Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2017

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2017.09.019

● *Original Contribution*

REPEATABILITY OF CONTRAST-ENHANCED ULTRASONOGRAPHY OF THE KIDNEYS IN HEALTHY CATS

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(*Received* 21 *March* 2017; *revised* 20 *September* 2017; *in final form* 25 *September* 2017)

Abstract—Contrast-enhanced ultrasound can be used to image and quantify tissue perfusion. It holds great potential for the use in the diagnosis of various diffuse renal diseases in both human and veterinary medicine. Nevertheless, the technique is known to have an inherent relatively high variability, related to various factors associated with the patient, the contrast agent and machine settings. Therefore, the aim of this study was to assess week-to-week intra- and inter-cat variation of several perfusion parameters obtained with CEUS of both kidneys of 12 healthy cats. Repeatability was determined by calculating the coefficient of variation (CV). The contrast-enhanced ultrasound parameters with the lowest variation for the renal cortex were time-to-peak (CV 6.0%), rise time (CV 13%), fall time (CV 19%) and mean transit time (24%). Intensity-related parameters and parameters related to the slope of the time-intensity curve had a CV of >35%. Lower repeatability was present for perfusion parameters derived from the renal medulla compared with the renal cortex. Normalization to the inter-lobar artery does not cause a reduction in variation. In conclusion, time-related parameters for the cortex show a reasonable repeatability; whereas poor repeatability is present for intensity-related parameters and parameters related to in- and outflow of contrast agent. Poor repeatability is also present for all perfusion parameters for the renal medulla, except for time to peak, which has a good repeatability. (E-mail:) © 2017 World Federation for Ultrasound in Medicine & Biology. All rights reserved.

Key Words: **Contrast-enhanced ultrasound, Repeatability, Variability, Cat, Kidney.**

INTRODUCTION

Contrast-enhanced ultrasound (CEUS) allows assessment of tissue perfusion in a variety of clinical conditions. It enhances the diagnostic accuracy of B-mode ultrasound by adding the use of an intra-venously administered contrast agent. The contrast agent is composed of microbubbles consisting of a high molecular-weight gas encapsulated by a stabilizing shell (Haers and Saunders 2009). The technique has several advantages: it is extremely safe, has no nephro- or hepatotoxicity, and uses no ionizing radiation (Dietrich et al. 2011; Haers and Saunders 2009; Seiler et al. 2013). CEUS shows great potential for the diagnosis of diffuse renal disorders. Quantitative CEUS has been shown to be useful in the diagnosis of humans with renal transplant rejection, diabetic nephropathy and chronic renal disease (Dong et al. 2014; Fischer et al. 2008; Kay et al. 2009; Ma et al. 2012; Tsuruoka et al. 2010). In dogs, CEUS is capable of measuring perfusion changes in iatrogenically induced ischemic renal disease and iatrogenic hypercortisolism (Dong et al. 2013; Haers et al. 2013). Our research group demonstrated the utility of CEUS to detect renal vasoconstriction induced by angiotensin II infusion in cats (Stock et al. 2016).

Chronic kidney disease is a major health issue, both in human and veterinary medicine. The prevalence of this disease in human adults in the United States has been reported to be 13% (Coresh et al. 2007). An even higher prevalence has been reported in the cat population, in which 30%–80% of the geriatric population is affected (Lulich et al. 1992; Marino et al. 2014). In both species, important structural (decreased capillary number) and functional (vasoconstriction) loss of

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micro-vascularization occurs in chronic kidney disease (Ballermann and Obeidat 2014; Brown et al. 2016; Schmiedt et al. 2016; von Stillfried et al. 2016), therefore, evaluation of renal perfusion may deliver important new insights in the disease and its diagnosis.

The major challenge in CEUS is the relatively high degree of variability, which originates from several factors related to the patient, the contrast agent, machine settings and the operator (Tang et al. 2011). Machine settings and, to a lesser extent, operator-dependent factors can be controlled. However, contrast agent composition and patient factors such as blood pressure, heart rate and respiratory rate cannot be controlled. It is important to know the magnitude of these variations in and between healthy patients to estimate to what extent quantification of the feline kidney perfusion with CEUS is reliable, before the technique can be used for the diagnosis of disease in human patients. Therefore, this repeatability study is designed to determine normal inter- and intra-individual variation in healthy cats.

MATERIALS AND METHODS

Twelve healthy European Shorthair cats, 9 neutered females and 3 neutered males, were included with the permission of the local ethical committee of Ghent University (EC 2014/148, Merelbeke, Belgium). The median age of the cats was 6 y and 9 mo (range 3 y and 11 mo–13 y and 2 mo). Their median weight was 3.28 kg (range 2.75–3.55 kg). The cats were deemed healthy on the basis of physical examination, blood pressure measurement, complete blood count and serum biochemical analysis, urine analysis and abdominal ultrasound. The cats were free from medication for at least 2 mo and received the same commercial food at least 2 mo before the study and during the study period.

CEUS of both feline kidneys was performed at 3 time points with a 7-d interval. At every time point, a catheter was placed in one of the cephalic veins. Anesthesia was induced with a bolus of propofol (Propovet 10 mg/mL, Abbott Laboratories, Lake Bluff, IL, USA), 4–8 mg/kg intravenously, given to effect and maintained with additional boluses (1 mg/kg) as necessary. The hair was clipped over the ventrolateral portion of the abdomen. Alcohol and coupling gel were applied to the skin. The ultrasound examinations were performed with the cat in dorsal recumbency.

The left kidney (for the 1st and 2nd injections) and the right kidney (for the 3rd injection) were centered on the screen and imaged in a longitudinal plane. The transducer was manually positioned by the same person during each imaging procedure and was maintained at the same position during the CEUS examination.

Sulfur hexafluoride-filled microbubbles (Sonovue, Bracco, Milan, Italy), provided in a commercially available kit, were reconstituted according the manufacturer's guidelines. The contrast agent was used within a time interval of 6 h. Before each injection, the suspension was rehomogenized by gently agitating the vial from top to bottom. A 0.05 mL/kg (±0.01 mL and ±0.01 kg) bolus of ultrasound contrast agent was manually administered intravenously over approximately 3s, followed by injection of a 1.5 mL (±0.1 mL) saline bolus. A three-way stopcock, with a dead volume of 0.3 mL, was used to avoid any delay between the injection of contrast agent and saline. The same person injected all cats, giving 3–4 injections of contrast. The first one was not used for further quantification because this injection often results in lower enhancement (Stock et al. 2017). Between subsequent injections, to avoid artifacts, remnant microbubbles were destroyed by setting the acoustic power at the highest level (mechanical index at 0.47) and scanning the caudal abdominal aorta, external and internal iliac arteries for 2 min.

All examinations were performed using a linear transducer of 12-5 MHz on a dedicated machine (iU22, Philips, Bothell, WA, USA) with contrast-specific software. Basic technical parameters included (i) a single focus placed directly under the kidney, (ii) persistency off, (iii) mechanical index 0.09, (iv) dynamic range C50, (v) gain 85% and (vi) side-by-side imaging. These settings were repeated during each injection. All studies were digitally registered as a movie clip at a rate of 7 frames per second, and this during 90 s.

The movie clips were exported as digital imaging and communications in medicine and analyzed using specialized computer software (VueBox, Bracco Suisse SA, Manno, Switzerland) for objective quantitative analysis. Curve fitting was based on a Bracco proprietary model optimized for bolus kinetics (Bracco Suisse SA). Analyses were based on linearized video data. Seven regions of interest (ROIs) were manually drawn: one on an interlobar artery (1–1.5 mm), three in the renal cortex (4 mm), two in the renal medulla (3 mm) and one surrounding the entire kidney. The ROIs for the cortex and medulla were identical in size and shape and drawn at approximately the same depth. An illustration of the ROI placement is provided in Figure 1. For every ROI, the software determined mean pixel intensities and created a time-intensity curve. Time-intensity curves were analyzed for blood flow parameters representing blood volume (peak enhancement [PE]), wash-in area under the curve (WiAUC), wash-out area under the curve (WoAUC) and total area under the curve (AUC) and blood velocity (rise time ([RT]), mean transit time (mTT), time to peak (TTP), wash-in rate (WiR), wash-in perfusion index (WiPI), fall time (FT) and wash-out rate (WoR). Time-related parameters are RT, mTT, TTP and FT. Parameters related to the slope are WiR, WoR and WiPI. The values for the three ROIs for the cortex and two ROIs for the medulla were averaged. Peak enhancement and