Intermediate Volume on Computed Tomography Imaging Defines a Fibrotic Compartment that Predicts Glomerular Filtration Rate Decline in Autosomal Dominant Polycystic Kidney Disease Patients

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Total kidney and cyst volumes have been used to quantify disease progression in autosomal dominant polycystic kidney disease (ADPKD), but a causal relationship with progression to renal failure has not been demonstrated. Advanced image processing recently allowed to quantify extracystic tissue, and to identify an additional tissue component named “intermediate,” appearing hypoenhanced on contrast-enhanced computed tomography (CT). The aim of this study is to provide a histological characterization of intermediate volume, investigate its relation with renal function, and provide preliminary evidence of its role in long-term prediction of functional loss. Three ADPKD patients underwent contrast-enhanced CT scans before nephrectomy. Histological samples of intermediate volume were drawn from the excised kidneys, and stained with hematoxylin and eosin and with saturated picrosirius solution for histological analysis. Intermediate volume showed major structural changes, characterized by tubular dilation and atrophy, microcysts, inflammatory cell infiltrate, vascular sclerosis, and extended peritubular interstitial fibrosis. A significant correlation (r = −0.69, P < 0.001) between relative intermediate volume and baseline renal function was found in 21 ADPKD patients. Long-term prediction of renal functional loss was investigated in an independent cohort of 13 ADPKD patients, followed for 3 to 8 years. Intermediate volume, but not total kidney or cyst volume, significantly correlated with glomerular filtration rate decline (r = −0.79, P < 0.005). These findings suggest that intermediate volume may represent a suitable surrogate marker of ADPKD progression and a novel therapeutic target.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common renal hereditary disorder and the fourth leading cause of end-stage renal disease (ESRD) in adults.1,2 ADPKD can arise from mutations in either the PKD1 gene (which encodes the protein polycystin 1) or the PKD2 gene (encoding polycystin 2). PKD1 is more severe than PKD2-associated disease, the mean age of onset of ESRD being 54 years and 74 years for PKD1 and PKD2, respectively.3 The clinical hallmark phenotype of this inherited condition is the progressive and marked enlargement of the kidneys caused by sustained expansion of multiple fluid-filled cysts originating from the tubule wall4 leading to crowding of adjacent nephrons and to injury of normal parenchyma.5–9 In ADPKD, renal function remains normal for a long period of time before starting to decline inexorably when the noncystic parenchyma has incurred serious damage.10 This often leaves patients who have normal renal function with the false belief that little renal
damage has occurred. This points out the urgent need of reliable markers of disease progression other than glomerular filtration rate (GFR), capable of detecting existing damage and possibly predicting progression over a relatively short period of time while renal function, as measured by serum creatinine or estimated GFR (eGFR), is normal or relatively preserved. The identification of such markers is deemed necessary both for clinical follow-up and for testing new therapeutic strategies in the context of clinical trials.

Since renal enlargement due to cyst growth is the underlying macroscopic process, image-based measurements have been used in the literature to quantify kidney disease progression in ADPKD.11–14 The CRISP (Consortium for Radiological Imaging Studies of Polycystic Kidney Disease) study showed that in ADPKD patients, total kidney volume and cyst volume increase exponentially with age11 and that magnetic resonance imaging can reproducibly detect this change.12,13 However, a causal relationship between kidney or cyst volume increase and the ultimate progression to renal failure has not been demonstrated. On the other hand, changes in noncystic tissue components, which may in principle be closely connected to GFR decline in ADPKD, are not necessarily directly dependent on changes in kidney or cyst volume. The increasing resolution of imaging systems now available allows one to reliably discern cystic and noncystic tissue components.15 In a recent study,15 we took advantage of advanced image processing techniques to characterize extracystic tissue on contrast-enhanced computed tomography (CT). In that work, we subdivided extracystic tissue into two separate components, fully enhanced parenchyma and hypoenhanced tissue, which we called “intermediate.”15 We found that in patients with ADPKD and normal renal function to severe renal dysfunction, the ratio of intermediate volume relative to parenchymal volume significantly correlated with both baseline GFR and GFR changes over the 6-month follow-up period.15 In that small cohort of patients, neither cyst nor parenchymal volumes correlated with GFR and GFR decline. Thus, quantification of the noncystic fraction of the polycystic kidney may provide an effective image-based method to gauge renal function loss16 and predict the rate of decline more reliably than total kidney or total cyst volume. However, the nature of intermediate volume and its role in long-term renal function loss remain ill defined. Actually, there are a number of preclinical studies showing that diffuse interstitial fibrosis occurs within renal parenchyma in ADPKD.17–19 Moreover, the presence of interstitial fibrosis and tubular atrophy in patients with ADPKD and incipient renal failure has been documented in the context of a clinical study.20 The purpose of the present study was then to characterize the structural nature of intermediate tissue on kidneys excised from three ADPKD patients with end-stage renal disease already on hemodialysis who underwent contrast-enhanced CT before surgery, relating radiological findings with histological findings. In addition, we investigated the relation between intermediate volume and renal function. This was done in two independent cohorts of ADPKD patients, enrolled in two distinct studies, the SIRENA (Sorilimus Treatment in Patients with Autosomal Dominant Polycystic Kidney Disease: Renal Efficacy and Safety) study21 aimed to assess the effects of mTOR inhibition on disease progression (SIRENA cohort) and the clinical study we performed22 to assess the effect of somatostatin treatment on disease progression (somatostatin cohort). Specifically, the SIRENA cohort data were used to further investigate the previously reported correlation between intermediate volume and GFR.15 The somatostatin cohort data, augmented by long-term clinical follow-up observations, were used to assess the power of intermediate volume to predict renal function decline over a 5-year follow-up.

Materials and Methods

Patients

Between October 2008 and September 2009, three consecutive ADPKD patients underwent bilateral nephrectomy at the Nephrology Unit of the Azienda Ospedaliera Ospedali Riuniti di Bergamo, after providing their written informed consent. Patient 1 (female, 67 years old) had polycystic kidney disease, known since the age of 29, with both kidney and liver involvement, and a family history of ADPKD (mother). At the age of 65, she developed ESRD and started renal replacement therapy with hemodialysis. A few months later, she had repeated episodes of sepsis secondary to cholelithiasis and/or infections of liver and kidney cysts. She underwent cholecystectomy and bilateral nephrectomy. Patient 2 (male, 38 years old) had polycystic kidney disease, known since the age of 17, and a family history of ADPKD (mother). Six months after starting hemodialysis due to ESRD (at the age of 35), he underwent binephrectomy due to repeated macrohematuria episodes in preparation for renal transplantation. Patient 3 (male, 48 years old) had polycystic kidney disease, known since birth, and a family history of ADPKD (brother). He started hemodialysis treatment at the age of 48 and, after 2 months, underwent binephrectomy due to recurrent episodes of abdominal pain that was thought to be due to his cystic kidneys. No complications arose during the nephrectomies in patients 1 and 2; patient 3 had significant bleeding, and the overall blood loss (about 700 mL) was reintegrated. Before surgery, all patients underwent a contrast-enhanced CT scan.

CT Acquisition

CT acquisition was performed using a 64-slice CT scanner (Lightspeed VCT; GE Healthcare, Milwaukee, WI). A single breath-hold scan (120 kV; 150 to 500 mAs; matrix 512 × 512; collimation 2.5 mm; slice pitch 0.984; increment 2.46 mm) was initiated 80 seconds after injection of 100 mL (nominal dose, adjusted by patient weight) of an iodinated nonionic contrast agent (Iomeron 350; Bracco, Italy) at the rate of 2 mL/second. Once acquired, images were transferred in DICOM 16-bit format from the clinical scanner onto PC workstations for subsequent processing.
Intermediate Volume Identification on Late-Stage ADPKD CT Images

We previously developed an automatic procedure to identify and quantify intermediate volume based on the presence of three different renal tissue components (cyst, intermediate, and parenchymal volumes). To determine intermediate volume on late-stage ADPKD CT scans, where residual parenchyma is not detectable, we computed the intensity range of intermediate volume in the 21 ADPKD baseline CT scans of the SIRENA cohort (ADPKD patients with GFR ≥40 mL/minute), acquired using the same CT scanner and acquisition protocol as adopted in the present study. The minimum intermediate volume intensity in the previously acquired CT scans was 41 ± 12 Hounsfield Units (HU), whereas the maximum intensity was 91 ± 23 HU. Intermediate volume intensity (in terms of HU) was found to be independent of patient weight, in line with the fact that contrast agent dose was determined based on patient weight. No significant difference was found between mean intermediate volume intensity in patients with low (≤300 mL) and high (>300 mL) parenchymal volume, suggesting that intermediate volume intensity does not depend on the amount of residual parenchyma. The average intermediate volume intensity range (41 to 91 HU) was restricted to (50 to 80 HU) to exclude partial volume effects, and the latter range was used to identify intermediate volume in the three patients considered in the present study.

Intermediate Tissue Identification on Excised Kidneys

After nephrectomy, kidneys were fixed in formalin by immersion and stored at 4°C for 24 hours. To collect tissue samples from excised kidneys that corresponded to intermediate volume on CT scans, an image-guided harvesting procedure was set up. For each patient, kidney outlines were traced on CT images by a trained operator (A.C.), using an interactive image editing software (ImageJ; NIH, http://rsbweb.nih.gov/ij). Three-dimensional (3D) renderings of the outlined CT images were then generated using the 3D Slicer software (http://www.slicer.org). At the time of sample collection, the 3D rendering, displayed on a laptop at the harvesting site, was rotated to match the orientation of the excised kidney on the bench. Kidneys were then transversally sectioned, and the corresponding reformatted CT slice was generated in the rendering software to guide kidney tissue harvesting. Tissue samples were then excised from kidneys under image guidance as shown in Figure 1. Samples from kidney tissue (approximately 1 cm³) located in areas of the reformatted image identified as intermediate on CT scans and from cyst walls were collected (Figure 1).

Histological Characterization

Kidney tissue samples corresponding to intermediate volume were processed for light microscopy using standard techniques. Each sample was post-fixed in 10% neutral formalin, dehydrated in alcohols, and then embedded in paraffin. Three-micron-thick sections (Ultratome V; LKB, Bromma, Sweden) were stained with H&E reagent for histological evaluation and with saturated picrosirius solution (0.1% Sirius Red in picric acid) for collagen staining. For Sirius Red staining, slides were taken through graded ethanol solution (100%, 90%, 80%) into distilled water. Slides were then stained for 1 hour in saturated picric acid with 0.1% Sirius Red (Aldrich Chemical, Milwaukee, WI). After incubation with Sirius Red, slides were washed in 0.01 N hydrochloric acid for 2 minutes, rapidly dehydrated through graded ethanol solution and toluene, and finally mounted with a coverslip in Eukitt mounting medium (GmbH, Freiburg, Germany). Descriptive morphological evaluation was performed for the different tissue components, namely glomerular, tubular, and vascular structures, as well as renal interstitium, including matrix deposition and cell infiltrates. As control condition, normal renal tissue from kidney sections excised because of adenocarcinoma was processed and evaluated in parallel.

Renal infiltrating macrophages were identified by immunoperoxidase staining with an antibody directed against a specific monocyte/macrophage antigen, CD68 (mouse anti-human macrophages; DakoCytomation, Glostrup, Denmark). The paraffin-embedded kidney sections (3 mm) were deparaffinized and rehydrated. For immunoperoxidase analysis of CD68 antigen, the sections were incubated for 30 minutes with 0.3% H₂O₂ in methanol to quench endogenous peroxidase and permeabilized in 0.1% Triton X-100 in PBS 0.01 mol/L (pH 7.2) for 30 minutes. Before quenching endogenous peroxidase, kidney samples were treated with proteinase-K (20 mg/mL; Sigma-Aldrich, Milan, Italy) for 10 minutes at 37°C, instead of Triton X100, followed by microwave [twice for 5 minutes in citrate buffer for 10 mmol/L (pH 6) at an operating frequency of 2450 MHz and 600-W power output] and citrate buffer (15 minutes) incubations. Pri-
mary antibody was diluted (1:100) and added overnight at 4°C. Subsequent steps included incubations with the secondary biotinylated antibody [sheep anti-mouse IgC (Chemicon International, Temecula, CA), avidin-biotin peroxidase complex solution, and finally, development with diaminobenzidine. The sections then were counterstained with Harris hematoxylin (Biooptica, Milan, Italy). Negative controls were obtained by omitting the primary antibody on adjacent sections.

**Correlation between Intermediate Volume and Renal Function**

We retrospectively considered the 21 ADPKD patients of the SIRENA cohort. Using the same quantification method previously described, we computed Pearson’s correlation between the volume of each tissue component, including relative intermediate volume (defined as the ratio of intermediate volume over residual parenchymal volume, as described in our previous work) and GFR, determined by iohexol plasma clearance. To further validate the relationship between intermediate volume and renal function at the time of CT scan, we also pooled the data from the SIRENA cohort with data from the somatostatin cohort, on which this correlation had been first identified.

**Long-Term Prediction of Renal Function Decline by Intermediate Volume**

To investigate the potential long-term predictive power of intermediate volume on renal function decline, we evaluated the follow-up of the somatostatin cohort from 2001 to 2010. Long-term GFR decline in individual patients was estimated using longitudinal data available in electronic outpatient medical records at the Nephrology Unit of Azienda Ospedaliera Ospedali Riuniti di Bergamo. GFR estimation was performed for each clinical observation using plasma creatinine and the abbreviated MDRD (Modification of Diet in Renal Disease) formula (four variables). The individual slope of GFR decline (ie, the slope of the regression line of eGFR versus observation time) was computed. Because some patients started dialysis treatment during the follow up period, only pre-dialysis follow-up data were considered. Patients with observation times shorter than 30 months or eGFR lower than 30 mL/minute/1.73 m² at baseline were excluded from the analysis. Thus, these patients had normal renal function or moderate renal insufficiency at the time of CT imaging. The relationship between relative intermediate volume at the time of CT acquisition and slope of GFR decline (mL/minute/month) over the observation period was then evaluated. The same analysis was repeated to investigate the relationship between either total kidney or cyst volume and the slope of GFR decline.

**Statistical Analyses**

The relationship between intermediate volume and renal function was evaluated by Pearson’s correlation. Individual GFR decline was computed by linear regression of eGFR versus observation time. The relationships between intermediate volume, total kidney or cyst volume, and regression slopes were evaluated by Pearson’s correlation. All statistical analyses were performed using the R statistical software (http://www.r-project.org).

**Results**

**Histological Characterization**

Renal samples for histological characterization of intermediate volume were collected from tissue areas matching the image identified as intermediate volume on CT scans. Representative images of the histological analysis performed on tissue sections stained with H&E and Sirius Red are reported in Figures 2 and 3, respectively. In all ADPKD samples (A–F), the renal parenchyma structure was severely altered as compared to normal kidney tissue (G and H). Tubular dilatation and microcyst are the major changes documented (Figure 2, A–D), and marked tubular atrophy was also found (Figure 2, B, D, and F). Tubular enlargement was associated with dilation of glomerular Bowman’s space (Figure 2B). Most of the tissue...
sections showed important interstitial fibrosis localized predominantly in the peritubular area. Deposition of fibrotic material was more pronounced near the wall of small cysts. Focal cellular inflammatory infiltrates were also found (Figure 2, C–E), occasionally including fibroblasts (Figure 2E). Vascular sclerosis and lumen narrowing were consistently documented (Figure 2F). This pattern of structural changes consistently characterized all samples collected in the tissue areas between large cysts and belonging to the intermediate volume as identified at CT scan. The localization of important renal fibrosis in these samples was confirmed by Sirius Red staining of collagen deposition (Figure 3). Several glomeruli showed marked thickening of Bowman’s capsule associated with concentric collagen deposition in periglomerular fibrotic areas (Figure 3, A and F). Marked peritubular collagen staining was found in all samples and involved both dilated and small atrophic tubules (Figure 3 B, D, and E). Sirius Red staining also localized in vascular wall confirming severe vascular fibrosclerosis (Figure 3, E and F). Important fibrosis was present within the cyst wall. As shown in detail in Figure 4, A and B, organized fibrils of collagen characterize cyst wall. Immunohistochemical analysis of the cellular infiltrate revealed that CD68+ monocytes were largely present in all biopsies. As shown in Figure 4, C and D, a marked infiltration of CD68+ cells was observed in fibrotic intermediate renal volume and around atrophic tubules.

Correlation between Intermediate Volume and Renal Function

The analysis of the correlation between relative intermediate volume and GFR in the SIRENA cohort confirmed the significant correlation found in the original work involving the somatostatin cohort (SIRENA: \( r = -0.69, P < 0.001 \), regression line: \( y = 107.57 - 0.22x \); somatostatin: \( r = -0.79, P < 0.005 \), regression line: \( y = 106.30 - 0.21x \)) with very similar correlation and slope. Again, no correlation was found between either total kidney or cyst volume and GFR (total kidney volume: SIRENA, \( r = -0.41, P = 0.07 \); somatostatin, \( r = 0.10, P = 0.75 \); total cyst volume: SIRENA, \( r = -0.43, P = 0.05 \); somatostatin, \( r = 0.21, P = 0.51 \)). As shown in Figure 5, we also found a significant correlation between relative intermediate volume and GFR in the pooled patient cohort (\( r = -0.78, P < 0.001 \), regression line: \( y = 106.34 - 0.21x \)). The regression slopes in the independent and pooled samples were very similar, despite differences in disease stage and CT acquisition protocol in the two cohorts. In the pooled patient cohort, absolute intermediate volume also significantly correlated with GFR (\( r = -0.46, P < 0.01 \), but the correlation was weaker than for relative intermediate volume (Figure 6). No correlation was found between either total kidney or cyst volume and GFR. Residual parenchymal volume significantly correlated with renal function, although the strength of such correlation was low (\( r = 0.37, P < 0.05 \)).

Prediction of GFR Decline by Intermediate Volume

Out of the 13 ADPKD patients enrolled in the somatostatin cohort, one was excluded from the longitudinal analysis.
due to the limited follow-up (24 months), which led to an unreliable slope of GFR decline (GFR versus time: \( r = 0.40, P = 0.13\)), and another due to the advanced stage of renal disease at baseline, proximal to dialysis (eGFR = 24 mL/minute/1.73 m\(^2\)), which led to low residual parenchymal volume and, consequently, a large intermediate/parenchymal volume ratio. In the remaining 11 patients, initial eGFR averaged 57 ± 19 mL/minute/1.73 m\(^2\) (range, 34 to 85). The mean observation period after first CT acquisition was 65 ± 26 months (range, 30 to 97). As expected, renal function progressively declined in all patients (Table 1), and the slope of eGFR ranged from −0.0790 to −0.5576 mL/minute/1.73 m\(^2\)/month. At CT scan, intermediate and parenchymal volumes were on average 503 ± 197 and 239 ± 62 mL, respectively. Intermediate volume relative to parenchymal volume ranged from 85% to 311% (mean 217% ± 68%). Both absolute and relative intermediate volume (Figure 7A) at the time of first CT scan significantly correlated with the slope of eGFR decline (\( r = −0.63, P = 0.037\); Vint/Vpar, \( r = −0.79, P < 0.004\); where Vint is the intermediate volume and Vpar is the parenchymal volume). Conversely, no significant negative correlation was found between either total kidney (Figure 7B) or cyst volume (Figure 7C) and long-term GFR decline. Baseline cyst volume showed a positive significant correlation with GFR decline, whereas total kidney volume showed no significant correlation. Additional analyses, performed including all 13 patients, confirmed the original results, showing that Vint/Vpar (%) is significantly associated with slope of GFR decline (\( r = −0.68 \) and \( P = 0.010 \) versus \( r = −0.79, P < 0.005 \)) whereas total kidney and cyst volumes are not (total kidney: \( r = 0.12, P = 0.698 \); cyst volume: \( r = 0.22, P = 0.475 \)).

### Discussion

In the present study, we investigated the structural nature of what we have previously defined “intermediate” volume, radiologically identified as hypoenhanced regions of extracystic tissue in ADPKD kidneys on contrast-enhanced CT. Intermediate volume has specific image intensity on contrast-enhanced CT (ranging from 50 to 80 HU), independently of patient size and of the amount of residual parenchyma. This, together with the availability of automatic algorithms for separating different tissue components, ensures the possibility to robustly and accurately identify it on CT scans. We found that histological samples of tissue corresponding to intermediate volume on CT images were consistently characterized by sparse dilated tubules, microcysts, and peritubular interstitial fi-

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Table 1. Individual Data Related to ADPKD Patients Included in the Investigation of Long-Term Prediction of Functional Loss by Intermediate Volume

<table>
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<tr>
<th>Patient number</th>
<th>eGFR at CT</th>
<th>Predialysis follow-up (months)</th>
<th>eGFR monthly decline</th>
<th>Vint/Vpar (%)</th>
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<td>42.7</td>
<td>30</td>
<td>−0.5350</td>
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<td>3</td>
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<td>5</td>
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<td>33</td>
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</table>

Patients were enrolled in the previous CT-based somatostatin study.\(^{22}\) eGFR monthly decline was defined as the slope of eGFR regression (mL/minute/1.73 m\(^2\)/month) versus time of observation. eGFR, glomerular filtration rate estimated using plasma creatinine and the abbreviated MDRD formula (mL/minute/1.73 m\(^2\)); Vint, intermediate volume; Vpar, parenchymal volume.
brosis, in line with previous histological findings showing the presence of interstitial fibrosis in ADPKD. From an imaging standpoint, hypoenhancement of intermediate volume on contrast-enhanced CT could then reflect the uptake of contrast agent in the sparse, dilated tubules and the reduced uptake throughout the fibrotic tissue, where peritubular capillaries are stretched with secondary hypoperfusion, globally leading to a loss of enhancement on CT images at the macroscale.

Interstitial fibrosis, the main histological finding we documented in the samples harvested from tissue having a hypoenhanced tissue appearance on CT may derive from cortical ischemia as suggested by the severity of vascular lesions and lumen narrowing. Our results, showing marked infiltration of macrophages in the context of fibrotic tissue, suggest that the tubulointerstitial damage may be the consequence of cytokines/chemokines and growth factors release by inflammatory cells known to contribute to interstitial fibrosis. At histological examination, a peculiar finding was the prominent deposition of fibrotic material adjacent to the wall of small cysts as well as diffusely in the interstitium. Indeed, evidence is available that cyst-lining epithelial cells produce large amounts of structural (collagen I and III, laminin) and soluble extracellular matrix-associated proteins (TGF-β, periostin) that accumulate around the cysts. Human and animal models of polycystic kidney disease also showed abnormal expression of matrix-degrading enzymes and inhibitors of metalloproteinases necessary for remodeling of interstitial extracellular matrix. Together, these observations point to an important role of epithelial cells in the development of surrounding fibrosis in ADPKD. Abnormal expression of growth factors TGF-β and PDGF by tubular epithelial cells may stimulate epithelial to mesenchymal transition and, ultimately, fibroblast generation in the cyst wall. This would account for the marked fibrosis of the cyst wall we found at histological examination of the removed ADPKD kidneys. Fibrosis within the cyst wall may be also induced by mechanical stimulation of cells due to hydrostatic pressure and development of organized structure of the wall to increase mechanical resistance. Tubular atrophy and interstitial fibrosis are known to occur concurrently to cyst development even in ADPKD patients and normal renal function, suggesting that characterization and monitoring of these phenomena could take place beginning in the earliest stages of the disease.

The compression of expanding cysts on residual parenchymal tissue and the stretching of kidney microves-sels with secondary hypoperfusion have been suggested to play a role in the disruption of the normal tissue architecture that eventually sustain the progressive renal functional loss. In light of this hypothesis, the sequential measurement of total kidney and cyst volume has been put forth as a surrogate marker of disease progression and total kidney and cyst volume have been often adopted in clinical trials as outcome measures. Our group, among the others, recently found a significant effect of somatostatin and sirolimus on total kidney and cyst volume increase, further supporting the adoption of total and cyst volumes as outcome variables in the search for new therapies in the context of clinical studies.

Although it could be intuitively anticipated that some link between cyst burden and renal functional impairment exists, the relationship is not straightforward, as documented by the great diversity in total kidney and cyst volumes associated with any given eGFR in the ADPKD patient we studied. Moreover, evidence of slowing renal growth in the absence of a stabilizing effect in GFR decline in ADPKD patients receiving everolimus further highlights the limits of total kidney volume changes as surrogate marker of renal functional loss and points out the need of novel biomarkers of functional loss, representative of disease progression. The loss of renal function in patients with ADPKD is insidious because it can remain undetected for several decades as a consequence of the ability of surviving nephrons to increase glomerular filtration rate in the face of the advancing renal structural damage. Tubulointerstitial disease is now recognized as an indispensable and prominent participant in the progression of renal disease. Landmark studies in the ‘60s were the first to highlight that the severity of renal fibrosis is the single best histological correlate of the decline in renal function and long-term prognosis.
sides cyst enlargement and parenchymal compression, fibrotic transformation of the cyst wall and the adjacent interstitium in ADPKD may play a role in determining renal functional loss. Current findings that intermediate volume, as determined by CT, is mainly constituted by interstitial fibrosis allows one to consider this imaging parameter as a strong candidate marker for monitoring disease progression and to anticipate the long-term renal functional outcome in ADPKD patients.

Here, histological findings on the nature of intermediate volume have been coupled to further investigations on the relationships between intermediate volume and renal function. In an independent cohort of 21 ADPKD patients (SIRENA cohort), we found a strong correlation between intermediate volume relative to parenchyma and GFR measured at the time of CT imaging. Furthermore, in follow-up data on 13 patients, originally enrolled in the somatostatin study, with normal renal function or moderate renal dysfunction at the time of CT imaging, intermediate volume significantly correlated with the slope of GFR decline over the 3- to 8-year follow-up period.

These findings indicate that the most severe condition for a patient seems to be associated with the presence of large portions of fibrotic tissue relative to the preserved parenchyma and that this volume ratio is more readily related to both GFR and GFR decline than the absolute volumes alone. This is particularly relevant for the potential that intermediate volume may have for early stratification of ADPKD patients, in addition to monitoring disease progression. In fact, although GFR is a late marker of kidney damage in ADPKD, the possibility of stratifying patients according to their rate of disease progression based on an early image-based evaluation of relative intermediate volume opens new prognostic and monitoring perspectives, as well as new windows for evaluating early treatment strategies.

There are a number of limitations to be considered in the interpretation of these results. Present findings were obtained in a small number of patients and are therefore to be regarded as hypothesis generating, needing confirmation. The small number of patients may also explain the lack of a significant relation between total kidney or cyst volume and GFR, at variance to the findings of the somatostatin study, with normal renal function or moderate renal dysfunction at the time of CT imaging. Intermediate volume significantly correlated with the slope of GFR decline over the 3- to 8-year follow-up period.

identified as intermediate in our previous studies, we assumed that such radiological pattern within the ADPKD kidney would be reflected by similar histopathological findings in other stages of the disease. This is consistent with a previous study, showing the presence of fibrosis both in early and in late ADPKD stages. Finally, it has to be taken into account that use of contrast agent for CT renal imaging has a potential risk especially in patients with reduced GFR.

In conclusion, we provided evidence that kidney tissue identified in ADPKD patients as intermediate on contrast-enhanced CT mainly corresponds to regions of interstitial fibrosis at histological characterization and that intermediate volume is tightly connected to GFR and its long-term decline. As such, this parameter may represent a promising marker for early stratification of ADPKD patients, as well as for monitoring disease progression. Further studies on larger cohorts are now needed to establish intermediate volume as a biomarker of disease progression and interstitial fibrosis as a potential therapeutic target in ADPKD.

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References


