BOLD imaging: a potential predictive biomarker of renal functional outcome following revascularization in atheromatous renovascular disease

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Abstract

Background. Stenting of the stenosed renal artery is commonly employed in atheromatous renovascular disease (ARVD) in order to revascularize the affected kidney. However, it is still far from clear which patient subgroups should be revascularized as stenting carries small but significant risks. We have previously demonstrated that the ratio of magnetic resonance-measured renal volume to isotopic single kidney glomerular filtration rate (isoSK-GFR) is higher in kidneys which show functional improvement after revascularization. Blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) does not require contrast administration and is sensitive to changes in tissue concentration of deoxyhaemoglobin.

Methods. In this study, we test the hypothesis that baseline BOLD R2* map signal and R2*:isoSK-GFR ratio will provide an additional independent predictive biomarker of response to revascularization.

Results. Studies were performed in 28 subjects (16 ARVD and 12 controls). All subjects had R2* mapping and isoSK-GFR measured at baseline and at 4-month follow-up. MRI data were collected on a 3 T whole-body MRI scanner using a coronal dual-echo, 2D gradient-echo breath-hold acquisition. Parenchymal regions of interest (ROIs) were drawn on a representative slice through the middle of the kidney. Parametric maps of R2* were generated and mean values of R2* were calculated for every ROI. The ratio of R2*:isoSK-GFR at baseline was significantly greater in kidneys where renal function improved (5.91 ± 6.51) versus stable (1.78 ± 1.11), deteriorated (2.15 ± 1.79) or controls (1.5 ± 0.91), P = 0.003. R2*:isoSK-GFR ratio that was greater than 95% confidence interval of the control kidneys was 66.7% sensitive, but 85.7% specific in predicting a positive renal functional outcome.

Conclusions. These pilot data show that BOLD R2* imaging, presumably by detecting intra-renal deoxyhaemoglobin in still viable ‘hibernating’ parenchyma, coupled with isoSK-GFR may provide an effective predictive biomarker for positive renal functional response to revascularization. R2* imaging is non-invasive, quick to perform and could provide further insight into reversible parenchymal changes in ARVD kidneys.

Keywords: atheromatous renovascular disease; blood oxygen level dependent; magnetic resonance imaging; non-contrast imaging; R2*

Introduction

Atheromatous renovascular disease (ARVD) is a common disorder that presents considerable challenges for management. In most cases, ARVD develops as part of systemic atheromatous disease with accompanying macrovascular pathology in other important vascular beds, notably the coronary [1], iliofemoral [2] and cerebrovascular [3] circulations. Renal artery stenosis (RAS) is usually focal but can be more diffuse. It is of great clinical importance not only due to the potential blood flow-limiting effects upon the renal circulation and consequent intra-renal ischaemia but also because of downstream neurohormonal and cytokine imbalances resulting in hypertension, renal impairment and fluid and salt retention. The management of ARVD, particularly the role of renal revascularization, provides a dilemma as it might be expected to improve patient outcomes, which is often not the case.

Unfortunately, renal functional outcomes after revascularization in ARVD are unpredictable with only 30% of patients showing significant improvement in renal function [4]. The recently published ASTRAL trial [5], which compared endovascular revascularization plus medical therapy with medical therapy alone in patients with significant anatomical ARVD but with heterogeneous clinical presentation, showed no overall clinical benefit from revascularization. However, these overall grouped results may obscure the existence of subgroups of patients who did experience benefit from revascularization. Furthermore, almost 7% of patients experienced...
a serious adverse event related to revascularization. Consequently, the ability to predict beneficial response to revascularization is increasingly important if groups of potentially responsive patients are not to be denied effective treatment [6], while conversely patients who will not benefit can still be subjected to a treatment that is not risk free.

Investigative and predictive imaging tools currently used in ARVD all have limitations [7]. In recent years, there has been growing interest in magnetic resonance imaging (MRI), which can provide a wealth of functional information within a short period of time, without the use of ionizing radiation. However, heightened awareness of the risk of gadolinium-induced nephrogenic systemic fibrosis in patients with severe renal impairment has led to renewed interest in methods of non-contrast MRI [8].

Blood oxygen level-dependent (BOLD) MRI is a well-established and validated method that forms the basis of functional brain imaging techniques. BOLD R2* mapping uses MRI sequences sensitive to microscopic variations in the strength of the local magnetic field, variations of which result in rapid loss in MRI signal. These microscopic variations can be produced by the presence of paramagnetic substances: molecules that have a small but positive magnetic susceptibility (magnetizability—tendency to align along the magnetic field). While oxyhaemoglobin is diamagnetic, deoxyhaemoglobin has four free iron electrons and is paramagnetic and thus has a high susceptibility. The measured magnetic resonance (MR) increase in signal loss due to T2* effects will therefore be accelerated in the presence of deoxyhaemoglobin. This effect is measured by calculating the rate constant (R2* = 1/T2* ) of the free induction decay resulting from loss of phase coherence among spins oriented perpendicular to the static magnetic field. Tissue R2* is thus related to, and dependent on, the concentration of deoxyhaemoglobin in the tissue [9, 10]. The magnitude of the R2* mapping effect is directly related to magnetic field strength and previous workers have found that measurements in humans at high-field strength (3 T) better distinguish discrete parenchymal regions of the kidney and detect an impaired response to alterations in oxygen consumption more effectively than at 1.5 T [11].

In rat models with transient acute renal artery occlusion, a decreased R2* is observed within the ipsilateral medulla in parallel with a decreased medullary blood flow [12]. In swine models of RAS, BOLD signal correlates with invasive microelectrode measurements of oxygen partial pressure [13, 14]. Higher cortical and medullary R2* values are produced as renal blood flow decreases with increasing RAS [15]. No previous study has looked at the prognostic significance of R2* mapping in humans, and whether different R2* values predict a better or worse outcome post-revascularization.

**Aims and objectives**

**Bold imaging**  It is now well known that the more commonly measured parameters in assessment of ARVD (degree of RAS, 2D ultrasonic size) are poorly correlated with the degree of renal functional impairment. We have previously shown that parenchymal volume is the most accurate structural correlate of isotopically measured single kidney glomerular filtration rate (isoSK-GFR) [16]. In addition, the ratio of MR-measured renal volume:isoSK-GFR provides some predictive information about which kidneys might improve post-revascularization [17]. However, measurement of renal volume requires manual intervention and provides no specific information about the physiological status of residual renal tissue. In addition, manual volumetry is both time consuming and operator dependent. We therefore designed the current study to test the hypothesis that baseline BOLD R2* mapping will provide an additional, non-contrast requiring, independent predictive biomarker of response to revascularization, based upon the levels of deoxyhaemoglobin within ischaemic kidneys. To complement the R2* calculations, we coupled these values with isoSK-GFR to account for individual baseline renal function, as our previous work had shown that renal volume:isoSK-GFR can allow selection of those kidneys with the potential to improve after stenting [17].

**Materials and methods**

**Patient selection**

The study was approved by the local ethical committee (REC reference number 07/Q1/40521) and all patients gave fully informed consent prior to inclusion. Patients presenting to our tertiary referral centre who were deemed suitable for renal revascularization were requested to consider entry into a separate prospective study of revascularization in ARVD, of which this study formed a subset. The clinical decision to consider revascularization was based on (i) clinical grounds (e.g. poorly controlled hypertension on four or more anti-hypertensive agents, deteriorating renal function or flash pulmonary oedema) or (ii) randomization into the revascularization arm of an ongoing clinical trial, namely either the ASTRAL [5] or CORAL [18] trials. These two multicentre randomized controlled trials randomised patients with significant RAS to either medical therapy alone or medical therapy and renal stent placement. All patients underwent MRI and isoSK-GFR at baseline and at 4 months after treatment.

Control patients were those attending the clinic in whom there had been clinical suspicion of ARVD (i.e. signs and symptoms suggestive of renovascular disease such as poorly controlled hypertension, chronic kidney disease (CKD) or discrepant kidney sizes) but who had no evidence of RAS detectable by Magnetic resonance angiography or Computer tomography angiography. Control subjects underwent BOLD MRI R2* mapping and isoSK-GFR at baseline only.

**BOLD methodology**

Data were collected on a 3 T MR scanner (Philips Achieva; Philips Healthcare, Best, The Netherlands) using a dual-echo 2D gradient-echo breath-hold acquisition. The MRI parameters were: 10 oblique-coronal slices through both kidneys and 2 TE values, 1.9 and 25 ms; FOV = 400 × 400 mm, 128 × 90 matrix, slice thickness = 8 mm, flip angle = 60°, TR = 271 ms. All images were acquired in breath-hold and the process took <20 s in total.

Parenchymal regions of interest (ROIs) were drawn on a representative slice through the middle of the left and right kidneys of patients and controls. The ROIs included both the renal cortex and medulla (parenchymal regions) and excluded the renal collecting system and any incidental renal cysts. Parametric maps of T2* and R2* (1/T2*) were generated on a pixel-by-pixel basis using the dedicated software package Mistar (Apollo Medical Imaging Technology, Melbourne, Australia) and are illustrated in Figure 1. Mean T2* and R2* values (ms) were calculated for every ROI. The slope of natural logarithm of signal intensity versus echo time equals relaxation rate R2* (1/T2*) and is related to the concentration of deoxyhaemoglobin, thus R2* was the parameter used in our analysis.

**Renal function**

IsoSK-GFR was calculated using radioisotope techniques with 51Cr-ethylenediaminetetraacetic acid clearance and 99mTc-dimercaptosuccinic acid scintigraphy for the assessment of the differential static radioisotope uptake of each kidney [19]. Following revascularization, kidneys were...
initially classified as having improved, stable or deteriorated based upon the following thresholds:

- **Improved**—isoSK-GFR increased by at least 1 mL/min and by >15% compared to baseline.
- **Deteriorated**—isoSK-GFR decreased by at least 1 mL/min and by >15% compared to baseline.
- **Stable**—changes between the above two definitions.

**Statistical analyses**

Statistical analyses were performed using SPSS version 16.0. Normality of data was tested by histogram visualization of the data and analysis of confidence intervals (CIs). Continuous data following a normal distribution was described in terms of the mean, SD and range and compared using analysis of variance (ANOVA) (e.g. R2*). Discrete data were described as number and percentage of the grouping and analysed by chi-square analysis, with Bonferroni correction for groups of n < 5. Pre- and post-revascularization imaging results were compared within groups using a paired samples t-test and across groups using ANOVA as data was normally distributed.

With renal damage and shrinkage, post-stenotic kidneys demonstrate variable levels of R2* [20, 21]. Hence, the value of R2* and ratio of R2* to isoSK-GFR has not been defined for varying levels of isoSK-GFR. The control kidneys were used to define reference values for a given isoSK-GFR, so as to avoid under or overestimation of the R2*:isoSK-GFR ratio. Hence, control kidneys were grouped into six categories according to their isoSK-GFR. RAS kidneys were considered to have a high R2*:isoSK-GFR ratio when this exceeded the upper limit of the 95% CI for control kidneys for a given volume group.

All statistical analyses performed were considered significant at a level of \( P < 0.05 \).

**Results**

Complete data were available in 12 of 16 patients who were successfully recruited as control subjects, and 16 of 19 patients recruited to undergo renal revascularization. In the patient’s whose kidneys were not suitable for analysis of BOLD imaging, the reasons related to distorted anatomy (the kidneys were severely shrunken and distorted) and ROI definition could not be reliably achieved, producing biologically non-meaningful values. These kidneys were thus rejected from further analysis. Complete data were therefore available for 28 subjects (16 ARVD and 12 control subjects). Six of these patients underwent revascularization according to randomisation within the ASTRAL or CORAL trials, and the remainder due to clinical indications. Seven ARVD patients underwent bilateral revascularization, providing 23 kidneys in which analysis of BOLD results pre- and post-stenting was available.

Baseline clinical demographics are shown in Table 1. Control subjects were younger than the ARVD group. All other clinical comorbidities and biochemical parameters were similar, barring lower diastolic blood pressure in the group undergoing revascularization.

**Renal functional response to stenting**

Individual stented kidneys were classified as improved, stable or deteriorated depending on their renal functional response post-revascularization (Table 2). There was no significant difference in baseline individual kidney function between each group, but numbers were low. Using ANOVA analysis, the R2* values of the improved or stable kidneys were significantly higher than those of kidneys of control patients (greater concentration of deoxyhaemoglobin), \( P = 0.007 \) and \( P = 0.020 \), respectively. Kidneys that remained stable or improved had higher R2* values than those kidneys which deteriorated, although this was not statistically significant. Furthermore, kidneys that improved
had a significantly greater mean R2*:isoSK-GFR ratio than kidneys in other groups (P = 0.003), including controls (P = 0.002). Control kidneys had R2*:isoSK-GFR values that were similar to those of kidneys that had stable function after revascularization. Interestingly, contralateral kidneys displayed a similar pattern, in that those with a higher R2*:isoSK-GFR ratio, were more likely to improve renal function post-procedure even though they were not revascularized (Table 3).

Control kidneys were divided into glomerular filtration rate (GFR) group categories, which allowed calculation of the mean (95% CI) reference ranges for R2* and R2*:isoSK-GFR (Table 4). Values of R2* and R2*:isoSK-GFR in ARVD kidneys that exceeded the 95% CI of the controls were regarded as disproportionately larger than expected. Using this method, the sensitivity of R2* and R2*:isoSK-GFR in predicting a positive renal functional outcome after renal stenting was 40%, and 66.7% respectively, specificity 85.7%, with a positive predictive value of 80% and negative predictive value of 50%.

Discussion

In this prospective pilot study, we show that BOLD measurements combined with isoSK-GFR have the potential to act as prognostic markers to differentiate renal functional response post-revascularization. R2* values on their own have moderate sensitivity in predicting renal functional outcome. R2*:isoSK-GFR ratio improves the specificity of the prediction, with higher values providing increased specificity in predicting a positive renal functional outcome.

The R2* measurements are thought to be detecting higher deoxyhaemoglobin values produced by abnormal oxygen metabolism within ischaemic RAS kidneys. At baseline, tissue that is metabolically active and ‘suffering’ from impaired oxygen supply will produce more deoxyhaemoglobin and has a high R2* value. We postulated that

| Table 1. Baseline demographics of ARVD patients and controls
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<td><strong>Revascularization group, n = 16</strong></td>
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<tr>
<td>Age (years), mean (SD), range</td>
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| aDM, Diabetes Mellitus.                                     |
| bPrevious cardiovascular event includes either/or acute coronary syndrome, congestive cardiac failure, stroke, peripheral vascular disease. |
| cMean (SD), range.                                          |
| dNumber (%).                                                |
these biological conditions and hence such high R\textsuperscript{2*:isoSK-GFR} values would be present in kidneys supplied by a haemodynamically significant RAS, yet without having undergone significant irreversible renal structural change. Interestingly, the majority of kidneys had higher R\textsuperscript{2*} values post-revascularization than at baseline. The explanation for this is uncertain as we had postulated that once an ischaemic kidney had been reperfused the deoxyhaemoglobin would be washed out with an accompanying decrease in R\textsuperscript{2*}, although this was not uniformly the case. One suggestion might be that revascularization allowed an increased metabolic activity of tissues resulting in higher deoxyhaemoglobin. Further evaluation is required in a larger study.

During ischaemia, organs tend to extract more oxygen, decreasing oxygen saturation in capillaries and venous blood. Hypoxaemia also promotes vascular dilatation by diverse mechanisms including reduction in small vessel resistance and direct nitric oxide-mediated vasodilatation caused by regional hypoxia and acidosis. This vasodilatation can cause an increase in vascular volume with a consequent increase in absolute tissue deoxyhaemoglobin content. Both the increase in blood deoxyhaemoglobin concentration and blood volume fraction will directly and synergistically affect the BOLD signal. Under normal circumstances, the renal medulla operates at a state of near-hypoxia, due to a gradient of oxygen tension between it and the renal cortex. Prasad et al. [9] have shown that the BOLD signal is able to clearly discern the oxygenation gradient existing between the normally well-oxygenated renal cortex and the hypoxic medulla, and this has been applied to transplanted kidneys to detect changes in renal oxygenation post-transplant [22] and in acute [23] and chronic [24] allograft nephropathy.

Renal hypoxia leads to both desired and undesired effects that in most cases perpetuate the progression of renal decline and fibrosis by triggering transcription factors, hypoxia inducible factor and a host of other effectors. Our previous MRI studies in ARVD have shown that the renal volume:i-soSK-GFR ratio helps to select the functional response of kidneys to revascularization [17], with kidneys having the largest ratio showing improved function. We have assumed that a high ratio reflects kidneys with preserved renal volume yet low GFR i.e. haemodynamically significant RAS but
subject to the deleterious cascade of ischaemic events that results in irreversible structural change. Interestingly, the two contralateral kidneys that manifested improved renal function displayed a similarly increased \(R^2*:\text{isoSK-GFR}\) pattern. As unilateral RAS can result in systemic hypertension and associated neurohormonal changes (e.g. renin and angiotensin release), these can also affect the contralateral kidney. We postulate that contralateral kidneys are subject to hypertension and downstream injury as a consequence. It is plausible that these renin–angiotensin-mediated changes can be partly or fully reversed by revascularization of the affected kidney. Contralateral kidneys that are metabolically active and display higher \(R^2*\) values are thus also more likely to improve post-revascularization of the RAS lesion. This is supportive of ‘systemic’ neurohumoral activation and adaptation induced by RAS.

This study has a number of limitations, the greatest of which was the small patient sample size. However, this was a hypothesis-generating pilot study which aimed to explore the usefulness of \(R^2*\) mapping in predicting renal functional outcome post-revascularization, and this had not previously been tested to our knowledge. The results generated reached statistical significance and warrant a larger prospective study aiming to strengthen the methodology and practical usefulness of BOLD imaging in ARVD. A methodological limitation was that only one MR slice was analysed for BOLD data. The slice that was used, however, was a representative central slice within the kidney. In addition, \(R^2*\) maps were calculated only from two echo times which we would justify by trying to reduce MRI scan time to a minimum, given the biological status of our patients and their inability to breath-hold for long periods of time. As both blood flow and oxygen bioavailability are dynamic physiologic processes, further dynamic studies are needed to determine whether use of a single time point or a series of time points could improve the sensitivity of BOLD.

We also did not apply a stimulus (e.g. furosemide, water load) to challenge the intra-renal microcirculation. Instead, the clinical intervention of revascularization was the ‘applied stimulus’. We acknowledge that use of furosemide with BOLD imaging might improve identification of potential responder kidneys as furosemide increases medullary oxygenation by reducing tubular workload and oxygen consumption. \(R^2*\) mapping may then distinguish between severely compromised but viable parenchyma (high basal values of \(R^2*\) which would fall after the administration of furosemide) present in those kidneys likely to improve post-revascularization and non-functional scarred tissue (low basal levels, unaffected by furosemide). BOLD is also able to show a decreased renal tubular response to furosemide in kidneys supplied by a stenotic RAS as opposed to non-affected kidneys [21, 25].

However, our analyses were performed in an unselected ARVD population and the \(R^2*\) maps were generated from images obtained on a 3 T magnet, which has been shown to better distinguish discrete cortical and inner medullary regions of the kidney and measured differences in oxygen tension [11].

In summary, \(R^2*\) mapping is non-invasive, requires no exogenous contrast, and is quick to perform (one breath-hold) and may be a valuable addition to the standard MR

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**Fig. 2.** (a-c) Control subject’s \(R^2*\) map showing \(R^2*\) values of 20.06 and 21.87 s\(^{-1}\) in the left and right kidney, respectively.
investigative protocol for ARVD. R2* measurements coupled with isoSK-GFR may be a useful predictor of renal functional response post-revascularization, presumably by detecting intra-renal hypoxia relative to renal function in preserved ‘hibernating’ parenchyma. Despite certain methodological limitations, this pilot study extends the potential for BOLD MR to be applied as a functional tool to examine systemic renal disease effects in the renovascular kidney. R2* maps coupled with isotopically calculated GFR have an improved specificity rather than R2* values on their own. Further and more extensive analysis is now warranted in order to determine whether this method may allow appropriate selection of those patients most likely to benefit from revascularization, and at the same time, reduce the likelihood of those who will not benefit from being exposed to the risks associated with interventional procedures.

Acknowledgements. Funding. This work was made possible by a grant from Kidney Research, UK (Grant reference number: RP24/1/2006).

Conflict of interest statement. None declared.

References


Received for publication: 19.4.11; Accepted in revised form: 9.6.11