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FULL PAPER

Ultrasmall superparamagnetic iron oxide nanoparticle uptake as noninvasive marker of aortic wall inflammation on MRI: proof of concept study

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Objective: Radiation therapy for cancer can lead to atherosclerosis by inducing inflammatory changes in the vascular wall. It is difficult to quantitatively measure inflammation on CT and MRI studies. The purpose of this study was to assess the use of ferumoxytol, an ultrasmall superparamagnetic iron oxide nanoparticle, as a noninvasive marker of vessel wall inflammation secondary to radiation therapy in pancreatic cancer patients in comparison with healthy volunteers.

Methods: MRI of upper abdomen (T_1 , T_2 , multi echo T_2^* weighted imaging) was performed on 3 T magnet before and 48 h after intravenous administration of ferumoxytol in pancreatic cancer patients who underwent radiation therapy ($n = 8$) and in healthy volunteers ($n = 8$). R_2^* value was obtained by drawing regions of interest outlining the aortic wall directly on the T_2^* medic image and subsequently transposed to the R_2^* image using

Amira software (v. 5.3.2, FEI, Bordeaux, France). The change in R_2^* values was analyzed by student's t -test.

Results: The average change in R_2^* value of the pancreatic cancer patients was determined to be 216.1 ms^{-1} . The average change R_2^* value of the control patients was determined to be 54.6 ms^{-1} . Thus, pancreatic cancer patients following radiation therapy had a greater uptake of ferumoxytol ($p = 0.0082$) in their aortic wall as compared to healthy controls.

Conclusion: This proof of concept study suggests that greater uptake of ferumoxytol in the aortic wall in cancer patients without visible atherosclerosis may be the expression of increased inflammation.

Advances in knowledge: Ultrasmall superparamagnetic iron oxide enhanced MRI can offer an imaging biomarker for quantitative estimation of aortic inflammation preceding atherosclerosis.

INTRODUCTION

Radiation therapy for cancer through increased inflammation may promote the accelerated development of atherosclerosis.¹ Specifically, vasculature exposed to significant amounts of radiation, which incites cell death and oxidative damages, alters the homeostasis between the proinflammatory M1-macrophages and anti-inflammatory M2-macrophages, triggering increased inflammation. It is universally accepted that increased inflammation accelerates atherosclerosis and in turn this leads to an increased risk to suffer from cardiovascular events.^{2,3} Current MRI techniques are very limited to quantitatively measure inflammation, mostly driven by lack of suitable contrast agents. Vascular inflammation

can be depicted by fludeoxyglucose-positron emission tomography (FDG-PET)⁴ but the technology is limited in its spatial resolution and the limited specificity of FDG as it is taken up not only by macrophages but also by other metabolically active cells.⁵ However, due to high phagocytic activity, macrophages and monocytes are ideal targets for ultrasmall paramagnetic iron oxide (USPIO).⁶ Ferumoxytol, a USPIO, is a negative contrast agent on T_2^*/R_2^* weighted imaging with selective uptake by macrophages apart from its expanding vascular applications particularly in pediatrics and patients with renal failure.^{7,8} The purpose of this proof of concept study was to assess the uptake of ferumoxytol in pancreatic cancer patients as compared to healthy volunteers.

METHODS AND MATERIALS

Patient and control population

This institutional review board approved single center, Health Insurance Portability, and Accountability Act compliant study drew the pancreatic cancer patients who underwent preoperative neoadjuvant therapy (with 25 Gy of proton beam radiation and concurrent capecitabine) ($n = 8$) and healthy volunteer controls ($n = 8$) from two different larger prospective studies. The pancreatic study⁹ was aimed at detection of lymph node metastasis following administration of USPIO ferumoxytol. Healthy volunteer controls were drawn from the study aimed at detection of pancreatic inflammation in Type 1 diabetes¹⁰ (NCT01521520). Abdominal CT scans of pancreatic cancer patients were screened to exclude any focal atherosclerotic plaques in the abdominal aorta to limit the evaluation of R_2^* changes to the aortic wall itself. The duration between completion of preoperative neoadjuvant therapy and USPIO enhanced scan was also recorded.

Contrast agent

The patients and controls both received 6 mg kg⁻¹ of ferumoxytol (AMAG Pharmaceuticals, Lexington, MA) with a maximum dose of 510 mg (17 ml). Ferumoxytol is a USPIO nanoparticle with average particle size of 30 nm and molecular weight of 750 kDa. The agent is a semisynthetic carbohydrate polyglucose sorbitol carboxymethylether coated non-stoichiometric magnetic core, available as a sterile, neutral PH containing 30 mg of elemental iron per ml. Ferumoxytol is currently approved for treatment of iron deficiency anemia in chronic renal disease. The dose dependent $t_{1/2}$ is approximately 14.5 h at a dose of 4 mg kg⁻¹. Ferumoxytol was administered intravenously by hand injection at 1 ml per second rate followed by a saline flush of 10 ml. Following the study visit completion, the patients and controls were evaluated for adverse events.

MRI and image analysis

MRI of the upper abdomen was performed at 3 T system (Magnetom Trio, A Tim System; Siemens, Erlangen, Germany) using an 8-channel phased array body coil, before, and 48 h after the intravenous administration of ferumoxytol. The 48 h time point post-ferumoxytol administration was chosen to allow its accumulation in the tissues. Pancreatic cancer patients were imaged within 2 weeks of undergoing neoadjuvant therapy. Imaging duration ranged from 30 to 45 min. T_1 volumetric interpolated breathhold examination (T1 VIBE) (echo time = 0.9 ms, repetition time = 2.9 ms, Voxel size $1.4 \times 1.4 \times 3.5$ mm), a T_2^* map (repetition time = 534 ms, echo time = 4.8, 9.5, 14.3, 19.1, 23.8, and 28.6 ms, voxel size = $1.86 \times 1.86 \times 4$ mm) and a multiecho data image combination (MEDIC) (voxel size = $1.86 \times 1.86 \times 4$ mm) sequences were used. Wall of the entire abdominal aorta from diaphragmatic hiatus to iliac bifurcation was segmented in consensus by two radiologists (with 8 and 23 years of experience in reading aortic and abdominal MRI scans, respectively) on either the MEDIC or a single echo of the T_2^* map manually in Amira software (v. 5.3.2, FEI, Bordeaux, France) and transposed onto the R_2^* map, an inverse of the T_2^* map, image. Nearest neighbor interpolation was used if the Cartesian coordinates were altered between scans. The segmentation was reviewed on

the MEDIC or single echo T_2^* image and on the R_2^* map image by a board-certified radiologist and the mean R_2^* value was calculated. The two-dimensional image visualization was performed in Osirix (Pixmeo SARL, Geneva, Switzerland) by overlaying the segmented aortic wall R_2^* data in pseudocolor over the registered T_1 VIBE image. The three-dimensional image visualization was performed in Amira using a volume rendered T_1 VIBE registered with the segmented aortic wall, also in pseudo color.

Statistical analysis

The analysis was performed using GraphPad Prism (v. 7.0c, La Jolla, CA). Categorical variables were expressed as frequencies and percentages. Continuous parametric variables were expressed as mean $\pm$ standard deviation. The change in R_2^* values was analyzed by unpaired t -test with Welch's correction. p -Value < 0.05 was considered as statistically significant.

RESULTS

The pancreatic cancer group comprised of four females and four males with an average age of 69 years. Within this group the average age of the four females was 71 ± 21.7 years and the average age of the four males was 67 ± 14.3 years. The healthy volunteer control group included two females and six males with an average age of 29.5 years. In the control group, the average age of females was 29 ± 1.4 years and the average age of males was 29.7 ± 2.9 years. The baseline aortic wall R_2^* in pancreatic cancer group was 83.8 ms^{-1} [95% confidence interval (CI) (160.8–106.8 ms^{-1})]; similar to 89.42 ms^{-1} in healthy volunteers [95% CI (73.1–105.8 ms^{-1})]. The R_2^* of the aortic wall 48 h following ferumoxytol administration was 300 ms^{-1} [95% CI (184.6–415.4 ms^{-1})] in pancreatic cancer group and 144.1 ms^{-1} [95% CI (95.3–192.8 ms^{-1})] in control group. The average change in R_2^* value of the pancreatic cancer patients was determined to be 216.1 ms^{-1} [95% CI (113.6–318.8 ms^{-1})]. The average change R_2^* value of the healthy volunteer control was determined to be 54.6 ms^{-1} [95% CI (6.9–102.4 ms^{-1})] (Table 1). The average change in R_2^* was significantly higher in pancreatic cancer patients in comparison with healthy volunteers ($p = 0.0082$) (Figures 1 and 2). No adverse events following administration of ferumoxytol were reported in the study population.

DISCUSSION

Following non-lethal radiation doses, there is more local than systemic effect on vascular wall as indicated by animal model

Table 1. Demographic comparison between pancreatic cancer patient and healthy volunteer groups

	Case	Control	p -value
Male ($n=$)	4	6	–
Female ($n=$)	4	2	–
Average age	69	29.5	–
Average BMI	26.54	25.56	–
Average baseline R_2^*	83.83	89.42	0.1644
Average delta R_2^*	216.2	54.67	0.0082

BMI, body mass index;

Figure 1. Pseudocolored images showing aortic wall R_2^* map superimposed on the T_1 weighted axial images. (A) baseline and (B) at 48 h show no significant aortic wall uptake (arrows) in 31-year-old male who was a healthy volunteer in comparison with a 62-year-old male with pancreatic cancer patient [baseline, (C) and 48 h, (D)] who showed significant ferumoxytol uptake.

studies showing unchanged systemic inflammatory markers.^{11,12} This leaves the scope of developing non-invasive methods to detect the vascular inflammation in humans before this inflammation progresses to full-blown atherosclerosis, thereby leading to major vascular events. Our study demonstrates increased accumulation of ferumoxytol as a surrogate marker of inflammation in aortic wall post-neoadjuvant therapy as early as 2 weeks in comparison with healthy volunteers. There was no significant difference in the baseline R_2^* in two groups, which is expected given the absence of focal abdominal atherosclerotic disease on abdominal CTs. Because radiation is targeted to a particular anatomical segment, we decided to focus on the abdominal aortic wall, which lies in the radiation field for pancreatic cancer patients. As the evaluation was focused on the abdominal aortic wall without any focal atherosclerotic lesion, we decided to segment entire aortic wall and compare

the average R_2^* . The average change in the R_2^* was significantly different in pancreatic cancer patients post neoadjuvant therapy when compared to the healthy volunteers, a trend similar to prior studies performed on carotid arteries wherein there was significant difference in healthy vs segments of carotid arteries not affected by plaque in patients with carotid stenosis.¹³ The USPIO uptake was able to distinguish between symptomatic vs asymptomatic plaques, predict future cardiovascular events, and was correlated with number of macrophages in endarterectomy studies.^{14–19} A prospective multicenter study recently demonstrated the utility of ferumoxytol in detecting cellular inflammation in aortic wall in patients with abdominal aortic aneurysm. The degree of USPIO accumulation predicted the rate of aneurysm growth as well of risk of rupture.²⁰ USPIO can also play a role in assessing atherosclerotic plaques at the risk of rupture. Kooi et al demonstrated accumulation of ultrasmall

Figure 2. Bar graph (E) shows R_2^* values pre, 48 h post-ferumoxytol injection and R_2^* difference (delta) between healthy controls and pancreatic cancer cases.

superparamagnetic iron oxide nanoparticles in macrophage rich rupture prone and ruptured carotid plaques.²¹ USPIO uptake in absence of plaque in post neoadjuvant therapy patients is indicative of indirect evidence that the macrophages start infiltrating the aortic wall as early as 2 weeks post neoadjuvant therapy, an important observation from this study.

The change in R_2^* in healthy volunteers can be explained by ferumoxytol in the blood pool within the vasa vasorum supplying the aortic walls.¹³ Two potential observations remain to be explored are how these macrophage accumulate evolve overtime and the average duration from the radiation to plaque formation. A larger sample size study with multiple time points of USPIO-enhanced MRI scans post-radiation would be ideal to fill our knowledge gaps in this regard. USPIO-enhanced imaging offers an assessment of pre-clinical atherosclerosis beyond mere identification and categorization of the degree of stenosis. When used with clinical scanners, R_2^* quantification of the aorta on USPIO-enhanced scans is feasible and can serve as a surrogate marker of inflammation, a predecessor of atherosclerosis. Because of this, radiation therapy-induced atherosclerosis could potentially be diagnosed earlier on due to its inflammatory nature.

We did not encounter any adverse events after intravenous administration of ferumoxytol. Although the data on adverse events after intravenous ferumoxytol as an off-label imaging indication is limited, Vasanawala et al. reported a single adverse event out of approximately 2000 patients.²²

Small sample size and single center study are some of the major limitations of our study. The aortic wall is between 0.5 and 2 mm and the contouring required zooming of images, which have affected the image resolution. The magnitude of change in R_2^* is likely under-represented in our study as the peak inflammation in arterial walls happen in 22–34 weeks as opposed to within 2 weeks post-treatment in our study.¹² We did not assess small arteries that were in the field of radiation therapy such as mesenteric vessel, however, radiation-induced atherosclerosis typically affects medium to large vessels.²³ Comparison of age matched aorta was not performed in our study. Such comparison may show different degree of average change in R_2^* between the two groups as shown by Smits et al in their observation on segments of carotid arteries with no plaques compared to segments with plaques both from same patient.¹³

Ferumoxytol can offer a noninvasive, quantitative assessment of vascular inflammation following radiation therapy in cancer patients. In conclusion, ferumoxytol enhanced MRI offers a novel, quantitative radiation-free imaging marker of inflammation to inform therapeutic decisions and image therapy-mediated changes.

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REFERENCES

1. Halle M, Gabrielsen A, Paulsson-Berne G, Gahm C, Agardh HE, Farnebo F, et al. Sustained inflammation due to nuclear factor-kappa B activation in irradiated human arteries. *J Am Coll Cardiol* 2010; 55: 1227–36. doi: <https://doi.org/10.1016/j.jacc.2009.10.047>
2. Gabriels K, Hoving S, Gijbels MJ, Pol JF, te Poele JA, Biessen EA, et al. Irradiation of existing atherosclerotic lesions increased inflammation by favoring pro-inflammatory

- macrophages. *Radiother Oncol* 2014; **110**: 455–60. doi: <https://doi.org/10.1016/j.radonc.2014.01.006>
3. Teresa Pinto A, Laranjeiro Pinto M, Patrícia Cardoso A, Monteiro C, Teixeira Pinto M, Filipe Maia A, et al. Ionizing radiation modulates human macrophages towards a pro-inflammatory phenotype preserving their pro-invasive and pro-angiogenic capacities. *Sci Rep* 2016; **6**: 18765. doi: <https://doi.org/10.1038/srep18765>
 4. Figueroa AL, Abdelbaky A, Truong QA, Corsini E, MacNabb MH, Lavender ZR, et al. Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. *JACC Cardiovasc Imaging* 2013; **6**: 1250–9. doi: <https://doi.org/10.1016/j.jcmg.2013.08.006>
 5. Joseph P, Tawakol A. Imaging atherosclerosis with positron emission tomography. *Eur Heart J* 2016; **37**: 2974–80. doi: <https://doi.org/10.1093/eurheartj/ehw147>
 6. Alaarg A, Pérez-Medina C, Metselaar JM, Nahrendorf M, Fayad ZA, Storm G, et al. Applying nanomedicine in maladaptive inflammation and angiogenesis. *Adv Drug Deliv Rev* 2017; **119**: 143–58. doi: <https://doi.org/10.1016/j.addr.2017.05.009>
 7. Yancy AD, Olzinski AR, Hu TC, Lenhard SC, Aravindhan K, Gruver SM, et al. Differential uptake of ferumoxtran-10 and ferumoxytol, ultrasmall superparamagnetic iron oxide contrast agents in rabbit: critical determinants of atherosclerotic plaque labeling. *J Magn Reson Imaging* 2005; **21**: 432–42. doi: <https://doi.org/10.1002/jmri.20283>
 8. Toth GB, Varallyay CG, Horvath A, Bashir MR, Choyke PL, Daldrup-Link HE, et al. Current and potential imaging applications of ferumoxytol for magnetic resonance imaging. *Kidney Int* 2017; **92**: 47–66. doi: <https://doi.org/10.1016/j.kint.2016.12.037>
 9. McDermott S, Thayer SP, Fernandez-Del Castillo C, Mino-Kenudson M, Weissleder R, Harisinghani MG. Accurate prediction of nodal status in preoperative patients with pancreatic ductal adenocarcinoma using next-gen nanoparticle. *Transl Oncol* 2013; **6**: 670–5. doi: <https://doi.org/10.1593/tlo.13400>
 10. Gaglia JL, Harisinghani M, Aganj I, Wojtkiewicz GR, Hedgire S, Benoist C, et al. Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. *Proc Natl Acad Sci U S A* 2015; **112**: 2139–44. doi: <https://doi.org/10.1073/pnas.1424993112>
 11. Stewart FA, Heeneman S, Te Poele J, Kruse J, Russell NS, Gijbels M, et al. Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE-/- mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage. *Am J Pathol* 2006; **168**: 649–58. doi: <https://doi.org/10.2353/ajpath.2006.050409>
 12. Weintraub NL, Jones WK, Manka D. Understanding radiation-induced vascular disease. *J Am Coll Cardiol* 2010; **55**: 1237–9. doi: <https://doi.org/10.1016/j.jacc.2009.11.053>
 13. Smits LP, Tiessens F, Zheng KH, Stroes ES, Nederveen AJ, Coolen BF. Evaluation of ultrasmall superparamagnetic iron-oxide (USPIO) enhanced MRI with ferumoxytol to quantify arterial wall inflammation. *Atherosclerosis* 2017; **263**: 211–8. doi: <https://doi.org/10.1016/j.atherosclerosis.2017.06.020>
 14. Tang TY, Howarth SP, Miller SR, Graves MJ, U-King-Im JM, Li ZY, et al. Comparison of the inflammatory burden of truly asymptomatic carotid atheroma with atherosclerotic plaques in patients with asymptomatic carotid stenosis undergoing coronary artery bypass grafting: an ultrasmall superparamagnetic iron oxide enhanced magnetic resonance study. *Eur J Vasc Endovasc Surg* 2008; **35**: 392–8. doi: <https://doi.org/10.1016/j.ejvs.2007.10.019>
 15. Tang T, Howarth SP, Miller SR, Trivedi R, Graves MJ, King-Im JU, et al. Assessment of inflammatory burden contralateral to the symptomatic carotid stenosis using high-resolution ultrasmall, superparamagnetic iron oxide-enhanced MRI. *Stroke* 2006; **37**: 2266–70. doi: <https://doi.org/10.1161/01.STR.0000236063.47539.99>
 16. Howarth SP, Tang TY, Trivedi R, Weerakkody R, U-King-Im J, Gaunt ME, et al. Utility of USPIO-enhanced MR imaging to identify inflammation and the fibrous cap: a comparison of symptomatic and asymptomatic individuals. *Eur J Radiol* 2009; **70**: 555–60. doi: <https://doi.org/10.1016/j.ejrad.2008.01.047>
 17. Degnan AJ, Patterson AJ, Tang TY, Howarth SP, Gillard JH. Evaluation of ultrasmall superparamagnetic iron oxide-enhanced MRI of carotid atherosclerosis to assess risk of cerebrovascular and cardiovascular events: follow-up of the ATHEROMA trial. *Cerebrovasc Dis* 2012; **34**: 169–73. doi: <https://doi.org/10.1159/000339984>
 18. Metz S, Beer AJ, Settles M, Pelisek J, Botnar RM, Rummeny EJ, et al. Characterization of carotid artery plaques with USPIO-enhanced MRI: assessment of inflammation and vascularity as in vivo imaging biomarkers for plaque vulnerability. *Int J Cardiovasc Imaging* 2011; **27**: 901–12. doi: <https://doi.org/10.1007/s10554-010-9736-7>
 19. Kooi ME, Cappendijk VC, Cleutjens KB, Kessels AG, Kitslaar PJ, Borgers M, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* 2003; **107**: 2453–8. doi: <https://doi.org/10.1161/01.CIR.0000068315.98705.CC>
 20. MA3RS Study Investigators. Aortic Wall Inflammation Predicts Abdominal Aortic Aneurysm Expansion, Rupture, and Need for Surgical Repair. *Circulation* 2017; **136**: 787–97. doi: <https://doi.org/10.1161/CIRCULATIONAHA.117.028433>
 21. Kooi ME, Cappendijk VC, Cleutjens KB, Kessels AG, Kitslaar PJ, Borgers M, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* 2003; **107**: 2453–8. doi: <https://doi.org/10.1161/01.CIR.0000068315.98705.CC>
 22. Vasanawala SS, Nguyen KL, Hope MD, Bridges MD, Hope TA, Reeder SB, et al. Safety and technique of ferumoxytol administration for MRI. *Magn Reson Med* 2016; **75**: 2107–11. doi: <https://doi.org/10.1002/mrm.26151>
 23. Fajardo LF. Is the pathology of radiation injury different in small vs large blood vessels? *Cardiovasc Radiat Med* 1999; **1**: 108–10.