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Quantification of experimental venous thrombus resolution by longitudinal nanogold-enhanced micro-computed tomography

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ABSTRACT

Introduction: The assessment of thrombus size following treatments directed at preventing thrombosis or enhancing its resolution has generally relied on physical or histological methods. This cross-sectional design imposes the need for increased numbers of animals for experiments. Micro-computed tomography (microCT) has been used to detect the presence of venous thrombus in experimental models but has yet to be used in a quantitative manner. In this study, we investigate the use of contrast-enhanced microCT for the longitudinal assessment of experimental venous thrombus resolution.

Materials and methods: Thrombi induced by stenosis of the inferior vena cava in mice were imaged by contrast-enhanced microCT at 1, 7 and 14 days post-induction ($n = 18$). Thrombus volumes were determined longitudinally by segmentation and 3D volume reconstruction of microCT scans and by standard end-point histological analysis at day 14. An additional group of thrombi were analysed solely by histology at 1, 7 and 14 days post-induction ($n = 15$).

Results: IVC resident thrombus was readily detectable by contrast-enhanced microCT. MicroCT-derived measurements of thrombus volume correlated well with time-matched histological analyses (ICC = 0.75, $P < 0.01$). Thrombus volumes measured by microCT were significantly greater than those derived from histological analysis ($P < 0.001$). Intra- and inter-observer analyses were highly correlated (ICC = 0.99 and 0.91 respectively, $P < 0.0001$). Further histological analysis revealed noticeable levels of contrast agent extravasation into the thrombus that was associated with the presence of neovascular channels, macrophages and intracellular iron deposits.

Conclusion: Contrast-enhanced microCT represents a reliable and reproducible method for the longitudinal assessment of venous thrombus resolution providing powerful paired data.

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1. Introduction

Experimental models enable the cellular and molecular mechanisms that regulate the formation and resolution of venous thrombi to be studied and have afforded considerable insight into the molecular and cellular mediators of these processes [1–3]. However, previous studies have relied largely on end-point analysis of thrombus weight or 'size', or a combination of histology and image analysis of sections taken through the thrombus. Advances in imaging techniques have facilitated the *in vivo* longitudinal measurement of thrombus, with magnetic resonance imaging (MRI) using a clinical 3 T scanner for determination of thrombus volume in the experimental setting being recently reported [4,5]. MRI accurately quantifies thrombus size in murine models of venous thrombosis with high resolution, but access to these scanners is limited because of the cost of this imaging modality. High frequency ultrasound has also been used to study experimental models of venous thrombosis, but although affordable requires a high degree of technical skill and so far only provides two-dimensional imaging of the thrombus [6,7].

Contrast-enhanced computed tomography (CT) has been used to demonstrate the presence of thrombus in man. Technological advances in the field of CT have facilitated the development of high-resolution micro-computed tomography (microCT) imaging platforms suitable for pre-clinical use [8]. The availability of microCT facilities has increased considerably in the past decade and represents an affordable and readily accessible methodology for pre-clinical imaging. MicroCT has been used extensively in the study of murine models of cardiovascular pathologies including critical limb ischaemia, abdominal aortic

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aneurysm and myocardial infarction [9–11]. Visualisation of the vasculature in these pathologies requires intravenous administration of high molecular weight blood-pool contrast agents, similar to CT angiography. This imaging modality has also been used to identify the presence of thrombus in a murine model of inferior vena cava (IVC) thrombosis [3], however, a detailed characterisation for the longitudinal assessment of thrombus resolution has yet to be reported. The aim of the present study was to assess the applicability of contrast-enhanced microCT for longitudinal measurement of thrombus resolution.

2. Materials and methods

2.1. St. Thomas' model of venous thrombosis

Thrombi were induced in the inferior vena cava (IVC) of 8–10 week-old male Balb/c mice as previously described [12]. Briefly, a midline laparotomy was carried out under general anaesthesia and sharp dissection used to separate the IVC from the aorta inferior to the left renal vein. A piece of 4–0 mersilk suture (Ethicon, USA) was slung around the IVC and tied onto a piece of 5–0 prolene suture (Ethicon, USA) laid along the vessel which was then removed to generate a 90% stenosis of the inferior vena cava. A neurovascular clip (Fine Scientific Tools, Germany) was applied to the infra-renal segment of the IVC to induce endothelial dysfunction. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

2.2. MicroCT

Mice were anaesthetised under 3% isoflurane at an oxygen flow rate of 1 l/min. Aurovist 15 nm nanogold (nAu) contrast agent (Nanoprobes, USA, 200 mg/ml nAu) was diluted with saline to a concentration of 50 mg/ml nAu. A 200 μl bolus of contrast agent was administered by intravenous injection into the tail vein giving a nAu dose of 10 mg/mouse. Contrast was allowed to circulate for 5 min prior to commencement of the scan.

A nanoScan PET/CT 8W (Mediso Ltd., Hungary) was used to image the thrombosed IVC. The mouse was placed on the scanner bed in the prone position and a pressure transducer placed to measure respiration rate. An initial scout view was obtained to allow specification of the anatomical limits of the high-resolution scan. A high-resolution scan (maximum zoom, 360 projections, pitch 1, 45 kVP, 1000 ms exposure) was completed taking approximately 20 min.

2.3. Image reconstruction

Scans were reconstructed using VivoQuant software (v1.22, Invicro, USA) with a voxel size of 65 μm. Reconstructed scans were segmented and analysed using ITKsnap software (v2.4, Open Source) [13]. Image contrast was adjusted linearly to provide optimal parameters for subsequent analysis. Thrombi were reconstructed in 3D using a semi-automatic volumetric bubble propagation system with a balloon expansion force of 1.00 and a curvature term of 0.20. Manual corrections to the segmentation were made using the free-hand tool. When the segmentation was complete a 3D rendered mesh of the thrombus was generated and thrombus volume recorded. A representative example of segmentation of the thrombus from surrounding tissue has been provided in supplementary material (Fig. S1).

2.4. Histology

The thrombosed IVC was excised from the site of stenosis to the iliac bifurcation at 1, 7 and 14 days post-induction. Samples were fixed in

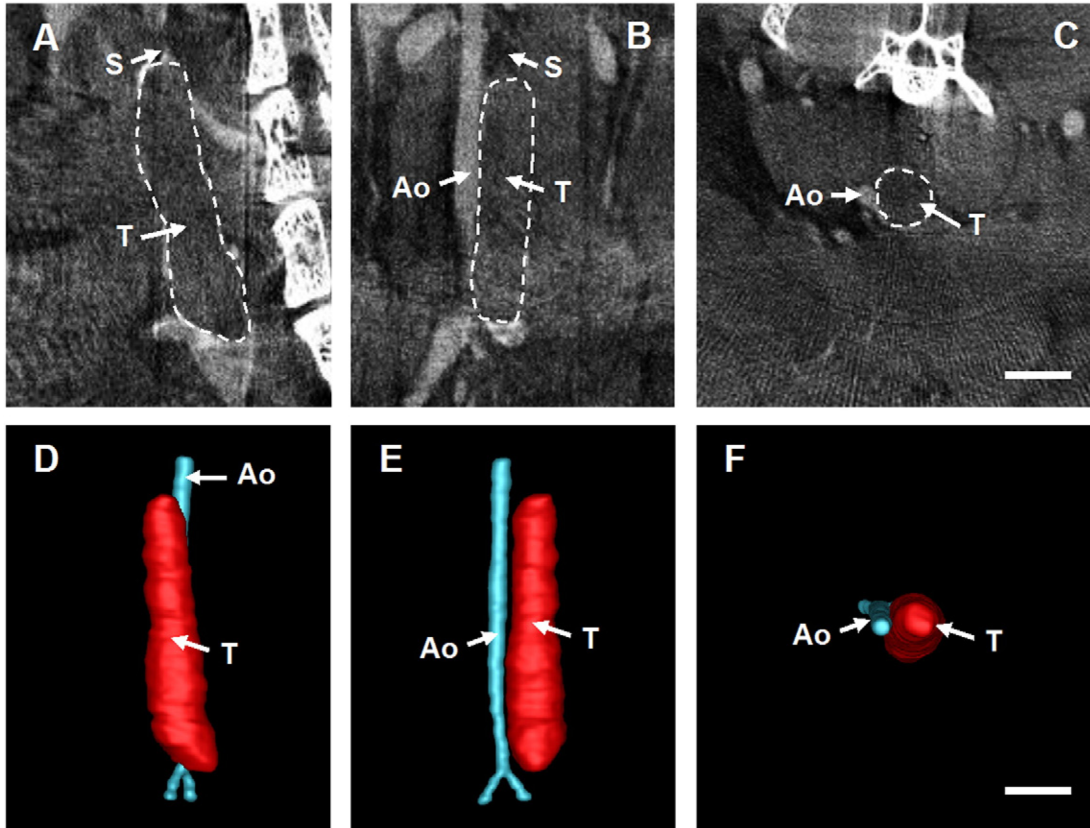

Fig. 1. Imaging of IVC thrombi by contrast-enhanced microCT. A representative day 1 thrombus imaged by contrast-enhanced microCT shown in transverse (A), sagittal (B) and coronal (C) planes (scale bar 2 mm). Segmentation of the scan allow for reconstruction of 3D volume renders (D–F). Images have been annotated with the position of the aorta (Ao), the site of stenosis (S) and the thrombus (T).

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