EXPERIMENTAL STUDIES

Quantification of Renal Blood Flow With Contrast-Enhanced Ultrasound

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OBJECTIVES	The goal of this study was to determine the ability of contrast-enhanced ultrasound (CEU) to quantify renal tissue perfusion.
BACKGROUND	The kinetics of tracers used to assess renal perfusion are often complicated by countercurrent exchange, tubular transport or glomerular filtration. We hypothesized that, because gas-filled microbubbles are pure intravascular tracers with a rheology similar to that of red blood cells, CEU could be used to quantify renal tissue perfusion.
METHODS	During a continuous venous infusion of microbubbles (SonoVue), regional renal perfusion was quantified in nine dogs using CEU by destroying microbubbles and measuring their tissue replenishment with intermittent harmonic imaging. Both renal blood volume fraction and microbubble velocity were derived from pulsing-interval versus video-intensity plots. The product of the two was used to calculate renal nutrient blood flow. Renal arterial blood flow was independently measured with ultrasonic flow probes placed directly on the renal artery and was increased using dopamine and decreased by placement of a renal artery stenosis.
RESULTS	An excellent correlation was found between cortical nutrient blood flow using microbubbles and ultrasonic flow probe-derived renal blood flow ($r = 0.82$, $p < 0.001$) over a wide range (2.5 fold) of flows.
CONCLUSIONS	Ultrasound examination during microbubble infusion can be used to quantify total organ as well as regional nutrient blood flow to the kidney. (J Am Coll Cardiol 2001;37:1135–40) © 2001 by the American College of Cardiology

The kidney is a complex structure involved in the excretion of metabolic waste products as well as the regulation of fluid volume, osmolarity and acid base status. These functions are influenced in large part by alterations in the regional distribution of blood flow between the renal cortex and medulla. The ability to accurately measure such variations in regional renal perfusion could provide important clinical insights into renal function.

Color and spectral Doppler are currently commonly used methods to assess renal perfusion noninvasively (1). These methods provide an assessment of only renal arterial blood flow (RBF) without any information on actual tissue nutrient blood flow (NBF). There are several disease states where regional NBF can be abnormal without significantly affecting RBF and vice versa. Currently used methods for quantifying regional renal NBF have significant limitations: they are either invasive, rely on the use of tracers that are

diffusible or have kinetics that are complicated by tubular transport or glomerular filtration (2,3).

Contrast-enhanced ultrasound (CEU) uses gas-filled microbubbles as a tracer to assess tissue perfusion with ultrasound. Because these microbubbles remain entirely within the intravascular space (4) and have a rheology similar to that of red blood cells (4,5), we hypothesized that measurements of their renal tissue kinetics could be used to quantify RBF as well as renal NBF. We have previously shown that, at steady state during a continuous infusion, measuring the rate at which microbubbles replenish tissue after their destruction provides an assessment of tissue microbubble velocity (6). When the tissue has been completely replenished with microbubbles, the signal from the microbubbles reflects tissue blood volume fraction (BVF) (6). The product of microbubble velocity and BVF reflects NBF (6). In this study, we evaluated the ability of this method to quantify regional renal NBF in a canine model.

METHODS

Animal preparation. The study was approved by the Animal Research Committee at the University of Virginia and conformed to the American Heart Association Guidelines for use of animals in research. After induction of anesthesia with 30 mg/kg^{-1} sodium pentobarbital (Abbott Laboratories, Abbott Park, Illinois), nine dogs were intubated and ventilated with a respirator pump (model 607, Harvard

Abbreviations and Acronyms

BVF = blood volume fraction
CEU = contrast-enhanced ultrasound
NBF = nutrient blood flow
PI = pulsing interval
RBF = renal arterial blood flow
VI = video intensity

Apparatus, South Natick, Massachusetts). Additional anesthesia was administered during the study as required. Both femoral veins were exposed and cannulated with 7F catheters for administration of microbubbles, fluids and drugs. A 7F catheter was placed in the femoral artery for continuous measurement of arterial pressure. An incision was made in the left flank to expose the left kidney and its vascular supply. A custom-designed screw occluder and an ultrasonic time-of-flight flow probe (series SB, Transonics, Ithaca, New York) were placed around the renal artery.

The flow probe was connected to a digital flow meter (model T206, Transonics). The femoral arterial catheter (connected to a fluid-filled pressure transducer) and flow meter were connected to a multichannel recorder (model ES2000, Gould Electronics, Valley View, Ohio). Mean RBF and central arterial pressure were acquired digitally into a personal computer via an analog-to-digital converter (Metrabyte Corp., Taunton, Massachusetts). The signals were also displayed on-line using Labtech notebook (Laboratory Technologies Corp., Wilmington, Massachusetts). **CEU.** Two milliliters of SonoVue (Bracco Diagnostics, Princeton, New Jersey) were diluted to a total volume of 50 mL and infused at approximately 100 mL/h^{-1} via the femoral catheter (7). When required, the infusion rate was adjusted to produce adequate renal opacification without attenuation of far-field structures.

Contrast-enhanced ultrasound was performed with a phased array system (HDI 5000, Advanced Technology Laboratories, Bothell, Washington) using transmit and receive frequencies of 1.66 and 3.3 MHz, respectively. The transmit power and compression were set at maximum. Gain settings were optimized at the beginning of each study and were then held constant. A saline bath acted as an acoustic interface between the transducer and the kidney. Ultrasound transmission was gated to the electrocardiogram and was progressively increased from a pulsing interval (PI) of 80 ms to 20 s. Up to eight images were acquired at each PI and were recorded on 1.25 cm S-VHS videotape (Panasonic AG-MD830, Matsushita Corp., Osaka, Japan) for later analysis.

Data were transferred from videotape to an off-line computer in a 320 × 240 × 8 bit format and analyzed using custom-designed software as previously described (8). At least five images acquired at baseline (pre-contrast) and at each PI were aligned using computer cross-correlation. Regions-of-interest were placed over the cortex and medulla of the kidney (Fig. 1). The cortex was identified by its
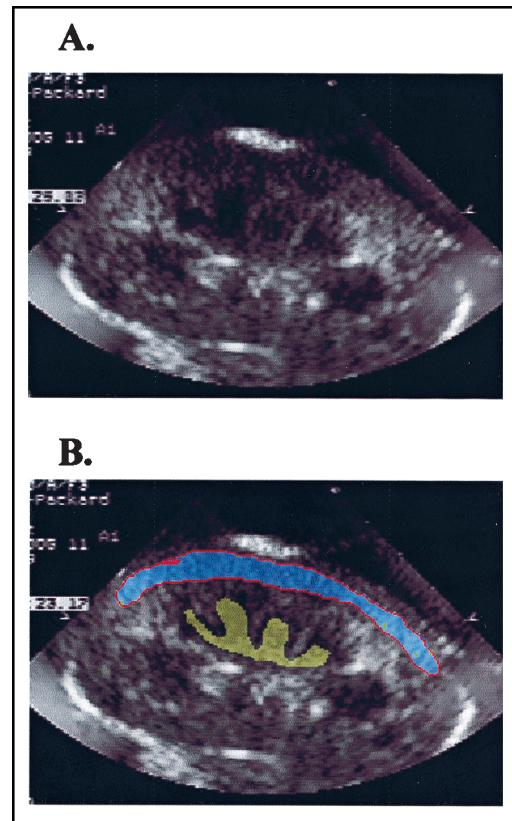
Figure 1. Gray-scale image of the kidney from an animal showing distinct differences in echogenicity between the cortex and medulla **(A)**. **(B)** The location of regions-of-interest drawn over the cortex **(blue)** and medulla **(green)** to derive regional video intensity measurements. See text for details.

echogenicity compared with the less echogenic medulla. The arcuate arteries, which separate the two regions, could be distinguished from renal parenchyma because the high blood flow velocity in these vessels allowed them to be opacified with microbubbles even during real-time imaging (PI = 33 ms). Care was taken to exclude the interlobar and arcuate arteries from the regions-of-interest. Video intensity (VI) in both regions were automatically measured from each aligned image and then background-subtracted. The resulting background-subtracted VI values from images at each PI were then averaged. Pulsing interval versus background-subtracted VI plots were generated and fitted to an exponential function: $y = A(1 - e^{-\beta t})$, where y is VI at PI of t, A is the plateau VI representing BVF, and β represents mean microbubble velocity. Nutrient blood flow was calculated by multiplying A by β (6).

In addition to the above analyses, color-coding was applied to averaged background-subtracted images to visually enhance regional differences in VI (8). The VI scale was first expanded to a dynamic range of 128 gray levels, where the pixel showing the greatest change was assigned a value of 128, and all other pixels were assigned proportionally lower values. All pixels with a gray scale value of >10 were then assigned a color based on the degree of contrast enhancement, where shades of red, progressing to hues of
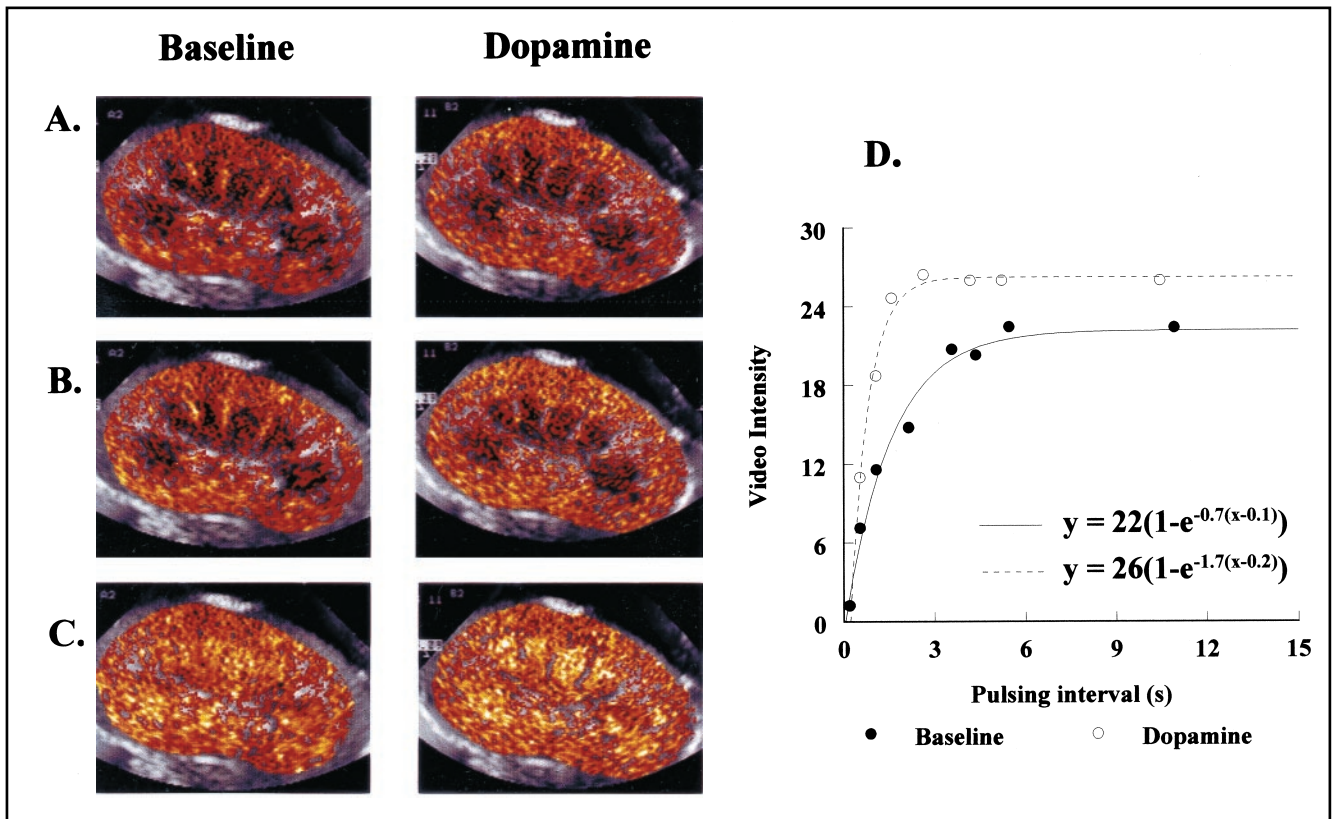
Figure 2. Background-subtracted color-coded images obtained from an animal at progressively longer pulsing intervals **(A to C)**, both at baseline and during infusion of dopamine. The corresponding pulsing interval versus video intensity curves from the cortex at both stages are shown in **D**. See text for details.

orange, yellow and then white represent incremental contrast opacification. Pixels with values ≤10 were considered to represent noise and were not assigned any color (8).

Protocol. In each dog, CEU was performed at baseline, and, after total RBF was either reduced with a flow-limiting renal artery stenosis or increased with an intravenous infusion of dopamine (2.5 to 5 $\mu g/kg^{-1}\cdot min^{-1}$). Hemodynamic variables were acquired at each stage.

Observer variability. Inter- and intra-observer variability were obtained by analysis of 12 random stages in dogs by two independent blinded observers and by the same observer at two different time points.

Statistical analysis. Data are expressed as mean ± standard deviation. Comparisons between two stages were performed using Student's *t* test, while those between >2 stages were made using repeated measures analysis of variance. Correlations were obtained using least squares fit linear regression analysis. Differences were considered significant at $p < 0.05$ (two-sided).

RESULTS

No significant differences were seen in mean aortic pressure or heart rate between baseline, stenosis and dopamine stages. At baseline, total RBF was 97 ± 26 mL/min^{-1} and, as expected, it decreased during placement of a flow-limiting stenosis on the renal artery (to 52 ± 30 mL/min^{-1})

and increased with the infusion of low-dose dopamine (to 125 ± 20 mL/min^{-1}). These changes in total RBF were highly significant ($p < 0.001$).

Because >90% of total RBF entering the kidney supplies the renal cortex (3), change in cortical microbubble velocity was used as a surrogate for alteration in total RBF. Changes in total RBF were mirrored by changes in cortical microbubble velocity during CEU. It was 0.8 ± 0.2 s^{-1} at baseline and changed to 0.6 ± 0.3 s^{-1} and 1.5 ± 0.3 s^{-1} during coronary stenosis and dopamine infusion, respectively ($p < 0.001$). An excellent correlation was found between ultrasonic flow probe-derived RBF versus microbubble velocity ($r = 0.82$, $p < 0.001$, SEE = 0.30).

Background-subtracted color-coded images obtained at three different PI, and the corresponding PI versus VI curves obtained from one dog at baseline and during infusion of dopamine are shown in Figure 2. Total RBF in this dog increased from 103 to 120 mL/min^{-1} during dopamine. A progressive increase in renal cortical VI is seen as the PI is increased from 1,016 ms (Fig. 2A) to 5,080 ms (Fig. 2B) to 15,000 ms (Fig. 2C). Brighter hues of orange and yellow are seen in the cortex at the shorter PIs during dopamine compared with the corresponding PI at baseline, indicating greater degrees of microbubble replenishment for each PI during dopamine infusion. Consequently, the rate of rise of VI is correspondingly higher (Fig. 2D) during dopamine on the PI versus VI curve (open circles) compared
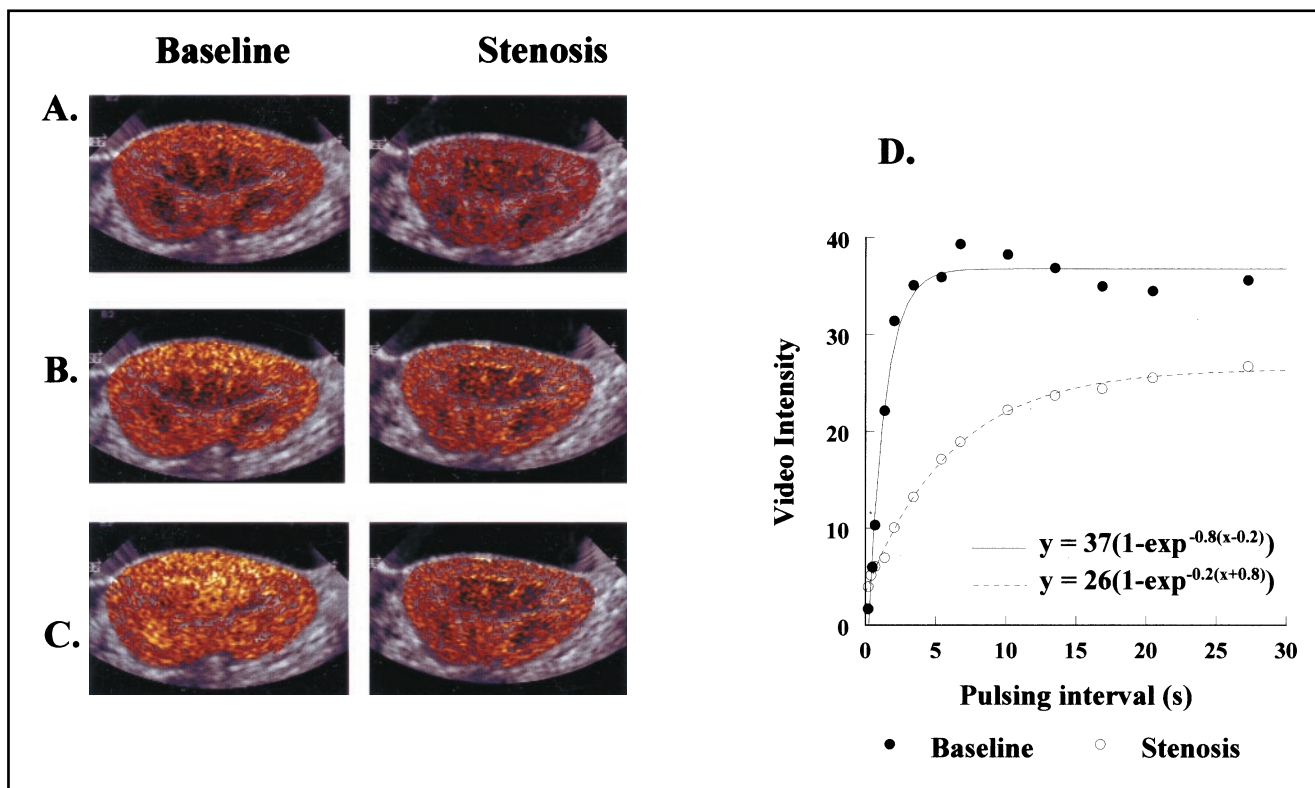
Figure 3. Background-subtracted color-coded images obtained from an animal at progressively longer pulsing intervals **(A to C)**, at baseline and in the presence of a flow-limiting renal artery stenosis. The pulsing interval versus video intensity curves obtained from the cortex during both stages are shown in **D**. See text for details.

with baseline (closed circles) and was reflected by an increase in microbubble velocity from 0.7 to 1.7 s^{-1}.

Background-subtracted color-coded images obtained at three different PI and the corresponding PI versus VI curves obtained from another dog at baseline and during a decrease in total RBF from 58 to 6 mL/min^{-1} after placement of a critical renal artery stenosis are shown in Figure 3. In the presence of a stenosis, a small change in cortical microbubble enhancement is seen as the PI was increased from Figure 3A to C, but the degree of enhancement is lower at all PI compared with baseline. The lower rate of rise of VI (open vs. closed circles) was reflected by a significant decrease in cortical microbubble velocity from 0.8 to 0.2 s^{-1} (Fig. 3D).

Observer variability. The inter- and intra-observer variability for the values *A* and *β* derived from VI versus PI curves from 12 separate random stages were excellent. They were: r = 0.88, p < 0.01 and r = 0.91, p < 0.01 for A, r = 0.91, p < 0.01 and r = 0.92 (p < 0.01) for *β*, respectively.

DISCUSSION

In this study, we have shown that the method of microbubble destruction and replenishment can be used to measure absolute RBF. The technique is easy to perform, robust and noninvasive. Therefore, this method could provide very useful information regarding renal tissue perfusion in individual patients, particularly when coupled with Doppler.

Together, these ultrasound methods could be used for routine noninvasive examination of the macro- and microvascular compartments of the kidney. In addition, they could provide unique insights on the effects of various interventions on total RBF as well as regional renal NBF.

Comparison of CEU with other methods of measuring renal NBF. Many techniques have been developed over the years in an attempt to quantify regional renal NBF. These include: regional washout of hydrogen or inert gases, heat diffusion, isotope trapping, radiolabeled microspheres, laser Doppler flow, computed tomography, magnetic resonance imaging and positron emission tomography (2,3). Many of these methods have limited clinical applicability because of their invasive nature or their limited availability. Others pose methodologic problems because the tracers themselves produce hemodynamic effects or are subject to tubular transport or glomerular filtration (2,3). Some tracers cannot be used simultaneously to assess both total RBF and regional renal NBF (3).

Microbubbles used with CEU are not subject to many of these limitations because they are hemodynamically inert and have a rheology similar to that of red blood cells (4,5). They are also small and uniform in size, and, therefore, not as prone to axial streaming or geometric exclusion as has been reported with the larger radiolabeled microspheres (9,10), which, in any case, cannot be used for assessing renal NBF in humans. Furthermore, the microbubbles remain

entirely intravascular, and, consequently, are not affected by glomerular filtration or tubular transport. Unlike many other techniques, repeated measurements of regional renal NBF can also be performed with CEU, as the microbubbles are rapidly cleared from the circulation after their administration. Additionally, ultrasound can provide simultaneous information regarding kidney size and structure.

Earlier attempts to quantify renal perfusion using CEU employed the use of direct intraarterial injections of air-filled microbubbles (11,12). The transit time of the microbubbles through the kidney could be measured, and total flow per unit volume could be quantified using classic indicator dilution curve theory. Although successful, this approach was invasive. Furthermore, unlike our method, absolute tissue flow could not be determined.

As shown by our results, CEU can be used to determine changes in total RBF. Because >90% of blood flow entering the kidney supplies the renal cortex (3), changes in cortical blood flow were compared with ultrasonic flow probe derived total RBF. Similar to the results from previous studies (13,14), infusions of low-dose dopamine resulted in increases in total RBF without affecting hemodynamic variables such as heart rate or blood pressure. In the absence of such changes in renal perfusion pressure with dopamine, no significant increase in renal cortical BVF would be expected. Thus, increases in RBF were reflected solely by increases in microbubble velocity. After total RBF was reduced with flow limiting stenoses, significant decreases in microbubble velocity were found. As shown in Figure 2, plateau VI was lower in the presence of a critical stenosis compared with baseline. This finding is in agreement with previous studies showing that reductions in renal perfusion pressure below the lower limit of autoregulation result in a dramatic decline in cortical BVF (15).

Detection of renal artery stenosis. One of the common conditions for which ultrasound examination of the kidney is performed is renal artery stenosis. As stated earlier, Doppler is currently used for this purpose. One of the main limitations with Doppler is inaccurate identification of the renal arteries, which can be exacerbated by anatomic variations such as the presence of multiple arteries. Identification of the renal artery is also tedious and time-consuming. Furthermore, correct estimation of renal artery stenosis severity from peak systolic velocity measurements requires that the angle of interrogation be parallel to the vessel, which is not always possible. Unlike Doppler, CEU does not require identification of the renal artery, nor is it angle-dependent.

Because a nonflow limiting stenosis does not affect resting RBF, its detection generally requires the use of some pharmacologic "stress," such as captopril (16). It has been shown, however, that even within the autoregulatory range, reductions in renal perfusion pressure are associated with decreases in cortical NBF without changes in medullary NBF (17). Thus, determining the ratio of cortical versus medullary NBF with CEU could potentially be used to determine the presence of nonflow limiting renal artery stenosis, even without recourse to a pharmacological maneuver.

Study limitations. One of the limitations of CEU is shadowing produced by microbubbles. Those nearest to the ultrasound probe act as a shield, thus preventing penetration of ultrasound to deeper structures. This artifact can be precluded by decreasing the microbubble infusion rate so that homogeneous opacification is produced even at long PI. Another common problem relates to lack of a homogenous ultrasound field, especially in a phased-array transducer, which was used for our study (18). Because the ultrasound energy falls off by the cosine of the angle from the center of the beam, less backscatter is noted in the lateral aspects of the sector. This can be overcome by using a linear-array transducer, which, in any case, is the preferred transducer for renal imaging. Despite the reduction in signal from the lateral aspects and the apparent reduction in plateau VI, however, the microbubble velocity will still be the same, which indicates that one is dealing with an artifact rather than an actual perfusion defect.

Conclusions. We have described a new method for measuring RBF and renal NBF noninvasively using ultrasound. The method is simple and easy to perform. Important and useful information can be obtained in a very short time. Repeated measurements can be performed to assess changes in RBF as well as renal NBF. The method has great potential for assessing patients with known or suspected renal disease. It also has potential for assessing NBF to other organs accessible to ultrasound.

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