Dynamic Tissue Perfusion Measurement: A Novel Tool in Follow-Up of Renal Transplants

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Background. The authors applied the novel method of noninvasive dynamic color Doppler sonographic parenchymal perfusion measurement to renal transplants.

Methods. Color Doppler sonographic videos of renal transplants from 38 renal transplant recipients were recorded under defined conditions. Specific tissue perfusion was calculated as mean flow velocity encoded by color Doppler signals of a region of interest during one full heart cycle.

Results. The authors could demonstrate significant differences of central versus peripheral cortical perfusion intensity (1.36 vs. 0.60 cm/sec) and a significant loss of perfusion intensity in the posttransplantation period in the peripheral cortex from 1.06 cm/sec in the first year to a minimum of 0.39 cm/sec in the 3- to 5-year interval, with stronger perfusion in longer surviving transplants: 0.71 cm/sec more than 9 years after transplantation. In the central cortex, a similar but less pronounced pattern could be demonstrated. A significant drop of parenchymal perfusion was found in patients with elevated serum creatinine (1.36 cm/sec in cases with normal and 0.82 cm/sec in those with elevated creatinine at the proximal cortical level). The perfusion ratio of the central 50% and the peripheral 50% shows marked changes over time: in the first year, the ratio was 2.99, climbing to 5.56 at the 3- to 5-year interval and declining later on.

Conclusions. Cortical tissue perfusion in renal transplants was quantified noninvasively from color Doppler signal data in an easily accomplishable manner. Renal transplants showed a marked decline in tissue perfusion after transplantation. Perfusion is significantly lower in transplant function loss with elevated serum creatinine.

Keywords: Tissue perfusion, Measurement, Color Doppler sonography, Kidney transplants, Software.

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As in native kidneys, function of renal transplants depends on sufficient perfusion. Renal transplants are subject to loss of function over time (*1*). Chronic rejection, recurrence of the transplant recipient's original disease, infections, arteriosclerosis, and drug toxicity may all contribute to loss of function. All of these disorders are accompanied by a change of transplant perfusion. It is important to measure renal perfusion in such cases to adapt therapeutic and immunosuppressive intervention. Evaluation of perfusion in renal transplants, however, is demanding, and scintigraphy is the method of choice today. This technique is not always available, is costly, and uses radiating substances. A new technique to quantify renal transplant parenchymal perfusion is desired. We developed a simple technique of noninvasive dynamic color Doppler sonographic parenchymal perfusion measurement and applied this to renal transplants of 38 recipients to evaluate changes of transplant perfusion at posttransplantation follow-up.

PATIENTS AND METHODS

Patients

We examined 38 renal transplant recipients (23 male and 15 female patients, aged 2.5–27 years, with a mean age of 15±4.9 years; mean body mass index, 21.6, ranging from 14.1–36.8; mean weight, 51 kg, ranging from 13–102 kg; mean height, 152 cm, ranging from 91–181 cm) from August 2000 to June 2002. Patients were routinely investigated according to clinical requirements. All patients from our hospital's pediatric dialysis department were included. Time after transplantation at investigation varied from 7 days to 11 years (mean, 3.4 years). Mean duration of follow-up was 10 months (range, 0 [single examination]–2 years). Every patient was investigated on average 4 times (range, 1–12 times). Causes of end-stage renal failure are listed in Table 1. During the observation period, for clinical reasons, seven biopsy specimens were obtained from four patients, revealing four acute and three chronic rejections.

Color Doppler Sonography

Color Doppler sonographic investigations of the renal transplants were performed with a 4- to 8-MHz curved array probe and Sequoia ultrasound equipment (Acuson, Mountain

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TABLE 1. Diagnoses responsible for end-stage renal failure in 38 renal transplant recipients

Diagnosis	No.
Glomerulonephritis	7
Polycystic kidneys	5
Hemolytic-uremic syndrome	5
Megaureter	3
Vesicoureteral reflux grade 4/5	2
Hydronephrosis	2
Juvenile nephronophthisis	2
Subvesical obstruction	1
Malformation	1
Cystinosis	1
Others	9
Total	38

View, CA). Longitudinal and transverse sections of the transplants were recorded. Care was taken to investigate vessels that run straight toward the transducer. This way, an angle correction of the Doppler signal could be avoided. A crucial point is the presetting of the ultrasound equipment. The same color Doppler frequency was always chosen for investigation. Otherwise, no comparison of different perfusion measurements is possible. The color Doppler frequency was 7 MHz. Color gain was fixed by the presetting and never changed. To avoid aliasing, color flow velocity settings were changed if necessary. Maximum color flow velocity was 4.3 to 8.6 cm/sec.

Dynamic Color Doppler Tissue Perfusion Measurement

We measured the intensity of perfusion automatically with a recently developed software package (2) (Pixelflux; Chameleon-Software, Leipzig, Germany). Color Doppler signals from digital color Doppler sonographic videos (Digital Imaging and Communications in Medicine [DICOM] format) were quantified in a novel dynamic way. "Dynamic" means that changes of blood flow during the heart cycle have been taken into account. This is important because blood flow velocity strongly pulsates even in the tiniest arteries of a kidney. Dynamic appreciation of flow at each single point of the heart cycle is therefore fundamental for refined understanding of tissue flow phenomena. The software calculates color pixel area and flow velocity—encoded by each pixel—inside a region of interest of a video sequence. This way, a quantification of blood flow inside the investigated tissue could be achieved. Videos with movement artifacts were excluded from perfusion quantification. The outputs were as follows:

1. Area (A) of depicted vessels inside the region of interest (ROI) (average of one whole heart cycle) (these measurements are not presented here).
2. Mean flow velocity (v) inside the ROI (average of one whole heart cycle) (results are not given here).
3. Indices of changes of these parameters during the heart cycle (not further outlined).
4. A measure of flow quantity inside called perfusion intensity (P) of the ROI (calculated by multiplication of vessel area (A) and mean velocity (v) in the ROI [A_{ROI}]): $P=A^*v/A_{ROI}$ (cm/sec).

The ROI was chosen in the renal parenchyma in the area between the outer border of the medullary pyramids and the kidney surface (Fig. 1) (3). The ROI was set to contain vessels running in a symmetric distribution pattern to the transducer. A parallelogram was placed to enclose a complete vascular segment fed by one interlobar artery. Digital videos containing 25 to 50 images and at least one full heart cycle were recorded.

Velocity calculation was carried out for each pixel automatically after calibration of the software with the color bar inside the image, which correlated color hues to their velocity values. Mean values of colored area and mean flow velocity were calculated automatically by reckoning average values of all pixels inside one single image's ROI and averaging these values for all images encompassing one full heart cycle. The detection of one full heart cycle was performed automatically by the software. These averaged values of flow velocity and area from an entire heart cycle inside the ROI were used for calculation of perfusion intensity.

Inside the ROI, the entire area occupied by colored pixels was calculated. This calculation was automatically repeated for the same ROI in a sequence of up to 50 consecutive images of a digital video. Each pixel defines a distinct velocity value because of its color hue. The velocity value is read out automatically and the mean velocity of all pixels inside the ROI is calculated. To achieve this, the software is calibrated initially: maximum flow velocity encoded by the end of color bar is the basis of automatic calculation of the hue velocity value of each. The color bar must be symmetric (i.e., both ends of the color bar must indicate the same color value). A linear progression of velocity values from zero to the given maximum value is correlated with red and blue color hues. This way, each hue is assigned a specific velocity value. Each pixel inside the ROI is then compared with the hues from the color bar and its perfusion intensity velocity is read.

This mean velocity (v) is multiplied by the area (A) of all pixels. Thereby, a flow value (P) is calculated, which defines the mean flow velocity of the whole ROI. This way, perfusion is computed that characterizes the whole ROI as if the ROI was homogeneously perfused with the same, calculated

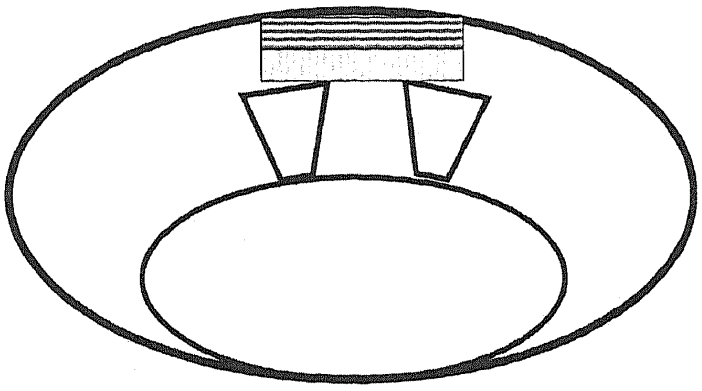

FIGURE 1. Schematic placement of the ROI and measurement regions. ROI encompasses a full segment fed by one interlobar artery. p50 (*vertically hatched*), Proximal 50% of the ROI; d50 (*horizontally hatched*), distal 50% of the ROI.

velocity. This is called the tissue perfusion intensity because the quantity of blood passing through a certain ROI is proportional to the mean flow velocity, and by this means perfusion is quantified.

Inside the parallelogram, which defined the ROI, measurements were performed at different levels as described below. The ROI was divided into two segments (p50 and d50). In p50, the entire width of the ROI but only the proximal 50% of the height were selected. Similarly, the distal 50% (d50) was selected and separate calculations were carried out (*3*). Altogether, 4,190 measurements from 907 videos have been performed and 104,750 single color Doppler images were analyzed automatically (2,757 images per patient).

Statistical Analysis

Means of groups were compared by using the Kruskal-Wallis H test, and between two independent groups by the Mann-Whitney *U* test. Values of $P<0.05$ were regarded as statistically significant. To investigate correlation, Pearson's correlation coefficient was calculated with the same significance assumptions.

RESULTS

Perfusion of renal transplant cortical parenchyma was significantly different between the layers (p50 and d50) under investigation. In the proximal 50% of cortex, mean perfusion intensity was 1.36 cm/sec (SD, 0.86 cm/sec), whereas in the distal 50%, a mean value of 0.60 cm/sec (SD, 0.52 cm/sec; $P=0.000$) was found. The time interval to transplantation significantly influenced perfusion intensity of renal transplant parenchyma. The highest perfusion was measured in the first year after transplantation, declining later to minimal values in the 3- to 5-year interval. We found a significant loss of parenchymal perfusion in the posttransplantation period in the peripheral cortex, from 1.06 cm/sec in the first year to minimal values of 0.39 cm/sec in the 3- to 5-year interval. In transplants surviving longer, perfusion was stronger: 0.71 cm/sec more than 9 years after transplantation. In the central cortex, a similar but less pronounced pattern could be demonstrated. In both regions, loss of perfusion intensity after the first year was highly significant, and in the peripheral cortex,

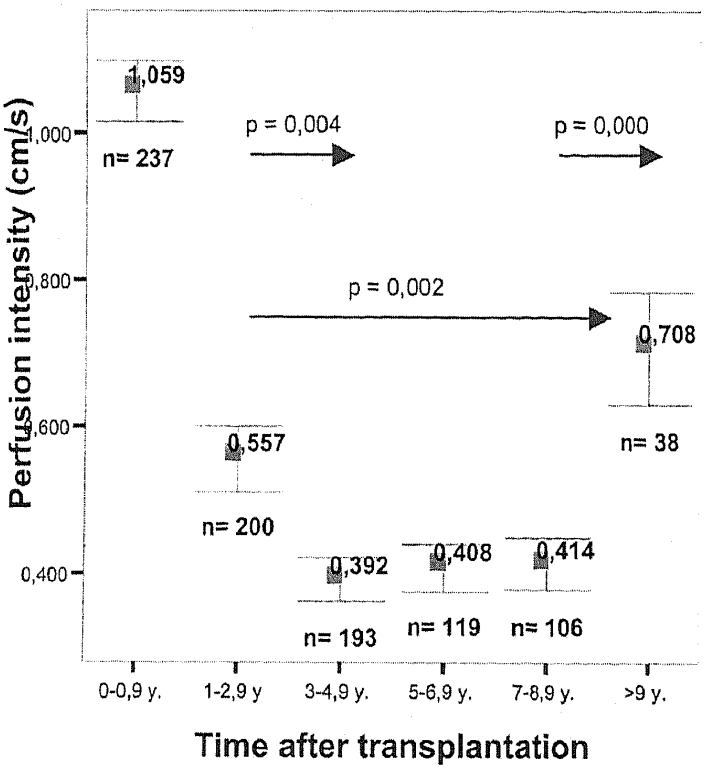

FIGURE 2. Course of parenchymal perfusion after transplantation; peripheral cortex. Significant differences of distal cortical renal transplant perfusion in various intervals to transplantation in 38 recipients in the distal cortex. (*bars*) Means and SEM. Additional significant *P* values are indicated. n, Number of investigations.

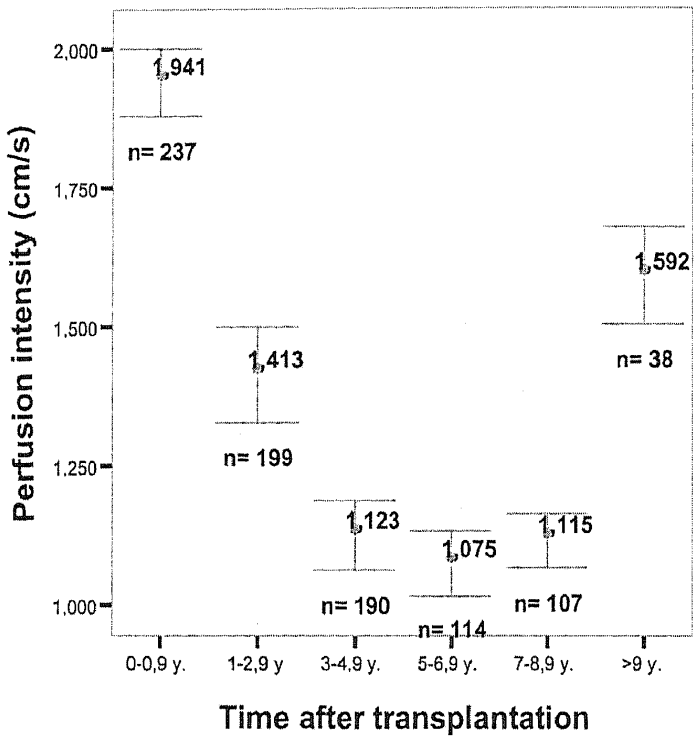

FIGURE 3. Course of parenchymal perfusion after transplantation; central cortex. Significant differences of distal cortical renal transplant perfusion in various intervals to transplantation in 38 recipients in the proximal cortex. (*bars*) Means and SEM. n, Number of investigations.

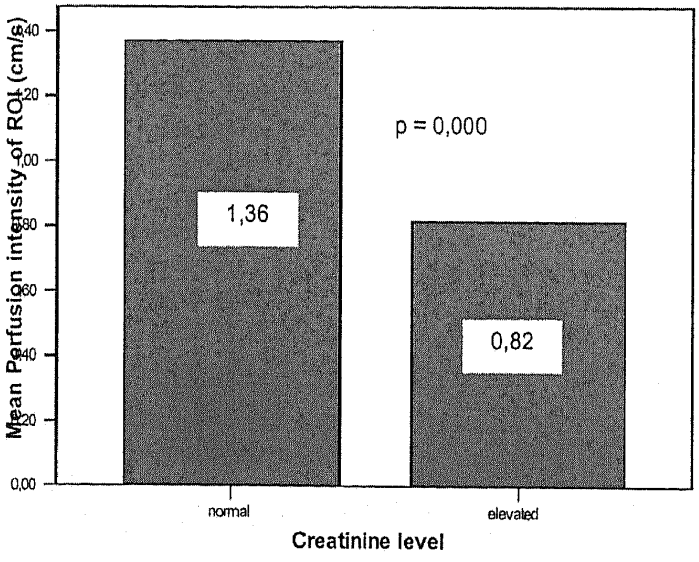

FIGURE 4. Significant differences of proximal cortical renal transplant perfusion in recipients with normal and elevated serum creatinine.

ongoing loss after 3 to 5 years was observed also. Older transplants showed a significant increase of tissue perfusion but did not reach initial values (Figs. 2 and 3). A significant drop of parenchymal perfusion was found in patients with elevated serum creatinine (1.36 cm/sec in cases with normal and 0.82 cm/sec in those with elevated creatinine at the proximal cortical level [p50]) (Fig. 4).

DISCUSSION

Perfusion is a crucial prerequisite for normal function in native and in transplanted organs. Chronic transplant function loss is, in the beginning, a creeping process and rarely assessed because of only faint sensations, symptoms, and laboratory signs (*4*). To prevent a silent loss of renal function, methods are necessary to monitor stealthy changes in renal parenchyma, especially in its vessels. An ideal method should be a cheap bedside technique that is informative in the preclinical stages of renal transplant diseases, and that is not harmful, radioactive, or invasive.

Scintigraphy, ultrasound, magnetic resonance imaging, and renal biopsy are commonly used tools for diagnosing renal transplant dysfunction, along with clinical and laboratory investigations. Scintigraphy and color Doppler sonography result in comparable evaluations in dysfunctioning transplants (*5*).

Scintigraphy is hampered by invasiveness, radiation, expense, and lack of availability. Ultrasound techniques are free of such limitations. Various methods are in use. Color Doppler ultrasound is used to calculate indices such as resistance index (RI) and pulsatility index (PI) of flow velocity to describe vascular resistance, which is caused by change with deterioration of perfusion in rejection. Measurements of peripheral vessel distance to renal surface and percentage of vascularization (compared with the entire parenchymal area) have been proposed to describe the extent and intensity of perfusion more comprehensively and in a tissue-related fashion (*6*). RI and PI are single-vessel parameters (*7, 8*) and suffer from the fact that, in decreased perfusion, only those vessels can be interrogated that are visible. This might lead to an erroneous favorable impression, because badly perfused vessels are not detectable (*9*). Doppler energy imaging (power Doppler) (*10, 11*) seems to offer additional information not given by other Doppler techniques—mainly, an overview of vascularization that is less angle dependent. Perfusion evaluation nevertheless remains subjective. New developments aim to detect changes in tissue elasticity—chronic fibrosis as a consequence of chronic rejection might increase the stiffness of transplant parenchyma (*12, 13*).

We developed a novel approach to this problem: a dynamic color Doppler sonographic perfusion measurement (*2*). This technique has been proven in healthy kidneys, in healthy bowel, in chronic inflammatory bowel disease, and in tumors (*3, 14–16*). It allows—the first time—to calculate tissue perfusion from conventional color Doppler investigations with respect to perfusion changes during heart action (thus "dynamic" perfusion measurement). No additional equipment is needed. Software calculates perfusion in a reproducible ROI, and long-term comparisons can be made in the time course of one patient and between different patients. The results are only a byproduct of a conventional color Doppler sonographic renal transplant examination and do not take additional investigation time.

In accordance with our prior results (*3*) in healthy kidneys, we could clearly distinguish perfusion intensity in standardized cortical tissue layers. Perfusion declines from the central to the peripheral cortex.

To achieve reproducible results, some indispensable preconditions must be followed. Relevant variables must be fixed (color Doppler frequency, color Doppler gain, wall filter, part of the kidney) and aliasing should be minimized. With these prerequisites, reproducible results can be expected. In a study with 37 kidneys and five measurements of renal tissue perfusion, we found a 2s-range of 8.41% of the mean values (results not shown here). In another precursor study, we could demonstrate a significant correlation of scintigraphic evaluation of renal function and dynamic color Doppler perfusion measurements in hydronephrotic kidneys (results not shown).

Compared with scintigraphic renal transplant evaluation, our new method could offer a valuable alternative. Each method has its peculiarities: scintigraphic techniques are technically more demanding but not as observer dependent as ultrasound techniques. In 1994, Mizuiri et al. (*16*) introduced a scintigraphic approach similar to ours: they calculated cortical (outer) versus juxtamedullary (inner) mean tracer transit time and could thus differentiate acute tubular necrosis from acute rejection. A similar approach was described for magnetic resonance renography with semiautomated signal analysis (*17*). Magnetic resonance imaging perfusion techniques still require sophisticated mathematical procedures of signal analysis but have the capacity to describe perfusion and glomerular filtration rate in animal models (*18*).

Our results demonstrate a decline in perfusion intensity from central to peripheral cortex. We obtained similar results in healthy kidneys (*3*), whereas the ratio of central to peripheral perfusion differs. In healthy kidneys, the central to peripheral perfusion ratio was calculated as 1.86 cm/sec÷0.51 cm/sec=3.64; in the group of renal transplants summarized here, it was1.36 cm/sec÷0.60 cm/sec=2.67. A direct comparison of these measurements is not allowed, however, because healthy kidneys were from children and adolescents and transplants stemmed from adult donors. Despite this, it is obvious that perfusion intensity measurements revealed comparable results in similar dimensions, stressing the power of the new method. Further investigations of healthy adult kidneys are necessary to relate transplant perfusion data to age-matched controls. Our study group consisted of patients with body weights up to 102 kg and body mass index up to 36.8. Transducer frequency should therefore be no limitation in adult studies.

We could demonstrate a decline of perfusion also, depending on the time interval to transplantation. The steepest decline occurred in the first year. Nankivell et al. (*1*) described histologically a distinct triphasic course of renal transplant glomerulosclerosis in the following stages: (1) "an intense but limited peak of damage in the first month," (2) "glomerulosclerosis then occurred as a late consequence of earlier immune-mediated tubular damage," and (3) "Subsequent progressive GS [glomerulosclerosis] occurred beyond 4 years." This description fits astonishingly well to our own perfusion

data, where a steep decline of perfusion occurred in the second year. In contrast to Nankivell et al. (*1*), we investigated transplants without selection of those that had undergone biopsy (i.e., in our population, the percentage of well-functioning transplants might be higher). This might explain diverging results in the later years. We observed in surviving transplants a slight increase of perfusion in the interval greater than 5 years. Some authors (*19*) describe characteristic patterns of vascular damage in renal transplant biopsies performed regularly over a 10-year period after transplantation. Beyond 1 year, a later phase of chronic allograft nephropathy was characterized by microvascular and glomerular injury. Vascular disease is the rationale for attempts to qualify allograft nephropathy by means of ultrasound Doppler techniques. Others used color Doppler images that have been fixed in systole (*20*) or single-power Doppler images (*21–23*). With these techniques, reduction of tissue perfusion was found in chronic allograft nephropathy and with other causes of transplant damage, such as acute rejection, acute tubular necrosis, and cytomegalovirus infection. Normal values, however, vary among different centers (normal transplant fractional colored area: 68% (*21*), >55% (*22*), and 29% (*20*)). This might be a consequence of differing imaging conditions (sensitivity of ultrasound equipment and other specifications of Doppler imaging). Higher values from power mode images may be caused by often less distinct vessel borders in this technique. Vessel borders tend to blur and pulsate less vividly in power Doppler imaging. Techniques published until now lack a time axis of perception. Today, real-time pulsatility measurement in single vessels is a mainstay of transplant perfusion evaluation. Higher pulsatility is a most important sign of transplant damage (*24*). This proven parameter is completely blinded out with still-image techniques. Our approach incorporates not only time-dependent changes of flow velocity (with conventional techniques referred to as RI and PI) but also simultaneous time-dependent changes of the perfused area. We combine changes of velocity and changes of perfused area—both over a full heartbeat—and calculate from these our parameter perfusion intensity. Therefore, it goes beyond existing Doppler techniques and adds relevant information. Nevertheless, future studies have to demonstrate the clinical use of these advantages.

Despite the encouraging results of our study, limitations and conditions must be discussed. The crucial point for obtaining reproducible results is a constant presetting of important parameters. Moreover, it is important to define the ROI with definite geometric patterns (we used a parallelogram whose side length was oriented to definite anatomic structures). It is useful to measure a segment that is fed by one interlobar artery that is running straight toward the transducer to avoid an angle bias of the entire segment under investigation. Another problem is prudent setting of color Doppler frequency and wall filter to minimize aliasing, on the one hand, and not to miss small vessels, on the other hand. Taking all these variables into account and fixed—whenever possible—the method of dynamic color Doppler perfusion measurement is capable of discriminating such tiny differences of perfusion intensity as encountered in proximal versus distal cortical renal parenchyma, as shown by results presented here and in healthy kidneys (*3*). We are, for this reason, optimistic to offer a valuable method for assessment of tissue perfusion, which might be applied in various renal diseases and functional disorders.

CONCLUSION

Links to clinical symptoms still need to be established. We found a significant drop of parenchymal perfusion in patients with elevated serum creatinine. An inverse correlation of serum creatinine and perfusion was also demonstrable—significant but weak. Larger studies are necessary to address the clinical impact of tissue perfusion measurements. To stimulate this, we invite colleagues to download and use Pixelflux software (www.chameleon-software.de).

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