Perfusion of kidney graft pyramids and cortex in contrast-enhanced ultrasonography in the determination of the cause of delayed graft function

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Summary

Background:
The aim of this study was to assess the usefulness of a new ultrasound technique – contrast-enhanced ultrasound examination (US-CE) – using sulphur hexafluoride in the early post-transplant assessment of graft perfusion. Time-intensity curves (TIC) were compared with hemodynamic flow parameters (resistive index: RI) in patients with good early graft function (EGF) and acute rejection (AR) or acute tubular necrosis (ATN) as a cause of delayed graft function (DGF).

Material/Methods:
US-CE was conducted in order to assess graft perfusion in the early period after kidney transplantation (72–120 hours) in 63 kidney allograft recipients: 35 with EGF and 28 with DGF. The DGF patients were later diagnosed based on graft biopsy as AR (n=10) or ATN (n=18). Time-intensity curves were compared with hemodynamic flow parameters typically assessed in post-operative graft diagnostics (e.g., RI).

Results:
In the examination with US-CE in EGF patients, the regular inflow of contrast medium was demonstrated in all regions of the graft. In patients with DGF, a delay in the inflow of the contrast medium was observed, as well as significant differences in the time of inflow to the regions of interest between those 2 groups. There was a significantly longer inflow time of the contrast medium to the cortex and renal pyramids in patients with AR than in ATN recipients.

Conclusions:
US-CE may be a valuable diagnostic tool in the determination of the cause of DGF.

Key words: contrast-enhanced ultrasonography • kidney graft perfusion • resistive index


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BACKGROUND

The introduction of ultrasound contrast enhancement (US-CE) opened up new directions in ultrasound diagnostics, especially in the assessment of tissue perfusion of parenchymal organs (e.g., in the diagnostics of focal liver lesions) [1–3]. Modern contrast media manifest distinctive high stability of microbubbles and perfusion through microcirculation. This examination enables the identification of regions with a limited number or absence of microcirculation vessels and performance of quantitative assessment of tissue perfusion in the evaluation of highly vascularized organs [4–6].

The use of ultrasound contrast enhancement is a highly promising diagnostic tool in kidney allograft recipients, but to date experience with its use in this clinical setting is very limited [7]. The decrease in tissue perfusion in the transplanted kidney directly affects its functions of glomerular filtration and urine production. Early detection of the cause of severe perfusion disorders in the graft parenchyma and early administration of the appropriate therapy play a pivotal role in the prevention of early graft loss and may affect its long-term function. Undisturbed good early graft function (EGF) is found in the post-operative period in only a fraction of kidney allografts. The vast majority of grafts manifest delayed graft function (DGF), which is influenced by a plethora of factors, and has required quite complex differential diagnostics thus far [8]. At present, the examinations routinely used in the post-operative graft diagnostics are real-time ultrasound (B mode) and color Doppler flow analysis (US-CD) [9–13]. These techniques allow morphological analysis of the transplant and indirect assessment of perfusion based on the distribution of vascular markings and parameters such as Resistive Index (RI) and Pulsatility Index (PI) [14,15].

The aim of this study was to assess the usefulness of contrast-enhanced ultrasound examination in the early post-transplant assessment of graft perfusion, and to compare time-intensity curves (TIC) with hemodynamic flow parameters (resistive index, RI) in patients with early and delayed graft function.

MATERIAL AND METHODS

Sixty-three graft recipients (31 women and 32 men, mean age 49±16 years) from deceased donors were examined in the early period after kidney transplantation (72–120 hours). The patients were divided into 2 groups based on renal graft function: 1 group consisted of 35 patients (mean age 38±15 years) with preserved good early graft function (EGF) who did not require dialysis therapy during the first week after transplantation, and the second group consisted of 28 patients with delayed graft function (DGF). DGF was characterized by requiring supportive dialysis therapy for at least 1 week in the early post-transplant period (assessed retrospectively). Despite the standard triple immunosuppressive therapy (tacrolimus, mycophenolic acid and steroids) with standard steroid regimen, in subsequent follow-up, routine biochemical markers of graft function such as serum creatinine and urea indicated continued worsening in 12 patients, raising a suspicion of rejection. In all of these 12 patients, a kidney biopsy was performed 10–28 days post-transplantation. In 10 patients (mean age 36±12 years) the biopsy confirmed acute graft rejection (AR). In the remaining 18 patients ATN was eventually diagnosed (mean age 40±15 years).

Ultrasound examinations were performed 1–2 hours before planned HD by the same expert radiologist, who was unaware of the purpose of the study, the results of laboratory measurements and patient’s clinical course with the Vivid 7 scanner (General Electric, Milwaukee, USA) using convex probe (type 3.5C), according to the protocol that included morphological assessment of the graft and of the surrounding tissues (B-mode), the assessment of the direction and distribution of the vessels (Color-Doppler, B-flow) and the assessment of flow parameters (PW) [13,15–20]. RI measurements were performed at the level of segmental arteries (3 measurements: upper and lower pole and the central part), and were expressed as means for calculations. In the examination with ultrasound contrast medium, the Sonovue Diagnostics preparation from Bracco Int. (Milan, Italy) was used at a dose of 2.4 ml per examination, administered as an intravenous bolus. Data acquisition was conducted using the protocol with low mechanical index (MI 0.1). Perfusion curves were traced based on the 30-second sequence obtained in the renal longitudinal axis immediately after the intravenous administration of contrast medium. The data were digitally recorded and analyzed on the external workstation (EchoPack, General Electric, USA), where local perfusion was assessed in the regions of interest (ROI) by means of time-intensity curves (TIC) [21]. For statistical analysis, temporal differences were determined in
the inflow until the maximum value of the time-intensity curve was reached (Δt), occurring between renal vessels (segmental arteries) and the parenchymal regions of interest. For the parenchymal region, the maximum time of inflow of the contrast to the renal cortex (Δt1) and to the renal pyramid (Δt2) was analyzed, and the pyramid demonstrating the largest swelling was selected for analysis.

All results are expressed as mean ±SD. Statistical significance was defined at p<0.05. The normality of data distribution was checked by Shapiro-Wilk test, and non-normally distributed data were logarithmically transformed before analysis. Between-group comparisons were performed using t test for independent samples or Mann-Whitney test for normally and non-normally distributed data, respectively. The Pearson or Spearman correlation coefficient was used to assess relations between the variables. Statistical analysis was performed using Statistica (license number SP7105488009951) for Windows (version 6.0, StatSoft, Tulsa, OK, USA).

All subjects gave their written informed consent for participation in the study, and the study protocol was approved by the Bioethics Committee of the Medical University of Lodz, Poland.

RESULTS

The mean RI in EGF patients was significantly lower than in patients with DGF (0.69±0.09 vs. 0.79±0.09, p<0.001) (Figures 1, 2). In the examination with the US-CE in EGF patients, the regular inflow of contrast medium was demonstrated in all regions of the graft. In patients with DGF, the delay in the inflow of the contrast medium was observed, as well as significant differences in the time of inflow to the regions of interest (ROI) between those 2 groups (Figures 3, 4). The mean Δt1 in the EGF group was significantly lower than in patients with DGF (0.92±0.53 s vs. 2.18±0.68 s, p<0.001). The same difference was observed in Δt2 (1.2±0.62 s vs. 3.12±0.83 s, p<0.001).

The outcomes in DGF recipients were disproportionately influenced by the results obtained
in patients with AR. The greatest differences in the inflow time of the contrast medium were between the cortex and renal pyramids in patients with acute allograft rejection (AR). In AR patients, a significant increase in RI was observed in comparison to patients with ATN (0.85±0.09 vs. 0.77±0.09, p<0.001), and a delay in the contrast medium inflow to the analyzed regions of interest in the graft (ROI) was noted in the contrast examination. Data from the regions of interest differed significantly between patients with AR and ATN: Δt1 was 2.35±0.69 s. vs. 2.08±0.69, p<0.01, Δt2 3.25±0.81 s. vs. 3.03±0.81 p<0.001.

We observed positive correlations between the RI values and Δt1 (EGF: r=0.449, p<0.01; DGF with AR (DGFA): r=0.475, p<0.166; DGF due to ATN (DGFB): r=0.693, p<0.01), as well as Δt2 (EGF: r=0.817, p<0.001; DGFA: r=0.449, p<0.193; DGFB: r=0.667, p<0.01). It is also of note that Δt1 and, in particular, Δt2 could much better differentiate the 2 groups (EGF and DGF) than RI index values, and a delay of contrast inflow of more than 2 seconds strongly indicates delayed graft function (Figures 5, 6).

**DISCUSSION**

In the monitoring of the grafts, the diagnostic protocol that included the application of contrast enhancement enabled an objective, quantitative and comparative analysis of tissue perfusion. Expansion of the diagnostic protocol with the application of contrast enhancement reveals a correlation between the values of resistance parameters typically assessed in post-operative graft diagnostics and, occasionally, of the inflow of contrast medium in the individual graft regions [16–18].

The assessment of resistance parameters is indirect, and it reflects the total changes taking place in the microcirculation, without the ability to assess the dynamics of tissue perfusion. The application of contrast enhancement made it possible to visualize parenchymal perfusion in the graft and the analysis of time-intensity curves (TIC), thus allowing quantitative assessment [7,16–18,21–23].

The relation between an increase in the resistive index (RI) and the pulsatility index (PI) is universally accepted [13,19,20]. Infrequent reports from the literature on the application of CE-US for the diagnostics of renal transplants indicate the occurrence of significant dependence of the flow resistive indices (RI) on the velocity of inflow of the contrast medium between renal vessels and the cortex layer. The data obtained in this study confirm earlier observations of Fischer et al that the time of flow of the contrast medium through the bed of microcirculation lengthens (time Δt1) with the increase in flow resistive indices (RI) [17,18,21,24]. An explanation to the described dependence may be that most pathologies occurring in the early post-operative period are vascular (eg, due to stenosis, thrombosis or obliteration of the vessels) [24,25]. Reduction in the flow through the vascular bed directly translates into the increase in resistance, which is reflected by the rise of the resistive index (RI), and results in the irregular and delayed contrast distribution in graft parenchyma in the contrast examination. This explains the differences in resistance parameters and the temporal delay in the contrast inflow to the layer of cortex and renal pyramids in the case of early (EGF) and delayed (DGF) function of the transplanted kidney observed in this study and also by other authors [16–18,21,22].
This paper includes a new and interesting observation of a delay in the contrast medium inflow to renal pyramids, and further demonstrates that this characteristic differentiates the groups with well-preserved and delayed graft function. Relative to the group with DGF, the patients with good graft function demonstrated efficient distribution of contrast medium in the parenchyma. The time necessary to contrast the regions of interest results from linear dependence on time necessary for the contrast agent to move along blood vessels. In the group with DGF, however, a statistically significant temporal delay in the contrast medium inflow between the cortex and renal pyramids was observed, and the result obtained in this group was greatly influenced by biopsy-confirmed acute rejection. In the group with ATN as the cause of DGF, the delay was markedly shorter. An explanation for the temporal delay in the flow of contrast medium between patients with AR and ATN may be found in the differences observed in the macroscopic image of the graft. One of the differences observed in the 2D ultrasound macroscopic image is the swelling of renal pyramids. Divergences in the distribution of the contrast medium between patients with AR and ATN may indicate the role played by the swelling pyramids as a mechanical factor causing delay in the flow through the compressed blood vessels at the intralobar level. The swelling of the pyramids that occurs at a low intensity in the ATN is temporary, and decreases after regeneration of the injured epithelium of renal tubes, exerting little influence on the increase in vascular resistance and contrast distribution. However, the swelling of renal pyramids that occurs in AR is caused by the intensified (immunological) reaction initiated by T lymphocytes activated by the HLA antibodies from the donor. The activated T lymphocytes proliferate and generate numerous lymphokines, which subsequently activate B lymphocytes. The activation of B lymphocytes triggers the cascade of the inflammatory process leading to the gradual destruction of the graft [23,24]. Unless appropriate therapy has been administered, this may extend the increase in resistance of the vascular bed and disturb the perfusion of contrast in the parenchyma, especially delaying the inflow of the contrast medium to the regions of renal pyramids affected by the swelling.

The explanation of the differences in the distribution of contrast medium between the determined regions of interest in kidney recipients with AR and ATN may be found at the molecular level. At the core of an acute immunological response is the occurrence of the anti-HLA antibodies, which damage the endothelium that lines the capillaries of microcirculation vessels and trigger the cascade of coagulation [26]. Multifocal microthrombosis and embolisms of microcirculation vessels are reflected in the increase in the resistance of the vascular bed, and could provide an explanation for the delay in contrast medium distribution.

There were statistically significant differences in the time of contrast medium perfusion in the regions of renal pyramids between the groups with distinctive EGF and DGF. The main finding of our study was a significant relation between the resistance parameters, and the inflow of contrast medium into the renal pyramids that distinguished patients with AR and ATN as a cause of DGF in the early phase after transplantation. If our observation of the slower inflow of the contrast medium to the pyramids affected by their swelling in cases of acute allograft rejection is confirmed by further research, this would broaden our understanding of early differentiation of the cause of delayed graft function, and would facilitate the choice of optimal therapy.

Conclusions

A delay of contrast medium inflow strongly indicates delayed graft function, and may be of use in the differential diagnosis of delayed graft function.

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