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Radiation induced atherosclerosis

Contrast enhancement of carotid adventitial vasa vasorum as a biomarker of radiation-induced atherosclerosis

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ABSTRACT

Purpose: Abnormal proliferation of adventitial vasa vasorum (vv) occurs early at sites of atherosclerosis and is thought to be an early biomarker of vascular damage. Contrast-enhanced ultrasound (CEUS) can detect this process. Its usefulness in irradiated arteries as a measure of accelerated atherosclerosis is unknown. This study investigates contrast intensity in carotid adventitia as an early marker of radiation-induced damage in head and neck cancer (HNC) patients.

Materials/methods: Patients with HNC treated with a wedged-pair and matched neck technique or hemi-neck radiotherapy (RT) (unirradiated side as control) at least 2 years previously were included. Patients had been prescribed a dose of at least 50 Gy to the neck. CEUS was performed on both carotid arteries and a region of interest was selected in the adventitia of the far wall of both left and right distal common carotid arteries. Novel quantification software was used to compare the average intensity per pixel between irradiated and unirradiated arteries.

Results: 48 patients (34 males) with median age of 59.2 years (interquartile range (IQR) 49.2–64.2) were included. The mean maximum point dose to the irradiated artery was 61.2 Gy (IQR 52.6–61.8) and 1.1 Gy (IQR 1.0–1.8 Gy) to the unirradiated side. The median interval from RT was 59.4 months (IQR 41–88.7). There was a significant difference in the mean (SD) contrast intensity per pixel on the irradiated side (1.1 (0.4)) versus 0.96 (0.34) on the unirradiated side ($p = 0.01$). After attenuation correction, the difference in mean contrast intensity per pixel was still significant (1.4 (0.58) versus 1.2 (0.47) ($p = 0.02$). Previous surgery or chemotherapy had no effect on the difference in contrast intensity between the 2 sides of the neck. Mean intensity per pixel did not correlate to traditional risk prediction models (carotid intima-medial thickness, QSTROKE score).

Conclusions: Proliferation of vv is demonstrated by increased contrast intensity in irradiated carotid arteries. This may be a useful, independent biomarker of radiation-induced carotid atherosclerosis when used as a tool to quantify neovascularization.

Vasa vasorum (vv) are a plexus of microvessels located in the adventitia of most medium and large arteries (including the aorta, coronary, femoral and carotid arteries). Their function is primarily related to the nutritional needs of veins and arteries. Researchers have demonstrated an increase in the density of adventitial vv before the development of atherosclerotic lesions in porcine coronary arteries [1]. Herrmann et al. [2] further reported that increase in the density of adventitial vv occurs before the onset of endothelial dysfunction (one of the first functional alterations in atherosclerosis). Studies in humans have identified plaque neovascularization as a key feature of vulnerable plaques, i.e. those plaques at risk of rupture [3,4]. Furthermore, abnormal proliferation of human adventitial vv occurs early at sites of atherosclerosis and is thought to be a precursor to atherosclerosis and an early biomarker of vascular damage [4,5].

Contrast-enhanced ultrasound (CEUS) imaging of the carotid arteries enables the acquisition of real-time carotid images with enhancement of the arterial lumen and plaque morphology, improved resolution of CIMT, and, importantly, direct visualization of the adventitial vv and plaque neovascularization [6–8]. This is an attractive diagnostic tool due to its non-ionizing nature and real-time imaging with good spatial resolution, relatively low cost and

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high accessibility. Contrast agents used in CEUS are microbubbles. These are small (2 to 8 μm) acoustically-active particles composed of a gaseous core of perfluorocarbons surrounded by a biocompatible shell composed of a combination of lipids, albumin, or polymers [6]. When exposed to an ultrasound beam, these particles expand and contract, creating backscatter and, consequently, enabling them to act as echo enhancers. Techniques with multi-pulse sequence transmission and signal processing have been widely used to improve the imaging specificity of microbubbles and have made it possible to image and quantify neovascularization in plaques and in adventitial vv [4,9–13]. However, current quantification of contrast enhancement is limited by spatially heterogeneous and patient-specific attenuation [14,15]. Accounting for these variables will allow for improved quantification of signal intensity from CEUS as a measure of adventitial neovascularization. Some studies have already attempted to correct for attenuation [14,16,17].

The aim of this study was to examine the contrast intensity in the adventitial vv of irradiated arteries and compare this to unirradiated arteries. We hypothesized that radiation-induced injury results in an inflammatory response in the endothelium and consequent proliferation of adventitial vv, which may be measured quantitatively using CEUS. The effect of surgery, as well as RT, to the neck was also studied and the difference in contrast intensity between irradiated and unirradiated carotid arteries was correlated to the interval from RT to see if contrast intensity increases early after RT. In addition, this study aimed to see if there is any correlation between contrast intensity and validated markers for risk prediction (carotid intima-medial thickness (CIMT) and QSTROKE score).

Materials and methods

Head and neck cancer patients treated with RT (minimum dose of 50 Gy) to one side of the neck prior to December 2009 were included in the study. Risk factor determination was performed as follows – medical history, blood pressure, body mass index, blood glucose, and lipid profile. Ethical approval was obtained from a regional Research Ethics Committee and the protocol for the study was reviewed by the Royal Marsden Committee for Clinical Research and registered on clinicaltrials.gov (NCT02060643).

Study assessments

Head and neck cancer patients treated prior to December 2009 were prospectively identified via the Royal Marsden Hospital head and neck RT database. Written informed consent was obtained from each participant and medication history and co-morbidities were recorded. Blood pressure, height and weight (to determine body mass index) were measured and electrocardiogram (ECG) was performed. Blood samples were taken for hemoglobin, plasma glucose, lipid profile, renal and liver profile.

Definitions

The following definitions were used in this study:

- Hypertension – systolic blood pressure (SBP) ⩾140 mmHg and/or diastolic blood pressure (DBP) ⩾ 90 mmHg.
- Diabetic – random serum glucose ⩾ 11.1 mmol/L, a glycosylated hemoglobin A1c (HbA1c) ⩾5.8% or current use of glucose-lowering agents or insulin.
- Hyperlipidaemia – fasting serum low density lipoprotein (LDL) ⩾ 2.6 mmol/L, high density lipoprotein (HDL) < 2.3 mmol/L or triglycerides (TG) ⩾ 2.3 mmol/L, or current use of cholesterol-lowering agents. Normal ranges: HDL cholesterol −1.0–2.3 mmol/L; LDL cholesterol – < 2.6 mmol/L; TG (fasting) – < 2.3 mmol/L (ideal = < 1.5 mmol/L); Total cholesterol – 3.6–8.0 mmol/L (non-fasting) (ideal = < 5.0 mmol/L (fasting)), Total cholesterol/HDL ratio <5.
- Smoking history – smoker (pack years) or non-smoker/ex-smoker >10 years.
- Normal BMI – 18.5–24.9 kg/m^2.
- Overweight – 25.0–29.9 kg/m^2.
- Obese – ⩾ 30 kg/m^2.
- Atrial fibrillation – standard ECG changes.
- Stroke – sudden onset of a neurological deficit persisting for ⩾24 h.
- Transient ischemic attack (TIA) – focal neurological symptoms lasting <24 h.

Ultrasound studies

CEUS image sequences were acquired on both sides of the neck with a clinical scanner (GE Vivid7 with a 9 MHz broadband linear array transducer) using a contrast-specific imaging pre-set. Contrast-enhanced ultrasound video loops were taken using a commercially available ultrasound contrast agent, SonoVue™ (Bracco, Milan) given as an intravenous infusion via a peripheral vein at a rate of 1.2 mL/min. The infusion was delivered over a total of 5–7 min. Imaging was performed in real-time prior to the arrival of and following the saturation of the carotid artery with SonoVue.

CEUS quantification

An attenuation correction and image normalization algorithm for CEUS carotid artery images was utilized and has been previously described [18] in order to reduce the effects of any non-uniform transducer-skin contact and heterogeneity in tissue attenuation on quantification. Analysis of CEUS video sequences was performed offline using software developed using Matlab (The Mathworks Inc., Natick, MA, USA). Regions of interest (ROIs) were selected manually, one to segment the lumen and the others to include regions in adventitia where quantification was required [18]. The motion of the lumen and adventitial ROIs in the video sequence was tracked and corrected by employing a piece-wise block matching algorithm [19]. As a result of motion correction, all images in the sequence were aligned to the first image. As the size of the ROIs differed for each video sequence, the average intensity per pixel was calculated by dividing the total intensity for the ROI by the ROI size (which was calculated in pixels). This was undertaken for video sequences obtained before (subtraction) and after contrast infusion.

CIMT

B-mode imaging using a 9 MHz linear array transducer prior to infusion of contrast agent was utilized to visualize the CIMT. The common carotid (CCA) and the proximal portion of the internal carotid (ICA) and external carotid arteries (ECA) on both sides of the neck were examined with the patient supine on an examination couch. Four-beat video loops (long-axis and short-axis) were stored for offline analysis.

CIMT was determined offline using semi-automated edge-detection software. Measurements were blinded as clinical details were not available at the time of analysis. Magnified still images were used for analysis and measurements were taken from the far wall of the CCA away from any atherosclerotic plaque, 1 cm proximal to the bulb. Mean CIMT was recorded and the average of 3 readings was taken for each measurement.

QSTROKE score

The QSTROKE score was specifically designed to aid general practitioners in predicting a patient's risk of developing a stroke