Advantages and Controversies in the Era of Intrarenal Volumetry

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Key Words
Animal models / Contrast-enhanced MRI / Kidney biopsy / Glomeruli / Microscopy / MRI volumetry / Pathology

Abstract
Background/Aims: Ultrasound is the preferred imaging modality in nephrology. In many kidney diseases, however, more accurate methods are needed to distinguish between relevant intrarenal structures. MRI could be a solution, although the use of MRI contrast has caused severe complications in some cases, and invasive kidney biopsies may follow, even though such small specimens traditionally provide inaccurate quantitative data. We evaluated the usefulness of MRI volumetry and quantitative kidney biopsies to assess glomerular number and volume as well as cortical volume.

Methods: We specifically highlight an experimental study in which different MRI scans were performed in healthy pigs as well as pigs with unilateral ureteral obstruction to assess intrarenal volume. Single-kidney glomerular filtration rate (GFR) was measured using ureteral cannulation and 51Cr-EDTA. Kidney biopsies were taken and evaluated employing stereological techniques to measure number and volume of glomeruli. Pigs were sacrificed and kidneys were removed for stereological analysis. Non-contrast-based MRI intrarenal volumes were – without significant difference to intrarenal volumes – obtained using contrast-enhanced MRI and ex vivo techniques. Results: Kidney biopsies gave valid estimates regarding quantitative parameters, such as mean number and volume of glomeruli in the cortex. Different structural parameters correlated with kidney GFR with high, although varying, correlation coefficients. Conclusion: Non-contrast MRI is suitable for estimating intrarenal volumes in healthy and diseased kidneys. We advocate further research in diagnostic modalities combining MRI and biopsies. Major challenges are the cortical architecture and heterogeneous distribution of glomeruli within the kidney.

Introduction

This article advocates the use of complementary MRI and stereology methods in nephrologic diagnostics. Today, ultrasound is the preferred imaging modality, and whole-kidney volume is based on a pole-to-pole length [1–3]. Kidney volume is a highly relevant parameter as many diseases and particularly chronicity are associated with changes in kidney size, whereas pole-to-pole length only gives a crude estimate of renal volume [4]. Further-
In this paper, we systematically communicate our experimental findings with combined intrarenal volumetry by MRI and stereological evaluations of renal biopsies, motivating this approach for clinical purposes. We will demonstrate how in vivo volume measures can be derived in a (semi-)automatic and user-independent way with high accuracy, and whenever both MRI and biopsy sam-

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If no contrast media are available, we will demonstrate how these seemingly different methods can provide complementary information about different structural parameters as well as renal function.

**Renal MRI Combined with Stereological Methods**

Absolute volume can be directly determined using several imaging techniques (e.g. MRI or CT), where the whole kidney, renal vessels, and renopathological structures can be depicted in three dimensions and entirely immersed in a spatial grid by virtual imaging techniques. Note, however, that the precise incidence of contrast-induced nephrotoxicity following intravenous contrast administration remains unclear in vivo both by MRI (due to the advent of the gadolinium-induced nephrogenic systemic fibrosis) and CT (iodinated contrast media). Thus, we propose a completely non-invasive MRI procedure, with a fast, efficient and reliable protocol, as the imaging method of choice to extract intrarenal volumes. In brief, differences in the intrinsic magnetic properties of water between renal segments can be used to selectively visualize intrarenal segments. MRI is capable of extracting these details on a three-dimensional (3D) grid by exploiting the magnetic relaxation properties of water in the renal cortex, medulla and pelvis using a strong T1-weighted sequence. Most available clinical MRI systems comprise acquisition techniques governed by inversion recovery spin echo sequences that are particularly sensitive to differences in T1 values. No contrast media are needed. Further, we introduce stereological methods based on sound mathematical and statistical principles to obtain quantitative data for the estimation of N_{glomerus} and V_{glomerus} [23, 26]. Stereology makes use of ‘the physical fractionator’, where the number of objects (e.g. N_{glomerus} in the whole renal cortex) is obtained from estimates of fractions sampled from that whole structure (e.g. a known fraction of the cortex); ‘the disector’ is a technique for unbiased sampling of objects using section planes (e.g. 2 consecutive slices of the renal cortex), and ‘the Cavalieri principle’ can be used for estimating the volume of structures divided into parallel planes (e.g. a kidney or a glomerulus divided into several parts, of which each profile area can be estimated).

**Methods**

**UO Model**

To demonstrate the usefulness of the proposed MRI and stereology means, we draw attention to our motivating findings reported in a UO animal model [27–29]. As mentioned, UO is followed by several renal complications and is known to upregulate the renin-angiotensin system, leading to intrarenal hypoxia and resulting in loss of nephrons over time [30]. In a reversible unilateral UO pig model, with ureteral ligature being induced by occlusion, we have previously observed marked changes in the cortical and medullary oxygenation in parallel with a significant reduction in the number of nephrons [31, 32].

**MRI: Cortical Volume**

Multi-slice MRI was performed in this UO pig model using a respiratory-gated 2D IRTSE MRI sequence (field-of-view = 320 × 320 mm², matrix = 256 × 256, slice thickness = 3 mm, time of recovery = 4,500 ms, echo time = 8 ms, time of inversion = 100–900 ms; 1.5-tesla MRI system). Volumetric data of a single MRI slice are demonstrated in figure 1, with a clear contrast between renal cortex and surrounding segments, allowing a straightforward semiautomatic segmentation of the renal cortex. In the same way, it is possible to define areas of the medulla and pelvis using dedicated volumetric segmentation software. Based on this technique, intrarenal areas were semiautomatically segmented using a 3D watershed-based algorithm. In this application, Vc could be calculated from the sum-of-areas principle [33]. Interestingly, we found that Vc in both healthy and UO kidneys could be measured...
accurately with MRI using semiautomatic as well as manual segmentation, and measurements were obtained with a high intra- and inter-observer reproducibility (covariance level <5%) [29].

**Stereology: Glomerular Number and Volume**

In the same UO porcine model, 6 biopsies were sampled from each kidney, and estimates of N_{glomerular} and V_{glomerular} of both healthy and obstructed kidneys were derived by stereology. In short, each biopsy was divided into a medullary part and a cortical part using a stereomicroscope. The mass of the cortical part was measured, and a volume for each cortical biopsy was calculated assuming that the mass density of renal cortex was 1.04 g/cm^3. The biopsies were embedded in plastic (Technovit 7100) and cut exhaustively into 20-µm-thick slices. Digital images were obtained from all slices using analysis software (newCAST, Visiopharm, Denmark), and glomeruli were sampled using ‘the disector principle’ (fig. 2).

The volume of a single glomerulus was estimated on the basis of the glomerular profile area and ‘the Cavalieri principle’. N_{glomerular} was then given by the equation: N_{glomerular} = N_{glomerular/cor} * V_{cor}, where N_{glomerular/cor} is the cortical glomerular density obtained from the N_{glomerular} in the biopsy and from the biopsy volume, and V_{cor} is the renal cortical volume. The total V_{glomerular} was then given by the equation: V_{glomerular} = V_{glomerular} * N_{glomerular}, where V_{glomerular} is the mean glomerular volume given by N_{glomerular} and V_{glomerular} within the biopsies. Ex vivo kidneys, processed for microscopy, underwent stereological analysis using the physical fractionator, the disector and test point systems for control methods regarding N_{glomerular} and V_{glomerular}. In this study, ex vivo estimates regarding V_{cor} and N_{glomerular} were used to calculate N_{glomerular} and V_{glomerular} from biopsy, respectively. Determination of both V_{cor} and N_{glomerular/cor} makes it possible to calculate total V_{glomerular}.

**Results**

**Volumetric Measures Are Associated with Intrarenal Function**

Both MRI and stereological means demonstrated pronounced intrarenal changes in the UO kidney compared to the control group [29]. For example, calculation of V_{cor} revealed a volume reduction of 28.9% in the obstructed kidney compared to V_{cor} of healthy kidneys. Stereology demonstrated that N_{glomerular} of UO kidneys was 18.2% reduced compared to the control group, suggesting that UO is followed by a substantial loss in nephrons. Interestingly, this 18.2% reduction in N_{glomerular} was accompanied by a 36.8% reduction in V_{glomerular} in the UO kidneys, suggesting loss of extracellular matrix. Next, we investigated the association between renal structural parameters and renal function. The structural parameters were renal V_{cor}, total renal volume, N_{glomerular}, and total V_{glomerular}, and renal function was expressed by the single-kidney GFR (skGFR) obtained by ^{51}Cr-EDTA clearance. Investigations were performed using the described UO pig model, and healthy pigs were considered as the control group. We found that skGFR overall correlated linearly with both N_{glomerular} and V_{glomerular}, V_{cor} and total kidney volume, with a coefficient of determination (based on linear fitting) in the range of 0.62–0.78 [28]. Figure 3 shows the derived linear prediction with 95% CI, and we believe that the observed correlations between structural parameters and renal function suggest that these parameters may potentially be useful as surrogate markers of renal function.

**Discussion and Conclusion**

Indeed, ultrasonography plays a critical role in many aspects of nephrological practice. The simplicity of this technique coupled with portability, low costs, and safety makes ultrasonography the modality of choice for kidney and vascular imaging. Nevertheless, disadvantages of ultrasonography include its operator dependence, limited imaging capability in patients with a large body habitus,
decreased intrarenal sensitivity, and complete inability to assess renal function. We believe that calculated volumes of either the whole kidney or intrarenal segments represent the sum of general kidney growth and loss of nephron mass caused by associated renal disease. In current clinical practice, volume measurements are not usually used in routine examinations of renally impaired patients. Nonetheless, we refer to several renopathological conditions where volumetry would be clinically important because renal size gives insight into renal functional reserve or the extent of renal injury, including glomerulonephritis [8, 9], allograft nephropathy [10], parenchymal renal diseases [11], renal artery stenosis [12–14], and UO [19]. High-resolution MRI is capable of delineating particular intrarenal structures without radiation exposure and provides excellent tissue contrast. In addition, since multi-slice dynamic contrast-enhanced MRI with sufficient temporal resolution is now feasible by many clinical MRI devices, assessments of renal blood flow and renal function (clearance) can be evaluated in subjects with regional renal dysfunction [34]. A combination of intrarenal volumetric measures (such as $V_{\text{glom}}$) with functional measures (filtration capacity per unit mass) allows a novel imaging-based method for important clinical data, such as skGFR [34].

We therefore suggest a complementary MRI volumetric approach without the need of contrast enhancement, combined with stereological analyses of biopsy samples. We have demonstrated that intrarenal measurements of $N_{\text{glom}}$, $V_{\text{glom}}$ and $V_c$ help to detect important structural

**Fig. 3.** Prediction of skGFR from the parameters $V_{\text{cor}}$ (a), total renal volume ($V_{\text{total}}$) (b), $N_{\text{glom}}$ (c), and total $V_{\text{glom}}$ (d). The full-drawn line indicates the best prediction, the long dashed lines indicate the 95% CI limits, and the dotted lines indicate the 95% prediction interval limits.
and functional changes in a renally impaired animal model (UO pig model). In conclusion, we advocate a paradigm shift in current nephrological diagnostics where underlying structural and renophysiological changes in kidney disease are diagnostically evaluated on a quantitative intrarenal level.

References


Disclosure Statement

The authors declare that they have no conflicts of interest.

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Am J Nephrol 2011;33(suppl I):40–45

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