



## Transplantation

### CKJ REVIEW

# When is contrast-enhanced sonography preferable over conventional ultrasound combined with Doppler imaging in renal transplantation?

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### Abstract

Conventional ultrasound in combination with colour Doppler imaging is still the standard diagnostic procedure for patients after renal transplantation. However, while conventional ultrasound in combination with Doppler imaging can diagnose renal artery stenosis and vein thrombosis, it is not possible to display subtle microvascular tissue perfusion, which is crucial for the evaluation of acute and chronic allograft dysfunctions. In contrast, real-time contrast-enhanced sonography (CES) uses gas-filled microbubbles not only to visualize but also to quantify renal blood flow and perfusion even in the small renal arterioles and capillaries. It is an easy to perform and non-invasive imaging technique that augments diagnostic capabilities in patients after renal transplantation. Specifically in the postoperative setting, CES has been shown to be superior to conventional ultrasound in combination with Doppler imaging in uncovering even subtle microvascular disturbances in the allograft perfusion. In addition, quantitative perfusion parameters derived from CES show predictive capability regarding long-term kidney function.

**Key words:** contrast-enhanced sonography, kidney transplantation, renal allograft perfusion, ultrasound

### Introduction

Conventional ultrasound is still the diagnostic standard procedure in renal allograft recipients. Apart from immunological tolerance, one of the most important factors to ensure a stable allograft function is renal blood supply, which is mainly influenced by parenchymal blood perfusion. Over 90% of renal blood flow in the renal cortex is provided by small renal arterioles and capillaries [1, 2]. Disturbances in the perfusion of these small vessels might cause acute or chronic graft dysfunctions. Thus, methods to estimate renal tissue perfusion in-depth can provide important diagnostic evidence for the evaluation of renal

allograft function. Conventional ultrasound in combination with Doppler imaging (CDUS) due to its technical limitations is not suitable to display renal tissue perfusion in more detail. However, real-time contrast-enhanced sonography (CES) is an easy to perform and non-invasive imaging technique to provide further information on microvascular tissue perfusion.

### Imaging modalities for the measurement of renal blood flow

Several attempts have been made to achieve a technique for measuring renal blood flow *in vivo*: regional washout of inert or

Received: September 30, 2014. Accepted: July 10, 2015

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hydrogen gases, heat diffusion, isotope trapping, radiolabeled microspheres and positron emission tomography [2, 3]. For most of these methods, clinical applicability is limited due to their invasive nature, radioactive exposure or their restricted availability.

At the moment, B-mode ultrasonography and Doppler ultrasonography are the most frequently used imaging modalities to evaluate morphology and vasculature of renal allografts. Doppler ultrasonography is a non-invasive procedure [4] and was shown to be predictive of allograft failure in renal transplant recipients [5]. However, the capability of conventional Doppler imaging is limited to the evaluation of large blood vessels. Renal perfusion can only be visualized up to the level of segmental and interlobular arteries as well as large parenchymal vessels. Doppler ultrasound identifies blood flow by measuring velocity and differentiating this signal from surrounded tissue. This technique is not suitable for small blood vessels because red blood cells are poor reflectors of ultrasound [6] and this difficulty is even aggravated in capillary beds where blood flow is too slow to be recognized [7].

### Ultrasound contrast agent and imaging technique

First efforts to display renal blood flow with the help of ultrasound contrast agent were made in the 1980s. In an animal model, gas-filled microbubbles were injected directly into the descending aorta and visualized by renal ultrasound [8]. Today, CES is a non-invasive routine imaging technique that allows the assessment of microvascular tissue perfusion.

The ultrasound contrast agent consists of gas-filled microbubbles stabilized by a supporting shell of biocompatible material like protein, lipid or polymer [9–11]. First-generation agents were filled with room air, which had the disadvantage to diffuse quickly into surrounding plasma. Ultrasound contrast agents of the second generation enclose gases of low solubility like sulfohexafluoride or octafluoropropane. These gases do not leak from the protecting shell for several minutes. Due to their small size with a range from 1 to 10  $\mu\text{m}$  [12], microbubbles pass to capillaries and enable visualization even of the microvascularization. Regarding the kinetics of microbubbles in the blood flow, it was shown that they have a similar rheology to red blood cells [13] and more important that they remain intravascular [14]. Microbubbles act as contrast agent by creating a boundary surface between the fluid phase in blood at the outside and the gaseous phase at the inside. The change of impedance leads to a reflection of emitted ultrasound waves. This increases ultrasound backscatter and enhances echogenicity of blood by a factor of 500–1000 [15].

The gas is exhaled through the lungs within 20–30 min after injection [16] and the shell is metabolized in the liver. Microbubble-based contrast agents are not nephrotoxic and do not interact with thyroid function [17]. Overall, it is a safe examination with an incidence of life-threatening anaphylactoid reactions in 0.001% [18].

Besides the injectable contrast agent, a contrast-specific imaging mode on the ultrasound device is required. This software suppresses the background signal from surrounding tissue and by that enhances the signal from the microbubbles. The standard imaging mode for CES is the low mechanical index mode in pulse inversion technique. This guarantees a long preservation of microbubbles for an appropriate observation time. If ultrasound is transmitted at a higher mechanical index, the bubbles are destroyed much earlier. Furthermore, low mechanical index techniques improve suppression of surrounding tissue signals [19, 20].

### Qualitative and quantitative assessment

In the evaluation of CES-derived data, it is useful to differentiate between the qualitative and the quantitative assessment. The qualitative assessment is based on the visible vascularization of parenchymal areas. This approach mainly provides morphological and anatomical information, e.g. the identification of areas with decreased tissue perfusion and the differentiation between benign cysts and vascularized lesions. This assessment is easy to perform and demands no special experience of the investigator (Figure 1).

However, the quantitative assessment is conducted via computer after the patients' examination. It requires a specific analysing software and basic experience in the handling of it. The quantitative assessment is a haemodynamic approach to evaluate parenchymal microperfusion. For that, it is necessary to measure blood flow velocity and blood flow volume, which is realized by destruction–replenishment technique. A single flash of ultrasound with high transmission power destroys the microbubbles [21]. After clearance of microbubbles, the replenishment of the contrast agent in the allograft and the changes in echogenicity can be followed and evaluated in a predefined region of interest (ROI). The analysing software then calculates a time/intensity curve to estimate renal blood flow (in dB/s) (Figure 2).

### Clinical feasibility of CES

The implementation of CES into clinical everyday course appears unpretentious. This applies particularly for the qualitative assessment, which is easy to perform and rather less time consuming than CDUS. Moreover, there are several practical points that argue for CES as a first diagnostic approach in renal transplant patients (Table 1).

For an initial assessment of allograft perfusion, the patient does not have to be transported to the CT or magnetic resonance imaging (MRI) department. Instead, the examination can be done bedside, in the intensive care unit or even in the operating theatre. Moreover, in contrast to CT or MRI examination with contrast agents, CES can also be executed in transplanted patients with impaired kidney function without the additional risk of allograft worsening—a setting that appears to be relevant particularly in the first weeks after transplantation. No special preparation of the patient is needed. The contrast agent is simply administered via the usual peripheral venous access or via a central venous catheter, and the examination can easily be repeated, without jeopardizing renal function. Allergic cross-reactions with iodinated contrast agents are not to be feared. Finally, CES examination is cheaper than most other imaging techniques that are based on contrast agents.

### Limitations of CES

Owing to the need of a special imaging mode being installed on the ultrasound machine, CES is limited to those devices with the required configuration. When we talk about the renal allograft perfusion measured by CES, this is not to be mistaken with the real and exact tissue perfusion, expressed in mL/unit time/unit mass of tissue. For the evaluation of CES-derived data, the analysing software provides one or more perfusion parameters that are considered.

It is also worthwhile to mention that the ultrasound contrast agent is not excreted in the urinary tract. Thus, in contrast to CT imaging with a contrast agent, CES cannot provide an excretory urography. Although CES is a safe examination, as a precaution, it should not be performed in patients with severe cardiopulmonary

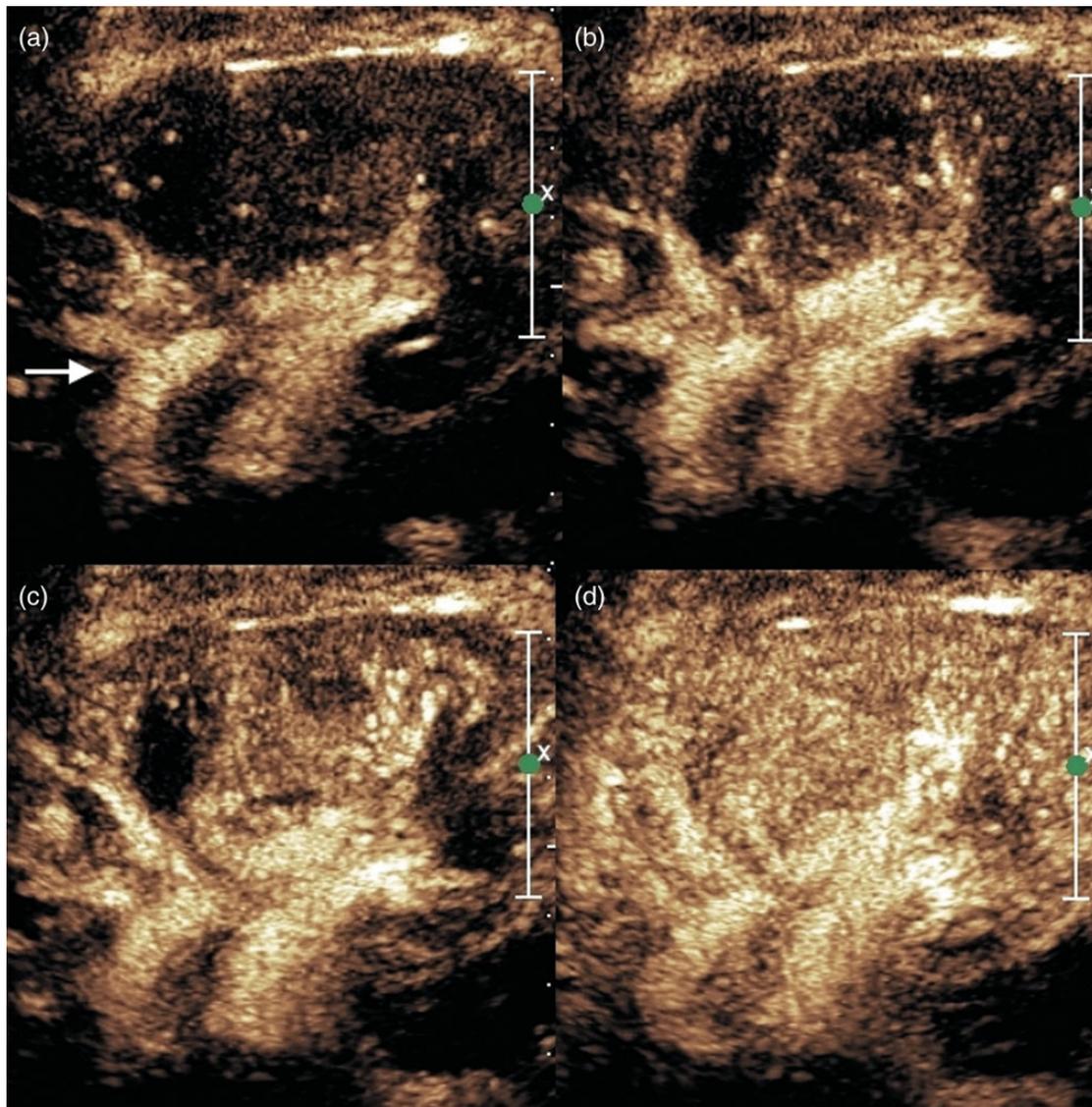


Fig. 1. Qualitative assessment of contrast agent replenishment in a renal allograft visualized by CES mode. First, only large vessels at the renal hilum (arrow) are perfused (a). Gradually also small parenchymal vessels show contrast agent uptake (b and c) and present a homogeneous allograft perfusion (d).

disease. The reason for that is an announcement of the U.S. Food and Drug Administration from 2007 that reported the death of four patients, which were temporally related, but not causally attributable, to a special perflutren-containing ultrasound agent (Table 1) [22].

### Indications for CES in kidney transplant recipients

There are two substantial arguments for the application of CES in the evaluation of renal allograft function. First, the anatomical position of the transplanted organ is superficial and organ movements due to respiration are reduced to a minimum. This facilitates examination with a contrast agent enormously while the organ should be kept in a stable position for the assessment of renal blood flow. Second, and this is the main argument, renal allograft underlies a progressive vascular remodelling process in the time period after transplantation. Vasculopathy and disturbances in allograft perfusion can occur in acute or chronic

processes and account for the majority of allograft failure [23, 24]. Most of these vascular insults affect small parenchymal arteries and arterioles, which cannot be assessed by Doppler sonography. Thus, there is a necessity of evaluating microperfusion to augment diagnostic evidence and to permit early administration of the appropriate therapy.

### Stenosis and thrombosis

Acute vascular events in the early period after transplantation are transplant artery stenosis and transplant vein thrombosis. Transplant renal artery stenosis (TRAS) is one of the most frequent vascular post-transplantation complications and occurs in 1.5–12.5% of patients [25–27]. Conventional colour Doppler imaging has shown to be the diagnostic mainstay for TRAS [28]. Its limitations are owed to anatomical idiosyncrasy of transplant vessels. Transplant renal arteries are often tortuous with kinking phenomenon, which may lead to elevated peak systolic velocity and thereby to false-positive diagnosis of TRAS [29].

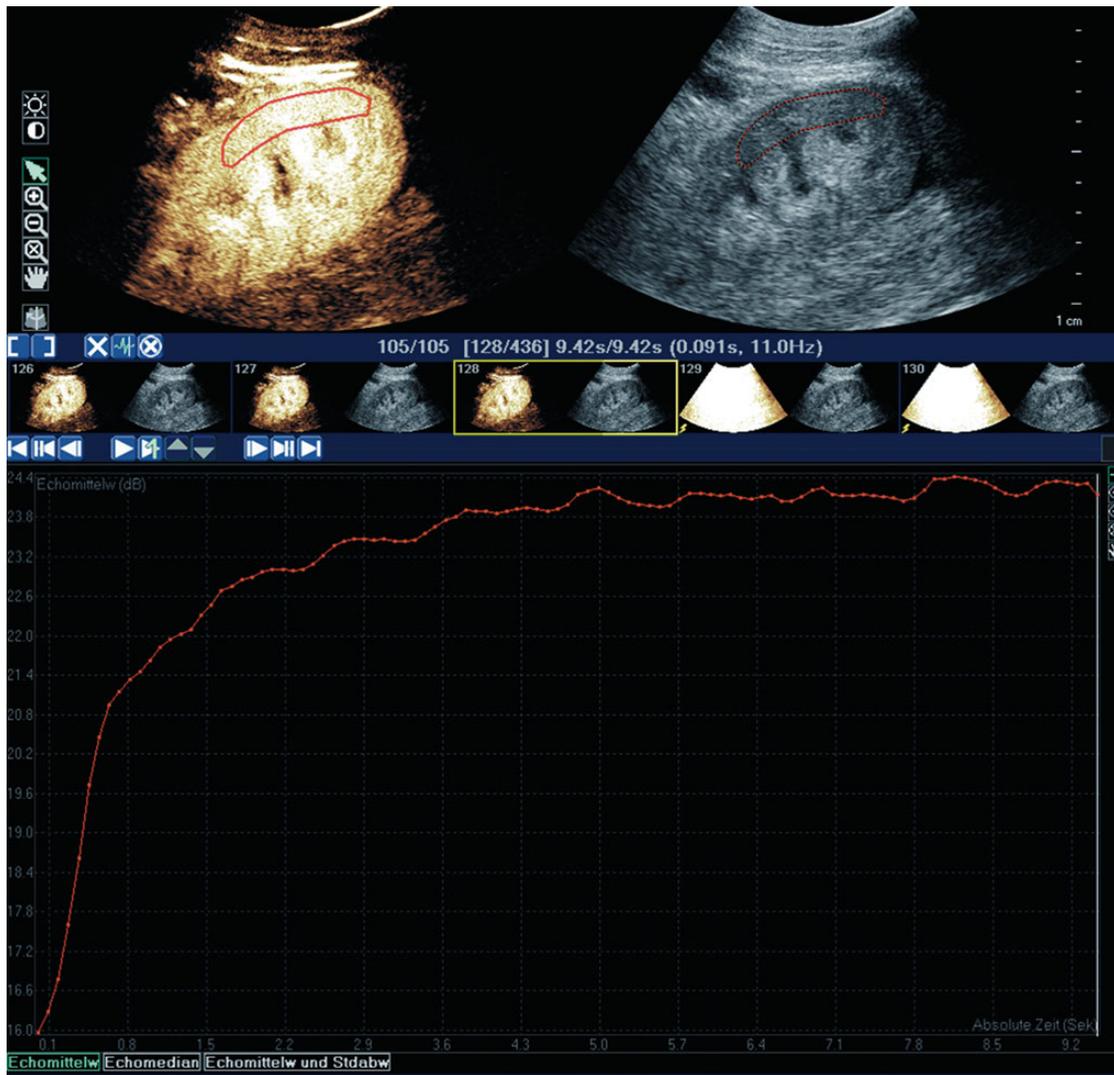


Fig. 2. Screenshot of the analysing software for the quantitative assessment. The upper screen shows on the left side an image from CES mode and on the right from B-mode. The red labelled area is the ROI, which should exclude major vessels and the peripheral ending of the cortex. The lower half of the screen displays the corresponding destruction–replenishment curve. The y-axis indicates the contrast intensity (dB) and the x-axis indicates time (s).

**Table 1.** General advantages and disadvantages of CES versus conventional ultrasound in combination with colour Doppler imaging in the kidney transplant context

**CES advantages**

- Impaired kidney function is no contraindication
- Displays microvascular tissue perfusion and allows renal blood flow to be quantified
- Qualitative assessment demands no special experience of the investigator

**CES disadvantages**

- Quantitative assessment demands special analysing software
- Examination with contrast agent is more expensive

Arterial stenosis can also be assessed by CES imaging. In kidney transplanted patients with TRAS, a longer time of contrast agent inflow compared with patients without perfusion defects

was observed and contrast agent inflow was correlated with severity of stenosis [30]. Nevertheless, according to our experiences in most of the cases, TRAS can still be diagnosed by conventional colour Doppler imaging.

Transplant renal vein thrombosis is a vascular event in the early period after transplantation requiring immediate surgical therapy [31]. Owing to the potentially life-threatening character of the disease and the importance of a prompt diagnosis, we recommend CDUS followed by MRI or CT as diagnostic gold standard. CES does not provide any additional diagnostic evidence in this context.

**Infarction**

The potency of CES arises in the follow-up examination for the assessment of postoperative microvascular graft perfusion. To determine a homogenous allograft perfusion, CES is a valuable tool, whereas conventional ultrasound is not able to show microvascular perfusion accurately [7]. This is of particular importance when graft function is delayed despite regular colour Doppler

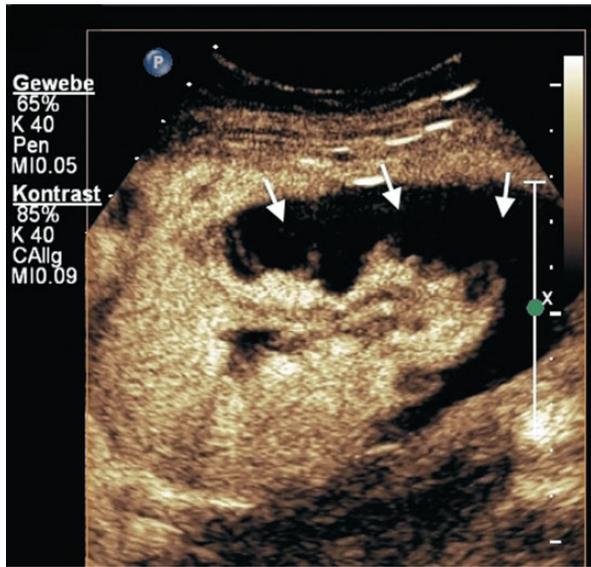


Fig. 3. Kidney allograft with a thrombus in the renal transplant artery. CES mode reveals a missing tissue perfusion in two-thirds of the organ (arrows). Blood supply of the cranial renal pole is ensured by a segmental artery that originates proximal from the thrombus.

indices and no detectable signs of disturbed allograft perfusion. Then, the qualitative assessment with CES technique enables even small areas of postoperative disturbed perfusion to be uncovered as well as local parenchyma infarction, which may be a feasible explanation for delayed graft function (Figure 3). In a comparative study, CES technique was superior in the identification of parenchyma perfusion disturbances to conventional ultrasound more perfusion disturbances and visualized them more precisely [32].

Conversely, with colour Doppler, it is sometimes not possible to evaluate if a visualized vascular disturbance like a reduced perfusion of one renal pole is significant or due to technical limitations. It is known that diagnostic findings in Doppler ultrasound are limited when the allograft lies deep within the iliac fossa [33]. In these cases, CES can differentiate between a real and an apparent perfusion defect and spares a further examination by MRI or CT (Figure 4).

A rare but serious vascular complication with irreversible function loss of renal allografts is acute cortex necrosis. In a retrospective study of five patients with pathology-proven acute cortex necrosis, only CES imaging but not CDUS was able to diagnose acute cortex necrosis. CES detected an unenhanced peripheral cortical continuous band in the transplanted kidney, a similar finding to the peripheral rim sign in CT or MRI, which is pathognomonic of a cortical necrosis [34].

#### Acute tubular necrosis and acute rejection episode

Acute tubular necrosis (ATN) and acute rejection episode (AR) are the most common causes of early graft dysfunction. Both modalities are characterized by unspecific or absent clinical and laboratory findings. Doppler ultrasound can provide evidence of ATN and AR by measuring an increase in resistance indices. For the interpretation of resistance indices, it should be considered that Doppler indices depend on several factors influencing vascular stiffness of the graft recipient, e.g. pulse pressure, intima media thickness and pulse wave velocity [35]. Unfortunately, a definitive

discrimination of ATN and AR by Doppler ultrasound is not possible.

In contrast, the CES technique provides further diagnostic information. In patients with AR episode, a delayed parenchymal perfusion in the renal cortex was observed [36]. An examination of a small study group led to the suggestion that with the help of CES-derived parameters of the quantitative assessment, it could be possible to distinguish ATN from AR [37]. Whether CES can further differentiate between ATN and AR on the one side and cyclosporine A (CsA) toxicity on the other side is not known.

In the context of acute graft dysfunctions, CES might probably be a tool to gain additional prognostic information, but to obtain a definitive diagnosis, performing a biopsy is still indispensable.

#### Prognostic value for allograft function

The general consideration of conducting CES in kidney allografts is to display (qualitative assessment) and to objectify perfusion parameters (quantitative assessment) that indicate graft function. It is accepted that a decreased tissue perfusion is directly related to graft function by affecting glomerular filtration and urine rate. Some groups have investigated the correlation between graft perfusion and established laboratory markers of renal function. Lebkowska et al. [38] were able to show that renal perfusion visualized by microbubble contrast agent correlates with the estimated glomerular filtration rate (eGFR) at 5–10 days after transplantation. This correlation was confirmed for eGFR at 3 months after transplantation [39].

A main cause of chronic renal transplant insufficiency ultimately resulting in graft loss is described by the former term chronic allograft nephropathy [40], which is characterized by and now called interstitial fibrosis and tubular atrophy (IF/TA). Hence, early diagnosis of IF/TA plays a crucial role in long-term allograft survival. IF/TA is frequently underestimated due to technical limitations of non-invasive methods. In this context, CES is a feasible easy to perform diagnostic option. It has been shown that CES can reflect IF/TA in transplant recipients by the quantitative assessment and is indicative of IF/TA even before the increase of serum creatinine and possibly before the onset of irreversible damage; hereby, CES had a higher diagnostic accuracy compared with colour Doppler ultrasound [41].

Recently, we were able to demonstrate the predictive capability of CES examination regarding long-term kidney allograft function. Kidney transplant recipients were investigated with CES and conventional CDUS 1 week after transplantation. Renal blood flow, quantitatively estimated by CES, revealed that patients with a renal blood flow higher than 12 dB/s developed a significantly better kidney allograft function 1 year after transplantation in comparison with patients with a lower renal blood flow. Interestingly, determination of renal blood flow correlated to donor but not to recipient age, whereas conventional resistive index, estimated with CDUS, was correlated to recipient age [42]. This finding indicates that renal blood flow reflects the intrinsic vascular condition of the allograft and not just the 'pretransplant' vascular stiffness of the allograft recipient, which is reflected by the resistive index. A substantial component of anti-rejection therapy after kidney transplantation is the application of calcineurin inhibitors (CNI). While the incidence of acute rejection episodes has decreased after the introduction of CsA, the consequences of long-term immunosuppression have become more obvious [24, 43]. Long-term application of CsA and tacrolimus can cause vascular remodelling processes that may result in chronic allograft failure [23]. In addition, the intake of CsA has been described to induce an acute renal vascular vasoconstriction

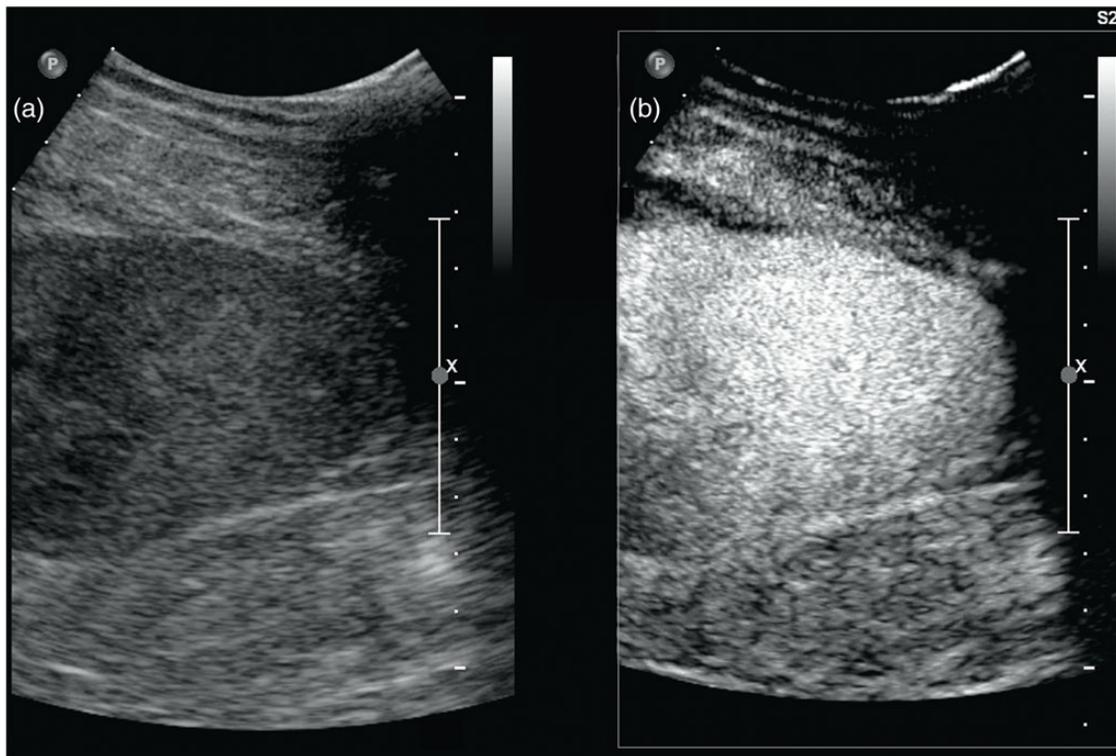
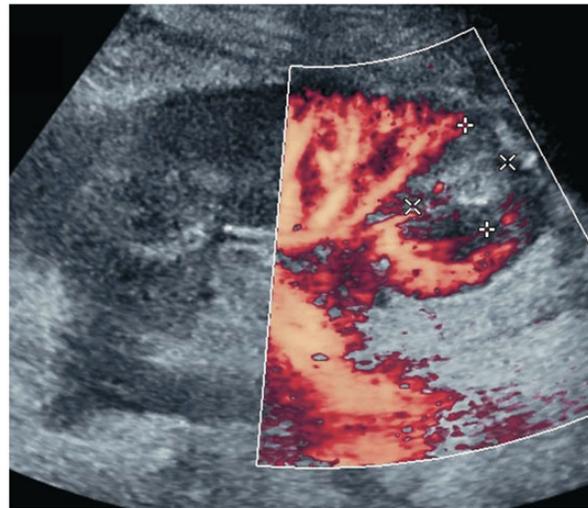


Fig. 4. (A) Perfusion of a kidney allograft visualized by power Doppler ultrasound shows a vascular disturbance at the caudal pole (delineated by × and +). (B) Same allograft examined with CES. (a) B-mode image of the caudal renal pole; (b) CES examination reveals a homogenous microvascular perfusion without any disturbances.

[44, 45], whereas this effect is supposed to be less distinct for tacrolimus [46, 47]. Using the CES technique, we were able to visualize and to quantify acute changes of allograft microperfusion caused by the administration of CsA and tacrolimus. In contrast to tacrolimus, which did not impair graft perfusion significantly, CsA led to a 49% reduction of kidney allograft microperfusion 2 h after intake [48]. It can only be speculated if these acute disturbances in microperfusion might have a prognostic impact on long-term allograft function and survival. Supporting evidence is provided by a prospective comparison of renal tissue perfusion in randomized allograft recipients treated either with CsA or with mammalian target of rapamycin inhibitor everolimus. Microvascular perfusion quantitatively assessed with CES was best maintained

by immunosuppressive therapy with everolimus. Moreover, the impairment in microperfusion due to therapy with CsA was reversible after a switch of the immunosuppressive agent from CsA to everolimus, leading to an improved allograft perfusion after 12 months, which was consecutively associated with an increase in eGFR [49]. Further studies are needed to evaluate the long-term impact of CNI administration on renal blood flow and allograft survival (Table 2).

#### Tumour diagnosis

Similar to non-transplanted patients with renal cysts, the need of differentiating a benign cyst from solid lesion also applies to

Table 2. CES versus CDUS for specific indications in the kidney transplant context

Indication	Preferable technique	Reason
Delay of graft function after transplantation	CES	Displays subtle perfusion disturbances and small areas of infarction
Discrimination of benign cystic masses from other lesions	CES	Benign cystic masses do not show contrast agent enhancement
Suspected TRAS or vein thrombosis	CDUS	Large vessels can be evaluated properly with this technique

transplanted kidneys. With the help of CES, it is possible to distinguish a benign cystic mass from other lesions. Benign cystic masses do not show any enhancement of the contrast agent or at most a few microbubbles of contrast material travelling in a few hairline thin septa. In contrast, even hypovascular tumours show at least a minimal intraparenchymal vascular enhancement. In this regard, CES is more sensitive than contrast-enhanced CT for detecting slight tumour blood flow, and thereby very useful in diagnosing malignant hypovascular renal tumours [50].

Moreover, the microvasculature of renal tumours differs from that of normal parenchyma. This is helpful when differentiating suspicious renal masses from normal variants, e.g. from a septum or from a physiological contour. In this setting, a pseudo-tumour, which is striking in B-mode, would enhance parallel to renal parenchyma [51]. Any area that enhances differently should be considered as suspicious and needs further investigation.

Apart from the above-named cases, CES is—contrary to liver studies—currently not used for differentiating between benign and malignant kidney lesions [52]. A definitive discrimination between benign and malignant renal masses is still not feasible because solid tumours do not show specific perfusion patterns after injection of CES contrast agents (Table 2) [53].

### Special indications for CES

Another minor indication for CES might be urinary tract infection. Kidney transplanted patients under immunosuppression are predisposed to infection diseases, especially for urinary tract infections. A major complication is the progression of an acute pyelonephritis. In a small study group, the diagnosis of acute pyelonephritis in kidney transplant patients was made with the help of a qualitative assessment with CES through the identification of visible areas with decreased perfusion with a sensitivity of 95% [54].

Other indications for CES are perirenal haematomas, which can occur in the early period after kidney transplantation and after allograft biopsy. In this context, CES was shown to increase the detectability of haematomas and allows a more detailed assessment of haematoma size compared with conventional B-mode ultrasound [55]. With the help of CES, it is even possible to visualize active bleeding.

Not only iatrogenic post-biopsy haematomas are an indication for CES but also accidental trauma. As the transplanted kidney is superficially located, it is more exposed to abdominal trauma than native kidneys. When graft damage is suspected, CES can be a first approach in order to avoid CT examination with iodine contrast exposure.

### Future perspectives

Future applications for CES include among others the direct targeting of predefined structures in the allograft for therapeutic and diagnostic purpose. With the help of modified contrast agent, it would be possible to directly bind to certain surface

molecules in the vasculature [56]. Thereby, pathologic processes could be identified and visualized with ultrasound technique [57, 58]. In a next step, therapeutic interventions could also be done with this technique when, for example, a pharmaceutical is bound to the shell of the contrast agent and directly brought to the target point.

### Conclusion

CES has emerged as a complementary and feasible tool for the evaluation of renal allografts. The greatest potency of this imaging technique arises in the assessment of detailed qualitative and quantitative information on renal microvascular perfusion. Whereas conventional colour Doppler sonography still plays an important role in diagnosing TRAS and venous thrombosis, CES imaging reveals even subtle microvascular damage, e.g. minor local parenchyma infarction, and thereby enables a more comprehensive and detailed statement on allograft function. But still, more studies are needed to evaluate the significance of CES examination in diagnosing episodes of acute graft rejection.

### Authors' contributions

M.Z., L.P.K., M.Z. and V.S. participated in the writing of the manuscript. F.D. attained and edited images.

### Conflict of interest statement

None declared.

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