control	27*	3*
7b	52	M	RCC	L2	Bone	Opioid	No	No	Yes	1	7	7	Radioresistant histology, pain control	27*	3*
8a	69	F	NSCLC-EGFR +	T1	Brain, bone. lung	Non-Opioid	No	No	Yes	1	3	5	Oligometastasis	24	3
8b	69	F	NSCLC-EGFR +	T10	Brain, bone. lung	Non-Opioid	No	No	Yes	1	3	4	Oligometastasis	27*	3*
8c	69	F	NSCLC-EGFR +	T12	Brain, bone. lung	Non-Opioid	No	No	Yes	1	3	6	Oligometastasis	27	3
9	51	F	Breast Invasive ductal carcinoma	T4	Nil	Nil	Yes	No	No	1	0	2	Oligometastasis	27	3
10	72	M	Prostate adenocarcinoma	L3	Nil	Nil	No	No	No	2	3	2	Oligometastasis	24	2

ECOG = Eastern Cooperative Oncology Group; F = female; LN = lymph nodes; M = male; Nil = nihil; NSCLC-EGFR - = non-small cell lung cancer without epidermal growth factor receptor mutation; NSCLC-EGFR + = non-small cell lung cancer with epidermal growth factor receptor mutation; RCC = renal cell carcinoma; SINS = spinal instability neoplastic score; VAS = visual analogue score

*Patient only completed 2 out of 3 fractions

(complete response (CR), partial response (PR), progressive disease (PD), stable disease (SD)) by a MSK radiologist (15 years' experience) who was blinded to the treatment (SS). Local recurrence was assessed based on available clinical imaging at the last follow-up. Data was censored at the time of last follow-up. Acute toxicity was assessed at 1 week post RT and late toxicity was assessed at 3 months and during further clinical follow-up, using the Common Terminology Criteria for Adverse Events version 5.0.

Magnetic resonance imaging protocol

All MRI examinations were performed using a 3-T MRI scanner (Siemens Biograph mMR) located at clinical imaging research centre (CIRC), National University of Singapore. MRI was performed at baseline (during time of CT simulation, approximately 2 weeks prior to the start of SBRT), 1-week after completing SBRT (median 8 days, range 6 to 13 days) and at 12 weeks post SBRT (median 92 days, range 82 to 100 days). MRI was obtained in the region of interest (target vertebral level, with 2 levels superior and inferior). The following conventional MRI sequences were acquired prior to contrast administration: sagittal T2, axial T2 (2 mm slice thickness), axial T2 TIRM (3 mm slice thickness), pre-contrast axial T1 VIBE with fat-saturation (2 mm slice thickness).

DCE-MRI was performed using a T1-weighted three-dimensional fast field-echo sequence in the axial plane. Before contrast injection, the pre-contrast T1-weighted fast field echo sequences were acquired at 4 flip angles (5, 10, 15 and 20 degrees) according to the same geometry to calculate the baseline T1 maps. DCE-MRI was performed after an intravenous bolus injection Dotarem (gadoterate meglumine), at 3 ml/s and dose of 0.2 ml/kg. This was done using an automatic injector and was

TABLE 3. Clinical outcomes

Patient ID	Post-SBRT VAS (Change from pre-SBRT baseline)	Acute toxicity	Late toxicity	Response assessment at 3 months (MD Anderson criteria)	Follow-up duration (months)	Local recurrence at last follow-up	Status of patient at last follow-up
1	0 (-8)	G1 esophagitis	Nil	PR	39	No	Dead
2	0 (-3)	Nil	Nil	PR	54	Yes	Alive
3	0 (-5)	Nil	G1 compression fracture	PR	50	No	Alive
4	0 (-8)	G1 esophagitis	Nil	SD	15	No	Dead
5	3 (-3)	G1 esophagitis	Nil	PR	22	No	Dead
6	0 (-3)	Nil	Nil	SD	13	No	Dead
7a	2 (-5)	Nil	G1 compression fracture	PR	42	No	Alive
7b	2 (-5)	Nil	Nil	PR	42	No	Alive
8a	-	Nil	N/A	-	N/A	N/A	Dead
8b	-	Nil	N/A	-	N/A	N/A	Dead
8c	-	Nil	N/A	-	N/A	N/A	Dead
9	0	Nil	G3 compression fracture	SD	37	No	Alive
10	0 (-3)	Nil	Nil	PR	22	No	Alive

Nil = nihil; PR = Partial response; SD = Stable disease; VAS = Visual analog scale

followed by a 15 ml saline flush. Seventy phases were acquired over 5 minutes with a temporal resolution of 4.3 s (flip angle 15 degrees), using parallel imaging. Delayed post-contrast axial T1 VIBE sequences with fat-saturation (2 mm slice thickness) were then acquired after DCE imaging.

The same sequences were performed at 1-week and 12-weeks post SBRT. The length of the aorta or major artery was included in the scan where possible, to minimize inflow artefacts.

Assessment of correlative plasma biomarkers

Blood was collected, in EDTA tube, at baseline (within 2 weeks prior to SBRT) and immediately after the last fraction of SBRT. Samples were centrifuged for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection, and plasma was removed to be stored at -80°C. Once plasma was collected from all patients, ASM (Human Acid Sphingomyelinase ELISA Kit, Elabscience) and S1P (Sphingosine 1-phosphase ELISA kit, Echelon Biosciences, UT, USA) was batch evaluated using a semi-quantitative method, using the Sandwich-ELISA method. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of S1P or ASM. The concentration of S1P and ASM were calculated by comparing the OD of the samples to the standard curve.

Analysis for DCE MRI metrics

Tumour regions of interest (ROI) were drawn in consensus by spine radiation oncologist (BV 12 years' experience) and two musculoskeletal radiologists (SS 15 years' experience, LCH 12 years' experience) on the non-contrast T1 and T2-weighted sequences. The soft tissue component of the metastatic deposits was delineated. For tumours without a predominant soft tissue component, the area showing T1 signal change was delineated.

Control ROI were drawn in the red marrow outside of the radiation volume. The same process was repeated for post-treatment imaging taking into account tumour regression.

All ROIs were drawn taking care to avoid venous structures, hemangiomas, disc spaces, cortical bone and spondylotic changes. ROIs drawn on anatomic reference images were simultaneously and automatically transferred to the corresponding location on the DCE parameter maps.

Raw blood perfusion data, obtained from DCE-MRI was processed and analysed using an in-house programme written on Matlab (MathWorks, Natick, MA, USA). Pre-processing steps included background spatial & temporal smoothing, noise removal. AIF was individually calculated for each acquisition of every patient, using a 3 x 3 (voxel averaged) window placed over an adjacent large vessel (aorta, or large calibre artery). Linear assumption between change in signal intensity and

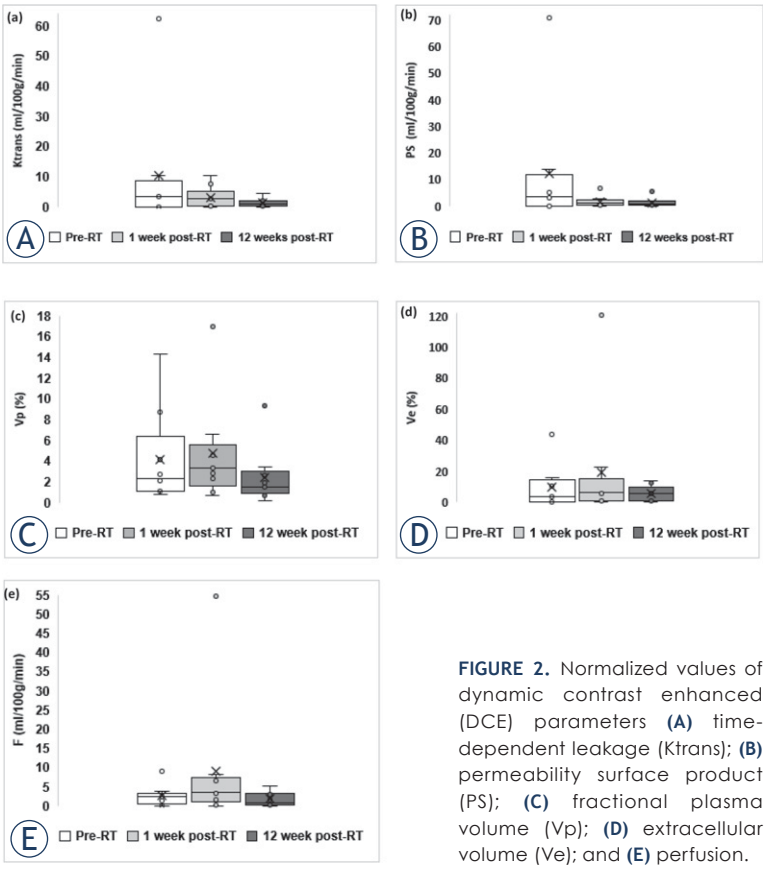

FIGURE 2. Normalized values of dynamic contrast enhanced (DCE) parameters **(A)** time-dependent leakage (Ktrans); **(B)** permeability surface product (PS); **(C)** fractional plasma volume (Vp); **(D)** extracellular volume (Ve); and **(E)** perfusion.

gadolinium concentration was made to convert the signal intensity curve to the concentration-time curve. The "Distributed Parameter" model was used for the calculation of quantitative perfusion parameters.

Both tumour and control ROIs were subjected to the same pre-processing, processing steps and analysis. Parameters were normalized using the readout from the control ROI on each scan. The median parameter value in the tumour, and control, ROIs were taken as the representative for each ROI. For quantitative parameters, including perfusion parameters and correlative plasma markers, mean, median, minimum, and maximum values were calculated. Quantitative perfusion parameters were also compared with the control cases.

Statistical analysis

We hypothesized a 30% reduction in DCE-parameters post SBRT. Working with a power of 80%, and alpha of 5%, we estimated a sample size of 8 - 10 patients. Differences between pre- and post-treatment values (of DCE-MRI metrics and plasma biomarkers) were compared using a paired T-test. Two-sided p-values of ≤ 0.05 were considered statistically significant. Statistical analysis performed using STATA v 14.

Results

Patient demographics and clinical presentation

Twelve participants were consented for the prospective study. Two patients withdrew consent before SBRT. Ten patients (with 13 lesions) were treated with SBRT. All 10 patients underwent blood sample collection. Nine of the ten patients underwent MRI assessment at 1 week and 3 months post SBRT. One patient died 1-month post SBRT secondary to pneumonia (unrelated to treatment). Overall, the median age was 62 years (range 51–75 years) and 70% were male. Majority of the patients (46%) had lesions in the thoracic spine, followed by 30% involving the lumbar spine. Kidney and lung were the most common primary tumour sites (30% each respectively). Median follow-up for alive patients was 42 months (range 22.3–54.3 months). Patient demographics and treatment related details are shown in Table 2. The most common indication for SBRT was oligometastasis (8/10). Median prescribed dose was 27 Gy (24–27 Gy) delivered in 3 fractions, with detailed breakdown of the dose and fractionation in Table 2. A majority of participants had prior systemic therapy (6/10). Three patients had prior immunotherapy, and none had prior anti-VEGF therapy.

DCE-MRI results

The normalised mean and median DCE-parameter values at baseline, 1 week and 12 weeks post SBRT are reported in Figure 2A-E. Representative images of Patient 7a are shown in Figure 3.

K trans (mL/100g/min): The normalised mean, median value at baseline was 12.08, 3.65 (range 0.03–62.49), at 1-week post SBRT, 3.10, 2.66 (range 0.01–10.55), and at 12-weeks post SBRT, 1.30, 0.92 (range 0.001–4.60) respectively. P-value comparing baseline & 1 week ($p = 0.14$), comparing baseline & 12 weeks ($p = 0.13$), and comparing 1 week & 12 weeks ($p = 0.08$) were obtained.

Permeability surface product PS (mL/100g/min): The normalised mean, median value at baseline was 13.89, 3.59 (range 0.02–70.83), at 1-week post SBRT, 1.66, 1.21 (range 0.01–6.56), and at 12-weeks post SBRT, 1.29, 0.66 (range 0.001–5.60) respectively. P-value comparing baseline and 1 week ($p = 0.12$),

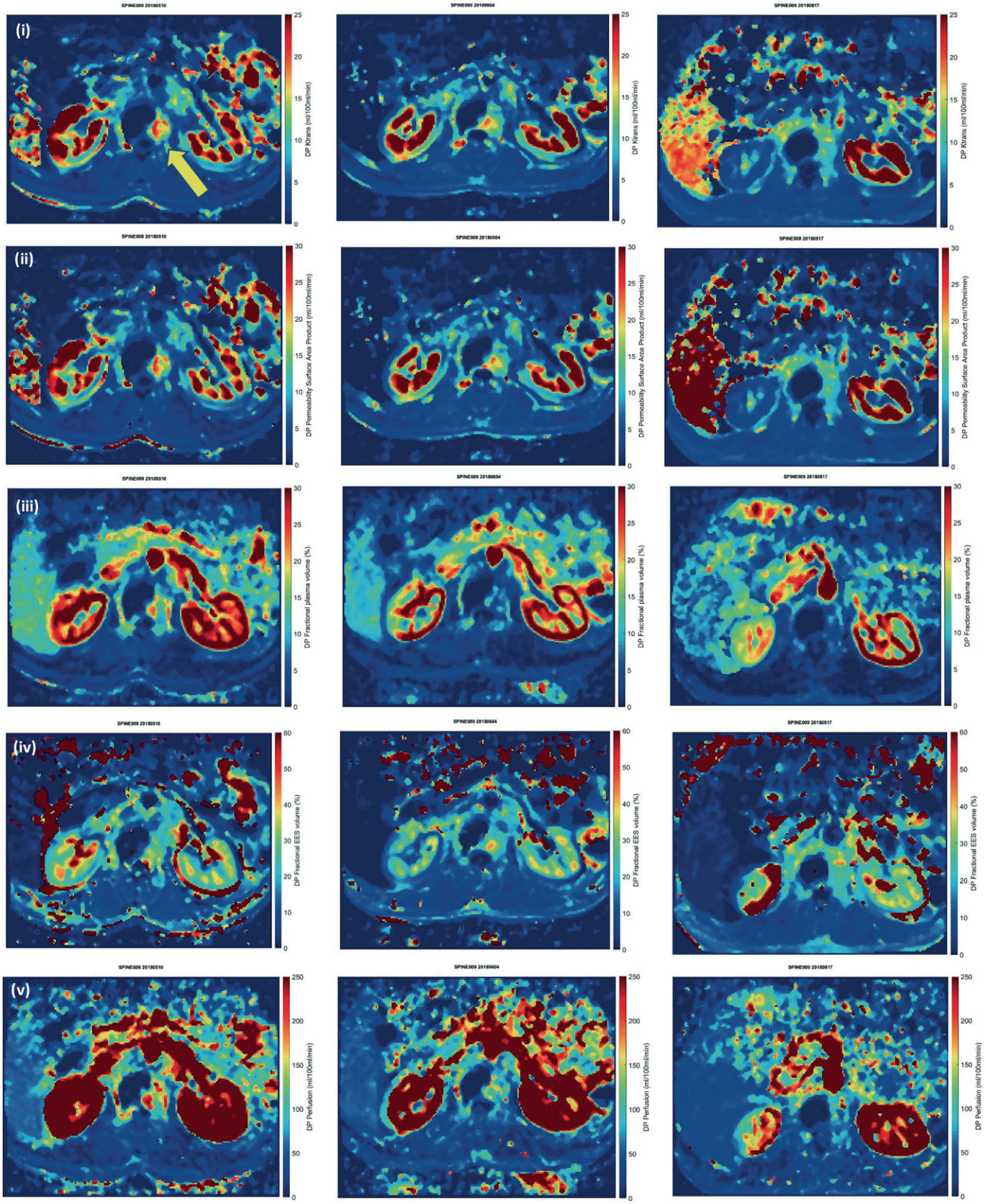

FIGURE 3. (A) Representative images for Patient 7a showing at L1 with reduction in dynamic contrast enhanced (DCE) parameters [i] time-dependent leakage (Ktrans); [ii] permeability surface product (PS); [iii] fractional plasma volume (Vp); [iv] extracellular volume (Ve); and [v] perfusion (F) across the 3 time points.

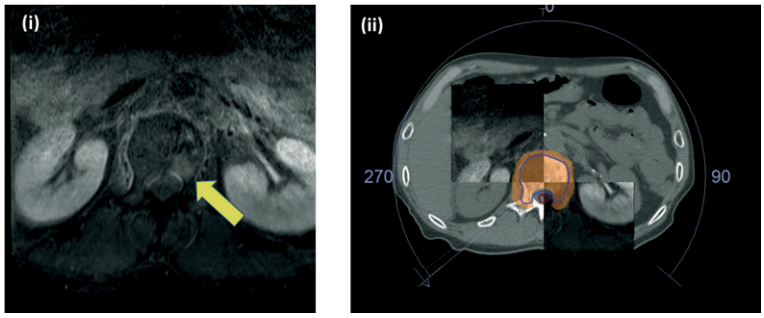

FIGURE 3. (B) [i] Metastatic deposit in L1 vertebral body (yellow arrow), as shown on T1 Axial MR (with gadolinium contrast); [ii] stereotactic body radiotherapy (SBRT) planning image (CT, MRI fused). SBRT 27 Gy in 3 fractions, delivered using volumetric modulated arc therapy. Clinical target volume (CTV) (blue outline), planning organ at risk volume (PRV)_cord (red outline), 95% isodose (orange colourwash).

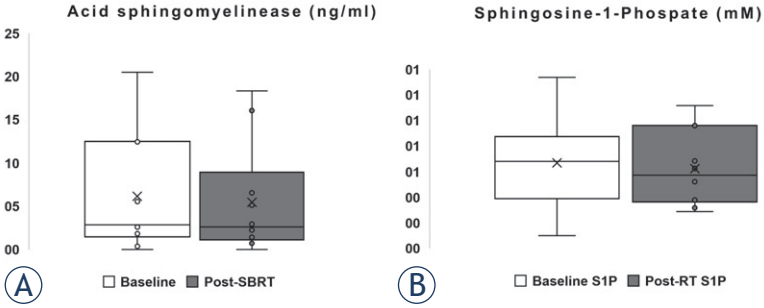

FIGURE 4. Correlative plasma markers **(A)** acid sphingomyelinase (ASM); and **(B)** sphingosine-1-phosphate (S1P).

comparing baseline and 12 weeks (p = 0.12), comparing 1 week and 12 weeks (p = 0.16).

Vp (%): The normalised mean, median value at baseline was 4.14, 2.21 (range 0.82–14.31), at 1-week post SBRT, 4.73, 3.27 (range 0.67–16.90) and at 12-weeks post SBRT, 2.40, 1.45 (range 0.20–9.30) respectively. P-value comparing baseline and 1 week (p = 0.41), comparing baseline and 12 weeks (p = 0.14), comparing 1 week and 12 weeks (p = 0.13).

Ve (%): The normalised mean, median value at baseline was 11.04, 4.03 (range 0.29–43.46), at 1-week post SBRT, 18.88, 5.92 (range 0.06–22.44) and at 12-weeks post SBRT, 6.01, 5.86 (range 0.65–13.51) respectively. P-value comparing baseline and 1 week (p = 0.11), comparing baseline and 12 weeks (p = 0.17), comparing 1 week and 12 weeks (p = 0.15).

Perfusion, F (mL/100g/min): The normalised mean, median value at baseline was 2.65, 2.37 (range 0.03–9.03), at 1-week post SBRT, 3.62, 3.54 (range 0.08–8.10) and at 12-weeks post SBRT, 1.77, 0.93 (range 0.07–5.23) respectively. P-value comparing baseline and 1 week (p = 0.25), comparing baseline and 12 weeks (p = 0.20), comparing 1 week and 12 weeks (p = 0.01).

Correlative plasma biomarker results

ASM levels did not show any significant change post SBRT (baseline mean 6.15 ng/ml *vs.* post SBRT mean 5.46 ng/ml, p = 0.71). Similarly, S1P levels did not show any significant change (baseline mean 0.67 μM *vs.* post-SBRT 0.63 μM, p = 0.52) (Figure 4)

Clinical outcomes

All patients tolerated SBRT well, with 3 patients experiencing mild acute toxicity (Grade 1 esophagitis). Three patients developed vertebral compression fractures (VCF) approximately 6 months post SBRT (23% on a per-lesion analysis). Of these, 2 were mild (Grade 1) and pre-existing, and did not require any intervention. One patient developed severe (Grade 3) symptomatic VCF and required stabilisation surgery. All patients had pain improvement 3 months post SBRT, with a median reduction of VAS of 5 (range 3 to 8). Response (MD Anderson criteria) at 3 months – with all patients, who were alive, demonstrating Partial Response (70%) or Stable disease (30%). There was one local recurrence (at 54 months) in the cohort, with data censored at last follow-up. These results are summarised in Table 3.

Discussion

To our knowledge, we are the first to prospectively quantify vascular changes, post SBRT (which uses large doses per fraction), in spine metastases. A controlled setting is important, as variabilities in baseline and follow-up assessment timepoints, and variability in scanner settings, may influence the interpretation of data.

We used a non-invasive method, using DCE-MRI, to quantify the vascular parameters at 1-week and 12-weeks post SBRT. We found an overall trend of reduction at 12 weeks in all the parameters (Ktrans, PS, Vp, Ve, F). Parameters such as Ktrans and PS showed a reduction as early as 1 week. Parameters (Ve, Vp, F) exhibited a slight rise 1-week post-SBRT before reducing below the baseline value – suggesting that there could be a short-term inflammatory response post RT. However, our findings can only be considered as hypothesis-generating, as majority of the P values were >0.05 (due to the small sample size). We did not find any

significant differences in DCE-MRI parameters between known vascular primaries (such as renal cell carcinoma versus other primary histologies)

Previous groups have utilised DCE-MRI to determine treatment response. Chu and colleagues, from Memorial Sloan Kettering Cancer Centre, performed DCE-MRI before and after radiotherapy for 15 patients.[8] They reported that changes in Vp were an early predictor of treatment response. In their study, both Vp and Ktrans were reduced post radiotherapy. However, the type of RT that the patients had undergone was not reported, and it remains unclear if patients were treated with conventionally fractionated RT or SBRT. The time point of assessment of the vascular parameters were also highly variable – baseline assessment ranged from 2 – 115 days, and post-treatment assessment ranged from 10 – 187 days. Another distinction from our study, is that they had utilised absolute parameter values, as there were limitations in drawing ROIs in normal marrow. To mitigate this variability, we normalised all our values, using an internal (patient level) control, by identifying non-irradiated marrow above or below the spine segment.

Another study from MSKCC, performed DCE-MRI, 1-hour post SBRT on 6 patients.[10] Similar to Chu *et al*., they only reported on Vp and Ktrans, which was derived using Toft's pharmacokinetic model.[8] The authors normalized the parameters using adjacent non-irradiated marrow. Authors observed a significant drop in Vp within 1-hour post SBRT, with a mean decrease of 65.2%. Ktrans was reduced as well, but to a lesser extent (pre-treatment mean 4.84, post-treatment mean 2.3). In contrast, we did not find a drastic drop in Vp at 1-week post RT. It remains unclear if these differences can be explained by the use of Toft's model (versus distributed parameter model). Although it is possible that these values are dynamic and may fluctuate with time, this is less likely to be the case as both Lis *et al.* and our study report a sustained and continuous reduction in Vp (baseline mean 4.14 *vs*. 2.4 at 12 weeks). We had planned to use the distributed parameter model a priori in view of the previous findings within our research group. The Toft's model was described in 1999, primarily based on intra-cranial conditions.[17] This is classified under the umbrella of "compartmental PK model", where the assumptions are that the compartments are homogenous at any given time, and the output of contrast agent is directly proportional to its concentration. The Toft's model has been more widely used due to its simplicity. In contrast, the distributed parameter model is classified as a "spatially distributed kinetic model". Unlike compartmental models, DP model accounts for both spatial and temporal variations in the administered contrast agent. These are believed to be closer to reality, taking into account underlying physiology. Interested readers can find more information about the various models in the referenced review article.[18]

These vascular changes seen post SBRT are suggested to be a result of endothelial cell apoptosis and mediated by the acid sphingomyelinase (ASMase) pathway. Radiation, particularly in doses above 8 Gy per fraction, induce translocation of the secretory ASMase from cytosol into glycosphingolipid contained in the plasma membrane, which in turn break downs sphingomyelin to ceramide.[5] Ceramide is a pro-apoptotic molecule. Endothelial cells have a high level of secretory ASMase, and therefore are susceptible to ceramide-mediated apoptosis with radiation. We had hypothesized for plasma ASM to increase, and S1P to decrease post SBRT. However, we did not find any significant changes. One possibility is that changes seen within the cellular micro-environment may not be reliably assessed in the serum, due to a lack of sensitive assays. To our knowledge, we are the first to report on the plasma ASM and S1P levels in patients undergoing SBRT. A previous study by Dubois *et al*., reported that the ceramide levels (and their sub-species), measured by LC–ESI-MS/MS, were elevated at 3- and 10-days post SBRT in responders.[19]

In our cohort, SBRT was well tolerated and provided a local control rate of 90%. Patients with baseline symptomatic SM, had a good pain response at 3 months (assessed using VAS). Patients are typically followed up every 3 – 6 months with MRI imaging for surveillance. The effectiveness of SBRT is in keeping with multiple other cohorts and randomized controlled trials.

The implications of our study support the preceding data that tumour vasculature is predominantly reduced with SBRT. We had initially conceptualised the idea of pre-operative spine SBRT, in order to reduce intra-operative blood loss. This can potentially be performed in lieu of embolization, and in addition this may reduce intra-operative tumour dissemination. However, from our study, not all vascular parameters were reduced at 1 week, and therefore it remains unclear if this will translate clinically. The concept of pre-operative SBRT is already being investigated for brain metastases in a randomized Phase III trial (NCT03741673).

The main strengths of our trial are as follows – prospective design, where assessment time points were pre-defined. In addition, the same scanner, and settings were used for repeated measurements, thereby reducing variability. SBRT was carried out following our department protocol by a specialized team (radiation oncologist, dosimetrist, radiation therapist), where the doses and prescription practices are uniform. In addition, we determined the arterial input function, on an individual basis, instead of using general population estimates. Lastly, we used the distributed parameter model to quantify the normalised vascular estimates. This is expected to be closer to reality compared to other similar studies.

We fully acknowledge our study's limitations. Firstly, we have a small sample size of patients with varying primary cancers – and thus our results may be under-powered and subject to inter-histology variations. Secondly, the serum assay that we had utilised to quantify ASM/S1P may not have been sensitive enough to pick up small changes. Thirdly, as only one patient developed local recurrence at 54 months, we could not investigate the difference in DCE-MRI parameters between responders and non-responders.

In conclusion, our study has shown that vascular changes post-SBRT can be quantified by DCE-MRI using the distributed parameter model. The tumour vascular micro-environment (measured by various metrics) shows a general trend towards downregulation post SBRT, and it is hypothesised that this is one of the reasons for improved local control.

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