pISSN 1738-2637 / eISSN 2288-2928 J Korean Soc Radiol 2016;75(6):487-494 https://doi.org/10.3348/jksr.2016.75.6.487



# Detection of Parenchymal Abnormalities in Experimentally Induced Acute Pyelonephritis in Rabbits Using Contrast-Enhanced Ultrasonography, CT, and MRI

가토에서 실험적으로 유발한 급성 신우신염의 조영증강 초음파검사, 컴퓨터단층촬영과 자기공명영상을 이용한 실질 이상 소견의 발견

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**Purpose:** We evaluated the efficacy of contrast-enhanced ultrasonography (CEUS) in detecting acute pyelonephritis (APN) using the rabbit kidney model and compared it with CT and MRI.

**Materials and Methods:** This study was approved by the Institutional Review Board. In a total of 20 New Zealand White rabbits, APN was induced experimentally. CEUS, CT, and MRI were performed on the first, third, and seventh postoperative days. After imaging studies, the subjects were sacrificed and the pathological diagnosis of APN was confirmed in each animal by a pathologist.

**Results:** Imaging studies were obtained in eight animals, including eight CEUS, four computed tomography (CT), and four magnetic resonance imaging (MRI) images. CEUS depicted diffuse renal enlargement (7), diffuse heterogeneous parenchymal enhancement (6), and focal areas of decreased parenchymal enhancement (6). These findings were well correlated with the CT and MRI findings in five cases in which these studies were available. CT and MRI showed diffuse renal enlargement, diffuse heterogeneous parenchymal enhancement, focal areas of decreased parenchymal enhancement, focal contour bulging, and the finding of perinephric spread of infection.

**Conclusion:** In a rabbit model, CEUS could depict the parenchymal lesions of APN similar to CT or MRI; however, it was limited in depicting the perinephric extension of inflammation.

#### Index terms

Kidney Pyelonephritis Rabbits Ultrasonography Magnetic Resonance Imaging Computed Tomography

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# **INTRODUCTION**

Ultrasonography (US) is an easily accessible modality and it can be used for the detection of hydronephrosis and renal abscess in the patients suspected of having acute pyelonephritis (APN). Especially in children and pregnant women, US is the most important diagnostic modality that does not use ionizing radiation (1).

The detection of renal parenchymal lesions in APN is important because it is diagnostic of APN in itself in the patients with atypical symptoms and signs, especially in children, pregnant women, elderly, diabetics and immunocompromised patients such as those with HIV, transplant recipients on immunosuppressant therapy and patients receiving cytotoxic chemotherapy, and also because early treatment of APN is effective in preventing parenchymal scarring especially in young children and precise differentiation from lower urinary tract infection is essential for optimal management (1, 2).

The parenchymal lesions of APN can be seen as patchy hypoechoic or hyperechoic areas on US. However, the changes are usually subtle and they are often difficult to differentiate from other lesions such as infarction, scar, or pseudolesions. When the inflammation is severe, US may demonstrate multiple, small, wedge-shaped hypoechoic defects similar to those seen on computed tomography (CT) (2, 3).

In humans, contrast-enhanced ultrasonography (CEUS) has been reported to better depict renal parenchymal lesions than conventional gray scale and color Doppler US, although it is operator-dependent and susceptible to various artifacts (4). In a recent study, CEUS was equally accurate in detection of APN compared to CT (5). In some animal studies, CEUS has been reported to be accurate in depicting the renal perfusion defects and the changes of renal blood flow during acute ureteral obstruction (6, 7). In a few other animal experimental models of APN, magnetic resonance imaging (MRI) was well correlated with pathological findings (8, 9).

In this study, we investigated the efficacy of CEUS in depicting renal parenchymal lesions of experimentally induced APN in rabbit kidneys, and compared these findings with CT and MRI findings.

# MATERIALS AND METHODS

#### Animals

Our experimental protocol was approved by the Institutional Animal Care and Use Committee at our hospital. A total of 20 New Zealand White rabbits with a mean  $\pm$  SD weight of  $3.15 \pm$ 0.68 kg were included in this study. Animals were housed in single metal cages and they had access to tap water and standard balanced rabbit chow. Room temperature was controlled between 18°C and 22°C, relative humidity was maintained between 55% and 65% and the light-dark cycle was 7 am–7 pm. All the animals were handled and cared for in accordance with the recommendations of the National Research Council Guidelines for the Care and Use of Laboratory Animals.

#### Experimentally Induced APN in Rabbits

Animals were initially sedated by an intramuscular injection of 35 mg/kg of ketamine hydrochloride (Ketamine; Yuhan, Seoul, Korea) and 15 mg/kg of xylazine (Rumpun; Bayer Korea, Ansan, Korea). Anesthesia was maintained by intramuscular administration of 15 mg/kg of xylazine at a 30 min time interval.

APN was induced by a surgically induced ascending infection, with a modification of the protocol described by others (9, 10). Through a lower midline abdomino-pelvic incision, the pelvic cavity was entered and the urinary bladder was opened. The ureteral orifice was identified, and via the orifice, a 3 French catheter was inserted into the distal ureter. Then, the cannulated distal ureter was ligated with a black silk non-absorbable suture. A total of  $0.5 \times 10^9$  colony-forming-units/mL of the pathogen (E.coli K149, 1 × 109 colony-forming-units/mL) at a volume of 1.0 mL were injected via the cannulation catheter into the distal ureter, just proximal to the suture. After injection, the catheter was removed from the distal ureter and the suture was left in place for 1 minute, and then it was removed. The peritoneum and the abdominal wall were closed in layers. The animal was taken to a recovery area, and after recovery, it was returned to its cage in the animal care facility. Intramuscular antibiotic (Amikin, Bristol-Myers Squibb Holdings Limited, Uxbridge, UK; 4 mg/ kg) was administered every 12 hours for prevention of septic shock.

#### CEUS, CT, and MRI

We performed CEUS, CT, and MRI on the first, third, and seventh post-inoculation days; on the first day, one rabbit underwent CEUS only, another rabbit underwent CEUS and MRI, and the other rabbit underwent CEUS, CT, and MRI; on the third day, one rabbit underwent CEUS only, another rabbit underwent CEUS and CT, and the other rabbit underwent CEUS, CT, and MRI; and on the seventh day, one rabbit underwent CEUS only, and the other rabbit underwent CEUS, CT, and MRI.

US images were obtained by a single radiologist using 5- to 12-MHz convex transducer (Logic700MR; GE Medical Systems, Milwaukee, WI, USA). Both kidneys were scanned with conventional gray scale and color Doppler US technique, and then CEUS was performed after the administration of the microbubble ultrasonographic contrast agent (Levovist; Schering, Berlin, Germany). The contrast agent was administered into the right ear vein by hand injection at a concentration of 300 mg/ mL, at a dose of 0.1 mL/kg and at a rate of 0.2 mL/s. Pulse repetition frequency was set at 700 Hz, wall filter was set at 100 Hz, the mechanical index was set at 0.7 units, and the gray-scale gain was set at 0 dB. The transducer-to-kidney surface distance was approximately 0.3–0.5 cm in each rabbit. The CEUS images were obtained on a coronal scan along the kidneys.

CT was performed using a HiSpeed Advantage scanner (GE

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Animal	Wt (kg)	Postop (d)	Kidney	CEUS	СТ	MRI	Pathology
1	3.6	1	Left	0	-	-	0
2	3.0	1	Right	0	-	0	0
3	3.5	1	Left	0	0	0	0
4	3.9	3	Left	0	-	-	0
5	3.3	3	Right	0	0	-	0
6	3.6	3	Left	0	0	0	0
7	3.1	7	Left	0	-	-	0
8	3.0	7	Right	0	0	0	0
Total	3.4	-	-	8	4	4	8

Table 1. Objectives' Characteristics

CEUS = contrast-enhanced ultrasonography, CT = computed tomography, MRI = magnetic resonance imaging





Е Fig. 1. Acute pyelonephritis involving the left kidney of a rabbit on the first post-inoculation day.

A. In the CEUS longitudinal image, the kidney shows diffuse heterogeneous enhancement. The large perfusion defect in the near field seems to be an artifact (arrowheads); however, the parenchyma on the opposite side and bilateral renal poles show multifocal areas of parenchymal perfusion abnormality (arrows). Renal hilum is not enhanced (asterisk).

B. T2-weighted axial image shows a normal right kidney of this rabbit having a normal renal size, cortical signal intensity and clear perinephric fat.

C. T2-weighted axial image of the left kidney shows increased parenchymal signal intensity, diffuse renal enlargement, hydronephrosis, and perinephric strands due to renal inflammation (arrows).

D. Delayed phase contrast enhanced CT axial image of the normal right kidney shows normal excretion.

E. In the same phase of (D), the left kidney shows diffuse decreased parenchymal enhancement and delayed renal excretion. Renal enlargement and hydronephrosis are also noted in the axial image.

CEUS = contrast-enhanced ultrasonography, CT = computed tomography, MRI = magnetic resonance imaging

Medical Systems) within 2 hours after CEUS. Precontrast and postcontrast spiral CT scans of both kidneys were obtained with 2.5-mm collimation and 2.5-mm/s table speed. For contrast enhancement, 3 mL/kg of intravenous contrast medium (Iopamidol; Bracco Diagnostics, Milan, Italy) was administered by hand injection at a rate of 1 mL/s. Scanning was performed 30 seconds and 90 seconds after administration of contrast material to obtain corticomedullary and nephrogra-phic phase images, respectively. The CT images were obtained on an axial scan along the body of rabbits.

MRI was performed on 1.5-tesla MR system (Vision; Siemens Medical Systems, Erlangen, Germany) within 4 hours after CEUS, using torso surface coil (20 cm internal diameter). Axial and coronal T1-weighted turbo spin echo images (TR 405 msec and TE 12 msec, matrix number  $256 \times 192$ ), and axial and coronal T2-weighted turbo spin echo images (TR 2400 msec and TE 120 msec, matrix number  $256 \times 256$ ) were obtained; 4-mm section thickness, 1-mm gap, and a 20 × 20-cm field of view. Contrast enhanced T1-weighted images were obtained in axial and coronal planes after bolus intravenous injection of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist; Schering, Berlin, Germany).

Two uroradiology fellowship-trained radiologists reviewed all of the images independently, and then the imaging findings were evaluated by consensus.

#### Pathological Diagnosis of APN

After imaging, animals were sacrificed using a barbiturate



Fig. 2. Acute pyelonephritis involving the left kidney of a rabbit on the third post-inoculation day.

**A.** The CEUS longitudinal image shows diffuse heterogeneously decreased parenchymal enhancement. Renal hilum is not included in this view. **B.** In the T2-weighted coronal image, the kidney shows a focal area of increased signal intensity in the lower pole (arrows). There is an adhesion between the renal capsule at the upper portion and small bowel loops (arrowheads).

C. Gadolinium enhanced T1-weighted coronal image also shows the loss of corticomedullary differentiation in the lower portion (arrows).

**D.** Delayed phase contrast enhanced CT axial image shows a large, sharply demarcated area of decreased enhancement. Renal enlargement and adhesion between the upper renal pole and small bowel loops are visualized (arrowheads).

CEUS = contrast-enhanced ultrasonography, CT = computed tomography

overdose technique and their kidneys were harvested prior to gross examination. A central cross-sectional incision was made through the midline in the craniocaudal direction, and gross findings were observed. Microscopic pathologic analysis was also performed and pathological diagnosis of APN was determined by a pathologist.

## RESULTS

Among the twenty rabbits who received pathogen inoculation into their ureter, twelve animals expired within 24 hours of bacterial inoculation in spite of prophylactic antibiotic injection. Finally, a total of eight animals were included in this study (Table 1). We tried to obtain the images of CEUS, CT, and MRI on the first (Fig. 1), third (Fig. 2), and seventh (Fig. 3) days after pathogen inoculation. Therefore, we obtained a total of eight CEUS, four CT, and four MR images from eight animals (Tables 2, 3).

CEUS showed diffuse heterogeneous parenchymal enhancement (Figs. 1, 2) or focal areas of decreased parenchymal enhancement in the parenchymal phase (Figs. 3, 4). Focal parenchymal lesions were correlated with focal APN lesions on CT, and the margins were ill- defined and the contours were wedgeshaped or mass-like. These parenchymal abnormalities were visualized in the images obtained on the first, third, and seventh post-inoculation days, and the typical wedge-shaped, sharply defined parenchymal lesions were also visualized in the images obtained on the first day and the seventh day.

CT showed diffuse renal enlargement, diffuse heterogeneous



Fig. 3. Acute pyelonephritis involving the right kidney of a rabbit on the seventh post-inoculation day.

A. In the CEUS longitudinal image, a large, sharply defined wedge shaped perfusion defect is noted in the mid portion of the kidney (arrows). Renal hilum is not included in this view.

**B.** Delayed phase contrast enhanced CT axial image shows a large, sharply demarcated area of decreased enhancement in the midportion of the right kidney (arrow). The renal pelvis shows markedly increased enhancement, suggestive of acute pyelitis (arrowheads). Right renal enlargement is definite in contrast to the normal sized left kidney.

**C.** In the T2-weighted axial image, the right kidney shows diffusely increased cortical signal intensity. CEUS = contrast-enhanced ultrasonography, CT = computed tomography

Animal	Decton (d)	CEUS	CEUS	СТ	СТ	MRI	MRI
	rostop (a)	Diffuse Lesion	Focal Lesion	Diffuse Lesion	Focal Lesion	Diffuse Lesion	Focal Lesion
1	1	-	-	Not done	Not done	Not done	Not done
2	1	-	-	Not done	Not done	-	-
3	1	+	+	+	+	+	-
4	3	+	+	Not done	Not done	Not done	Not done
5	3	+	+	+	+	Not done	Not done
6	3	+	+	+	+	+	+
7	7	+	+	Not done	Not done	Not done	Not done
8	7	+	+	+	+	+	+
Total	-	6	6	4	4	3	2

#### Table 2. Parenchymal Lesion Detection of CEUS, CT, and MRI

Diffuse lesion: diffuse heterogeneous parenchymal enhancement or signal intensity or attenuation, Focal lesion: focal areas of parenchymal decreased enhancement or abnormal signal intensity or attenuation, -: not detected, +: detected, not done: imaging study not performed. CEUS = contrast-enhanced ultrasonography, CT = computed tomography, MRI = magnetic resonance imaging

Table 3. Imaging Findings of the Experimentally Induced Acute Pyelonephritis in Rabbit in CEUS, CT, and MRI and the Detected Frequencies

Modality	Finding				
CEUS	Diffuse renal enlargement	7			
( <i>n</i> = 8)	Hydronephrosis	5			
	Diffuse heterogeneous parenchymal enhancement	6			
	Focal parenchymal decreased enhancement areas	6			
	Well delineated parenchymal abscess	0			
	Perinephric extension of infection	0			
	Intraparenchymal gas or gas in the collecting system, calculi	0			
CT	Diffuse renal enlargment	4			
( <i>n</i> = 4)	Hydronephrosis	3			
	Diffuse heterogeneous parenchymal enhancement	4			
	Focal parenchymal decreased enhancement areas	4			
	Focal contour bulging with parenchymal abnormalities	2			
	Well delineated parenchymal abscess	1			
	Perinephric stranding, thickening of Gerota fascia,	4			
	fluid collection				
	Intraparenchymal gas or gas in the collecting system, calculi	0			
	Pvelitis showing thickening and enhancement	3			
	of central collecting system				
MR	Diffuse renal enlargement	4			
(n = 4)	Hydronephrosis	2			
	Diffuse heterogeneous parenchymal enhancement	3			
	or signal intensity				
	Focal parenchymal decreased enhancement	2			
	or abnormal signal intensity				
	Focal contour bulging with parenchymal abnormalities	1			
	Well delineated parenchymal abscess	1			
	Perinephric stranding, thickening of Gerota fascia,	2			
	fluid collection				
	Loss of corticomedullary differentiation	3			
	Pyelitis showing thickening or enhancement	1			
	of central collecting system				

CEUS = contrast-enhanced ultrasonography, CT = computed tomography, MRI = magnetic resonance imaging

parenchymal enhancement, wedge-shaped sharply defined focal areas of decreased parenchymal enhancement with or without focal contour bulging, perinephric inflammatory changes, and hydronephrosis with pyelitis.

MRI revealed diffuse renal enlargement, diffuse heterogeneous parenchymal enhancement, and loss of corticomedullary differentiation. The focal parenchymal pyelonephritis lesions were also depicted as focal areas of decreased parenchymal enhancement with or without focal contour bulging. All of the imaging findings have been described in detail in Tables 2, 3.

Each renal lesion was confirmed by gross pathologic and microscopic examinations. Gross examination of the affected kidneys showed diffuse renal swelling, parenchymal abscess formation, subcapsular abscesses, renal sinus edema, pyonephrosis, and circumscribed areas of parenchymal nephritis. Microscopically, the harvested kidneys were extensively infiltrated by numerous inflammatory cells with hemorrhage, necrosis, and microabscesses. The renal collecting ducts and glomeruli, and renal sinus revealed inflammatory cell infiltration and cellular debris. APN was diagnosed pathologically in all of the harvested kidneys.

## DISCUSSION

The diagnosis of APN in humans is made based on a combination of clinical symptoms of flank pain, fever, dysuria, and laboratory findings including bacteriuria or pyuria (1). Imaging studies are usually performed in the patients with atypical clinical features or suspicion of complications, patients with diabetes, and elderly and immunocompromised patients (2).

CT has been known to be diagnostic of APN, and it is the preferred modality because of its superiority in evaluating perinephric inflammation and complications as well as the parenchymal lesions (1, 3). In this study, CT demonstrated parenchymal lesions very clearly.

US and MRI have an advantage over CT especially in the patients with pregnancy, renal failure, diabetes, or iodine allergy (2, 4). On MRI, the typical APN lesions have low signal intensity on T1-weighted images and increased signal intensity on T2weighted images; a loss of corticomedullary differentiation in the T1 image, perinephric fluid, and renal enlargement. After gadolinium administration, focal areas of decreased enhancement and striated pyelogram pattern have been reported (2, 8).

The major limitation of this study is that we could not match the pathologic specimen and images point-to-point because of the unpredictable death of animals. Also, we could obtain only a small number of imaging studies because of the lack of experience in anesthesia and poor understanding of the rabbit APN pathophysiology. However, in spite of the small number of subjects in this study, CEUS showed quite consistent findings. CEUS depicted diffuse renal swelling, diffuse heterogeneous paren-



Fig. 4. Parenchymal abnormalities of acute pyelonephritis in rabbits.

**A.** In the CEUS longitudinal image of the kidney in a rabbit on the third post-inoculation day, multifocal areas of decreased parenchymal enhancement are noted in the upper pole and deep side of the parenchyma (arrows). Calyceal dilatation is noted in the lower pole (arrowheads). A portion of the renal pelvis is noted (asterisk).

**B.** In the CEUS longitudinal image of the same kidney, a sharply demarcated focal parenchymal perfusion defect in the lower portion is noted, and it seems to be an abscess (arrows). Calyceal dilatation (arrowhead) and dilated renal pelvis (asterisk) are also visualized.

**C.** In the CEUS longitudinal image of the kidney in the other rabbit on the first post-inoculation day, diffuse heterogeneous parenchymal perfusion is noted along the kidney. Renal hilum is not visualized in this view.

CEUS = contrast-enhanced ultrasonography

chymal enhancement and focal areas of decreased parenchymal enhancement. These findings were well correlated with the CT and MRI findings in all five cases.

There was a difference between the findings of CEUS in a rabbit model and human studies. In human studies, CEUS frequently showed discrete, wedge-shaped, decreased enhancement lesions (4), and focal parenchymal swelling, or hypoechoic area with increased rim enhancement (5). These lesions tended to be more localized than the parenchymal lesions of rabbit APN in this study. We inferred that this difference may be related to the characteristics of renal anatomy and physiology, duration of infection, and subjective immune reactions.

The second limitation is that we did not transmute the MR parameters or gadolinium dosage throughout the study. Our MRI findings were almost similar to those of the previous study in the rabbit APN model (9). The authors reported mildly increased T2-weighted signal intensity and transient positive contrast enhancement; however, in our study, positive contrast enhancement along the margins of the parenchymal lesion was not definitely observed.

In conclusion, CEUS was able to depict the parenchymal lesions of rabbit APN similar to CT or MRI; however, it had a limitation in depicting the perinephric extension of inflammation.

#### Acknowledgments

This study was supported by a Bayer-Schering Radiological

Research Fund of the Korean Society of Radiology for 2001.

The authors thank to professor Kwan Hyun Park for his urological and surgical instructions to prepare the pyelonephritis animal model, and professor Hyo K. Lim for his instruction about contrast-enhanced ultrasonography.

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# 가토에서 실험적으로 유발한 급성 신우신염의 조영증강 초음파검사, 컴퓨터단층촬영과 자기공명영상을 이용한 실질 이상 소견의 발견

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**목적:** 가토에서 실험적으로 유발한 급성 신우신염의 실질 이상 소견을 조영증강 초음파검사를 이용하여 진단할 수 있는지 알아보고 컴퓨터단층촬영과 자기공명영상 소견과 비교하였다.

대상과 방법: 20마리의 가토에서 실험적으로 급성 신우신염을 유발한 뒤 1일, 3일, 7일에 조영증강 초음파검사, 컴퓨터 단층촬영과 자기공명영상을 시행하였다. 영상 검사 후 가토를 희생하여 급성 신우신염을 병리학적으로 확진하였다. 각 영 상 검사에서 관찰되는 소견을 두 명의 영상의학과 의사가 평가하였다.

**결과**: 총 8마리의 가토에서 총 8예의 조영증강 초음파, 4예의 컴퓨터단층촬영과 4예의 자기공명영상을 얻었으며 8예의 조영증강 초음파 검사상 미만성 신종대(7), 미만성 불균일 조영증강(6), 국소적 조영증강의 감소(6) 소견이 관찰되었으 며, 이들은 컴퓨터단층촬영과 자기공명영상에서 관찰된 실질 이상 소견과 일치하였다. 컴퓨터단층촬영과 자기공명영상에 서는 실질 이상 소견 외에도 국소적 형태 돌출과 신주변 조직의 염증 파급 소견들이 관찰되었다.

**결론:** 가토 모델에서 조영증강 초음파검사는 급성 신우신염의 실질 이상 소견을 보여줄 수 있으나 신주변 조직에의 염증 파급 소견의 발견은 제한적이다.

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