ARFI-based tissue elasticity quantification and kidney graft dysfunction: First clinical experiences


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Abstract. Background and purpose: Beyond the medical history, the clinical exam and lab findings, non-invasive ultrasound parameters such as kidney size and Doppler values (e.g. the resistive index) are important tools assisting clinical decision making in the monitoring of renal allografts. The gold standard for the diagnosis of renal allograft dysfunction remains the renal biopsy; while an invasive procedure, the justifiable necessity for this derives from its definitive nature a requirement beyond the synopses of all non-invasive tools.

“Acoustic Radiation Force Impulse Imaging” (ARFI)-quantification is a novel ultrasound-based technology measuring tissue elasticity properties. So far experience related to this new method has not been reported in renal transplant follow-up. The purpose of this study was to evaluate changes in ARFI-measurements between clinically stable renal allografts and biopsy-proven transplant dysfunction.

Methods: We employed “Virtual Touch™ tissue quantification” (Siemens Acuson, S2000) for the quantitative measurement of tissue stiffness in the cortex of transplant kidneys. We performed initial baseline and later disease-evaluative ultrasound examinations in 8 renal transplant patients in a prospective study design. Patients were first examined during stable allograft function with a routine post-transplant renal ultrasound protocol. A second follow-up examination was carried out on subsequent presentation with transplant dysfunction prior to allograft biopsy and histological evaluation.

All patients were examined using ARFI-quantification (15 measurements/kidney). Resistive indices (RI) were calculated using pulsed-wave Doppler ultrasound, and transplant kidney size was measured on B-mode ultrasound images. All biopsies were evaluated histologically by a reference nephropathologist unaware of the results of the ultrasound studies. Histopathological diagnoses were based on biopsy results, taking clinical and laboratory findings into account.

Finally, we calculated the relative changes in ARFI-quantification, resistive indices and the absolute change of kidney size on a percentage basis at these defined assessment times and compared the results with the final pathologic diagnosis.

Results: Histological results enumerated five cases of acute T-cell-mediated rejection, one case of calcineurin inhibitor toxicity and two cases of acute tubular necrosis. Calcineurin inhibitor toxicity and acute tubular necrosis were subsumed as “other pathologies”. Mean ARFI-values showed an average increase of more than 15% percent in transplants with histologically proven acute rejection whereas no increase was seen in transplants with other pathologies. Mean RI-values showed no increase either in the diagnostic group of acute rejection, nor in the group with other pathologies. Kidney size showed a mean absolute increase of 0.5 centimetres in allografts with acute rejection, whereas a mean decrease of 0.17 centimetres was seen in the group with other pathologies.

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Conclusion: As shown before in other studies, RI values and kidney size are of doubtful utility in the evaluation of kidney allograft dysfunction. ARFI-based elasticity measurement shows promise as a complementary non-invasive parameter in follow-on diagnosis of renal allograft rejection.

Keywords: Ultrasonography, elasticity imaging techniques, kidney, kidney transplantation, Doppler ultrasonography, ARFI, graft rejection

1. Introduction

Acute allograft rejection impacts on the survival of renal transplants [14]. After an acute rejection some renal allografts do not recover their earlier function – even undertaking maximum target-specific therapy. Moreover, episodes of acute allograft rejection promote the development of chronic allograft dysfunction [2].

Clinical symptoms of acute renal allograft rejection include an acute rise of the creatinine level (a late sign of rejection), a decreased urine output and an increased blood pressure. However, unfortunately, acute rejection may be clinically asymptomatic [5].

The gold standard for the diagnosis of renal allograft dysfunction is the renal biopsy; an invasive procedure with a low risk of bleeding and hematoma [23]. It is indicated when the results from all non-invasive tools including medical history, the clinical exam, lab findings and ultrasound parameters suggest rejection as a realistic possibility. The renal biopsy is regarded as mandatory for establishing a histology-based diagnosis important for the appropriate selection of differential drug therapy for acute renal allograft rejection. Some transplant units perform protocol-driven biopsies of the renal transplant to preclude subclinical rejection evading detection [11].

Non-invasive ultrasound parameters of B-mode kidney size and Doppler values (e.g. the resistive index) are still important tools informing clinical decision making in the monitoring of renal allografts in daily practice; even though the real clinical impact of Doppler values has changed in the last fifteen years. Initial Doppler studies showed encouraging results that the resistive index (RI-value = (peak systolic velocity-enddiastolic velocity)/peak systolic velocity) would become a powerful tool in differentiating acute renal allograft rejection from acute tubular necrosis (ATN) and cyclosporine-associated toxicity [17, 18]. Further studies did not confirm these data, so the resistive index is nowadays regarded as inapplicable for the diagnosis of renal allograft dysfunction due to this lack of discriminatory power [1, 9, 12, 15, 26]. However, RI-values greater than 0.8 were shown to be predictive of death and poor long term prognosis for the renal allograft [16].

New ultrasound methods such as contrast-enhanced ultrasound are under evaluation and have shown a prolonged perfusion time of the renal cortex in patients with acute rejection [8, 10].

Two pilot studies of novel ultrasound-based elasticity imaging using shear-waves or transient elastography limited at a role for non-invasive elasticity imaging in predicting the histology-based fibrosis score in renal allografts [4, 22], although a third group describes interobserver variability problems using this method [24, 25]. The effect of other pathologies – besides fibrosis- on renal parenchymal elasticity has not yet been reported and is the subject of ongoing histopathological studies.

The potential role of ultrasound-based elasticity imaging for establishing the diagnosis of renal allograft dysfunction has not been reported in renal transplant follow-up, although these non-invasive measurements can be easily incorporated in the clinical routine, using a standardized protocol. The purpose of this pilot study was to evaluate changes in ARFI-measurements between stable renal allografts and biopsy-proven transplant dysfunction.
2. Material and methods

All patients qualifying for our prospective study were first admitted to our specialized nephrology-ultrasound unit during stable allograft function for routine transplant kidney ultrasound (according to our standardized clinical routine ultrasound protocol for renal transplant patients, including B-mode, Doppler ultrasound and elasticity measurements).

A second follow-up ultrasound examination was done following presentation with renal allograft dysfunction at our outpatients clinic. We performed ultrasound-guided kidney biopsy for histopathological examination in these patients within a narrow time frame (<3 days). This prospective pilot study was approved by the ethics committee of the Technical University of Munich and, being conducted in accordance with its guidelines, informed consent was obtained from all patients [7].

2.1. Patients

Within a period of six months (July 2009 to December 2009) 8 patients (6 male, 2 female) were eligible to be included in our prospective study (Table 1). Age ranged from 17 to 66 years, mean age 43.0 years (±19.7 years), mean weight was 74.6 kg (±13.1 kg) and mean body-mass-index 27.1 (±5.3).

Patients presented with stable renal allograft function after a median time of 1.7 months after kidney transplantation (IQR: 0.8–4.3 months) for the first “ultrasound-study-exam” (baseline measurement). The second ultrasound exam was performed after a median time of 4.7 months (IQR: 2.9–7.4 months). Mean time between first and second exam was 2.4 months (±1.5 months).

Seven patients received a cadaver kidney donation and one patient a living kidney donation. Median time for cold ischemia in the transplanted kidneys was 10 hours (IQR: 8.5–13.8 hours) and median time for warm ischemia was 25 minutes (IQR: 20–30 minutes).

2.2. Technical ultrasound features

2.2.1. Ultrasound device

All ultrasound and elasticity examinations were performed on a Siemens-Acuson S2000 ultrasound machine (Siemens, Erlangen, Germany) with a curved array multifrequency transducer (4-1 MHz).
2.2.2. Doppler studies and calculation of the resistence indices
We used colour Doppler for the evaluation of transplant kidney perfusion. The gain and the pulse repetition frequency were adapted individually for each patient.

We examined three arteries in the renal cortex (a. arcuatae) one in each third of the transplant kidney, also using pulsed-wave Doppler for the registration of angle-independent resistive indices. The resistive index of the three a. arcuatae was calculated according to the formula: \( \text{RI-value} = \frac{\text{peak systolic velocity} - \text{end-diastolic velocity}}{\text{peak systolic velocity}} \).

2.2.3. Principle and clinical procedure of ARFI-based quantification
The ultrasound elasticity modality “Virtual Touch™ tissue quantification” (S2000, Siemens, Erlangen) –used in our study- applies “Acoustic Radiation Force Impulse (ARFI)” technology. This comprises an acoustic “push” pulse which is transmitted into the target tissue. Consequently the tissue experiences a small displacing mechanical force leading to the propagation of shear waves traveling in a direction perpendicular to the push pulse induced displacement of tissue. The shear wave’s velocity (metres per second, m/s) can be measured within a user-placed region of interest (ROI-size: 1 cm) box. With ultrasound tracking beams numerical measurements (“ARFI-values”) can be derived, corresponding to tissue stiffness at user-defined anatomical locations (the stiffer the tissue, the higher the shear wave’s velocity). We measured five ARFI-values in the parenchyma of the upper pole (Fig. 1), the middle third and the lower pole of the patient’s transplant kidney, resulting in 15 ARFI-values for each transplant kidney. The patient was examined in dorsal position. In the event of a rejected/non valid measurement (internally determined by a shear wave velocity estimation algorithm) the ultrasound machine displayed a measurement error, prompting a repeat measurement. The elbow of the ultrasound-performing physician was rested remotely to avoid any additional compression with the ultrasound transducer on the renal allograft. The collection of the 15 ARFI-values lasted between 2–5 minutes, depending on the number of rejected measurements encountered.
2.3. Ultrasound examination protocol

All patients were examined with conventional B-mode ultrasound, high-frequency ultrasound, colour-coded Doppler sonography and finally by the elasticity method “Virtual Touch™ tissue quantification” (encompassing “Acoustic Radiation Force Impulse” (ARFI)-technology) by three experienced full-time ultrasound-physicians (ultrasound unit, Department of Nephrology).

Firstly, the length measurement of the transplant kidney was obtained with B-mode ultrasound examination.

We registered transplant kidney perfusion and the resistive indices by employing Doppler ultrasound. Further to this, we performed ARFI-quantification measurements to evaluate the tissue stiffness of the kidney.

All 8 kidney biopsies were performed under ultrasound guidance from the lower pole of the transplant kidney, employing a 16-G-Magnum needle with a high-speed puncture device. There were no complications reported in these patients following renal biopsy.

2.4. Histological examination

Histology of the transplant kidneys was studied in the “nephropathology reference unit” of the Institute of Pathology of the University of Erlangen/Germany.

In our patient group the grade of fibrosis of the transplant kidney biopsies was documented, converted into a percentage, and then classified according to the BANFF-classification for chronic allograft injury [19, 20].

The histologically proven acute renal allograft rejection was classified according to the BANFF-classification of acute renal allograft rejection [5, 19, 20]:

1. Normal
2. Antibody-mediated changes
3. Borderline changes
4. T-cell mediated rejection
   Type I A– significant interstitial inflammation and foci moderate tubulitis
   Type I B– significant interstitial inflammation and severe tubulitis
   Type II A– Mild to moderate arteritis
   Type II B– Severe arteritis
   Type III– Transmural arteritis
5. Interstitial fibrosis and tubular atrophy, without evidence of any specific etiology
6. other

2.5. Statistical analysis

We used PASW Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA) for the data processing. All descriptive results were presented as mean values with the standard deviation as “±” or, if more appropriate, as median and interquartile range (IQR).
Table 2
Results of the nephropathology

<table>
<thead>
<tr>
<th>Patient</th>
<th>Grade of fibrosis (%)</th>
<th>BANFF-score</th>
<th>Histology</th>
<th>BANFF-classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>I</td>
<td>Drug-related toxicity</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Acute rejection</td>
<td>IA</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>Acute rejection</td>
<td>IA</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>Acute tubular necrosis</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>Acute tubular necrosis</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>I</td>
<td>Acute rejection</td>
<td>BANFF-Borderline</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>Acute rejection</td>
<td>IB</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>I</td>
<td>Acute rejection</td>
<td>BANFF-Borderline</td>
</tr>
</tbody>
</table>

BANFF-score: according to the BANFF-classification of chronic allograft injury. BANFF-classification: according to the BANFF-classification of acute renal allograft rejection.

Table 3
Clinical parameter and ultrasound findings in follow-up exams after renal transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exam 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARFI-mean-value (m/s)</td>
<td>E 1</td>
<td>3.27</td>
<td>2.14</td>
<td>2.98</td>
<td>2.13</td>
<td>3.01</td>
<td>2.59</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>E 2</td>
<td>2.80</td>
<td>2.46</td>
<td>2.99</td>
<td>2.32</td>
<td>3.02</td>
<td>2.93</td>
<td>2.99</td>
</tr>
<tr>
<td>RI-mean-value</td>
<td>E 1</td>
<td>0.79</td>
<td>0.66</td>
<td>0.83</td>
<td>0.70</td>
<td>0.55</td>
<td>0.76</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>E 2</td>
<td>0.60</td>
<td>0.84</td>
<td>0.68</td>
<td>0.57</td>
<td>0.77</td>
<td>0.80</td>
<td>0.65</td>
</tr>
<tr>
<td>Kidney size (cm)</td>
<td>E 1</td>
<td>13.7</td>
<td>12.3</td>
<td>10.0</td>
<td>10.4</td>
<td>10.8</td>
<td>12.2</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>E 2</td>
<td>13.3</td>
<td>13.0</td>
<td>11.4</td>
<td>10.5</td>
<td>10.6</td>
<td>12.1</td>
<td>13.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>E 1</td>
<td>2.1</td>
<td>1.3</td>
<td>1.7</td>
<td>1.9</td>
<td>1.3</td>
<td>2.3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>E 2</td>
<td>2.2</td>
<td>1.4</td>
<td>3.8</td>
<td>4.1</td>
<td>1.8</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>E 1</td>
<td>34</td>
<td>&gt;60</td>
<td>32</td>
<td>42</td>
<td>54</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>E 2</td>
<td>32</td>
<td>&gt;60</td>
<td>13</td>
<td>17</td>
<td>37</td>
<td>26</td>
<td>19</td>
</tr>
</tbody>
</table>

Yellow = acute rejection; GFR = glomerular filtration rate; AF = atrial fibrillation; E = exam.

3. Results

The histology of the eight kidney biopsies showed five cases of acute T-cell-mediated rejection, one case of calcineurin-inhibitor toxicity and two cases of acute tubular necrosis (Table 2).

For our analysis the patients with Calcineurine-inhibitor toxicity and acute tubular necrosis were subsumed in the group “other pathologies”.

We compared in our pilot study the group “acute rejection” (n = 5) versus the group “other pathology” (n = 3) on behalf of the changes in ultrasound parameters in the follow-up-situation (Table 3).

B-mode-kidney size showed a mean absolute increase of 0.5 centimetres in allografts with acute rejection, whereas a mean decrease of 0.17 centimetres was seen in the group with other pathologies.

Mean RI-values showed no increase neither in the diagnostic group of acute rejection, nor in the group with other pathologies.
Mean ARFI-values showed an average increase of more than 15% percent in transplants with histologically proven acute rejection whereas no increase was seen in transplants with other pathologies. (Table 4).

4. Discussion

A non-invasive imaging tool such as ultrasound should help to support the putative diagnosis of renal rejection and hence adding more selectively in the justification for renal biopsy, would be greatly advantageous. The renal biopsy is required to establish the diagnosis (“gold standard”) and to choose the best medical treatment in each individual [6].

As there is nephropathologic variety in the causes and types of acute rejection reflected in different changes in blood vessels, blood flow, interstitial fluid (resulting in edema) and anatomical structures, ultrasound will –at the moment- only be able to depict some indirect signs, the “collateral damage” of this acute processes [19].

Modern ultrasound machines provide a wide range of utilities. The “ultrasound toolbox” comprises traditional B-mode and Doppler ultrasound, and more lately contrast-enhanced ultrasound and integrated elasticity tools. Standardized protocols implementing these newer options for the follow-up of renal transplants are in use in many transplant units with specialized ultrasound units.

Our especial interest was directed towards the additional diagnostic impact of ARFI-based elasticity measurements in the evaluation of renal allograft rejection, not least as this noninvasive and quickly achievable parameter is so easily obtained. Nevertheless no experiences with this method for monitoring renal allografts have been published.

We first studied ARFI-quantification in renal transplant fibrosis and our pilot study showed a correlation between the ARFI-values and the grade of fibrosis in a small number of patients [22]. Another research group from Berlin was able to show similar results using transient elastography [4]. A pilot study from a research group from Oslo –with ten more patients then in each of the other two studies- reported no significance for ARFI in low grade fibrosis, yet still in a small number of patients and comprising a group of different ultrasound investigators [25].

Aware of possible technical limitations of the method, we translated our first experiences in renal allograft elastography into a standardized ultrasound protocol for the transplant kidney in follow-up exams. Our first data – in a small group of patients- showed differences between the course of the Mean-RI-values and the Mean-ARFI-measurements in the patient group with “renal transplant rejection”. While Mean-RI-values were not helpful to confirm the clinical diagnosis of “acute allograft rejection” we noted
a slight increase of the Mean-ARFI values (+17 percent), highlighting the importance of follow-up measurements. The absolute mean-ARFI-values seemed not to have such an importance per se. This is consistent to the published role of the RI-value in the diagnosis of rejection [1, 9].

The slight rise of the mean-ARFI-values in the “rejection group” might lead to the hypotheses that the kidney stiffness is elevated in acute rejection. The possible influence of inflammatory activity on tissue stiffness might be usefully compared to experiences in hepatology where elasticity tools (Fibroscan and recently ARFI-quantification) have been intensively evaluated. Liver stiffness values measured by Fibroscan were elevated in acute hepatitis [3]. One study analysing the role of ARFI for the noninvasive assessment of fibrosis in chronic hepatitis C noted a moderate correlation between ARFI-values and histologic markers of inflammatory activity [13]. Further studies with larger patient populations are required to better understand the influence of histologic changes on ARFI-measurements in acute renal transplant rejection as there is no published data available to date.

The follow-up-kidney size was slightly larger in the “acute rejection group”, according to the expected increase in kidney size clinically known in patients with acute renal failure and rejection [21].

5. Conclusion

As documented in previous studies, RI values are of doubtful use in the evaluation of kidney allograft dysfunction. ARFI-based elasticity measurement shows promise as a complementary non-invasive selective parameter within a follow-up evaluation for the diagnosis of renal allograft rejection.

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References


