Prediction of the severity and outcome of acute tubular necrosis based on continuity of Doppler spectrum in the early period after kidney transplantation

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Abstract

Background. Doppler flow spectrum, quantified in the segmental arteries of the graft early after kidney transplantation (KTx), reflects the exacerbation of interstitial oedema. In some patients, the spectrum is characterized by the absence of blood flow during part or during the whole diastole of the cardiac cycle. We have previously observed that such discontinuous flow is associated with a more severe clinical course of acute tubular necrosis (ATN). In order to further verify this hypothesis, we have quantified prospectively the timing of blood flow in Doppler spectrum within the cardiac cycle.

Methods. Doppler sonography was performed in 173 recipients between 2 and 4 days after KTx. A total of 18 patients with a diagnosed episode of acute rejection or primary graft non-function were excluded from the analysis. Fifty-three out of 155 patients (34%) developed ATN, defined as a requirement for more than one haemodialysis session after KTx. In patients with a discontinuous spectrum of flow, we have quantified the ratio of time of the whole cardiac cycle in which the flow is present [flow time index (FTI) expressed as %].

Results. The discontinuous spectrum of flow was present in 35 out of 53 (66.0%) patients with ATN but only in 6 out of 102 (5.9%) patients with immediate or slow graft function. The relative risk of ATN occurrence for patients with discontinuous spectrum of flow was 5.98 (3.83–9.34) and the duration of ATN in these patients was twice as long—12 (10–14) versus 6 (5–8) days. In patients with ATN a significant correlation was found between FTI and duration of ATN (r = −0.357, P = 0.035).

Conclusion. The discontinuous spectrum of flow in the segmental arteries of the kidney graft in the early period after KTx is typical for ATN and predicts its duration.

Keywords: acute tubular necrosis; discontinuous flow; Doppler spectrum; kidney transplantation

Introduction

Renal transplantation is recognized as the optimal method of treatment in patients with end-stage renal disease with regard to morbidity, mortality and quality of life [1]. One of the main factors affecting long-term graft survival is the occurrence of delayed graft function, which may be caused by inferior graft quality or the necrosis of tubular epithelial cells with subsequent exacerbation of interstitial oedema [2–4]. Several important clinical issues in addition to acute tubular necrosis (ATN) are responsible for modification of kidney graft function in the early post-transplant period, i.e. vascular complications, rejection episodes and acute toxicity of immunosuppressive drugs, particularly calcineurin inhibitors (CNI). Ultrasound measurement of intrarenal blood flow in transplanted kidneys by Doppler is a non-invasive method that helps to differentiate the above-mentioned causes of kidney graft dysfunction after kidney transplantation (KTx) [3,5]. The evaluation of pulsatility index (PI) and resistive index (RI) in segmental arteries of the kidney graft within the first days after KTx estimates the degree of interstitial oedema. These parameters depend mainly on the arterial blood flow during the diastolic phase, which is disturbed by increased intrarenal resistance, caused largely by ATN in the early post-transplant period [5]. In some patients with severe ATN, the Doppler spectrum is characterized by the absence of blood flow during diastole [6,7]. We have observed that a flow spectrum with a long discontinuous flow phase is associated with a more severe clinical course of ATN. To further verify this hypothesis, we have prospectively quantified the timing of blood flow in Doppler spectrum in a non-selected cohort of kidney transplant recipients.

Subjects and methods

A total of 173 adult kidney transplant patients who were consecutively operated on in our centre and who received a kidney graft between October 2005 and April 2008 were enrolled in the study. Eighteen patients with a diagnosed episode of acute rejection or primary graft non-function were excluded from further analysis. Protocol biopsies were not performed. Acute rejection episodes in the excluded...
patients were diagnosed based on clinical signs and symptoms along with kidney biopsy. Out of 155 patients, 53 (34\%) had developed ATN, defined as the requirement for more than one haemodialysis procedures after transplantation. All 155 patients received triple immunosuppression therapy, consisting of cyclosporine (N = 99) or tacrolimus (N = 56), mycophenolate mofetil (N = 124) or mycophenolic acid (N = 3) or sirolimus (N = 3) or everolimus (N = 7) or azathioprine (N = 18) and steroids (N = 155). Seven patients received basiliximab (Simulect\textsuperscript{®}, Novartis, Basel, Switzerland) and 7 antithymocyte globulin (ATG\textsuperscript{®}, Fresenius, Bad Homburg, Germany) as induction therapy (12 patients in the continuous flow group and 2 in the discontinuous flow group). The study protocol did not interfere with the immunosuppressive regimen.

In all patients, Doppler sonography of the kidney graft was performed between 2 and 4 days post-transplantation. All examinations were performed by two experienced sonographers with a Siemens machine (Sonoline Antares, Mountain View, CA), equipped with a 2.5–4.0 MHz convex-array transducer. In each patient, after visualization of three to five segmental arteries, the Doppler spectrum was recorded and analysed. During the examination, patients were asked to refrain from forced inspiration, since this can modify the intra-abdominal pressure. According to the end-diastolic flow, patients were divided into two groups: with continuous flow (group 1) or, with lack of flow during or during the whole diastole of the cardiac cycle; such a spectrum was considered as discontinuous (group 2). The characteristics of patients with continuous and discontinuous flow pattern is given in Table 1. In patients with a discontinuous spectrum of flow in segmental arteries, we have quantified the ratio of time during the whole cardiac cycle in which orthograde flow is present [flow time index (FTI) expressed as \%), using the formula: FTI (%) = time of orthograde flow/time of whole cardiac cycle × 100. The exact time measurements were performed during the examination, by firstly signalling the beginning and the end of one whole cardiac cycle on spectrum recording—to calculate the whole cardiac cycle time, and then by signalling the beginning of the cardiac cycle and the moment of orthograde flow cessation—to calculate the orthograde flow time (Figure 1). A supplementary method of measurement was the calculation of both distances in millimetres using standard linear measure and printed recording of the Doppler flow spectrum. Using this supplementary method, we also compared and verified the results of both sonographers performing the Doppler examinations and parameter calculations during the scan. As expected, both above measurements gave concurrent results. Doppler sonography was repeated after 5 days. A further shortening of flow time was considered as overlapping with acute rejection, and patients were then excluded from the study.

Statistical analyses were performed by the STATISTICA 7.0 PL for Windows software package (StatSoft Polska, Kraków, Poland). Values are presented as means and 95\% confidence intervals (in brackets). Statistical significance between compared groups, i.e. patients with discontinuous and continuous flow patterns and, in the discontinuous subgroup, in patients with and without ATN was estimated using non-parametric chi-square and Mann–Whitney U-tests.

The Spearman rank-order test was used to calculate the univariate coefficients between FTI and recipient and donor age, donor serum creatinine concentration, donor history of hypotensive episodes, as well as doses of dopamine and noradrenaline used before organ recovery, recipient BMI and time on dialysis prior to transplantation, doses and blood concentrations of CNI, the percentage of panel-reactive antibodies (PRA), cold ischaemia time (CIT), mismatches at the HLA-A, B and DR loci and duration of ATN. A receiver operator curve (ROC) was used to calculate sensitivity and specificity of the prediction of ATN occurrence. Relative risk was calculated based on logistic regression. The results were considered as significant with a P-value of <0.05.

Results

Generally, the discontinuous spectrum of flow was present in 26.5\% of all transplant patients. The discontinuous spectrum of flow was present in 35 out of 53 (66.0\%) patients with ATN. In contrast, it was observed only in 6 out of 102 (5.9\%) patients without ATN. According to ROC analysis, the discontinuous spectrum of flow discriminated ATN with 66.0 (51.7–78.5\%) sensitivity and 94.1 (87.6–97.8\%) specificity. A similar ROC analysis was performed for RI values in patients with and without ATN. The cut-off value of RI was 0.83 with 85.3 (76.9–91.5\%) sensitivity and 77.4 (63.8–87.7\%) specificity. The discontinuous spectrum of flow was characterized by lower sensitivity but higher specificity than RI > 0.83 in the detection of ATN.

The relative risk of ATN occurrence in patients with a discontinuous spectrum of flow was 5.98 (3.83–9.34). Moreover, the duration of ATN in these patients was twice as long: 12 (10–14) versus 6 (5–8) days (Figure 2). The comparison of both groups revealed that patients with a discontinuous spectrum of flow had higher BMI and received kidneys from older donors, who had worse renal excretory function before organ recovery (Table 1). There was no difference in CIT and percentage of PRA, CNI regimen, doses and blood concentrations, but a borderline difference in the HLA class II mismatch and in the dose of dopamine used in donor care was found (P = 0.06).

The FTI was lower in patients who developed ATN [65\% (59–71) versus 87\% (83–90), P = 0.0014] than in patients without ATN. There was no significant correlation between recipient age, BMI, time on dialysis prior to transplantation or percentage of PRA and FTI. Moreover, we were unable to prove that HLA mismatch and CIT influenced FTI. A borderline correlation between donor age and FTI was found (r = −0.268, P = 0.09), while no correlation between donor serum creatinine before organ recovery and FTI was observed.

Of note, in the subgroup of patients with ATN, a significant correlation was found between FTI and the duration of ATN (r = −0.357, P = 0.035) (Figure 3).

Discussion

This study demonstrates that patients with a discontinuous pattern of Doppler flow spectrum measured in segmental...
### Table 1. Characteristics of patients from the continuous and discontinuous flow groups, statistical significance for continuous versus discontinuous group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 155)</th>
<th>Continuous flow (n = 114)</th>
<th>Discontinuous flow (n = 41)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 (43–46)</td>
<td>43 (41–46)</td>
<td>47 (44–50)</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>92/63</td>
<td>68/46</td>
<td>24/17</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (23.6–24.9)</td>
<td>23.8 (23.1–24.5)</td>
<td>25.4 (24.0–26.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Duration of dialysis (months)</td>
<td>39 (32–46)</td>
<td>37 (29–45)</td>
<td>44 (30–59)</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>74.2</td>
<td>77.2</td>
<td>65.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Duration of hypertension (years)</td>
<td>9 (8–10)</td>
<td>9 (8–11)</td>
<td>8 (6–10)</td>
<td>0.62</td>
</tr>
<tr>
<td>Previous cardiovascular episode (%)</td>
<td>5.2</td>
<td>3.5</td>
<td>9.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.0</td>
<td>8.8</td>
<td>17.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Mismatch HLA I</td>
<td>2.51 (2.35–2.67)</td>
<td>2.46 (2.26–2.65)</td>
<td>2.65 (2.38–2.92)</td>
<td>0.43</td>
</tr>
<tr>
<td>Mismatch HLA II</td>
<td>0.98 (0.87–1.09)</td>
<td>0.91 (0.78–1.05)</td>
<td>1.17 (1.01–1.33)</td>
<td>0.06</td>
</tr>
<tr>
<td>CIT (hours)</td>
<td>16 (15–17)</td>
<td>16 (15–17)</td>
<td>16 (14–18)</td>
<td>0.67</td>
</tr>
<tr>
<td>PRA (%)</td>
<td>3.2 (1.3–5.1)</td>
<td>3.6 (1.1–6.1)</td>
<td>2.0 (0.3–4.4)</td>
<td>0.87</td>
</tr>
<tr>
<td>Highly sensitised pts (PRA &gt; 30%) (%)</td>
<td>4.6</td>
<td>5.4</td>
<td>2.4</td>
<td>0.44</td>
</tr>
<tr>
<td>Calcineurin inhibitor (CyA/Tc)</td>
<td>99.56</td>
<td>71.43</td>
<td>28.13</td>
<td>0.49</td>
</tr>
<tr>
<td>CyA dose (mg/day)</td>
<td>517 (494–540)</td>
<td>518 (490–547)</td>
<td>513 (477–550)</td>
<td>0.93</td>
</tr>
<tr>
<td>Tc dose (mg/day)</td>
<td>11.6 (10.8–12.5)</td>
<td>11.3 (10.2–12.3)</td>
<td>12.8 (11.3–14.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>CyA C0 (ng/ml)</td>
<td>187 (170–204)</td>
<td>184 (163–206)</td>
<td>193 (162–224)</td>
<td>0.47</td>
</tr>
<tr>
<td>CyA C2 (ng/ml)</td>
<td>996 (941–1052)</td>
<td>994 (928–1061)</td>
<td>1001 (983–1109)</td>
<td>0.71</td>
</tr>
<tr>
<td>Tc C0 (ng/ml)</td>
<td>12.2 (10.3–14.1)</td>
<td>11.9 (9.7–14.2)</td>
<td>13.1 (10.1–16.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>43 (41–45)</td>
<td>42 (39–44)</td>
<td>47 (43–50)</td>
<td>0.03</td>
</tr>
<tr>
<td>Donor serum creatinine (µmol/l)</td>
<td>105 (95–116)</td>
<td>97 (87–107)</td>
<td>128 (99–156)</td>
<td>0.03</td>
</tr>
<tr>
<td>Donor hypotensive episode (%)</td>
<td>62.7</td>
<td>61.8</td>
<td>65.0</td>
<td>0.87</td>
</tr>
<tr>
<td>Donors receiving dopamine infusion (%)</td>
<td>86.6</td>
<td>89.3</td>
<td>79.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Dose of dopamine used in donor (µg/kg/h)</td>
<td>6.8 (5.8–8.2)</td>
<td>5.8 (4.6–7.1)</td>
<td>9.8 (5.7–13.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>Donors receiving noradrenaline infusion (%)</td>
<td>64.1</td>
<td>62.1</td>
<td>69.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Dose of noradrenaline used in donor (µg/kg/h)</td>
<td>0.29 (0.11–0.46)</td>
<td>0.32 (0.08–0.57)</td>
<td>0.2 (0.06–0.34)</td>
<td>0.61</td>
</tr>
<tr>
<td>ATN (%)</td>
<td>34.2</td>
<td>15.8</td>
<td>85.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of ATN (days)</td>
<td>10 (8–12)</td>
<td>6 (5–8)</td>
<td>12 (10–14)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data shown as means ± 95% CI or frequencies.

BMI: body mass index, HLA: human leukocyte antigen, CIT: cold ischaemia time, PRA: panel-reactive antibodies, CyA: cyclosporine, Tc: tacrolimus, C0: cyclosporine trough level, C2: cyclosporine blood concentration 2 h after oral intake, Tc C0: tacrolimus trough level, ATN: acute tubular necrosis.

**Fig. 1.** Time measurements performed during the Doppler examination: the vertical lines are placed at the beginning and at the end of one cardiac cycle, and than at the beginning and at the end of orthograde blood flow; the time was calculated automatically and shown below. (A) Doppler spectrum in a patient with moderately impaired end-diastolic flow (FTI = 76%); (B) Doppler spectrum in a patient with severely impaired end-diastolic flow (FTI = 44%).
arteries of a kidney graft in the early post-transplant period are at six times higher risk of ATN occurrence. Moreover, if ATN has occurred, its duration was twice as long in patients with a discontinuous flow pattern than in patients with continuous flow.

ATN and, in consequence, delayed graft function after transplantation are caused by kidney graft damage occurring before organ procurement, during its conservation prior to KTx and after reperfusion (ischaemia/reperfusion injury) [8]. Many risk factors for developing ATN after KTx have already been identified, including the donor and recipient age, recipient overweight, CIT, the presence of arteriosclerosis in both donor and recipient and the occurrence of donor hypotensive episodes before organ procurement [8–10].

In our study, the discontinuous flow pattern group consisted of patients with a higher BMI at the time of transplantation than those with continuous flow ($P = 0.04$). Moreover, kidney grafts in the discontinuous flow group were recovered from older donors receiving higher doses of dopamine, whose renal excretory function (measured by serum creatinine concentration) before organ procurement was significantly worse ($P = 0.03$). In addition to this inferior initial organ status in this group, a trend towards worse HLA class II antigens mismatch was observed in patients in the discontinuous flow group ($P = 0.06$). Other analysed factors such as CIT, PRA, CNI regimen used, CNI doses and blood concentrations, recipient age and sex, pre-transplant duration of dialysis therapy and recipient concomitant morbidity did not differ between the two groups. All the above differences may partially explain the substantially higher occurrence of ATN in the discontinuous flow group.

Of great importance, the duration of ATN was twice as long in patients in this group. Thus, the Doppler spectrum analysis in the first few days after KTx was useful in predicting the clinical course in an early post-transplant period, and to make an informed decision with regard to a dialysis procedure, with an impressive relative risk of 5.98.

The newly introduced FTI quantitatively expresses the ratio between the time of orthograde flow and the time of the whole cardiac cycle in segmental arteries of the kidney graft (Figure 1). It helps to define kidney graft dysfunction and prognosis in a significant proportion of patients with a discontinuous flow pattern after transplantation (in our study 26.5% of all patients). A calculated RI value using Doppler in these patients has limited value. Regardless of the severity of end-diastolic flow impairment, the calculated RI value is 1.0 (Figure 1A and B). Thus, FTI seems to be superior. The higher the ratio (with an optimal value of 100%, as in the case of continuous flow), the better the prognosis of graft function and lower probability of ATN occurrence. Patients who developed ATN had significantly lower FTI than those without ATN (65% versus 87%, $P = 0.0014$). Moreover, in the ATN patients, a significant correlation was found between FTI and the duration of ATN ($r = -0.357$, $P = 0.035$). On the other hand, there was no significant correlation between recipient age, BMI, time on dialysis prior to transplantation, HLA mismatch, CIT, PRA or CNI doses and blood concentrations and FTI, while only borderline correlation between donor age and FTI was found. Thus, FTI seems to be a valuable new parameter for kidney graft blood flow measurement during the early post-transplant period.

To date, we are unaware of any previous report, concerning a quantitative method of blood flow measurement in patients with discontinuous flow pattern in Doppler examination. In such patients, the calculation of traditional flow resistance indices—PI and RI—is not very precise due to the lack of end-diastolic flow within intrarenal arteries. As a consequence, the highest range of resistive indices seems not to be accurate for estimation of intrarenal resistance. Nevertheless, what was shown by the correlation between the FTI and ATN duration time, using FTI was helpful in predicting the time span of ATN based on Doppler examination performed within the first few days after KTx. This method could be useful in the early planning of dialysis treatment in patients without hyperkalaemia and...
overhydration. Thus, we would recommend quantification of intrarenal resistance within the kidney graft by using this new and simple parameter—FTI.

There are, however, some limitations of our study. Kidney biopsy was not performed in the majority of patients with delayed graft function, when there was no clinical suspicion of acute rejection or thrombotic microangiopathy. Thus, we are unable to prove that there was no overlapping ATN and subclinical acute rejection in some of the cases.

In conclusion, a discontinuous pattern of Doppler flow spectrum measured in segmental arteries of the kidney graft within the first few days after transplantation is typical for ATN. Moreover, if ATN occurs, a longer duration of ATN in patients with a discontinuous flow pattern is expected. The newly introduced FTI enables better quantitative determination of ATN severity after KTx, especially in patients with a discontinuous flow spectrum.

Conflict of interest statement. None declared.

References


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The effect of rabbit anti-thymocyte globulin induction therapy on regulatory T cells in kidney transplant patients

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Abstract

Background. Prevention of alloreactivity by rabbit anti-thymocyte globulins (rATG) may not only result from immunodepletion but also from the induction of T cells that control allogeneic immune responses. In the present prospective and controlled study, we investigated the effect of rATG on the frequency, function and phenotype of peripheral immunoregulatory CD4+ T cells in kidney transplant (KTx) patients.

Methods. After transplantation, 16 patients received ATG-induction therapy and triple therapy consisting of tacrolimus, MMF and steroids. The control group (n = 18) received triple therapy only. By flow cytometry, T cells were analysed for CD25, FoxP3, CD127, CD45RO and CCR7.

Results. Pre-transplant levels of FoxP3+CD127−/low T cells were 6% of CD4+ T cells. One week post-ATG treatment, no measurable numbers of regulatory T cells were present (P < 0.01). After 4 weeks, the cell numbers

References


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