

# Renal microvascular assessment by contrast enhanced ultrasound

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## Abstract

**Introduction and objectives.** To identify the value of contrast-enhanced ultrasound (CEUS) assessment for renal microvascular perfusion damage in patients with essential arterial hypertension (AHT) and associated comorbidities, diabetes and chronic kidney disease (CKD).

**Materials and Methods.** 85 patients with AHT grade I-III (29 with diabetes and 12 with CKD), age=60+/-12, Males=45.8% and 10 healthy adults were investigated by CEUS. After intravenous administration of 1.2ml contrast agent the images were recorded for 3 minutes. Renal micro-vascular perfusion was evaluated in early cortical phase (N=10-14sec), late cortical phase (N=15/20-40sec) and medullar phase (N=45-120sec). Time-intensity curves (TIC) were analyzed by Contrast Dynamics software using: arriving time (AT), time to peak (TP), peak intensity (PI), area under the curve (AUC) and mean transit time (MTT).

**Results.** The enhancement times were progressively prolonged in the study group according to the grade of the hypertension and more in diabetes and CKD. TIC analyze were similar: AT in AHT group was 18sec, in diabetes 21sec and in CKD 25 sec vs. healthy 10 sec. TP, PI, AUC are also well correlated with the grade of the hypertension and associated comorbidities. No adverse effect was noted during the study. No changes in biological status were noted in the study group after CEUS.

**Conclusions.** CEUS is a reliable, non-invasive, simple and safe method to evaluate in real-time the renal microvascular perfusion damage in all grades of hypertension and associated comorbidities. TIC parameters (TP, PI and AUC) accurately assess the renal microvascular impairment in different stages.

**Keywords:** renal microvascular assessment, hypertension, contrast enhanced ultrasound, SonoVue®

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## Introduction

Essential arterial hypertension is the leading cause of morbidity and mortality from cardiovascular diseases, with an increasing prevalence, slightly influenced by current therapy. The hypertension induced renal damage is common and the degree of renal impairment is considered a major predictive factor of future cardiovascular events and mortality [9].

Adaptation of glomerular microcirculation to chronic increase of systemic blood pressure by increasing afferent arteriole resistance leads to maintaining normal values of intra-glomerular pressure, glomerular plasma flow and glomerular filtration rate / nephron. This adjustment results in maintaining normal renal function in hypertensive patient over a period of time [28]. Prolonged persistence of elevated blood pressure can cause hypertensive nephrosclerosis, an important cause of kidney failure. Currently, diagnosis of hypertension-induced renal changes is based on elements that emphasize reducing kidney function and / or urinary albumin excretion level detection. Chronic kidney disease is classified according to estimation of glomerular filtration rate (GFR), calculated using a series of formulas that take into account age, gender, ethnicity and serum creatinine (MDRD, Cockcroft-Gault, CKD-EPI) [9]. These formulas allow detection of kidney function changes when serum creatinine is normal. Focal segmental glomerular sclerosis (GSFS) is the main lesion in hypertension and consists in connective tissue proliferation in some nephrons from different kidney areas. Pathogenic mechanism is incompletely understood. Circulation factors are responsible for onset of focal segmental glomerular sclerosis [28]. The main lesion in nephrons is the podocyte alteration followed by mesangial, endothelial and epithelial proliferation, eventually collapse of capillaries. Structural and functional changes in renal microvasculature are the main determining factors in the onset of nephrosclerosis. These changes are highlighted in later stages of the disease being difficult to diagnose in early stages. The modalities by which changes in renal microvasculature and nephrosclerosis can be early identified are useful diagnostic methods in different stages of hypertension, as well as evaluating methods for effectiveness of therapy and assessment of disease severity.

CEUS evaluates macro and microvasculature "in real time", allowing exploration of blood vessels smaller than 100µm (below conventional Doppler exploration which is 1mm) [2,3]. The EFSUMB Guidelines for non-hepatic applications published in 2012 recommend using

CEUS in renal focal lesions (both in characterization and detection), in vascular nephropathy (renal parenchymal ischemia, renal infarction, cortical necrosis, parenchymal differentiation between non-perfused and hypo-perfused areas, renal artery stenosis) and in evaluation of percutaneous ablative therapies [1]. This "real time" imaging method can be used safely in patients with kidney failure, is fast (10 minutes), without exposure to ionizing radiation, without nephrotoxicity and fear of claustrophobia and with low cost as compared to other imaging methods [4-8, 10-26]. CEUS can uniquely provide additional information about the microcirculation of the renal parenchyma that no other imaging method offers [27].

## Objective

The current study aims to determine the value of CEUS in hemodynamic changes assessment of renal microcirculation in hypertensive patients, in different stages of the disease, the clinical significance of these changes and the effects of antihypertensive medication.

## Materials and methods

This prospective and observational study started in October 2014 and is ongoing, taking place in Urology Clinical Hospital "Prof. Dr. Th. Burghele" Bucharest. We estimate to enroll in this study over 100 patients with essential arterial hypertension in various stages of evolution. A control group of 20 healthy people will be evaluated in order to compare renal microvasculature with that of patients enrolled in the study. According to the funding resources the patients will be followed every 3 to 6 months.

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	
1.	Patients diagnosed with essential hypertension according to the European Society of Hypertension Guidelines in 2013: Blood Pressure (BP) > 140mmHg (systolic) and / or > 90mmHg (diastolic)
2.	Age: 25 -75 years for both gender
3.	Treated or without antihypertensive medication
4.	With or without kidney failure
5.	With or without diabetes
Exclusion criteria	
1.	Age <25 years and > 75 years

2. Secondary hypertension
3. Patients with neoplasia
4. Contraindications of SonoVue® administration (shunts right-left, severe pulmonary hypertension-pulmonary artery pressure>90mmHg, uncontrolled hypertension, acute respiratory distress syndrome, in association with dobutamine in patients whose clinical condition suggests cardiovascular instability and dobutamine is contraindicated, pregnant and breastfeeding women, known hypersensitivity to sulfur hexafluoride or any of the SonoVue® excipients)
5. Severe liver diseases

### CEUS technique

All the ultrasound examinations including CEUS were performed on Siemens S2000 equipment with dedicated included contrast software (CPS) using abdominal convex transducer of 3-5 MHz. The ultrasound contrast agent used is SonoVue® (Bracco, Italy), a second generation contrast agent with the following characteristics: spherical structures (microbubbles) with very small size of the bubbles ( $d = 2 - 6$  microns), an inert gas (sulfur hexafluoride) and capsule with high elasticity (phospholipids: macrogol 4000, polietilenglicol, distearoil-phosphatidyl-coline, di-palmitoil-phosphatidyl-glycerol). It is a non-toxic, non-allergenic and non-emboligenic contrast agent, strictly intravascular (without solubility and diffusion in tissues), with extemporaneous preparation, intravenous administration (preferably in a cubital vein), eliminated by expiration (in 8-15 minutes after injection – for the gas) with hepatic metabolism for the capsule. The doses which are usually used are very low: between 0.8 and 2.4 ml (depending of the sensitivity of the US equipment and the examined organ). The low doses determine low cost for this imaging method.

The quantitative analyzes of renal microvascular contrast enhancement – time intensity curves (TIC) were obtained with dedicated software (Contrast Dynamic) of this equipment.

At all patients we injected 1.2 ml of SonoVue® in a cubital vein (in bolus) using a 20 Gauge cannula, followed by 5-10 ml of saline solution. Microvasculature from the renal parenchyma was assessed continuously, in real-time, for about 3 minutes from the moment of injection. The timer button was activated when contrast injection started. A digital clip with dynamic contrast enhancement in renal vascular phases was recorded to be analyzed off-line. All grey-scale ultrasound characteristics of examined kidney were registered. CEUS examinations were performed and analyzed by two ex-

perts in this field, with experience in renal pathology in both conventional ultrasound and CEUS.

### Qualitative assessment of renal microvasculature

There are two vascular phases of contrast enhancement in kidney:

1. *Cortical phase* (90% of renal perfusion) - starts at 10 seconds from the injection time and lasts up to 20-40 sec. This vascular phase can be divided into two sub-phases:
  - *early arterial phase* (11 to 14 seconds from the moment of injection) with enhancement of the intra-renal segmental arteries;
  - *late arterial phase* (15- 20 to 40 seconds from the moment of injection) with enhancement of the renal cortex
2. *Medullar phase* from 40 - 45 to 110-120 seconds.

### Quantitative assessment of renal microvasculature

The renal perfusion enhancement curves (TIC) were analyzed by the following parameters: AT (arriving time) - the time of contrast arrival in renal arteries; TTP (time to peak) - time to achieve the maximum intensity of the renal cortex enhancement; PI (peak intensity) - maximum intensity of contrast enhancement; AUC (area under the curve); MTT (mean transient time) - average contrast transit time in renal parenchyma.

### Results and discussions

Up to the present we investigated by CEUS 85 patients with AHT grade I-III (29 with diabetes and 12 with CKD), age=60+/-12, Males=45.8% and 10 healthy adults. All patients had clinical and biological investigations and signed the informed consent form before any study procedure. All patients had normal BP (therapeutically controlled) before CEUS examination.

The initial data show that there are differences in dynamic contrast enhancement of renal parenchymal vascular phases for both qualitative and quantitative assessment (real-time observation of contrast enhancement, time of contrast agent arrival in renal cortex, the intensity of contrast uptake in both cortical and medullar phases and finally the "washing" of the contrast agent).

Table 2. CEUS – Renal perfusion quantification: mean time of enhancement in renal vascular phases. TIC analyses.

Group dg.	No. of Pts.	Early cortical phase (N=10-14 sec.)	Late cortical phase (N=15/20-40 sec.)	Medullar phase (N=45-120 sec.)	AT sec.	TP sec.	PI %	AUC %sec.	MTT sec.
<b>AHT Group</b>	<b>85</b>	<b>19-22</b>	<b>24-43</b>	<b>45-108</b>	<b>18</b>	<b>44</b>	<b>20</b>	<b>1444</b>	<b>65</b>
Grade I	7	17-20	22-43	45-115	16	37	24	1480	52
Grade II	28	17-21	22-40	43-104	16	42	21	1517	64
Grade III	50	20-24	25-44	47-109	19	46	18	1397	67
AHT+Diabetes	29	22-26	27-47	49-109	21	51	18	1623	77
AHT+CKD	12	26-30	31-49	51-105	25	42	26	1109	62
<b>Control</b>	<b>10</b>	<b>11-13</b>	<b>14-39</b>	<b>43-114</b>	<b>10</b>	<b>42</b>	<b>26</b>	<b>1855</b>	<b>63</b>

The enhancement times were progressively prolonged in the study group according to the grade of hypertension and more in diabetes and CKD, especially for the early and late arterial cortical phase (Table 2).

In all AHT group the early cortical phase started in 19-22sec from the injection time as compared to 11-13sec in the control group. There are also differences in early cortical phase in different grades of hypertension (progressively increased from grade I to grade III): 17-20sec in grade I to 20-24sec in grade III and more in associated comorbidities (22-26sec in diabetes and 26-30sec in CKD).

In late cortical phase the enhancement times were 24-43sec for all AHT group (progressively increased from 22-43sec in grade I to 25-44sec in grade III) versus 14-39sec in control group.

In medullar phase we noted a slightly decrease of the wash-out phase from 45-108sec in AHT versus 43-114sec in control group.



Fig. 1. Qualitative analysis of the CEUS images at 3 patients in different vascular renal phases (early arterial phase -16sec, late arterial phase-30sec, early medullar phase-42sec, late medullar phase-1min and 42

sec).

The images from Fig. 1 show the comparison in qualitative analyzes obtained from three patients at the same time from the moment of injection. Note the significant differences in terms of contrast enhancement in different vascular renal phases regarding the contrast arrival in kidney parenchyma, homogeneity and intensity of contrast enhancement and also the transit time of contrast agent in renal microcirculation.

TIC analyzes were similar with qualitative assessment (Table 2). AT in AHT group was 18sec (16sec in grade I to 19sec in grade III), in diabetes 21sec and in CKD 25sec vs. healthy 10sec.

TP was 44sec in AHT (37sec in grade I to 46sec in grade II) versus 42sec in control group. In the group associated with diabetes TP is 51sec. No differences in CKD group vs. control for TP.

PI progressively decreased from 24sec in grade I to 18sec in grade III (with 20sec in all AHT group), and with 18sec in diabetes and 16sec in CKD versus 26sec in control group. AUC and MTT are also well correlated with the grade of the hypertension and associated comorbidities.

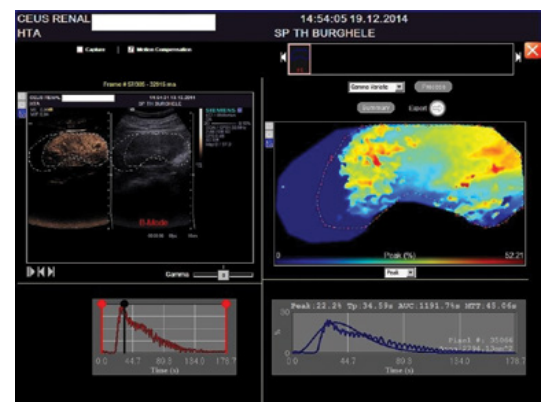


Fig. 2 TIC analysis in a patient with AHT gr. I



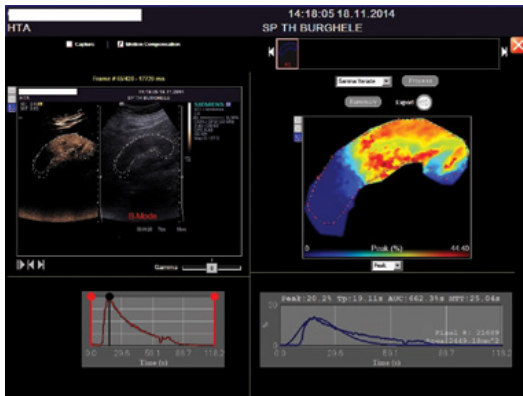


Fig. 3 TIC analysis in a patient with AHT gr. II

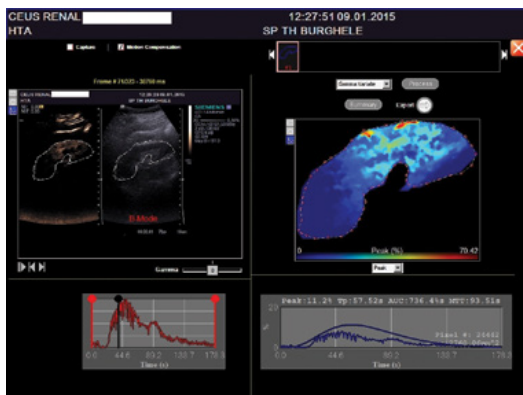


Fig. 4 TIC analysis in a patient with AHT gr. III

Fig. 2-4 show the quantitative analysis (TIC) of the CEUS images from three patients in different grade of hypertension (grade I – grade III). For TIC analyses we choose the region of interest (ROI) in which only include the renal parenchyma (cortical and medullar) and exclude the renal sinus in order to eliminate the macrovasculature from the quantitative analysis. TIC will reflect the microcirculation of ROI.

The statistical analyses of these data shall be proceeding after the study will be finalized.

### Safety

No patient had adverse effect during and after contrast agent injection. No changes in biological and hemodynamic status were noted in the study group after CEUS.

### Conclusions

Renal microvascular perfusion damage is present and progressively increases in all grades of hypertension and associated comorbidities (diabetes and CKD). CEUS is a feasible, well-tolerated, non-invasive, simple

and safe method to evaluate in real time the renal microvascular damage. Both qualitative and quantitative analyzes of the CEUS images seems to be a very important tool for diagnostic of renal microvascular perfusion damage. TIC parameters (TP, PI and AUC) could accurately assess the renal microvascular impairment in different stages. Further studies are required to establish whether there are correlations between renal microvascular perfusion changes and biological markers.

**Acknowledgement:** This work received financial support through the project entitled “CERO – Career profile: Romanian Researcher”, grant number POS-DRU/159/1.5/S/135760, co-financed by the European Social Fund for Sectorial Operational Program Human Resources Development 2007-2013

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