Contrast-enhanced ultrasonography in chronic glomerulonephritides: correlation with histological parameters of disease activity

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Abstract

Purpose To compare contrast-enhanced ultrasonography (CEUS)-derived time-intensity (TI) curves with histological findings in kidneys of patients affected by chronic glomerulonephritides (GN) in the early stage of disease.

Methods Research ethics committee approval and patient written informed consent were obtained. Thirty-one patients who showed clinical and laboratory signs of GN, with preserved renal function, were consecutively enrolled. They underwent kidney CEUS, from which TI curves were obtained, and kidney biopsy. TI curves were compared with clinical data, ultrasound (US) Doppler, and histological parameters.

Results The persistence of contrast agent signal during the wash-out phase was found to be correlated with the degree of disease activity ($p=0.016$) and in particular with the presence of mesangial hyperplasia ($p=0.008$). No correlation was observed between TI curves and clinical or Doppler US-derived parameters.

Conclusions The persistence of contrast agent signal in the wash-out phase of CEUS appears to reflect a disturbance of perfusion in glomerular capillaries in the early stages of GN. We found that the histological element directly correlated with the prolonged wash-out was mesangial hyperplasia.

Keywords CEUS · Kidney · Glomerulonephritis · Nephropathy · Ultrasonography

Sommario

Scopo Confrontare le curve intensità-tempo (TI) derivate dallo studio ecografico con mezzo di contrasto (CEUS) con i reperti istologici dei reni in pazienti affetti da glomerulonefrite (GN) cronica nelle fasi iniziali di malattia

Metodi Abbiamo dunque arruolato 31 pazienti con segni clinici e laboratoristici di GN e con funzione renale conservata. Costoro sono stati sottoposti a CEUS e biopsia renali. Si è quindi proceduto alla valutazione della curve TI rispetto ai parametri clinici, eco Doppler e istologici

Risultati La persistenza del mezzo di contrasto nella fase di wash-out correla con il grado di attività di malattia, ed in particolare con la presenza di iperplasia mesangiale. Non sono state trovate correlazioni tra le curve TI ed i dati eco Doppler e clinici

Conclusioni Nelle fasi iniziali delle GN, la persistenza del mezzo di contrasto durante la fase di wash-out sembra documentare una condizione di alterata perfusione nei capillari glomerulari, direttamente correlata all'iperplasia mesangiale

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Introduction

Glomerulonephritides (GN) represent a group of heterogeneous pathologic conditions, initially symptomless, characterized by non-specific clinical signs and laboratory test alterations [1–3]. Kidney ultrasound (US) is often performed as a first-level examination, whereas imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and urography are only used in a few selected conditions, providing overall morphological information about kidneys [4]. GN can be suspected, yet not diagnosed, by means of non-invasive techniques [5]. The histological evaluation of a bioptic sample is the only procedure that allows the establishment of a diagnosis.

US contrast agents (USCAs) nowadays in use are safe in patients with renal diseases [6] and have been used for functional evaluation of the transplanted kidney [7, 8]. CEUS (contrast-enhanced ultrasonography) performed with Sonazoid (Sonazoid™, Daiichi-Sankyo, Tokyo) as contrast medium has been tested on patients with chronic renal failure, showing that USCA enhancement decreases with the severity of renal function impairment [9].

On the basis of our previous experience, CEUS is a non-invasive, reproducible, and safe tool for studying native renal parenchyma by extracting time-intensity (TI) curves [10].

The histological alterations in the early stages of GN are specific for each disease condition, showing a different but often significant involvement of glomerular capillaries. In various experimental models, CEUS provided a detailed visualization of parenchymal microvessels and a measure of flow and perfusion of the tissue [11, 12].

We tested the hypothesis that USCAs, by remaining inside the blood vessels, and reflecting the efficiency of capillaries in ensuring tissue perfusion, can be used for the detection of GN microvascular alterations through TI curves analysis. By comparing CEUS with histology, we tried to correlate USCA transit with histological findings in the early stages of GN when renal function is still preserved.

Materials and methods

From January 2008 to May 2010, from our Internal Medicine and Nephrology and Dialysis Units, we consecutively enrolled 31 patients with clinical signs and laboratory tests that indicated a suspected GN. Exclusion criteria were: treatment with drugs able to modify the hemodynamic status of the kidneys, such as renin-angiotensin system blockers (i.e., angiotensin-converting enzyme inhibitors

or angiotensin type 1), non-steroidal anti-inflammatory and immunosuppressive drugs; cancer; ischemic or congestive heart failure and severe pulmonary hypertension; estimated glomerular filtration rate (eGFR) < 60 mL/min.

Medical history and clinical data (age, sex, body mass index, and physical examination) were collected from each patient. Blood and 24 h urine samples were collected to measure: creatinine, according to Jaffé reaction; blood urea nitrogen, using chemical colorimetric tests; chemical urine analysis. eGFR was calculated according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [13]. A 12-lead electrocardiogram was also obtained.

All subjects underwent kidney Doppler US and CEUS (US device: Esaote Technos MP) using a 3.5 MHz sector transducer. The target was the kidney chosen for the following biopsy. The contrast agent (sulphur hexafluoride SF₆, Sonovue®, Bracco, Italy) was infused by peripheral vein (25 mg/5 mL NaCl), at a rate of 2 mL/s, followed by a 10 mL bolus of NaCl 0.9%. A 5 min acquisition was recorded. A clip of the entire examination was stored in the DICOM format using the hardware of the ultrasonography machine.

In the post-processing phase, we defined a region of interest (ROI) in the cortex avoiding artefacts. The shape of the ROI was arciform, chosen in the portion of the parenchyma closer to the transducer, as large as at least three renal pyramids (Fig. 1). TI curves were generated according to a linear scale: intensity was a measure of the echogenicity defined as the mean of the gray-scale values within the ROI. TI curves were examined for the parameters indicated in Table 1 and Fig. 2.

Within 3 days from CEUS, each patient underwent ultrasound-guided percutaneous renal biopsy. Biopsies were performed by two operators with a standardized procedure, previously described [14]. The patient was placed in a prone position and, after preliminary US localization of the kidney, local anesthesia was induced around the renal capsule. The scanner head was placed on the back on a sagittal plane, to obtain a longitudinal scan of the kidney corresponding to its maximum axis. The biopsy needle (Biocut, Sterylab, Rho/Milan, Italy) was 11 or 15 cm long, with an inner diameter of 1.2 or 1.6 mm and a specimen notch 17 or 20 mm long, respectively. The needle was introduced through the needle guide, which was then positioned caudally to the scanner probe. Position of the needle and kidney was continuously visualized on the monitor screen. The introduction of the biopsy needle through the renal capsule and the biopsy were performed with the patient in mild inspiratory apnea. The biopsy specimens were taken from the centre of the lower pole of the kidney.

Specimens were fixed with phosphate buffered formalin, pH 7.2, for 4–18 h, embedded in paraffin, sectioned at 3–4 µm, and stained with hematoxylin and eosin, Masson's trichrome stain, methenamine–silver, and periodic

Fig. 1 Shape of the ROI for TI curves. The ROI is arciform, chosen in the portion of the parenchyma closer to the transducer, as large as at least three renal pyramids [10]

Table 1 TI curve parameters

Time points

Injection point: time at which Sonovue[®], was injected

Baseline point: time just before the start of enhancement

Starting point: time at which intensity in the ROI had increased more than 1 dB in gray scale above the baseline point

Peak point: time at which maximum intensity was reached

90" w-o (wash-out) point: time calculated 90" after the peak

Periods of time

w-i (wash-in) period: interval from the starting point to the peak point

w-o period: interval from the peak point to the 90" w-o point

Slopes

w-i slope: gradient of intensity from the start point to the peak point; rate of increase in enhancement

w-o slope: gradient of intensity from the peak point to the 90" w-o point; rate of decrease in enhancement

Areas

w-i area: area calculated under the w-i period

w-o area: area calculated under the w-o period

acid–Schiff. Only biopsy samples containing more than 7 glomeruli were considered. All specimens were tested for immunofluorescence as previously described [15].

Two blinded pathologists scored the specimens for activity and chronicity indices, modifying the classification of lupus nephritis by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) (2003) [16] which, in the absence of a single common classification for primitive and secondary GN, to the best of our

knowledge, represents the most comprehensive classification of active and chronic lesions [17] (Table 2).

Written informed consent was obtained from all patients, and the study was approved by the Local Ethics Committee of the Catholic University of Rome.

Continuous data were presented as mean $\pm$ standard deviation or median and minimum and maximum values; categorical data were presented as percent proportion.

Fig. 2 TI curve: profile and derived parameters. *w-i* wash-in, *w-o* wash-out

Table 2 Histological indices of activity and chronicity (modified from [16])

Activity indices	Chronicity indices
Mesangial hyperplasia	Glomerular sclerosis
Subendothelial deposits	Fibrotic crescents
Cellular crescents	Tubular atrophy
Fibrinoid necrosis	Interstitial fibrosis
Leucocyte infiltrate	
Interstitial infiltrate	

The presence of each of the following parameters was scored from 0 (no presence) to 3 (full presence) in each specimen, giving a total score for GN activity and chronicity

The correlation between CEUS-derived parameters and histology-based scores of disease activity and chronicity was analyzed with Spearman's rank correlation. When a CEUS parameter was found to correlate significantly with one of the disease scores, it was correlated with each histological component of the total score.

The accepted level for significance was $p < 0.05$. All statistical analyses were performed with Statistical Product and Services Solutions SPSS Inc., Chicago, IL, USA, version 15.0.

Results

The characteristics of the population are shown in Table 3.

No adverse reactions were observed after CEUS.

Table 3 Characteristics of population

Age (years)	40 ± 13
Males (<i>n</i> , %)	16 (55%)
Serum creatinine (mg/dL)	0.9 ± 0.2
eGFR (mL/min)	102 ± 15
Activity index	3 (1–7)
Chronicity index	1 (0–4)

Fig. 3 Total activity score is directly correlated with the wash-out area

Fig. 4 Total activity score is inversely correlated with the wash-out slope

We found a linear direct correlation between histological activity index of GN and wash-out area ($p_s = 0.43$, $p = 0.016$) (Fig. 3). An inverse correlation was also observed between

disease activity index and wash-out slope ($\rho_s = -0.39$, $p = 0.030$) (Fig. 4).

No correlation was found between chronicity index and CEUS-derived parameters.

Among all activity score components, we found a correlation between mesangial hyperplasia and wash-out area ($\rho_s = 0.47$, $p = 0.008$). The correlation between mesangial hyperplasia and wash-out slope showed a trend towards significance ($\rho_s = -0.34$, $p = 0.065$) (Table 4, Fig. 5).

No correlation was observed between CEUS-derived parameters and clinical- or Doppler-derived parameters.

Discussion

CEUS was developed to improve US images [18]. This technique utilizes microbubbles with an external shell made of protein, saccharide, or lipid, and filled with a gas. The main difference of USCAs, in comparison with contrast agents used in CT and MRI, is that the bubbles behave in the same way as red cells and, having a diameter below 6 μm , they do not diffuse out of the vascular space [17]. USCAs have proven to be safe also in patients with impaired renal function and to be free from hemodynamic effects [18–20].

CEUS has been extensively used in cardiology [20], oncology [21, 22], hepatology [23–26], gastroenterology [27–30], and in the study of vascular diseases [29, 30]. In nephrology, the indication of CEUS includes the detection and characterization of renal pseudotumors, complex cystic lesions, vascular disorders such as thrombosis, infarction, cortical necrosis, trauma [6, 31–36], and the evaluation of transplanted kidney [8, 37, 38]. In various experimental models, CEUS provided a detailed visualization of parenchymal microvessels and a measure of the flow and perfusion of the tissue [11, 12]. As previously mentioned, Tsu-ruoka and coll. observed an attenuation of renal contrast enhancement in patients with renal failure [9].

In diagnosing renal parenchymal diseases, US is used as a first-level imaging technique in patients with renal failure, haematuria, or proteinuria. In the recent years, many efforts were made to improve kidney diagnostic imaging through several novel US-based techniques, including elastography, ultrasensitive Doppler, and CEUS [39–41]. In the case of GN, biopsy remains the gold standard for

Fig. 5 Mesangial hyperplasia is directly correlated with the wash-out area

diagnosis. In the past, we obtained interesting data about the early renal involvement in diabetic patients using Doppler US [42]. Very recently, histological findings were correlated with Doppler US parameters in patients with glomerulonephritis [43].

In a previous study, we attempted to use CEUS to obtain TI curves for the evaluation of native kidneys, with the purpose of validating a feasible and reproducible method [10].

In the present study, we compared CEUS with histological pattern in chronic GN with preserved renal function.

In our population, we observed a direct correlation between the degree of disease activity and the area measured under the TI curve during wash-out phase. Consequently, we also found an inverse correlation between wash-out slope and GN activity score. This means that patients suffering from GN have a slower contrast agent wash-out during CEUS, suggesting a disturbance of perfusion in glomerular capillaries during the early stages of GN. Furthermore, the histological element directly correlated with the prolonged wash-out was mesangial hyperplasia, which is known to be one of the early ultrastructural alterations in the pathologic glomerulus, which ultimately leads to fibrosis and sclerosis [44, 45].

Table 4 Among all parameters that concern the total activity index, mesangial hyperplasia is the one correlated with the wash-out (w-o) area

	Mesangial hyperplasia		Leukocyte infiltrate		Subendothelial deposits		Cellular crescents		Interstitial infiltrate	
	ρ_s	p	ρ_s	p	ρ_s	p	ρ_s	p	ρ_s	p
w-o area	0.47	0.008*	0.27	0.144	0.22	0.246	0.02	0.913	0.02	0.908
w-o slope	-0.34	0.065	-0.23	0.220	-0.30	0.097	-0.08	0.662	-0.15	0.432

* significance level $p < 0.05$

In conclusion, CEUS appears to be a safe and promising tool in the study of renal parenchymal diseases. Our findings indicate how CEUS reflects microvascular alteration in the early stages of GN, and confirm how crucial the role of mesangial hyperplasia is in the progression of the disease. More efforts are necessary to clarify whether CEUS can be used as a non-invasive technique to predict GN severity and to investigate the effectiveness of therapies. For this last point, if the results will be confirmed, TI curves can be used as a response parameter to the therapy in GN.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed written consent was obtained from all patients included in the study.

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