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Careful clinical monitoring in comparison to sequential Doppler sonography for the detection of acute rejection in the early phase after renal transplantation

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Abstract Acute rejection is the most frequent cause of early graft failure. There is unanimity that Doppler sonography is a helpful method for the detection of complications after kidney transplantation. In the past, the indication for renal biopsy relied mainly on clinical assessment, although this assessment has not been standardised. Therefore, we conducted this prospective study to compare the value of sequential Doppler measurements with a standardised clinical rejection score, based on renal function, weight gain, graft swelling and tenderness. Fifty-eight patients (37 males, 21 females, mean age 46 ± 12 years) after kidney transplantation were consecutively enrolled into the study. Doppler investigations were obtained within the first 24 h after transplantation, followed by an interval of 48–72 h. At the same time, a clinical examination was scored by a transplant physician blinded to the Doppler results. Clinical score and Doppler results, both were referred to the histological results of renal biopsy. In 24 out of 58 patients 25 acute rejections occurred. In seven patients, acute re-

jection was superimposed on primary graft failure. The cut-off levels for rejection were set at $RI \geq 0.80$ and $PI \geq 1.70$ based on receiver-operator curves using data from 663 Doppler examinations. Sensitivity and specificity was 72 % for RI, and 72 % and 74 % for PI, respectively. The calculation of the intraindividual increase ($\Delta RI \geq 3\%$, $\Delta PI \geq 10\%$) did not improve these values. The clinical score revealed a sensitivity and specificity of 82 % and 87 %, respectively. The combined analysis of Doppler indices and clinical score showed a sensitivity of 96 % with a specificity of 66 %. Careful clinical monitoring alone using a clinical score is an appropriate procedure with which to decide about renal biopsy. Our data show that Doppler sonography should be performed within the first 24 h after transplantation to evaluate graft perfusion and baseline values. Afterwards, it should be used when clinical signs of rejection occur to underline the decision for renal biopsy even in borderline cases.

Key words Doppler sonography · Acute rejection · Renal transplantation · Clinical score

Introduction

Early graft function is a major determinant of graft survival after renal transplantation [5]. Thus, differential diagnosis of early graft failure is a challenge for every

transplant physician. There is unanimity that Doppler sonography is valuable for the evaluation of vascular complications which may induce early graft failure, e.g. renal artery stenosis and thrombosis of the graft [7, 9, 10].

Nevertheless, acute rejection is the most frequent cause of early graft failure. In the past, the indication for renal biopsy relied mainly on clinical assessment, although this assessment has not been standardised [1, 17]. It seems feasible that the Doppler technique can detect acute rejection, since there may be an increase of renal vascular resistance due to organ swelling during rejection [16]. Accordingly, many authors determined intrarenal Doppler parameters, e.g. the resistive index (RI) and the pulsatility index (PI), for the detection of acute rejection after renal transplantation [2–4, 8, 18–20]. Because both acute rejection and acute tubular necrosis are associated with elevated RI or PI, there are major problems in distinguishing these frequent causes of early graft failure by a “snap-shot-picture” [2, 6, 18]. Since Doppler indices depend mainly on recipient-related factors, sequential measurements are probably more reliable [12].

In consequence, some authors reported encouraging results by scanning the grafts sequentially after transplantation [7, 13, 21, 24, 25]. In these studies, acute rejection was diagnosed by intraindividual comparison of Doppler indices (Δ RI, Δ PI). The indices usually rise during rejection, while acute tubular necrosis is characterised by initially high but later decreasing Doppler indices [24]. From these studies, however, it cannot be decided whether time consuming sequential Doppler measurement is indeed the better approach to detect rejection than careful clinical monitoring [7, 13, 21, 24].

Therefore, we conducted this prospective study to investigate the value of sequential Doppler measurements for the detection of acute rejection and to compare this technique with a standardised clinical rejection score. Doppler and clinical results, both were referred to the histological results of renal biopsy.

Materials and methods

Patients

Fifty-eight kidney transplantations were consecutively performed in our unit. All patients (37 males, 21 females, mean age 46 ± 12 years, range 22–68) were enrolled into the study. The mean cold ischemic time was 25 ± 7 h (range 2–38). All subjects received standardised immunosuppressive therapy consisting of steroids (0.2–0.5 mg/kg per day in the first month), azathioprine (2.0 mg/kg per day in the first month) and cyclosporine A (target serum trough level 180–220 ng/ml). In the case of prior renal transplantations (13 subjects with second transplantation and two subjects with third transplantation), anti-T-lymphocyte-globulin of the rabbit (ATG-Fresenius 10 ml/day) was administered for the first 10 days. Primary graft failure occurred in 21 of the 58 patients (36%), so that hemodialysis was required for a mean period of 17 ± 11 days (range 1–48) in these patients. The mean heart rate during Doppler examination was 81 ± 13 beats/min (range: 48–130) and the mean arterial blood pressure was 108.5 ± 14.7 mmHg (range: 67–170) measured in the supine position.

Doppler measurements

During hospitalisation, all patients were prospectively scanned with a total number of 663 colour Doppler studies (mean: 11 ± 4 studies / patient). In each patient, the first Doppler examination was done within 24 h after transplantation. At intervals of 48–72 h, sequential Doppler measurements were carried out by the same investigator, who was blinded to the clinical course of the patients. The grafts were scanned with a phased array 2.5–3.5 MHz transducer (Acuson 128 XP10, Mountain View, Calif., USA) in the supine position. Interlobar and segmental renal arteries were visualised by the colour mode as previously described [11]. In each instance, RI and PI were calculated from the Doppler spectra using the following ratios: RI = peak systolic velocity – end diastolic velocity / peak systolic velocity; PI = peak systolic velocity – end diastolic velocity / mean velocity.

The values of six different spectral samples were averaged to the mean RI or PI of the graft, which were used for statistical evaluation. The intraindividual difference of two consecutive measurements (Δ RI, Δ PI) was calculated and referred for statistical evaluation. Additionally, RI and PI were corrected for heart rate according to the formula published by Schwert et al.; however, this correction did not influence sensitivity and specificity of the indices (data not shown) [22]. RI and PI correlated neither with the mean arterial blood pressure nor with the cyclosporine trough levels, so that these values were not considered for evaluation.

Clinical score

On the same day as Doppler examination, the patients were investigated by an experienced transplant physician, who was blinded to the results of Doppler sonography. This clinical investigation was scored with a maximum of 15 points accordingly to a created protocol. This protocol included consideration of serum creatinine level, daily diuresis, weight gain and body temperature of the recipient as well as the size and tenderness of the graft (Table 1).

Finally, a third physician, unaware of Doppler results and clinical score, examined the patients on his daily visit. This independent physician initiated renal biopsy in the case of suspected acute rejection before specific therapy was started. Biopsies were also routinely performed when prolonged primary graft failure occurred (> 10 days). The histological results were classified according to the Banff classification [23] and served as the gold standard to evaluate the accuracy of Doppler sonography and clinical score. The date of biopsy was defined as the day of rejection (day 0) for further calculations.

Table 1 The clinical score for the diagnosis of acute rejection in the early period after renal transplantation

Parameters	1 points	2 points	3 points
Size of the graft	Small	Normal	Large
Tenderness of the graft	Smooth	Normal	Firm
Body temperature (°C)	< 37	37–38	> 38
Increase of serum creatinine (mg/dl per day)	< 0.3	> 0.3	
Decrease of daily diuresis (ml/day)	< 300	> 300	
Increase of body weight (g/day)	< 500	> 500	

Statistical analysis

All values are expressed as means \pm SD. The Mann-Whitney rank sum test was used for the comparison of two not normally distributed groups. $P < 0.05$ was considered statistically significant. Univariate linear logistic regression analysis was performed to determine associations between Doppler indices, hemodynamic parameters and cyclosporine levels. Sensitivity, specificity and predictive values were determined using the four-fold-table. Receiver-operator-curves (ROC) were calculated for different cut-off values of the parameters, referred to the histological results of biopsy.

Results

Renal biopsy

During the study period, a total of 30 renal biopsies were taken from the 58 recipients. In a mean time of 10 ± 8 days after transplantation, 25 acute rejections occurred in 24 patients and were histologically classified (three mild, eight moderate and four severe interstitial lesions, six moderate vascular lesions and four mixed forms). In seven out of the 24 patients, acute rejection was superimposed on primary graft failure. Five renal biopsies revealed acute tubular necrosis without any signs of acute rejection. Four grafts had to be explanted due to arterial thrombosis (one), refractory acute interstitial rejection (one) and vascular rejection (two).

Doppler sonography

RI and PI

Based on 663 Doppler measurements in the total study group, the mean RI and PI was 0.75 ± 0.09 (range 0.44–1.00) and 1.71 ± 1.76 (range 0.47–35.1), respectively. In general, patients with acute rejection had significantly higher Doppler indices than patients without rejection (mean RI: 0.77 ± 0.09 versus 0.74 ± 0.09 ; $P < 0.05$, mean PI: 1.92 ± 2.47 versus 1.52 ± 0.57 ; $P < 0.0001$). The first Doppler values (< 24 h after transplantation) of the patients with primary graft failure ($n = 21$) were significantly higher than that of the patients with primary functioning grafts ($n = 37$) (mean RI: 0.75 ± 0.12 versus 0.68 ± 0.10 ; $P < 0.01$, mean PI: 1.86 ± 1.28 versus 1.27 ± 0.34 ; $P < 0.005$); however, there was a large overlap (Fig. 1).

The intraindividual follow-up of the patients with rejection revealed a significant rise of both RI and PI at the time of rejection (day 0) referred to the prior value (mean RI: 0.84 ± 0.10 versus 0.77 ± 0.11 ; $P < 0.05$, mean PI: 2.87 ± 3.80 versus 2.33 ± 3.70 ; $P < 0.05$) (Fig. 2a, b). Both RI and PI of the grafts without rejection did not differ intraindividually during the follow-up. The following cut-off values were determined by the ROC:

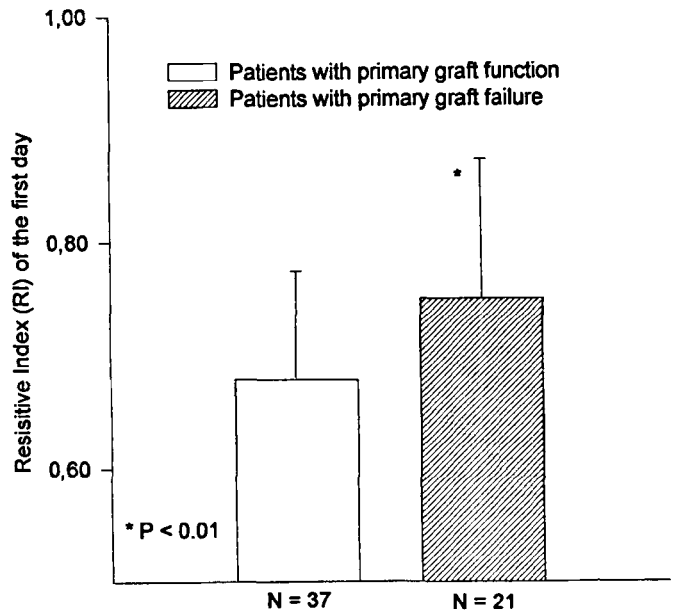


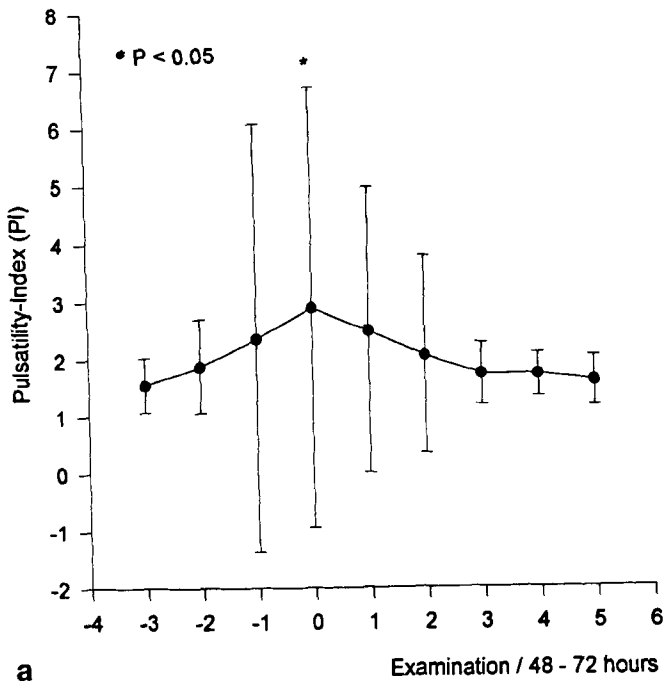
Fig. 1 The open bars show the means \pm SD of the resistive index (RI) measured within 24 h after transplantation in patients with primary graft function ($n = 37$). The haiched bars show the means \pm SD of the first RI of the patients with primary graft failure ($n = 21$). There is a significant difference ($P < 0.01$) between the two groups tested with the Mann-Whitney test

RI ≥ 0.80 with Δ RI $\geq 3\%$ and PI ≥ 1.70 with Δ PI $\geq 10\%$ (Fig. 3). The values of diagnostic accuracy for these thresholds are given in Table 2.

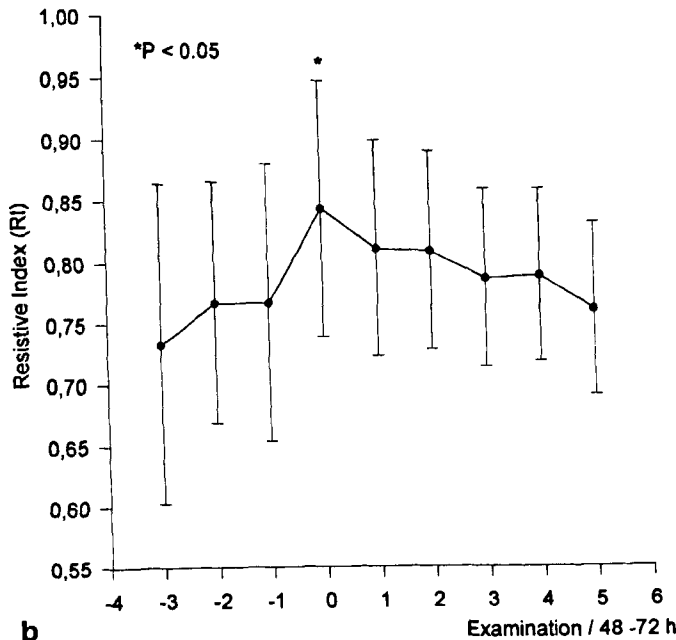
There was no correlation between Doppler indices and possibly influencing factors, e.g. cyclosporine trough levels, mean arterial blood pressure or heart rate (data not shown).

Clinical score

The mean clinical score of the patients with acute rejection ($n = 313$ clinical examinations) was higher than that of the patients without rejection ($n = 350$ clinical examinations) (8.2 ± 2.2 versus 7.8 ± 2.1 patients, $P < 0.01$). At the time of rejection (day 0), the mean score of the 24 patients was significantly increased compared with the previous value (11.1 ± 1.9 versus 8.0 ± 1.7 patients; $P < 0.001$) for the same patient (Fig. 4b). The ROC revealed the best cut-off value at ≥ 10 patients for the diagnosis of acute rejection (Fig. 3). Using this criterion, the sensitivity and specificity of the score was 88% and 82%, respectively; however, the positive predictive value was rather low (25.6%). The calculation of relative values, e.g. percentage of the increase of the score, did not improve the results (Table 2).



a



b

Fig. 2a, b The pulsatility index (PI) and resistive index (RI) of the 24 patients with acute rejections are shown as means \pm SD during the rejection period. Day 0 was defined as the day of rejection, when a biopsy was taken and antirejection therapy was started. The Doppler examinations were performed with an interval of 48-72 h. Mean PI and RI obtained on the day of rejection (day 0) differed significantly ($P < 0.05$) in comparison to the prior value

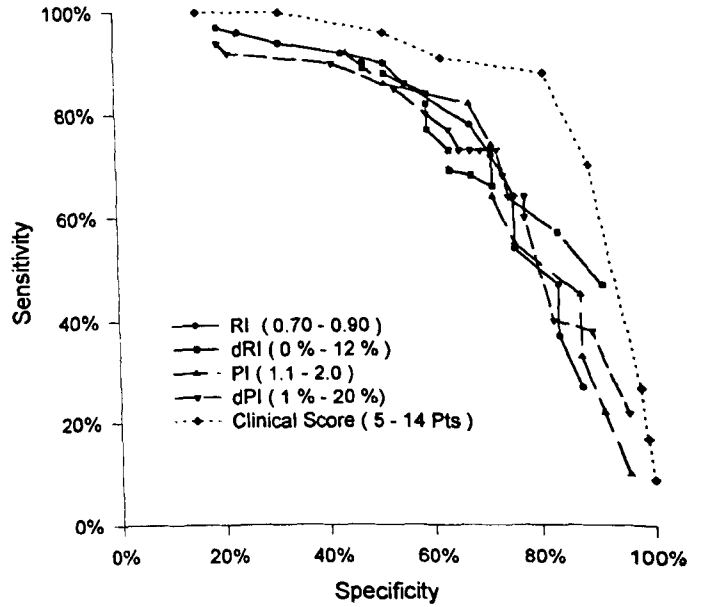


Fig. 3 Receiver-operator-curves (ROC) of the different parameters are shown; these were calculated for the number of histologically proven acute rejections. The ROC of the clinical score reveals the best diagnostic accuracy represented by the largest area under the curve

Combination of Doppler measurements and clinical score

The combined analysis of Doppler indices and clinical score showed an improved sensitivity of 96% for the detection of acute rejection. This improved diagnostic accuracy based on two patients with acute rejection, who had elevated RI and PI (≥ 0.80 and ≥ 1.70 , respectively) without significant clinical signs of rejection (score < 10 patients). The specificity of combined clinical and Doppler monitoring, however, was rather low, with 66% due to the high number of falsely elevated Doppler indices (RI ≥ 0.80 ; PI ≥ 1.70) in the 34 patients without rejection.

Discussion

We tested the utility of Doppler sonography in comparison to a clinical score for the detection of acute rejection early after kidney transplantation. Using sequential measurements we could not confirm the encouraging results of previous Doppler studies [7, 13, 21, 24]. With standardised intraindividual follow-up and defined cut-off values of Δ RI ($> 3\%$) and Δ PI ($> 10\%$), the sensitivity and specificity were rather low. However, the best but still rather disappointing sensitivity and specificity was calculated for absolute cut-off values of RI and PI (Table 2). Our findings are in clear contrast to previous

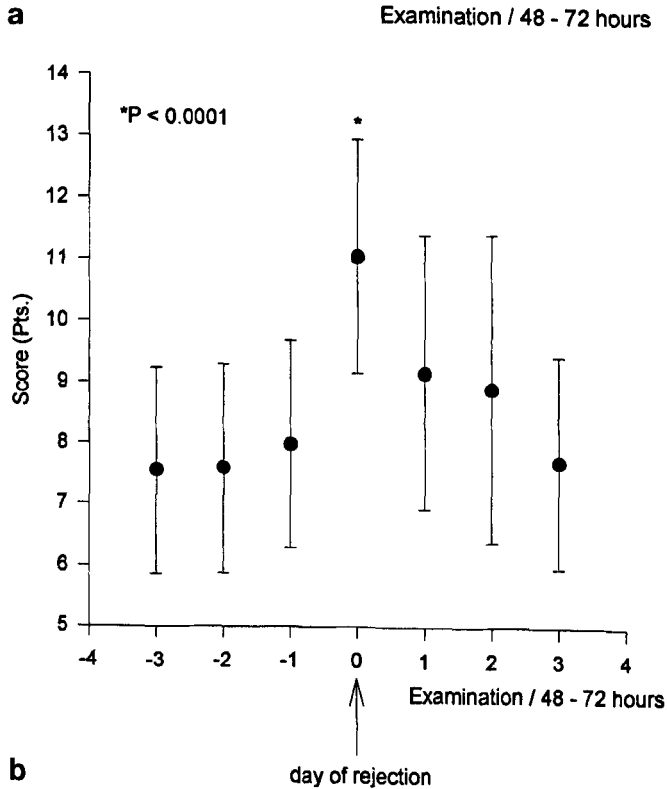
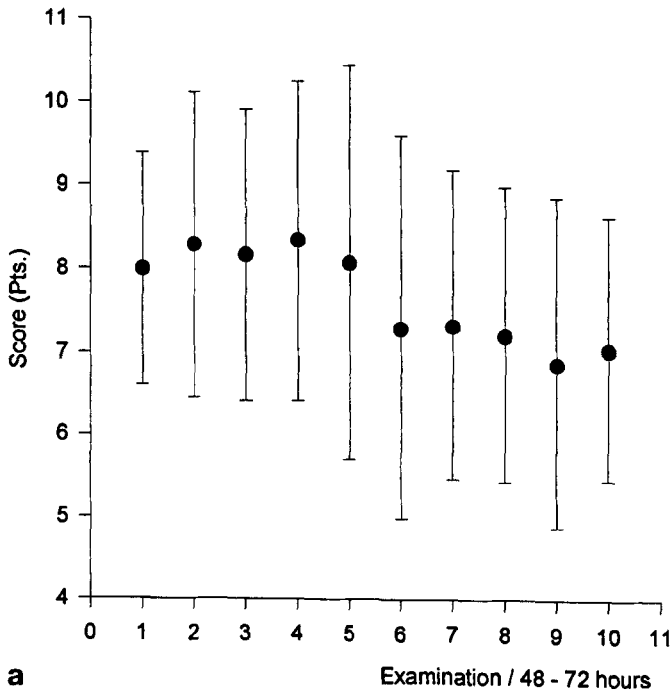


Fig. 4a, b The score of 34 patients without rejection and the score of 24 patients with rejection are shown as means \pm SD. Day 0 was defined as the day of rejection, when biopsy was taken and antirejection therapy was started. The clinical examinations were performed with an interval of 48–72 h. The mean score obtained at the day of rejection (day 0) differed significantly ($P < 0.0001$) in comparison to the previous value

Table 2 The cut-off values with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the clinical score and of the Doppler indices for the diagnosis of acute rejection in the early post-transplant period

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RI \geq 0.80	72	72	17.6	96.6
Δ RI \geq 3%	68	68	15.8	92.4
PI \geq 1.70	72	74	17.9	96.5
Δ PI \geq 10%	72	72	16.6	93
Score \geq 10 pts	82	87	25.6	98.2
Δ Score \geq 12%	74	74	20.1	96.4

published studies which considered follow-up values and reported increased sensitivities and specificities of the sequential Doppler technique [7, 13, 15, 21].

Hollenbeck et al. calculated the daily increase of RI and PI; however, the measurements were only performed twice a week [7]. In this particular study, a continuous rise of the indices was considered as a true positive result in patients with rejection. However, the magnitude of the rise of the indices is not specified. Our data suggest that there is a high rate of falsely positive scans due to elevated Doppler indices in normal functioning grafts without any signs of rejection.

Rob et al. [21] retrospectively calculated maximum differences of RI and PI values in the intraindividual course of patients during normal and pathological function. They found significant maximum differences in the indices of patients with several parenchymal disorders, e.g. acute tubular necrosis and vascular rejection. However, this procedure does not seem practicable, because the time of normal function with the lowest index as well as the time of worst function with the highest indices cannot be prospectively defined. Therefore these differences are of no clinical use for prospective Doppler monitoring in the daily routine. Leimenstoll et al. observed a significant rise of PI during rejection episodes when performing daily measurements. Comparing the Doppler results (increase of PI $>$ 20%) with the results of biopsies, they found an impressive sensitivity and specificity of 94% and 96%, retrospectively [13]. These authors calculated the baseline values as an average of five consecutive measurements during stable graft function. This means, on the one hand, that a longer period of stable graft function is necessary for comparison. On the other hand, the variation of the single examination is neglected by this procedure. Furthermore, daily scanning seems not to be practicable in the daily routine of a transplant unit.

In our study, the Doppler indices obtained at the first postoperative day were significantly elevated in the grafts with primary failure in comparison with that of the normally functioning grafts. This is in agreement with previous studies [14, 15, 24]. However, we found a

large overlap of the data, so that it seems difficult to identify acute tubular necrosis in an individual patient.

In contrast to all previous Doppler studies, we evaluated a clinical rejection score to assess the efficacy of Doppler monitoring. Surprisingly, the ROCs of the different parameters revealed the best accuracy for the clinical score (Fig. 3), as shown by the greatest area under the curve. The combination of Doppler sonography and clinical examination improved the sensitivity; however, due to the large number of falsely elevated Doppler indices in patients with normal functioning grafts, the specificity and positive predictive was rather low. This means that sequential Doppler sonography will induce many transplant biopsies, which will eventually show no histological signs of rejection. It is questionable whether this is indeed a disadvantage. A higher

sensitivity of 96% will ensure that less acute rejections will be overlooked by the combination of both procedures.

Our data show that careful clinical monitoring is at least an appropriate procedure to detect acute rejection and to decide about renal biopsy. Furthermore, time consuming sequential Doppler measurements were of no use to increase the accuracy. We conclude that Doppler sonography should be performed within the first 24 h after transplantation to evaluate graft perfusion and baseline values. Afterwards, it should be exclusively used when clinical signs of rejection occur. Acute rejection should be suspected if either $RI \geq 0.80$ or $PI \geq 1.70$ are measured. It has to be proven in further studies whether the created clinical score can be established in the daily routine.

References

- Chan GL, Hodge EE, Chang HH (1989) The use of routinely available clinical data in differentiating renal allograft rejection from cyclosporine nephrotoxicity. *Transplantation* 48: 1075-1076
- Frauchiger B, Bock A, Eichlisberger R et al. (1995) The value of different resistance parameters in distinguishing biopsy-proved dysfunction of renal allografts. *Nephrol Dial Transplant* 10: 527-532
- Genkins SM, Sanfilippo FP, Carroll BA. (1989) Duplex Doppler sonography of renal transplants: lack of sensitivity and specificity in establishing pathologic diagnosis. *Am J Roentgenol* 152: 535-539
- Gifford RR, Neuymer MM, Yang HC, Escobar FS, Thiele BL (1994) Successful identification of acute renal allograft rejection with duplex ultrasonography. *Clin Transplant* 8: 40-44
- Halloran PF, Aprile MA, Farewell V et al. (1988) Early function as the principal correlate of graft survival. A multivariate analysis of 200 cadaveric renal transplants treated with a protocol incorporating antilymphocyte globulin and cyclosporine. *Transplantation* 46: 223-228
- Hollenbeck M (1994) New diagnostic techniques in clinical nephrology. Colour coded duplex sonography for evaluation of renal transplants-tool or toy for the nephrologist? *Nephrol Dial Transplant* 9: 1822-1828
- Hollenbeck M, Hilbert N, Meusel F, Grabensee B (1994) Increasing sensitivity and specificity of Doppler sonographic detection of renal transplant rejection with serial investigation technique. *Clin Invest* 72: 609-615
- Kelcz F, Pozniak MA, Pirsch JD, Oberly TD (1990) Pyramidal appearance and resistive index: insensitive and non-specific sonographic indicators of renal transplant rejection. *Am J Roentgenol* 155: 531-535
- Krumme B, Gondolf K, Kirste G, Schollmeyer P, Keller E (1993) Farbko-dierte Duplexsonographie zur Diagnostik von Nierenvenenthrombosen in der Frühphase nach Nierentransplantation. *Dtsch Med Wochenschr* 118: 1629-1635
- Krumme B, Blum U, Benzing T, Keller E, Schollmeyer P, Rump LC (1996) Treatment of primary graft dysfunction after kidney transplantation by renal artery stent. *Nephrol Dial Transplant* 11: 208-210
- Krumme B, Blum U, Schwertfeger E et al. (1996) Diagnosis of renovascular disease by intra- and extrarenal Doppler scanning. *Kidney Int* 50: 1288-1292
- Krumme B, Grotz W, Kirste G, Schollmeyer P, Rump LC (1999) Determinants of intrarenal Doppler indices in stable renal allografts. *J Am Soc Nephrol* (in press)
- Leimenstoll G, Engemann R, Grigat KP, Bartholdy A, Niedermayer W (1990) Duplex ultrasound: monitoring of rejection episodes of renal allografts. *Transplant Proc* 22: 1392-1394
- Merkus JW, Hoitsma AJ, van Asten WN, Koene RA, Skotnicki SH (1994) Doppler spectrum analysis to diagnose rejection during posttransplant acute renal failure. *Transplantation* 58: 570-576
- Meyer M, Paushter D, Steinmuller DR (1990) The use of duplex Doppler ultrasonography to evaluate renal allograft dysfunction. *Transplantation* 50: 974-978
- Norris CS, Barnes RW (1984) Renal artery flow velocity analysis: a sensitive measure of experimental and clinical renovascular resistance. *J Surg Res* 36: 230-236
- Oh CS, Stratta RJ, Pirsch JD, Sollinger HW, Kalayoglu M, Belzer FO (1988) A simple and reliable way of differentiating acute rejection from cyclosporine nephrotoxicity in renal transplantation. *Transplantation* 46: 311-312
- Phillips AO, Deane C, O'Donnell PO, Bewick M, Hillis AN. (1994) Evaluation of Doppler ultrasound in primary non-function of renal transplants. *Clin Transplant* 8: 83-86
- Quarto Di Palo F, Rivolta R, Elli A, Castagnone D (1996) Relevance of resistive index ultrasonographic measurement in renal transplantation. *Nephron* 73: 195-200
- Rigsby CM, Taylor KJ, Weltin G et al. (1986) Renal allografts in acute rejection: evaluation using duplex sonography. *Radiology* 158: 375-378
- Rob PM, Jansen O, Richter V, Erbsloh-Moller B, Marienhoff N, Wiedemann G (1993) Diagnosis of renal transplant failure by real-time and duplex Doppler sonography. *Clin Invest* 71: 531-536
- Schwerk WB, Restrepo IK, Prinz H (1993) Semiquantitative analysis of intrarenal arterial Doppler flow spectra in healthy adults. *Ultraschall Med* 14: 117-122

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23. Solez K, Axelsen RA, Benediktsson H et al. (1993) International standardisation of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int* 44: 411-422
 24. Stevens PE, Gwyther SJ, Hanson ME et al. (1993) Interpretation of duplex Doppler ultrasound in renal transplants in the early postoperative period. *Nephrol Dial Transplant* 8: 255-258
 25. Tranquart F, Lebranchu Y, Haillet O, Pourcelot D, Grezard O, Pourcelot L (1993) The use of perioperative Doppler ultrasound as a screening test for acute tubular necrosis. *Transplant Int* 6: 14-17