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ORIGINAL ARTICLE

Noninvasive evaluation of renal allograft fibrosis by transient elastography – a pilot study

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Summary

Chronic allograft injury (CAI) is the most common cause of graft failure after the first year of transplantation. To date, only protocol biopsies can reveal subclinical disease. Transient elastography (TE) is a novel noninvasive technique that has demonstrated high reliability in the assessment of liver fibrosis. This study evaluates the feasibility of TE for the assessment of renal allograft fibrosis. Fifty-seven patients underwent TE by the FibroScan® device. Biopsies were performed in 20 patients. Measurement of parenchymal stiffness by TE was successful in 55 of 57 patients (96.5%). Stiffness was significantly correlated to the extent of interstitial fibrosis (Pearson r: 0.67, P: 0.002, R^2: 0.45) and inversely related to estimated glomerular filtration rate (eGFR) (Pearson r: −0.47, P: 0.0003, R^2: 0.22). Stiffness values of patients with an eGFR >50 ml/min were significantly lower than in patients with an eGFR ≤50 ml/min (22.2 ± 11.0 vs. 37.1 ± 14.2 kPa, P: 0.0005). The stiffness values of CAI Banff grades 0–1 differed significantly from grade 2 (P: 0.008) and grade 3 (P: 0.046). Parenchymal stiffness measured by TE reflects interstitial fibrosis in kidney allografts. A longitudinal assessment of parenchymal stiffness might be a powerful tool to identify patients with CAI who benefit from biopsy and consequent adaptation of the immunosuppressive regime.

Introduction

Chronic allograft injury (CAI) is the most common cause of graft failure beyond the first year after transplantation. The pathophysiology of this entity is incompletely understood. Following the recently revised Banff classification system, the former term 'chronic allograft nephropathy' has been replaced by 'interstitial fibrosis and tubular atrophy, without evidence of any specific etiology' [1]. This definition adequately describes the common final pathway of interstitial fibrosis and tubular atrophy as the consequence of various immunologic and nonimmunologic processes. Immunologic factors comprise both clinical and subclinical rejection episodes, human leukocyte antigen match, and panel reactive antibodies [2,3]. Nonimmunologic factors include hypertension, glomerular hypertension and hypertrophy, delayed graft function, and drug toxicity. The immunosuppressive regimen may affect interstitial fibrosis in a dialectic manner, as on the one hand an adequate level of immunosuppression provides protection against rejection episodes. On the other hand immunosuppressive agents like calcineurin inhibitors (CNIs) may promote vascular damage and interstitial fibrosis.

Progressive interstitial fibrosis and tubular atrophy finally lead to an increase in serum creatinine, which may prompt the clinician to perform an allograft biopsy revealing CAI. Unfortunately, the period between initiation of the progressive changes in the renal parenchyma and a rise of creatinine followed by the biopsy-based diagnosis is usually too long. Thus, at the time of diagnosis, there are advanced irreversible histomorphological

renoparenchymal changes. An early therapeutic intervention [e.g. CNI reduction or conversion to mammalian target of rapamycin (mTOR) inhibitors], however, requires a diagnosis of CAI before it becomes clinicially evident by a rise of creatinine. Therefore, some transplant centers established a protocol biopsy program, e.g. at 6, 12 and 26 weeks post-transplantion [4]. Protocol biopsies have indeed demonstrated that histological lesions of CNI toxicity can develop as early as 3 months post-transplantation. Many authors regard protocol biopsies as a valuable tool for detecting subclinical disease that can benefit from modification of therapy [5]. In a risk evaluation of >1000 protocol biopsies, the following complication rates were reported: Gross hematuria 3.5%, perirenal hematomas 2.5%, arterio-venous fistulas 7.3% and vasovagal reactions 0.5%. Major complications requiring invasive procedures such as blood transfusions or urinary catheter were seen in 1% of cases [4]. Therefore, the current dilemma is not whether protocol biopsies reveal insight in subclinically progressive disease, but whether the gain justifies the risk. The easiest solution of this dilemma would be a noninvasive technique to assess the development of progressive fibrosis post-transplantation. Ideally, this noninvasive technique should be accurate for the grading of fibrosis, easy to perform, reliable and inexpensive, thus allowing a longitudinal monitoring of the progression of fibrosis after transplantation.

Transient elastography (TE, FibroScan®; Echosens, Paris, France) is a novel noninvasive method that has been evaluated for the assessment of hepatic fibrosis in patients with chronic liver diseases by measuring parenchymal stiffness. TE is a rapid technique that can easily be performed at the bedside or in the outpatient clinic with immediate results and good reproducibility [6,7]. It has proven a good correlation to biopsy-proven grade of hepatic fibrosis and it is increasingly used in routine clinical care of hepatitis patients [8]. This study evaluates the feasibility of TE for the assessment of renal allograft fibrosis.

Patients and methods

Study population

Fifty-seven renal transplant patients were included in the study. Sixteen patients had stable graft function with an estimated glomerular filtration rate (eGFR) >50 ml/min, 41 had impaired graft function (eGFR < 50 ml/min). Inclusion criteria were age >18 years, ability to declare informed consent and a functioning renal allograft. The study was approved by the local ethics committee of the Charité – Universitaetsmedizin Berlin.

Patients' characteristics including gender, age, body mass index (BMI), proteinuria, immunosuppression and comorbidities are presented in Table 1. In those patients undergoing biopsy, the mean time span between first deterioration of graft function and biopsy was 21.6 ± 25.2 days. The modification of diet in renal disease formula was used for the estimation of eGFR.

Principle of transient elastography

Transient elastography was performed using the FibroScan® device as shown in Fig. 1. Briefly, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency (50 Hz) are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness [the elastic modulus E expressed as $E = 3\rho V^2$, where V is the shear velocity and ρ is the mass density (constant for tissues)]. The stiffer the tissue, the faster the shear wave propagates. TE measures parenchymal stiffness in a volume that approximates a cylinder 10 mm wide and 25–30 mm long (Fig. 1).

Protocol

The probands underwent an assessment of allograft stiffness by TE, an ultrasound-based measurement of resistance index (RI), and a measurement of serum creatinine. All these examinations were performed on the same day. In 20 of these patients, allograft biopsy was performed because of deterioration of graft function closely around the time of FibroScan® examination.

Table 1. Study population.

Number of subjects included	57
Female	16 (28.1)
Male	41 (71.9)
Age (years)	56.1 ± 11.8
Body mass index (kg/m^2)	26.2 ± 4.2
Proteinuria (mg/24 h)	560 ± 1041
Immunosuppression	
Triple immunosuppression	42 (73.7)
Mono/dual immunosuppression	15 (26.3)
Calcineurin inhibitors	44 (77.2)
mTOR inhibitors	3 (5.3)
Mycophenolic acid	48 (84.2)
Azathioprine	2 (3.5)
Steroids	53 (93.0)
Hypertension	48 (84.2)
Diabetes mellitus	16 (28.1)

mTOR, mammalian target of rapamycin.
Age, body mass index and proteinuria are presented as mean ± standard deviation. Values in parentheses are percentages.

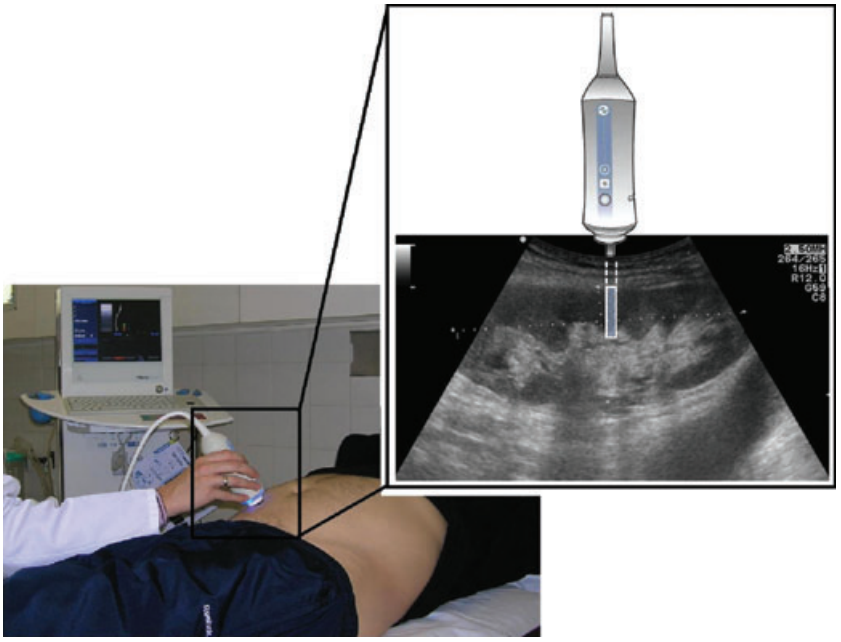

Figure 1 Performance of transient elastography. The renal allograft ultrasound illustrates the site and volume of measurement that approximates a cylinder 10 mm wide and 25–30 mm long.

Conduction of transient elastography

All the measurements were conducted by the same observer (R.A.), blinded to patient data. Measurements were performed with the patient lying in a supine position. Before starting the elastography, a conventional ultrasound examination of the renal allograft was performed to define an optimal position for the transducer probe. Criterion for the choice of this site was the possibility of a preferably orthograde positioning of the transducer on the renal surface and maximal broadness of the parenchyma. The distance between tip of the transducer and kidney surface was measured. In pretests we had shown that the impression depth with the TE probe exceeded the impression depth of the sonography probe by about 5 mm because of its smaller tip surface. Therefore, if the distance was <20 mm in sonography, probe 'S1' was selected (measurement depth of 15–40 mm). If the distance was ≥20 mm, probe 'S2' was selected (measurement depth of 20–50 mm). Both probes have a transducer frequency of 5 MHz. The tip of the transducer probe was covered with coupling gel. Once the area of measurement had been located, the operator pressed the probe button to begin an acquisition. He repeated the procedure until 10 valid measurements were performed in each patient. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The median value was considered representative of the elastic modulus of the tissue. The interquartile range of the 10 stiffness measurements was calculated as a measure of intra individual variation.

Assessment of resistance index

All color Doppler examinations were performed by a single investigator (S.S.), blinded to patient data, using a Siemens SONOLINE G50 machine (Siemens, Munich, Germany) with a 5-MHz convex-array transducer, with the patient lying in a supine position. Intrarenal Doppler spectra of the segmental arteries were obtained at three representative locations from the upper, middle and lower third of each kidney. RI was calculated as '(peak systolic velocity – end diastolic velocity)/peak systolic velocity'. The average of the three measurements was used for statistical analysis.

Histological examination of renal allograft

Kidney tissue obtained by needle biopsy was fixed in 4% buffered formaldehyde. After embedding in paraffin, 2-μm-thick serial sections were cut and hematoxylin and eosin staining, periodic acid schiff reaction and methanamine silver method was applied for assessment of tubular atrophy and other changes of chronic rejection. In addition, a Masson–Goldner's trichrome method was performed to quantify the percentage of blue stained collagen in the interstitium in relation to the red color of the cytoplasm, e.g. of the tubular cells. Stainings were performed according to standard protocols. Slides were analyzed independently by two investigators (C.L., M.G.) blinded to patient data and problematic cases were discussed over a multihead microscope until consensus was reached. Biopsy specimens were classified according to the Banff scheme for CAI (interstitial fibrosis and tubular atrophy,

Table 2. Banff classification of chronic allograft injury (CAI) [1].

Grade 1	Mild fibrosis of the interstitium (6–25% of the cortical area) and mild atrophy of the tubules (≤25% of the area of the cortical tubules) either with or without specific glomerular or vascular findings suggestive of CAI
Grade 2	Moderate interstitial fibrosis (25–50% of the cortical area) and moderate tubular atrophy (26–50% of the area of the cortical tubules) with or without specific changes as in grade 1
Grade 3	Severe interstitial fibrosis (>50% of the cortical area) and tubular atrophy (>50% of the area of the cortical tubules) with or without specific changes as in grade 1

without evidence of any specific etiology) [1] as presented in Table 2.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Statistical analysis was performed by NCSS 97 (NCSS, Kaysville, UT, USA). The association of graft function parameters and parenchymal stiffness was analyzed by Pearson correlation. Linear regression analysis was performed in order to describe the relation of the extent of fibrosis and parenchymal stiffness. Stiffness values of patients with stable and impaired graft function and stiffness values of the individual Banff grades were compared by Mann–Whitney *U*-test. All tests were performed with a two-tailed type-I error rate of $P < 0.05$.

Results

The assessment of renal allograft parenchymal stiffness was successful in 55 of 57 patients (96.5%). In two patients, the FibroScan® device was unable to provide any stiffness values (one with biopsy, one without biopsy). Mean stiffness was 32.7 ± 14.9 kPa with an interquartile range of 14.9 ± 12.3 kPa and a success rate of 73.7 ± 28.3% (Table 3). Mean distance of transducer tip and kidney surface was 17 ± 6 mm. Assessment of RI was successful in all the patients, the corresponding values are presented in Table 3. Allograft biopsy was performed in 20 patients. Biopsy specimen retrieval was sufficient for histological

Table 3. Graft characterization.

Donor age (years)	47.2 ± 15.4
Time since transplantation (months)	53.5 ± 70.4
Creatinine (μmol/l)	222.5 ± 142.1
eGFR (ml/min)	32.5 ± 17.9
Resistance index	0.75 ± 0.10
Parenchymal stiffness (kPa)	32.7 ± 14.9
Interquartile range of stiffness (kPa)	14.9 ± 12.3
Success rate of elastography (%)	73.7 ± 28.3
Interstitial fibrosis (%)	31.8 ± 18.2
CAI Banff grade (median and range)	2 (0–3)

Data for donor age, time since transplantation, creatinine, estimated glomerular filtration rate (eGFR), resistance index, parenchymal stiffness (assessed by FibroScan®), interquartile range and success rate of stiffness measurements are presented as mean ± standard deviation. Histological Banff grade of chronic allograft injury (CAI) is presented as median and range.

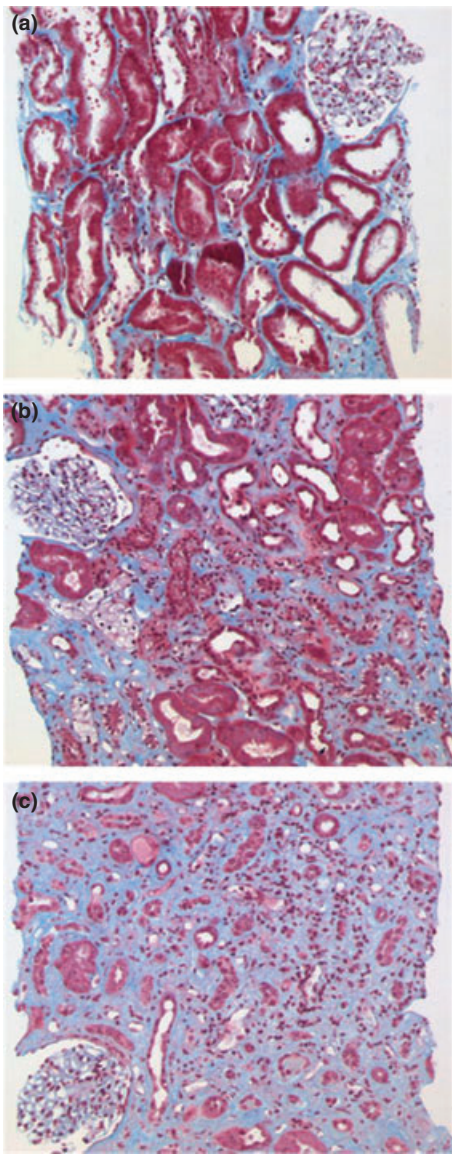

Figure 2 Representative renal biopsies with (a) mild fibrosis of the interstitium and mild atrophy of the tubules according to Banff grade 1, (b) moderate interstitial fibrosis and tubular atrophy according to Banff grade 2 and (c) severe interstitial fibrosis and tubular atrophy according to Banff grade 3; Masson–Goldner's trichrome staining (collagen stained blue).

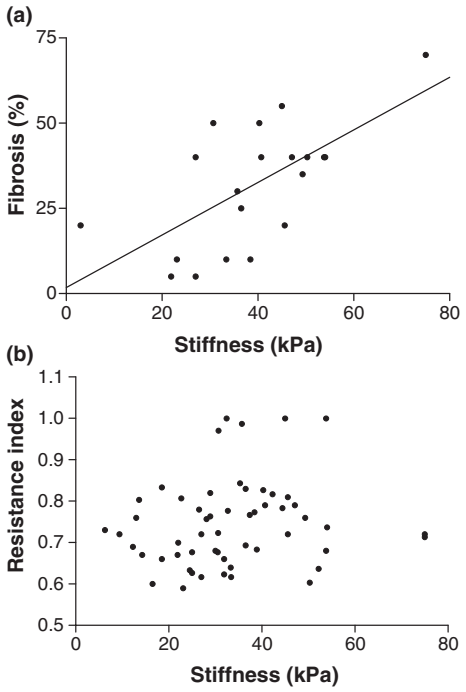

Figure 3 (a) Association of parenchymal stiffness (kPa) and extent of interstitial fibrosis (%). The highly significant correlation (Pearson *r*: 0.67, *P*: 0.002) is described in a linear regression model by the equation 'Fibrosis (%) = −1.58 + 0.80 × Stiffness (kPa)'. R^2, the proportion of the variation in fibrosis that can be accounted for by the variation of stiffness, is 0.45. (b) Association of parenchymal stiffness (kPa) and resistance index showing no significant correlation (Pearson *r*: 0.17, *P*: 0.23).

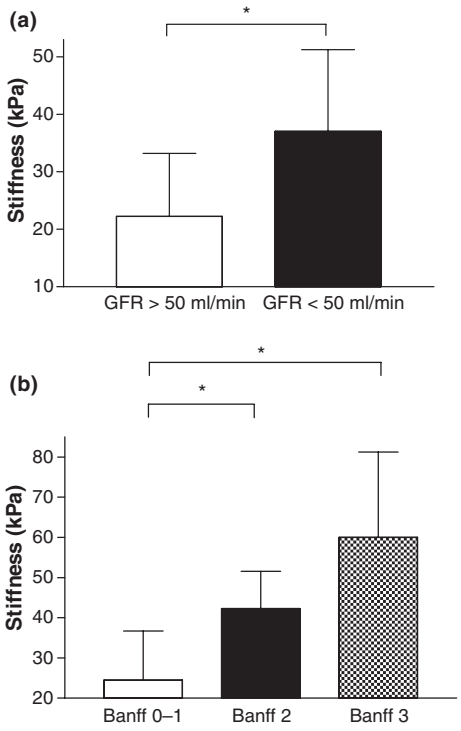

Figure 4 Parenchymal stiffness of (a) patients with eGFR >50 and ≤50 ml/min and (b) Banff grades 0–1, 2 and 3 of chronic allograft injury. Mann–Whitney *U*-test reveals a significant difference between patients with eGFR >50 vs. ≤50 ml/min (*P*: 0.0005), Banff grades 0–1 vs. grade 2 (*P*: 0.008), and grade 0–1 vs. grade 3 (*P*: 0.046). *P < 0.05.

examination in all the biopsies. The percentage of interstitial fibrosis in relation to the whole biopsy ranged from ≤5% to 70% (Table 3). The corresponding Banff grades for CAI ranged from 'absence of CAI' to grade 3. Figure 2 shows representative examples for CAI Banff grade 1 (Fig. 2a), grade 2 (Fig. 2b) and grade 3 (Fig. 2c) in Masson–Goldner's trichrome staining (collagen stained blue).

Parenchymal stiffness showed a highly significant negative correlation with eGFR (Pearson *r*: −0.47, *P*: 0.0003, R^2: 0.22). Furthermore, there was a highly significant positive correlation between parenchymal stiffness and the extent of interstitial fibrosis (Pearson *r*: 0.67, *P*: 0.002, R^2: 0.45). In a linear regression model, the association of stiffness and interstitial fibrosis is described by the following equation:

$$\text{Fibrosis}(\%) = -1.58 + 0.80 \times \text{Stiffness(kPa)}.$$

The value of R^2, proportion of the variation in fibrosis that can be accounted for by the variation of stiffness, is 0.45. Parenchymal stiffness and RI did not have a significant correlation (Pearson *r*: 0.17, *P*: 0.23). These results are displayed in Fig. 3. As shown in Fig. 4, parenchymal stiffness significantly differed between subjects with stable graft function and an eGFR >50 ml/min and subjects with impaired graft function and an eGFR ≤50 ml/min (22.2 ± 11.0 vs. 37.1 ± 14.2 kPa, *P*: 0.0005). Moreover, CAI Banff grade 0–1 differed significantly from Banff grade 2 (*P*: 0.008) and grade 3 (*P*: 0.046).

As this study examines TE in renal allografts for the first time, there are no data on the impact of interquartile range and success rate on measurement accuracy. Therefore, we did not primarily exclude any data, if 10 valid measurements could be performed. As described above, the median value of these 10 measurements was used for the analyses mentioned above. In a second analysis, we considered only measurements with a success rate >60%. The correlation of parenchymal stiffness with both eGFR (Pearson *r*: −0.46, *P*: 0.005) and interstitial fibrosis (Pearson *r*: 0.67, *P*: 0.01) remained significant.

Discussion

Transient elastography is a well-established noninvasive diagnostic tool for the evaluation of liver fibrosis. The

present study shows that this technique can be used for the assessment of parenchymal stiffness of kidney allografts as well. Our data suggest that allograft stiffness adequately reflects transplant fibrosis.

Parenchymal stiffness measurement was successful in 97.6% of the patients. The failure rate of 2.4% is lower than the same relating to such measurements in liver stiffness measurements with a mean reported failure rate of approximately 5% [9]. Liver stiffness measurements can be difficult in obese patients or in those with narrow intercostal space or ascites. The latter two aspects do not apply to renal transplants. Although all but one measurement of this study were successful in patients with a BMI >30 kg/m^2, massive abdominal adiposity complicated the examination and led to an increased duration of the procedure. The choice of the TE probe is of outstanding importance for the failure rate and validity of the results. Measurements in kidney allografts differ from liver measurements in two aspects: First, measurements are not performed in an intercostal space with the ribs adjusting impression depths of the small tip to a similar level even though impression pressure might vary from measurement to measurement. Second, the region of interest of the stiffness measurement is much smaller in kidneys compared with the liver, as we are only interested in the stiffness of the cortical parenchyma, not of the medulla or renal pelvis. Only small probes (measurement depth of 15–40 mm for 'S1' and 20–50 mm for 'S2') should be used in order to reduce the proportion of the pyelon in the measurement focus to a minimum. It has to be stated that even a small probe is not able to focus exclusively on the cortex, which limits the current accuracy of the method. A dynamic probe allowing an adjustment of measurement depth on the individual anatomy would be desirable for the future.

Parenchymal stiffness was higher in kidneys than in liver tissue. Healthy liver tissue without significant fibrosis usually has stiffness values of ≤10 kPa [7,10]. With regard to the differences of the histological structure of hepatic and renal tissue, these experiences cannot be one-to-one transferred to kidneys. Mean stiffness of renal parenchyma was 32.7 kPa in this study. The inverse correlation of stiffness and eGFR shows that TE is indeed able to reflect functional aspects of the graft. As an increase of parenchymal fibrosis leads to a deterioration of graft function, the inverse character of this association is plausible from a clinical point of view.

The present findings reveal a robust correlation of interstitial fibrosis of the renal allograft and parenchymal stiffness assessed by TE. The higher the percentage of interstitial fibrosis and tubular atrophy, the faster the shear wave propagates and the higher the resulting value for tissue stiffness. TE measures tissue stiffness in a volume that approximates a cylinder 1 cm wide and 2.5–3 cm long. This volume is at least 100 times bigger than a biopsy sample, and is consequently far more representative of the graft's parenchyma [6,7]. The reproducibility of TE is high. In liver stiffness measurements, the intra- and inter-operator standardized coefficients of variation have been described as 3.2% and 3.3% respectively [6]. In a large study by Fraquelli *et al.*, 800 examinations were performed by two operators in 200 patients with various chronic liver diseases. The authors found an excellent reproducibility for both inter- and intra-observer agreement with intraclass correlation coefficients of 0.98 [11]. Sensitivity and specificity of TE for the diagnosis of cirrhosis has been evaluated in hepatitis C patients: A recent meta-analysis of nine studies found a sensitivity of 87% and a specificity of 91% [12]. The present study in kidney allografts was not powered to define any cut-offs for the diagnosis of different grades of interstitial fibrosis. Consequently, we cannot provide sensitivity and specificity of TE in this context.

The mean stiffness values of allografts with stable and impaired function were significantly different (Fig. 4). Furthermore, stiffness values of the individual Banff grades were significantly different from each other (Fig. 4). Nevertheless, the sample size of the present study does not allow a definition of distinct ranges of 'healthy' and 'ill' or for the individual Banff grades. A larger study with more histological data might be able to define medians and confidence intervals for each grade.

A noninvasive technique like TE will never be able to achieve the diagnostic power of a histological examination. Therefore, it has to be emphasized that TE cannot replace biopsy in the situation of deteriorating graft function. The most promising and attractive application of this method, however, is its ability to monitor a change of allograft parenchymal structure in the course of time. With regard to the ongoing debate on whether to perform protocol biopsies or not, a continuous increase of parenchymal stiffness in consecutive TE measurements might be helpful to define patients who benefit from a kidney biopsy even though creatinine is stable. TE cannot detect subclinical rejection, recurrence of underlying disease or viral infection but it can be of high value to identify patients with subclinically progressive interstitial fibrosis. Furthermore, a noninvasive surrogate parameter of interstitial fibrosis might be of interest for clinical studies aiming at the reduction of CAI, e.g. by CNI reduction or withdrawal.

Protocol biopsies have been proven to be a powerful tool for the detection of subclinical CAI/CNI toxicity. Because of their invasive character, however, patients' acceptance of biopsies is often poor. TE is a rapid novel, noninvasive technique for the assessment of hepatic fibrosis with

excellent patients' acceptance, good reproducibility and immediate results. The present study shows that TE can detect interstitial fibrosis in kidney allografts as well. A longitudinal assessment of parenchymal stiffness in renal allografts might be a powerful tool to identify patients with subclinically progressive interstitial fibrosis who benefit from biopsy. Future studies should focus on the association of fibrosis and stiffness in a larger study cohort and possibly with a probe allowing individual adjustment of sample volume. The assessment of parenchymal stiffness will be of interest both in protocol biopsies and in patients with deterioration of graft function for specific reasons like rejection or acute tubular necrosis.

Authorship

RA, SS, CL, MG and MvdG: participated in the performance of the research. WZ: participated in research design. THW: research design, data analysis, writing of the manuscript.

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