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Assessment of renal allograft fibrosis by transient elastography

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Summary

Transient elastography (TE, Fibroscan) has been established as a noninvasive assessment tool of liver fibrosis. We evaluated potentials and limitations of TE for identifying renal allograft fibrosis. The technical possibility of kidney examination by TE was assessed in two 10-week-old German landrace pigs and kidney stiffness (KS) was evaluated in 164 renal transplant patients. KS could be determined in all animals at the pole and pars media (29 ± 10 kPa vs. 31 ± 17 kPa). In human renal allografts KS was successfully performed in 94.5% of the test series with reliable results in 72% of the measurements. Mean KS at the pole or pars media were comparable (35.0 ± 19.9 kPa vs. 33.2 ± 18.6 kPa). Significantly higher KS was detected in renal allografts with histologically confirmed advanced fibrosis. Body-mass-index, skin-allograft distance, and peri or intrarenal fluid accumulation were important confounders of successful KS measurements (BMI: $r = -0.31$; $P < 0.001$; distance: $r = -0.50$; $P < 0.001$). Notably, KS did not correlate with renal function. TE represents a noninvasive approach in selected transplant recipients to identify allografts with severe fibrosis. The heterogeneous kidney morphology and several other confounding factors negatively affect measurability of KS by TE. Further technical modifications are required to improve applicability of TE for kidney assessment.

Introduction

In renal transplantation chronic allograft dysfunction remains the major reason for late allograft loss [1–3]. Progressive renal allograft damage with increasing interstitial fibrosis and tubular atrophy is detected by an increase in serum creatinine. Biopsies performed because of creeping creatinine mostly demonstrate advanced irreversible histomorphological changes [4]. In addition, renal allograft biopsy is an invasive diagnostic tool which can cause severe complications such as bleeding [5].

For this reason, novel elastographic techniques either based on ultrasound or magnetic resonance imaging are increasingly studied to assess noninvasively renal allograft fibrosis [6–10]. Transient elastography (TE) is a rapid and noninvasive method to measure tissue stiffness. It has been first established as an excellent tool to assess liver stiffness, and multiple prospective trials and meta-analysis found a strong correlation between liver stiffness and fibrosis stage in patients with various liver diseases [6–8,10–12]. Factors such as liver congestion [13], inflammation [14–16], or cholestasis [13] also increase liver stiffness independent of fibrosis stage. Recently, TE has been applied to spleen and the kidney in smaller cohorts [17,18]. In the first clinical study on kidney, TE was found suitable to assess the progression of renal allograft fibrosis [17]. In a rat model of glomerulosclerosis ultrasonic shear wave elastography has been performed to detect renal cortex stiffness changes and prediction of histological development of fibrosis [19]. In this experimental study, increased cortical stiffness correlated with the degree of renal dysfunction.

The purpose of the present study was to evaluate the possibilities and limitations of TE to assess renal allograft stiffness first in an animal pilot project followed by a larger

patient cohort with biopsy proven fibrosis stage. In addition, we studied optimal conditions and probe positioning in humans.

Material and methods

Animal model

The kidneys of two 10-week-old German landrace pigs were investigated by TE *in vivo* and *ex vivo*. All pigs had a standardized narcotic protocol (premedication: Azaperon 8 mg/kg intramuscularly, Midazolam 0.5–0.7 mg/kg intramuscularly, Ketamin 5 mg/kg intravenously, Atropin 1 mg intravenously) and endotracheal intubation with pressure-controlled ventilation in a half-closed system. Arterial blood gases were controlled within a strict limit (pO_2, 100–150 mmHg, pCO_2, 35–42 mmHg). Pigs were anticoagulated with Heparin 5000 IU given intravenously. Cardio circulatory parameters were measured by catheters placed into the internal jugular vein and common carotid artery. Preparation of the kidneys was done after a longitudinal laparotomy. Renal parenchyma stiffness was determined at the pole and pars media of both kidneys in situ and after harvesting of the kidneys using the S-probe of the investigational FibroScan device (FibroScan®; Echosens, Paris, France). After the examination a tissue biopsy was done and histopathological analysis was performed by an external pathologist.

All animal experiments were approved by the local committee for Animal Welfare of the Regierungspräsidium Baden-Württemberg.

Patient study design

Altogether, 164 renal allograft recipients (aged ≥ 18 years) with stable renal allograft function (S-creatinine <3 mg) of the Department of Nephrology at the University Hospital Heidelberg, Germany, were included consecutively in this study. Kidney stiffness (KS, FibroScan®; Echosens), Ultrasound, and Doppler sonography (Sonosite® M-Turbo™; Bothell, WA, USA) of the renal allograft was assessed in each patient. Renal allograft function was determined by estimated glomerular filtration rate (eGFR) calculated by the Modification of Diet in Renal Disease (MDRD) formula [20]. Patients with deteriorating renal allograft function (increase of serum creatinine of >20% within the last 12 months) were allocated to renal allograft biopsy.

The institutional Ethics Committee of the University of Heidelberg, Germany, approved the clinical study protocol, and written informed consent was obtained from all enrolled patients. The study was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Study objectives

The primary objective of the present clinical study was to evaluate the possibilities and limitations of TE as noninvasive technique to detect renal allograft fibrosis. Optimal patient and allograft factors contributing to reliable stiffness results were clarified and renal stiffness assessed by TE was compared with histopathological analysis.

Transient elastography

In the present study, an investigational TE device was used (FibroScan®; Echosens). The operators of the TE device were trained by staff member of Echosens prior to study start. The M-probe (depth 25–65 mm, frequency 3.5 MHz) as well as the S-probe (depth 15–45 mm, frequency 5 MHz) was used to assess renal stiffness. Both probes had a signaling mechanism to avoid high external pressure on the tissue. The investigational device allowed focusing measurement on the area of interest given by the investigator; e.g., the skin-allograft distance (SCD) as well as the cortical thickness could be accounted. The stiffness (E) results were expressed in kilopascals (kPa). The median value of twenty acquisitions was considered for the analysis. KS values were divided into three categories as described for liver stiffness: (i) failure: no KS obtained, (ii) unreliable KS values: success rate <60% and/or IQR/M >30%, (iii) reliable KS values: success rate >60% and IQR/M <30% [21]. In contrast to liver stiffness measurements with 10 required measurements, the success rate (SR) was given in percentages (%) of 20 valid acquisitions.

Since the TE device (Fibroscan) was primarily designed to assess liver stiffness, we re-analyzed the elastographs in a computer-assisted manner using a special software tool provided by Echosens to determine correct shear wave velocity in the renal cortex. This tool allowed adaptation of the angle, depth, and length of the shear wave to the visualized anatomical structure. Thereafter, calculation of the stiffness could be limited to the area of interest. This computer-assisted optimized re-evaluation was established because of the high failure rate in successful stiffness measurements caused by the anatomical speciality and locality of renal allograft.

Renal allograft biopsy

Percutaneous renal allograft biopsies were performed under ultrasound guidance with a 14-gauge needle. The renal biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin as well as Masson's trichrome. An independent and blinded expert kidney pathologist analyzed all biopsy specimens. Fibrosis stage (interstitial fibrosis and tubular atrophy, IF/TA) of

renal biopsy samples was categorized using the 2007 updated Banff criteria [22]. Mild IF/TA below 25% of the cortical area was classified grade 1, moderate IF/TA with 26–50% of the cortical area was classified grade 2 and severe IF/TA with >50% of the cortical area was classified grade 3.

Statistical analysis

A sample size of at least 100 patients was chosen for this study with respect to the exploratory nature of the study, rather than being based on statistical power considerations. To avoid selection bias, all eligible renal allograft recipients were consecutively enrolled in this study.

Variables were expressed as mean ± standard deviation. Frequency distributions were provided for categorical variables. Differences between groups were analyzed using Wilcoxon signed-rank test and chi-squared test for continuous and dichotomous variables, respectively. Correlations between variables were assessed using Spearman rank correlation coefficient test. Logistic regression analysis was performed to identify independent determinants of renal TE. Factors showing co-linearity were excluded from analysis. A two-sided $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS software package (version 17.0; Chicago, IL, USA).

Results

Preliminary study of kidney stiffness in German landrace pigs

Altogether, KS data were obtained from eight test series (four *in vivo*, four *ex vivo*) in two German landrace pigs. *In vivo*, means KS assessed at the pole region was 29 ± 10 kPa with a SR of 49%; corresponding results at the pars media were 31 ± 17 kPa with a SR of 75%, respectively. *Ex vivo* measurements showed a higher mean stiffness of 47 ± 6 kPa with a SR of 62% at the renal pole. Mean stiffness at the pars media was also higher with 39 ± 9 kPa and SR 72%. Increased KS in *ex vivo* kidneys was most likely because of coagulation. These preliminary animal studies suggested to us that the pars media is the predominant location for reliable measurements of KS.

Prospective analysis of KS in patients in various parts of the kidney

Altogether, 164 renal allograft recipients were included in this nonrandomized trial (117 men (71.3%); mean age 49.9 ± 15.2 years). Patients' demographics are listed in Table 1. At least twenty successful single acquisitions per test series were achieved in 154 (93.9%) patients at the pole region and in 156 (95.1%) patients at the pars media. Thirty-eight (24.7%) and 44 (28.2%) unreliable KS values were obtained at the pole and pars media. KS could be reliably measured at the pole and pars media in 112 (72.7%) and 112 (71.8%) patients, respectively. The mean KS from all reliable measurements were 35.0 ± 19.9 kPa (pole) compared to 33.2 ± 18.6 kPa (pars media). KS of the renal pole and pars media correlated significantly ($r = 0.62$, $P < 0.001$). In 39 patients, renal allograft stiffness was assessed with the M- and the S-probe at three allograft regions: upper pole, pars media and lower pole. SR was significantly higher if the S-probe was used compared with the M-probe (74.7% ± 25.2% vs. 44.2 ± 27.5%) (Table 2). In conclusion, S-probe at the pars media is best suitable for KS measurements.

Table 1. Demographics of 164 renal allograft recipients assessed by transient elastography. Data are shown as mean and standard deviation or number and percentages.

Patients	$N = 164$
Gender (male)	117 (71.3%)
Age (years)	49.9 ± 15.2
Time after transplantation (years)	4.7 ± 5.5
Body-mass-index (kg/m^2)	25.6 ± 4.2
S-creatinine (mg/dl)	2.08 ± 1.35
eGFR MDRD (ml/min)	53 ± 16
Blood pressure (mmHg)	137 ± 15/85 ± 8
Resistance index	0.71 ± 0.08
Pole: Skin-allograft distance (cm)	2.49 ± 1.04
Pole: cortical parenchyma thickness (cm)	3.44 ± 0.82
Pars media: skin-allograft distance (cm)	2.29 ± 1.10
Pars media: cortical parenchyma thickness (cm)	1.75 ± 0.47

eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease.

Table 2. Renal allograft stiffness (E) and success rate (SR) in 39 patients assessed with the M- and S-probe at the upper pole, pars media, and lower pole of the kidney.

	E (kPa)	SR (%)
M-probe		
Upper pole	32.2 ± 18.4	36.4 ± 25.4
Pars media	24.7 ± 13.4	47.6 ± 30.0
Lower pole	21.6 ± 13.4	48.6 ± 27.2
S-probe		
Upper pole	30.0 ± 19.5	71.6 ± 24.4
Pars media	32.0 ± 21.2	75.1 ± 27.7
Lower pole	27.1 ± 19.3	77.4 ± 23.4

Intra and interobserver variability of KS assessment

To assess intraobserver variability, 12 patients with stable allograft function were measured twice within 3 months by one observer. KS of both measurements correlated significantly (pole: $r = 0.82$, $P < 0.0001$; pars media: $r = 0.71$, $P = 0.002$). KS varied by 3 ± 14 kPa between the first and

Table 3a. Confounding factors on the success rate of transient elastography measurement to assess the renal allograft (*r*, Spearman regression coefficient; *P*, significance).

	Pole		Pars media	
	r	*P*	*r*	*P*
Skin-allograft distance	−0.50	<0.00	−0.56	<0.00
Parenchyma thickness	−0.14	0.09	−0.15	0.06
Body-mass-index	−0.31	<0.00	−0.27	<0.00

second measurement. Interobserver variability was assessed in ten renal allograft recipients (time between visits 36 ± 32 days). KS of both measurements correlated significantly (pole: $r = 0.78$, $P = 0.01$; pars media: $r = 0.67$, $P = 0.03$). Pole and pars media KS differed with 6 ± 11 kPa and 1 ± 14 kPa between the measurements of the two observers. Thus, TE showed good intra and interobserver agreement in kidneys with best results obtained in the pars media region.

Confounders of measurement failure and accuracy

Both, body mass index (BMI), SCD and a small cortical parenchyma thickness negatively affected SR of KS measurements (Table 3a) with BMI directly correlated to SCD. Fluid collection (cyst, lymphocele, seroma) also decreased SR by 12.2% at the pole and 17.3% at the pars media. In addition, fluid accumulation was accompanied by an increase in KS by >20%. Peri or intrarenal fluid accumulation was observed in 37 of 164 patients (22.6%). Longer SCD, small parenchyma thickness, and peri or intrarenal fluid collection also negatively affected accuracy as indicated by an increased IQR (Table 3b). The precision increased with an approximately 15% lower IQR after computer-assisted re-evaluation compared to the original results.

KS is significantly increased in patients with creeping creatinine

There was no evidence for a significant correlation between KS and parameters of renal function such as S-creatinine, eGFR, blood pressure, and resistance index. However, patients with creeping creatinine (Δ 20% in the last year, $n = 32$) showed a statistically significant higher KS as compared to stable patients (pars media: 39.7 ± 21.3 vs. 31.9 ± 18.4, $P = 0.048$; pole: 43.1 ± 20.8 vs. 32.7 ± 18.7, $P = 0.009$).

KS is significantly increased in patients with severe allograft fibrosis

Based on the above results, the following inclusion criteria were used for further kidney studies using the commercially available Fibroscan: BMI ≤ 30 kg/m^2, SCD ≤ 3.5 cm, parenchyma thickness ≥ 1 cm. Only TE results showing a shear wave without interruptions were included in the histological analysis. Minimal requirements for renal allograft biopsies were a renal parenchyma sample length of at least 1 cm and at least seven glomeruli in the biopsy sample.

Using these criteria, 52 renal allograft recipients could be included for KS and histology analysis (39 male, age 48 ± 18 years, time after transplantation 1.6 ± 2.8 years). In 96.2% (50 of 52), valid, reliable KS data could be obtained both from the pole and the pars media. In this

Table 3b. Confounding factors in assessment of renal allografts by transient elastography.

	Original assessment				Computer-assisted reprocessment			
	E (kPa)		IQR (kPa)		E (kPa)		IQR (kPa)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Pole								
Skin-allograft distance	−0.17	0.04	−0.18	0.03	−0.27	<0.001	−0.20	0.01
Parenchyma thickness	0.07	0.39	−0.22	0.01	0.07	0.39	−0.12	0.14
Age	−0.13	0.11	−0.05	0.51	−0.03	0.69	−0.05	0.54
Body-mass-index	−0.13	0.11	−0.13	0.11	−0.12	0.15	−0.08	0.30
Time after transplantation	−0.11	0.19	0.03	0.73	−0.13	0.11	0.12	0.14
Pars media								
Skin-allograft distance	−0.06	0.49	−0.08	0.30	−0.26	<0.001	−0.04	0.63
Parenchyma thickness	0.09	0.25	−0.18	0.02	0.06	0.44	0.00	0.99
Age	0.01	0.91	0.00	1.00	0.02	0.79	0.00	0.97
Body-mass-index	0.02	0.79	−0.07	0.42	−0.11	0.19	0.01	0.89
Time after transplantation	−0.13	0.11	0.02	0.86	−0.11	0.18	0.12	0.14

E, stiffness or Young's modulus; IQR, interquartile range; kPa, kilopascal; *P*, significance; *r*, regression coefficient.

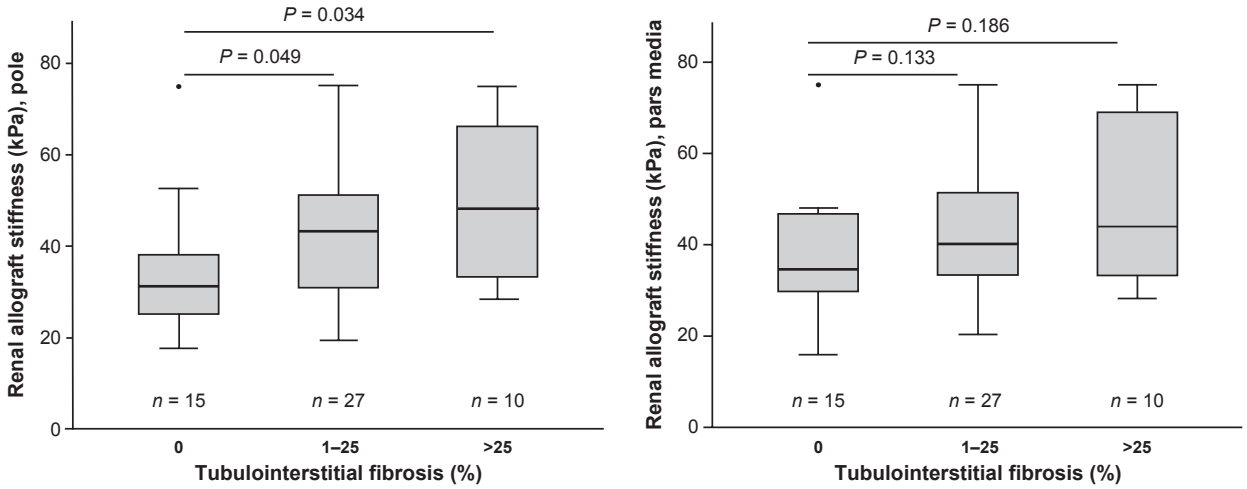

Figure 1 Renal allograft stiffness and tubulointerstitial fibrosis assessed by an independent pathologist and classified according Banff grades. because of small patient numbers in higher fibrosis categories Banff grade 2 and 3 (>25%) are shown in one box.

patient cohort mean KS at the renal pole was 42.0 ± 17.0 kPa and the KS at the pars media was 42.8 ± 15.7 kPa. KS at both parts of the kidney correlated significantly ($r = 0.56$; $P < 0.05$). Interstitial fibrosis and tubular atrophy of renal biopsy samples where categorized by a nephropathologist according Banff 07 class 5 criteria. Five renal allograft samples showed no signs of interstitial fibrosis or tubular atrophy, 27 samples showed IF/TA <25%, six samples IF/TA 25–50% and four samples IF/TA >50%. KS increased with enlarging tubulo-interstitial fibrosis at the pole and at the pars media (Fig. 1). Interstitial fibrosis of >25% was observed in 20 of 24 (83%) patients with a KS >40 kPa (pars media). The sensitivity and specificity to detect renal allograft fibrosis by TE with a cut-off of 40 kPa was 54% and 73%, respectively. In summary, KS allows identification of significant allograft fibrosis (>25%) in patients fulfilling defined criteria.

Discussion

We here study the potentials and limitations of TE to identify renal allograft fibrosis in a noninvasive bedside manner. Preliminary studies on German landrace pigs indicated that TE could be performed on kidneys with acceptable reproducibility. We then demonstrate reliable KS measurements in more than 70% of the 164 human renal allograft recipients. SCD, BMI and peri or intrarenal fluid collections are the major confounding conditions that negatively affect success rate and accuracy of KS measurements. Although KS did not correlate with renal function, age, blood pressure or resistance index, KS was significantly higher in patients with a creeping creatinine. Moreover, about 85% of patients with a KS >40 kPa demonstrated significant tubulo-interstitial fibrosis in renal allograft biopsies. Computer-assisted re-evaluation of elastographs further improved data accuracy and criteria for an optimal renal allograft assessment by TE could be established.

Our results suggest that TE allows the evaluation of the renal allograft if the confounding factors are considered and certain criteria are met such as BMI ≤ 30 kg/m^2, SCD ≤ 3.5 cm, parenchyma thickness ≥ 1 cm, and no fluid accumulation around the allograft. With a technical failure rate of 5.5%, renal allograft examinations were comparable to the assessment of liver stiffness [23,24]. In contrast to the liver, a significantly smaller number of reliable measurements were obtained on the kidney (approximately 72% vs. 85%).

With about 35 kPa, KS is significantly higher as compared with the stiffness of a healthy liver of 4 kPa [9,21]. KS of a normal human allograft was comparable to renal stiffness in 10-week-old landrace pigs without tubulointerstitial fibrosis and corresponded very well to previously published renal allograft stiffness of 32.7 kPa [17]. Thus, the regular KS even exceeds the stiffness of an advanced liver cirrhosis >20 kPa which is most likely because of the higher abundance of connective tissues and vascularization in kidneys under physiological conditions [25–27]. Considering the upper detection limit of the Fibroscan device (75 kPa), only a small measuring range from 30 to 75 kPa remains for the kidneys [8].

Although the inter and intraobserver variability of KS measurements is comparable to those reported in livers (3.2% vs. 2.2%) [8,23], several confounding factors negatively affect accuracy (IQR) and success rate as compared with liver stiffness measurements. Our study has identified the following major confounders: SCD, BMI, missing intercostal fixation, fluid accumulation, variation of the measuring angle, and small cortical parenchyma with heterogeneous calyxes. The recent introduction of the more powerful XL

probe has drastically improved stiffness measurements of livers [28] which had similar confounding factors namely in obese patients [25] or ascites [29]. Unfortunately, the XL probe cannot be used in kidneys because of the small and heterogenous cortex parenchyma. In fact, our study demonstrates that the small S-probe, originally designed for children allows for a better assessment of KS.

In contrast to a recent report, our data do not support a reliable assessment of renal allograft fibrosis in all renal allograft recipients without any patient selection criteria [17]. Following the results of our TE assessment, we identified the several criteria to obtain reliable KS values: BMI <30 kg/m^2, SCD <3.5 cm, parenchyma thickness >1 cm, absence of peri or intrarenal fluid accumulation. In a selected study cohort meeting these criteria, KS increased with raising renal allograft fibrosis although no correlation between KS and renal function or resistance index could be observed. This is in contrast to the previous pilot study that showed significant differences between KS of allografts with stable and impaired function but did not define and use criteria for reliable measurements [17]. It also clearly contrasts recent data obtained in liver where stiffness correlates well with liver function [30].

Although we used an investigational device that allowed the manual choice of the shear wave slope to calculate KS much more precisely, reliable KS values could only be obtained in about 70%. According to our experience, a further technical optimization is required to allow a broad and feasible application of TE for renal allograft assessment. Despite these limitations, it remains unclear why impaired renal function or interstitial fibrosis has a poor impact on KS, and only severe fibrosis is indicated by increased KS. First, most renal diseases affect several compartments of the kidney tissue. Second, interstitial fibrosis is only one factor contributing to deterioration of renal function next to glomerulosclerosis, rejections, and recurrence of glomerular diseases. Third, as mentioned above, the measuring range of KS is much more limited as compared with the liver stiffness since normal KS is already rather high exceeding by far cut-off values for liver cirrhosis.

Nevertheless, we would like to express our optimism that KS could be of additional value for the noninvasive assessment of kidney allograft transplants for the following reasons: (i) the present data clearly show that patients with creeping creatinine have an increased KS. (ii) The recent introduction of other imaging-based elastographic techniques such as magnetic resonance elastography, acoustic radiation force impulse imaging or real time shear force imaging, or the combination of TE and ultrasound guidance may solve one major obstacle: the heterogeneous texture and anatomy of the kidney compared with the liver. More studies using these novel technologies are required to answer these questions.

In conclusion, TE on renal allograft is limited by several confounding factors and the anatomy as compared with the liver. Future studies with optimized or upcoming elastographic techniques are needed to show whether KS could be a robust screening marker for allograft monitoring.

Authorship

CS: designed the research project, performed the study, analysed data and wrote the paper. MS and CS: performed the study, collected data and analysed the data. GM: designed the research project, performed the study and wrote the paper. HKS and MZ: designed the research project and wrote the paper. SM: designed the research project, analysed data and wrote the paper.

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