Original Article

Visualization of luminal thrombosis and mural Iron accumulation in giant aneurysms with *Ex vivo* 4.7T magnetic resonance imaging

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Abstract

Background: Better diagnostic tools to identify rupture-prone saccular intracranial aneurysms (sIA) are needed. Inflammation and luminal thrombus associate with degeneration and rupture of the sIA wall. Iron-uptake has been detected in the inflammatory cells of the sIA wall and thrombus is the likely source of this iron. We investigated *ex vivo* the use of magnetic resonance imaging (MRI) to detect iron accumulation and luminal thrombus in giant sIAs.

Methods: Giant sIAs ($n = 3$) were acquired from microsurgical operations, fixed with formalin, embedded in agar and imaged at 4.7T. Samples were sectioned maintaining the orientation of the axial plane of MRI scans, and stained (hematoxylin-eosin and Prussian blue).

Results: All three giant sIAs showed a degenerated hypocellular wall with both mural and adventitial iron accumulation and displayed different degrees of luminal thrombus formation and thrombus organization. Signal intensity varied within the same sIA wall and associated with iron accumulation in all tested sequences. Wall areas with iron accumulation had significantly lower signal to noise ratio (SNR) compared with areas without iron accumulation ($P = 0.002$). Fresh and organizing thrombus differed in their MRI presentation and differed in signal intensity of the aneurysm wall ($P = 0.027$).

Conclusion: MRI can detect *ex vivo* the accumulation of iron in giant sIA wall, as well as fresh and organizing luminal thrombus. These features have been previously associated with fragile, rupture-prone aneurysm wall. Further studies of iron accumulation as a marker of rupture-prone aneurysm wall are needed.

Key Words: Giant aneurysm, iron, magnetic resonance imaging, rupture risk, thrombus

INTRODUCTION

Despite advances in microsurgical and endovascular therapies, preventive treatment of saccular intracranial aneurysms (sIA) is not without risk of morbidity and mortality. Since unruptured sIAs are relatively common in the middle-aged population (approximately 3.5%),[8] identification of rupture-prone sIAs that require prophylactic treatment among those that can be followed, remains a significant clinical challenge.

Histopathological studies have demonstrated structural differences in ruptured and unruptured sIA walls,[1,2,6] as well as increased inflammatory cell infiltration in fragile, degenerated, rupture-prone sIA walls.[1,2,6] Recently it was shown that this inflammatory cell infiltration in the sIA wall can be imaged in the clinical setting with magnetic resonance imaging (MRI) using an iron-oxide (ferumoxytol)-based contrast agent that is taken up by macrophages.[4,5]

Iron accumulates in the aneurysm walls also without any iron-oxide contrast injection.[2]

The macrophages that take up the iron-oxide given as a contrast agent are likely to take up also the iron that accumulates in the aneurysm wall spontaneously, making it therefore possible that one might detect iron-loaded macrophages in degenerated sIA walls even without contrast. This could potentially be used to identify sIAs with a rupture-prone wall.

The iron that accumulated in the aneurysm wall may be derived from microhemorrhages in the aneurysm wall or from luminal thrombus, and possibly even from iron in the plasma. Microhemorrhages in the aneurysm wall are not frequently observed,[1,2,6] whereas luminal thrombus is common in aneurysms and associates with wall degeneration and tendency to rupture.[1,2,6] Thrombus contains iron and can be detected from the vessel wall with MRI.[3] MRI could potentially be used to identify sIAs with thrombus, which are mostly the ones with a degenerated, fragile and rupture-prone wall,[1,2,6] as well as the ones likely to have iron-loaded macrophages in their walls.

We investigated with *ex vivo* MRI and histopathology the wall of giant sIAs that often contain a lot of luminal thrombus of different ages, and therefore are likely to also have iron accumulation in their walls. Our aim was to test the concept that MRI without any iron-oxide contrast could be sufficient to detect iron accumulation and thrombus in the sIA wall, which might help in the detection of degenerated, rupture-prone sIA wall.

MATERIALS AND METHODS

Three giant sIAs were collected during microsurgical treatment. Samples were fixed in 10% formalin. Patient and sIA characteristics were obtained from medical records [Table 1]. The study was approved by the institutional review board and ethics committee.

4.7T MRI

Samples were embedded in 1% agar gel and imaged with a 4.7T MRI scanner (Bruker Pharmascan, Bruker, Germany) in a linear birdcage radiofrequency coil with inner diameter of 60 mm. After a pilot scan, the whole aneurysm was imaged with rapid acquisition with relaxation enhancement (RARE) sequence, with RARE factors 4 for T1-weighted (TR 600 ms, TE_{eff} 10 ms, number of excitations (NEX) 5, matrix size (MTX) 256 × 256), and with RARE factors 8 for T2-weighted (TR 2600 ms, TE_{eff} 40 ms, NEX 10, MTX 256 × 256) and with RARE factors 4 for proton density weighted (TR 3000 ms, TE_{eff} 10 ms, NEX 3, MTX 256 × 256) imaging. Additionally, fast low-angle shot sequence (FLASH) was used for T2* weighted images (TR 500 ms, TE 8 ms, NEX 4, MTX 256 × 256 or 512 × 512 depending on aneurysm size) and fast imaging in steady-state precession (FISP) T1/T2* weighted images (TR 14.226 ms, TE 7.113 ms, NEX 20, MTX 512 × 512) scans were acquired.

Histology

After imaging, while still embedded in agar, samples were cut parallel to the axial plane of MR images and reembedded in OCT Tissue Tek compound (Sakura, Netherlands). The distance of the plane of cut from the apex of the aneurysm dome was measured. Tissues were serial sectioned at 10 μm and the cut surface was photographed at 100 μm intervals. Each section and its respective distance from the initial cut was recorded. Sections were stained using either hematoxylin and eosin or prussian blue and nuclear fast red. MR images, photographs taken during sectioning, distance data and stained sections were compared to verify the relationship between MR images and histological sections. Examples are provided in Figure 1.

Analysis of MRI scans

MR images were reviewed on a Macintosh workstation using OsiriX software (OsiriX version 5.5.1, open source, http://www.osirix-viewer.com). In addition to visual

Table 1: Summary of patient and aneurysm characteristics

Case number	1	2	3
Age	64 years	60 years	67 years
Gender	Female	Male	Female
Smoking	No	Yes	No
Hypertension	No	Yes	No
Familial	No	No	No
Aneurysm location	Left PCom	Right MCA	Left MCA
Aneurysm size (length×width×neck)	22×16× 10 mm	46×37× 7 mm	40×30× 9 mm
Aneurysm rupture	Yes	No	No
Prior SAH	No	No	No

PCom: Posterior communicating artery, MCA: Middle cerebral artery, SAH: Subarachnoid hemorrhage
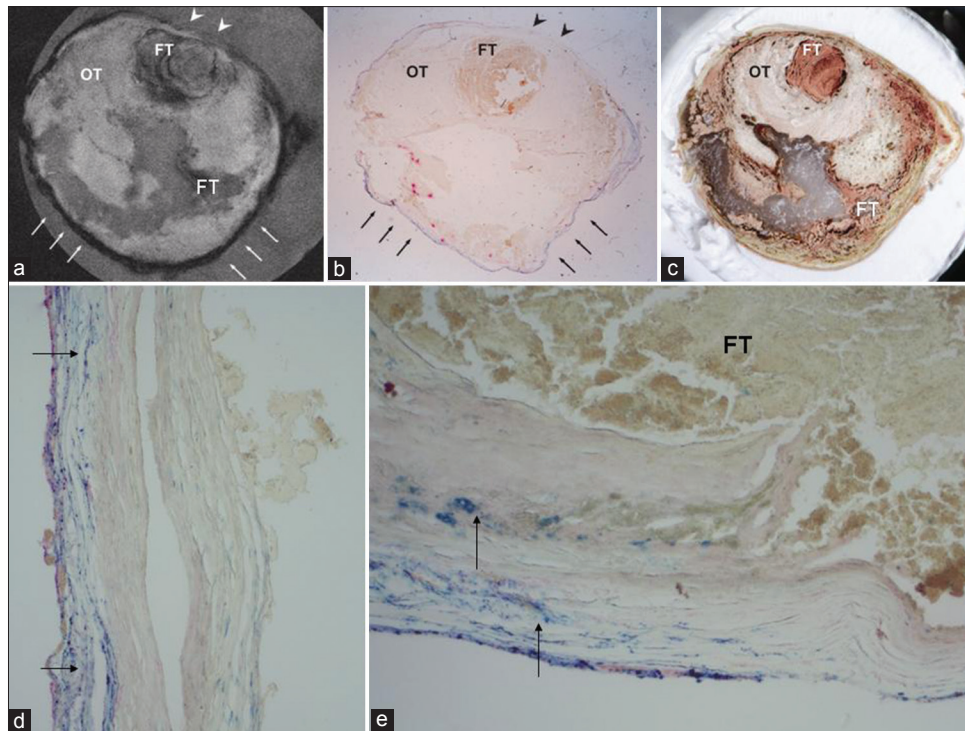
Figure 1: Representative T1/T2*-weighted MR image (a), histological section stained with Prussian blue (b) and the corresponding photograph taken during sectioning (c). FT = fresh thrombus, OT = organizing thrombus, arrow heads = wall with iron accumulation, arrows = wall without iron accumulation. Microphotographs D and E demonstrate accumulation of iron (blue, marked with arrows, Prussian blue staining) into giant aneurysm wall without (d) luminal thrombus or with (e) luminal thrombus

inspection of apparent signal intensity (SI), a region of interest (ROI) analysis was performed. Since the aim was to compare the SI of different tissue types in the aneurysm wall, and to make a correlation with the SI and the type of wall tissue, ROIs were drawn in each analyzed MR section on (i) wall with iron, (ii) wall without iron, (iii) fresh thrombus, and (iv) organizing thrombus, as identified in corresponding histological sections. Two histological sections and their corresponding MR images were analyzed per sample and 1-2 ROIs for each tissue type were drawn. Fresh thrombus was detected in only 2/3 cases. Mean SIs in each ROI was calculated. Tissue signal to noise ratio (SNR) was defined as:

$$SNR_{tissue} = \frac{SI_{tissue}^{mean}}{SD_{noise}}$$

SD_{noise} was the standard deviation of the signal measured from a ROI drawn in the background air (free of ghosting artefacts).

Statistics

Differences in SNR distributions were compared using related samples nonparametric Wilcoxon signed rank test. The level of significance was set at $P < 0.05$. All statistical analysis was performed with IBM SPSS Statistics software (IBM SPSS Statistics Version 19, IBM Corporation, New York, USA).

RESULTS

All three sIAs had luminal thrombus formation and thrombus organization and showed a degenerated hypocellular wall with focal luminal, intramural, and adventitial iron accumulation [Figure 1].

Aneurysm walls displayed varying SI in all the sequences and wall SI changed within a single sIA [Figure 1]. Wall areas with iron accumulation ($n = 6$ ROIs, 2 per aneurysm) had significantly lower SNR compared with areas without iron accumulation ($n = 4$ ROIs) in all tested sequences ($P = 0.002$, Wilcoxon sign test, SNR for iron+ and iron- areas for each sequence tested separately [Figure 2].

In histological analysis, organized thrombus was found in all 3/3 sIAs and fresh thrombus was found in 2/3 of the aneurysms. In T1-weighted images, fresh thrombus had high and homogenous SI, whereas organizing thrombus had low and homogenous SI. In T2 and T2* weighted images, fresh thrombus had mostly low and heterogenous SI, whereas organizing thrombus had high (although also heterogenous) SI [Figure 2]. Although fresh and organizing thrombus could be identified in the same MRI image according to differences in SI, the overlap in SI of fresh or organizing thrombus in the different areas of one sample and in the different samples was such that the median SI for fresh thrombus did not significantly differ from the median SI of organizing thrombus. Nevertheless, the SI of organizing
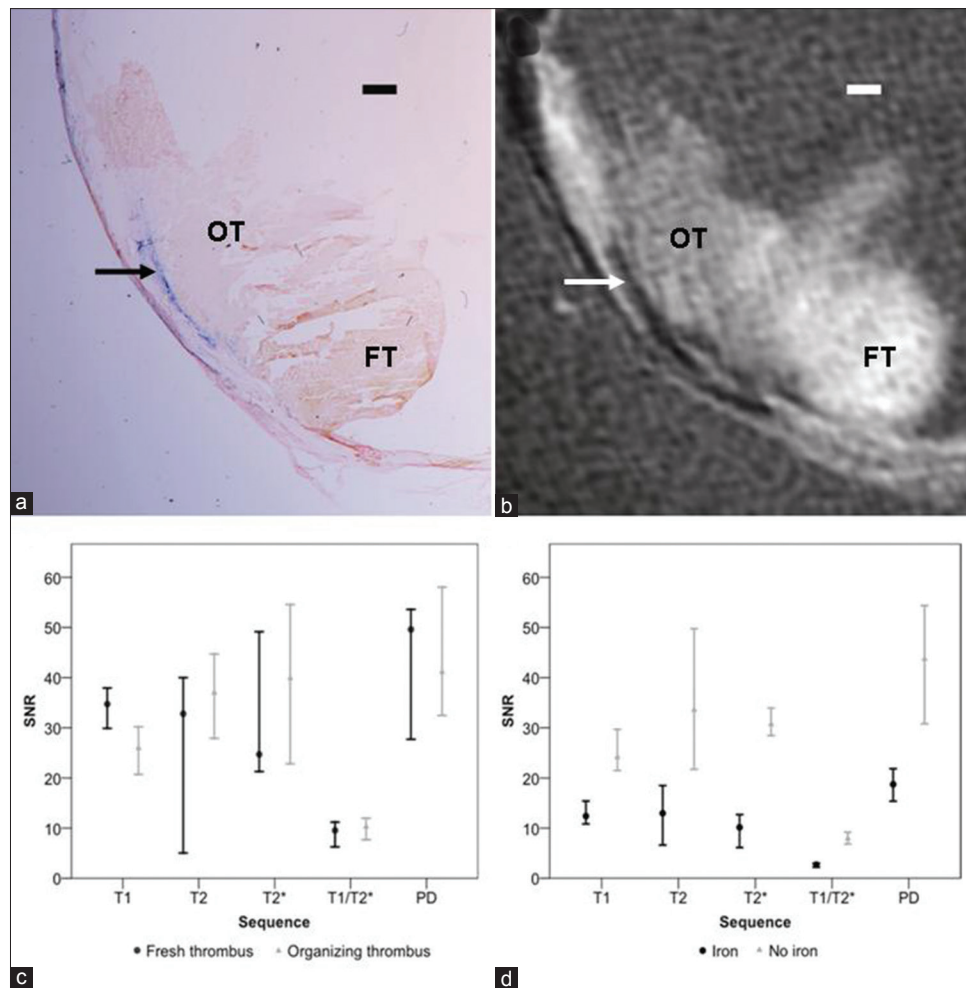
Figure 2: Histological view of sIA wall segment (a) and corresponding T1-weighted MR image (b) show a thin aneurysm wall with intramural iron accumulation (Arrows) and luminal organizing (OT) and fresh thrombus (FT). Scale bars = 1 mm. SNR analysis shows differences in median SNR and 95% confidence intervals of thrombi of different age (c) and wall segments with and without iron accumulation (d)

thrombus did significantly differ from the SI of the aneurysm wall with or without iron accumulation ($P < 0.001$ and 0.027, respectively, Wilcoxon sign test) demonstrating the capability of MRI to discriminate thrombus in the luminal surface of the aneurysm from the aneurysm wall itself.

DISCUSSION

This is a proof of concept study that demonstrates that MRI without contrast can be used to detect iron accumulation in degenerated, hypocellular giant sIA walls with histological structure similar to rupture-prone sIA walls.[1,2,6] Histopathological studies have already characterized the differences between ruptured/rupture-prone and unruptured sIA walls.[1,2,6] However, at the moment the only available method to detect these histological changes in the sIA walls *in vivo*, is the use of iron-oxide (ferumoxytol) as a contrast agent to visualize macrophage infiltration in the wall of sIAs.[4,5] This study demonstrates that at least in giant aneurysms, the sIA wall already has iron and it is questionable whether any

additional iron-oxide given as a contrast agent is needed. Rupture occurs because the sIA wall is degenerated up to the point that it cannot resist hemodynamic stress. Therefore the ability to evaluate the condition of the sIA wall with MRI that is sensitive to spontaneous mural iron accumulation may have direct implications for determination of the rupture-risk. In our samples from giant aneurysm walls, the amount of iron present in the sIA wall was high, which may explain that we could detect iron rich regions of the sIA walls with all sequences. In sIAs that have less iron in their wall, very iron sensitive sequences such as T2* might be required to detect the spontaneously occurring iron accumulation.

Luminal thrombus was detectable in all samples in all the MRI sequences we used. In addition, we found a visually clear difference in the SI of organized or fresh luminal thrombus in MR images [Figures 1a, c and 2a, b]. However, the SI or SNR differences between organized or fresh luminal thrombus did not reach statistical significance, probably due to high variation and small samples size. Thrombus is not only a source of hemoglobin metabolites

and iron in the sIA wall, but a source of matrix degrading proteases, as well as an inducer of inflammation in the aneurysm wall.[2,7] The biological effects that the luminal thrombus has on the sIA wall seem to have a major role in the degeneration of the sIA wall toward a rupture-prone type.[2] More importantly, luminal thrombosis associates with wall degeneration and sIA rupture.[1,2,6] It seems therefore possible that the ability to detect luminal thrombus with MRI could potentially be used to detect those sIAs that will develop a degenerated rupture-prone wall. This hypothesis merits further study.

CONCLUSIONS

MRI with T2* sensitive and other sequences can be used to detect spontaneous iron accumulation in the aneurysm wall. This finding is of potential importance for the detection of rupture-prone, aneurysm walls, as well as for imaging studies that use ferumoxytol or other iron-based contrast agents to detect inflammation in aneurysms, and merits further investigation in larger series.

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