Value of Contrast-Enhanced Ultrasonography in Evaluating Rheumatoid Arthritis: Preliminary Research Based on an Animal Model

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Background:
The aim of this study was to evaluate the effectiveness of grayscale ultrasound (GSUS), power Doppler imaging (PDI), and contrast-enhanced ultrasonography (CEUS) in early rheumatoid arthritis (RA) diagnosis through animal experiments.

Material/Methods:
A rabbit RA model was constructed. The animals were randomly divided into 2 groups, namely, the RA model group and the control group. GSUS, PDI, and CEUS were performed in the model group during early RA and were compared with pathology of synovial biopsies. The consistency of 3 types of ultrasonography was evaluated in tandem with pathological grading.

Results:
23 rabbits in the RA model group completed the experiment. GSUS showed that the synovial thickening of grades 1, 2 and 3 occurred in 12, 19, and 15 joints, respectively. The sensitivity, specificity, and accuracy of PDI in the diagnosis of knee joint synovitis in RA grades 1, 2, and 3 were 80.56% (29/36), 60.00% (6/10), and 76.09% (35/46), respectively, while those with CEUS were 94.44% (34/36), 90.00% (9/10), and 93.47% (43/46), respectively. The differences in diagnostic sensitivity, specificity, and accuracy of the 2 methods were statistically significant. Additionally, the thickness of the synovium measured with GSUS precontrast was greater than that of postcontrast.

Conclusions:
RA evaluated with GSUS is often more hypertrophied than when evaluated with CEUS, while evaluation by PDI is less hypertrophied than that by CEUS. However, from a practical view point, GSUS and PDI are of sufficient practical value, except for in a few special cases.

Keywords: Arthritis, Experimental • Microscopy, Acoustic • Models, Animal • Ultrasonography, Doppler

Abbreviations:
GSUS – grayscale ultrasound; PDI – power Doppler imaging; CEUS – contrast-enhanced ultrasonography; HRUS – high-frequency ultrasound; HCDU – high-frequency color Doppler ultrasound; RA – rheumatoid arthritis; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; Ova – ovalbumin; AIA – antigen-induced arthritis model; SE – sensitivity; SP – specificity; AC – accuracy; NPV – negative predictive value; PPV – positive predictive value; 95% CI – 95% confidence interval

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/931327
Background

Rheumatoid arthritis (RA) is an autoimmune-related chronic disease, with nonsuppurative joint synovitis as the major pathological feature. The pathological changes mainly involve the small joints of the hands and feet, but RA can involve joints in other regions, ultimately leading to impaired joint function and structural damage, with increased risks of disability and mortality.

Early diagnosis and active treatment of RA are recommended according to various guidelines. In fact, delayed treatment of RA can lead to a poor prognosis in patients [1,2]. Therefore, diagnosis and treatment evaluation of RA are very important for optimal patient outcomes. Recent studies have shown that no bone damage occurs in the absence of synovitis, and that the presence of synovitis is a prognostic indicator of bone damage; thus, early detection of vascularized synovia should be one of the primary goals in the assessment of RA [3-5]. Contrast-enhanced ultrasonography increases the intensity of Doppler signals from blood through the administration of microbubble contrast agents. It enhances the signal-to-noise ratio and can improve a non-diagnostic Doppler examination by raising the intensity of weak signals to a detectable level [6]; thus, it facilitates the early diagnosis of rheumatoid arthritis. CEUS also plays an important role in assessing disease activity. CEUS allows not only for significantly better differentiation of active articular synovitis, but also allows a better characterization of the synovitis around tendons. CEUS also provides better spatial resolution; therefore, this technique also allows better characterization of the pannus in terms of differentiating between hypervascularity, hypovascularity, and avascularity to evaluate synovial vascularity to determine prognosis and assess treatment response. The current evaluation for remission mainly includes clinical remission and imaging remission, in which the role of ultrasound is increasingly valued [7]. The use of US to monitor patients considered to be in remission can help predict those likely to suffer subsequent joint damage and flare-up of disease [8]. To estimate the degree of RA remission, ultrasound can be considered a semiquantitative assessment, mainly through the grayscale ultrasound (GSUS) presentation of the soft tissue around the joints and the detected blood flow signal. Based on the harmonic effect of the ultrasound contrast (contrast-enhanced ultrasonography, CEUS) agent, microbubbles, and their resolution on peripheral microcirculation, some scholars have applied CEUS in the diagnosis and evaluation of the remission of RA [9,10]. As with MRI and other techniques, these studies have often been based on serological tests [7,11], in which the clinical pathology of the diseased RA joints is often overlooked. The results of serological testing can be disturbed by many factors, both those relevant to RA activity and some uncorrelated interference factors, such as CRP and ESR. Currently, some studies have shown that clinical symptoms and serological markers in RA patients do not always match the pathological changes in involved joints [12,13]. Another study showed that ultrasound imaging changes can match pathological changes [14], but due to ethical issues, it is difficult to obtain human RA pathological specimens. Therefore, we used an animal model in this study. Studies have shown that the early pathological levels of RA detectable by ultrasound surpass serological findings [14]. Therefore, it is necessary to determine whether contrast-enhanced ultrasound can effectively reveal RA remission at early pathological levels. The purpose of our study was to assess the value of CEUS in the evaluation of RA pathology status based on an animal model.

Material and Methods

Animal Model

The animal use protocol listed below was reviewed and approved by the Animal Ethics and Welfare Committee of Fujian Medical University with the reference number SYXK (min) 2016-0008. The study animals included 30 male New Zealand (Fujian Medical University, Fuzhou, Fujian, China) white rabbits, ~6 months old, with an average body weight of 2.5 to 3.0 kg. The animals were randomly divided into either the RA model group (n=25) or the healthy control group (n=5), according to a randomization table. The RA model for rabbits was completed following the method of “Egg protein induction” introduced by Glynn [15]. Experimental reagents included albumin from chicken egg whites and Freund’s complete adjuvant (F-5881) supplied by Sigma (Merck KGaA, Darmstadt, Germany). The RA model group underwent ovalbumin-induced arthritis, as previously described by Atkinson et al [16], which mainly included basic sensitization and joint sensitization. For basic sensitization, 1 mL of 20 g/L Freund’s complete adjuvant solution was used for back subcutaneous sensitization, injected once every other week for 3 continuous weeks of sensitization. Joint sensitization was initiated 1 week after the last injection of basic sensitization (ie, the 4th week), at which time 1.6 mL (5 g/L) of dissolved ovalbumin solution was injected into the bilateral knee joints of each rabbit. For the control group, on each weekend for the first 3 weeks, 1 mL of saline was injected subcutaneously into the scapular area of the rabbits. During the 4th week, 1 mL of saline was injected into the bilateral knee joints of each rabbit. The feeding methods between the 2 groups were identical, and the time points for ultrasound observation and pathological specimen collection were selected in the 7th and 8th weeks.

Instruments and diagnostic methods

The instruments used included a Philips IU 22 Doppler ultrasound diagnostic apparatus (Philips, Best, the Netherlands), with the
Probe frequency setting of 7-12 MHz. Low-speed blood flow conditions were utilized for bone and muscle. Abdominal anesthetic was administered using 1% sodium pentobarbital at a dose of 6 mg/kg body weight. After the rabbits were fully anesthetized, they were placed in an appropriate knee flexion and extension position, and a scan of the bilateral knee joints was obtained according to the standard procedure. The main observations were: 1) joint cavity fluid thickness; 2) joint synovial thickness (the joints were scanned and checked, the thickness of each thickest synovium was measured 3 times to obtain the mean, and the means were graded as levels 0-3 according to the semiquantitative criteria for synovial hyperplasia proposed by Szkudlarek [17]; and 3) the color signal of synovial blood flow, measured by PDI, and the synovial vessels semiquantitatively divided into 4 grades (0-3) according to the method described by Szkudlarek [17].

After the conventional ultrasound examination, a deep vein catheter (obtained from the contrast agent package) was inserted into the rabbits’ ear veins for the administration of contrast agent. The contrast agent used was SonoVue (Bracco, Milan, Italy), prepared according to the manufacturer’s instructions. For each joint, a single dose of 1-1.5 mL and mechanical index (MI) of 0.08 were administered. PM/PI Philips contrast-enhanced ultrasound technology was used for the measurement.

In this study, synovial CEUS was semiquantitatively assessed according to 3 levels, as referenced by the grading criteria established by IACUS (International Arthritis Contrast Ultrasound) [18]:
Grade 0: no enhancement within the synovium; contrast agent filling signal visible in the surrounding tissue (eg, muscles, tendons, and ligaments);
Grade 1: visible enhancement in the synovium with lower intensity than the knee surrounding tissue;
Grade 2: synovial enhancement greater than knee surrounding tissue;
Grade 3: severe synovial inflammation, equivalent to that of hypertrophied synovitis (Figure 1).

The ultrasound results, including CEUS, were scored by 2 doctors independently, followed by a third evaluation performed in cases of inconsistent evaluations. Overall, there was high interrater reliability.

Pathological Specimens

An ultrasound-guided synovial biopsy was performed around the 8th week after CEUS. A Bard-Magnum 18-G biopsy needle (Bard-Magnum Biopsy Instrument, Covington, GA, USA) was used as the puncture needle. Under ultrasound guidance, the biopsy needle was inserted approximately 0.5 cm above the attachment point of the patellar ligament. When ultrasound confirmed that the biopsy needle had entered the diseased synovial membrane of the joint cavity, the synovial specimen was quickly extracted based on the synovial condition, with careful avoidance of large blood vessels and joint effusion sites. The animals used in this experiment were euthanized in a humane way (killing by venous thromboembolism during narcosis), following the Ethics Guide of Fujian Medical University.

Pathological Grading

Synovial pathological specimens were prepared by conventional methods and were stained with HE. Synovial hyperplasia and inflammatory cell infiltration of surrounding tissue was observed under a microscope. The specimens were scored according to the synovitis pathology scoring criteria proposed by Krenn et al [19]. Each specimen was scored by 2 pathologists 3 times, and the median score was recorded. Zero points indicated no inflammatory infiltration, 1 point indicated mild synovial inflammation and mild inflammatory cell infiltration, 2 points indicated moderate synovial inflammation and moderate inflammatory cell infiltration, and 3 points indicated severe synovial inflammation and severe inflammatory cell infiltration.
Statistical Analysis

All of the data were analyzed using the SPSS statistical software package. The paired $t$ test was used for measurement data, while the chi-square test was used for statistical analysis of discontinuous data. The efficacy of PDI and CEUS in diagnosing hypertrophied synovitis was evaluated using a 2×2 table. A $P$ value of 0.05 was used to define statistically significant differences between methods. Consistency of different ultrasound evaluations was determined by kappa testing.

Results

Rabbit RA Modeling

The RA model group included 23 rabbits that successfully underwent the experimental procedures. Two rabbits died of an overdose of anesthesia; thus, no ultrasound or pathology evaluation was performed on them. During the stage of basic sensitization, redness and ulceration appeared gradually at the back subcutaneous injection site of the posterior parts of the body, which healed gradually in approximately 2 months. After the 4th week, all of the rabbits assigned to the RA model group showed varying degrees of redness of the bilateral knee joints, increased limb temperature, and limited activity. The rabbits in the control group (a total of 5) exhibited a symmetrical body, strong muscles, smooth and dense bright hair, quick action and reactions, and normal eating, drinking, and living habits. Two rabbits were unable to complete a biopsy because of incomplete anesthesia.

RA synovitis Conventional Ultrasound Performance

Under GSUS, the distinction among the synovial membrane, joint capsule, and synovial fluid was not obvious among the healthy controls. In contrast, varying degrees of thickening synovium in the knee joint patellar capsule and joint cavity were observed in the RA model group. These observations were mainly low- or high-echo-based and could not be deformed and displaced when pressure was applied to the probe. Some joints showed small branching or villiform changes (Figure 2). Synovial thickening was observed in grade 1 (12 joints), grade 2 (19 joints), and grade 3 (15 joints). In this study, grade 1 PDI revealed that the thickened synovium demonstrated different degrees of blood flow signal, which could be divided into levels 0-3 according to the blood flow grading standards proposed by Chakr et al [20]. Different degrees of the blood flow signal in the thickened synovial membrane were visible by PDI (Figure 3). The 46 knee joints examined were graded as follows: grade 0: 1 (1/46, 2.17%); grade 1: 13 (13/46, 28.26%); grade 2: 14 (14/46, 30.43%); and grade 3: 18 (18/46, 39.13%). Interrater reliability was measured by the kappa index, which reached 0.79 (synovial thickening, $P<0.05$) and 0.80 (blood flow grading, $P<0.05$), showing good consistency between different observers. In animal experiments, because the animals did not coordinate like humans, it was easy to produce flash artifacts displaying as grade 1 PDI. In this evaluation, blood flow signals of 0 and 1 indicated non-disease states, while grade 2-3 blood flow was classified as disease states.

RA Performance Assessed by CEUS

Among the 46 knee joints examined, 1 was determined to be CEUS grade 1 (1/46, 2.17%), 8 were grade 2 (8/46, 17.39%), and 37 were grade 3 (37/46, 80.43%). According to the results of IACUS [18], active synovitis was defined as thickening of intra-articular tissue that is not displaceable and not at all or minimally compressible and exhibits PDUS signals or contrast enhancement at CEUS. Therefore, grade 1 or greater is defined as active synovitis. In total, the CEUS method diagnosed 45 knee joints as exhibiting active synovitis. The kappa index for evaluation of interrater reliability was 0.77 ($P<0.05$).
Twenty-three (23) of 25 animals (ie, 46 joints from the RA model group) completed the ultrasound-guided synovial biopsy (Figure 4), with a success rate of 92% (46/50). The length of the specimens was greater than 0.4 cm, and the specimen sampling was satisfactory. There was no instance of local or systemic infection after surgery. Synovial membrane semiquantitative scoring occurred as follows: 0 points for 1 joint; 1 point for 9 joints; 2 points for 20 joints; and 3 points for 16 joints. The average score of synovial inflammation was 2.14±0.78 points. The pathological manifestation of synovial hyperplasia is shown in Figure 5.

Correction of CEUS to Synovial Thickness Measured by GSUS

The thickness of the synovial tissue was measured by GSUS. By CEUS, the enhanced thickness of the synovial tissue could be measured when the maximum enhancement signal appeared. For 42 joints, the synovial thickness measured before contrast was greater than the thickness measured after contrast. For 4 joints, the synovial thickness before and after contrast was equivalent (within 0.2 mm). No joints exhibited a greater synovial thickness after contrast. The paired t test was performed on synovial thickness before and after contrast. The results are shown in Table 1.

The above statistical results demonstrated that contrast enhancement with GSUS significantly impacted measured synovial thickness.

<table>
<thead>
<tr>
<th>N=46</th>
<th>Synovial thickness</th>
<th>t</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>Before ultrasound contrast</td>
<td>6.41±1.79*</td>
<td>2.066</td>
<td>0.044</td>
</tr>
<tr>
<td>After ultrasound contrast</td>
<td>5.62±2.48*</td>
<td></td>
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</tbody>
</table>

* P<0.05 indicates statistically significant difference. N – number of joints.

Comparison of Synovitis Evaluated by PDI Before and After CEUS Contrast

A total of 32 knee joints were diagnosed as having hypertrophied synovitis by PDI, while a total of 37 were classified as hypertrophied synovitis cases by CEUS. Among the total of 46 knee joints evaluated, only 1 joint did not show obvious blood flow as detected by PDI without CEUS enhancement. Twenty-seven joints demonstrated a weaker blood flow signal by PDI with significant enhancement by CEUS, and 18 joints showed a rich blood flow signal by PDI (grade 2-3 blood flow) and obvious grade 2 enhancement by CEUS (Figure 1).

Comparison of PDI and CEUS with Pathology

Using the pathological results as the criterion standard, the sensitivity, specificity, accuracy, negative predictive value, and positive predictive value of PDI in the diagnosis of knee joint synovitis in this RA model were 80.56% (29/36, SE), 60.00% (6/10, SP), 76.09% (35/46, AC), 46.15% (6/13, NPV), and 87.88% (29/33, PPV), respectively, while those parameters according to the CEUS methods were 94.44% (34/36, SE), 90.00% (9/10, SP), 93.47% (43/46, AC), 81.82% (9/11, NPV), and 97.14 (34/35, PPV), respectively (Table 2). The diagnostic sensitivity, specificity, and accuracy of the 2 methods showed statistically significant differences (P<0.05) (Tables 3, 4).
Table 2. Comparative diagnosis of sensitivity, specificity, accuracy, negative predictive value, and positive predictive value between Doppler ultrasound and contrast-enhanced ultrasound.

<table>
<thead>
<tr>
<th></th>
<th>PDI (95% CI)</th>
<th>CEUS (95% CI)</th>
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<tbody>
<tr>
<td>SE (95% CI)</td>
<td>80.56% (63.98% to 91.81%)</td>
<td>94.44% (81.34% to 99.32%)</td>
</tr>
<tr>
<td>SP (95% CI)</td>
<td>60.00% (26.24% to 87.85%)</td>
<td>90.00% (55.50% to 99.75%)</td>
</tr>
<tr>
<td>AC (95% CI)</td>
<td>76.09% (62.64% to 89.05%)</td>
<td>93.47% (80.40% to 98.81%)</td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>46.15% (27.10% to 66.41%)</td>
<td>81.82% (53.53% to 94.62%)</td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>87.88% (76.94% to 94.03%)</td>
<td>97.14% (84.09% to 99.55%)</td>
</tr>
</tbody>
</table>

SE = sensitivity; SP = specificity; AC = accuracy; NPV = negative predictive value; PPV = positive predictive value; 95% CI = 95% confidence interval.

Table 3. Comparative diagnosis of knee joint synovitis by CEUS and standard clinical pathology (N: number of joints).

<table>
<thead>
<tr>
<th>CEUS</th>
<th>Pathology</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

The diagnostic sensitivity, specificity, and accuracy of the CEUS.

Table 4. Diagnosis of knee joint synovitis by Doppler ultrasonography (number of knee joints), using pathology as the criterion standard.

<table>
<thead>
<tr>
<th>Doppler ultrasonography</th>
<th>Pathology</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
</tr>
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</table>

Discussion

In this study, the synovial thickness of 46 knees joints in the RA model group was measured before and after contrast-enhanced ultrasound. It was found that there was significant difference in synovial thickness measured by CEUS and GSUS. Contrast enhancement with GSUS had a significant effect on the measured synovial thickness. In addition, the results of PDI and CEUS semiquantitative measurement of synovium in 46 knee joints were compared with the results of synovial pathological grading. It was found that CEUS was more sensitive than PDI in measuring synovial blood perfusion. This study found that RA evaluated with GSUS is often more hypertrophied than when evaluated with CEUS, while evaluation by PDI is less hypertrophied than that by CEUS.

Rabbit antigen-induced arthritis model (AIA) is a large-animal model that has been developed over the past 30 years [21], especially in the research on imaging. Qiu [22] reported a nearly 100% success rate when 8 mg of Ova was injected into the joints. In fact, the size of the rabbit knee is comparable with that of human peripheral joints, which are the most common lesions in RA. The following observed results also indicated pathogenesis similar to human RA. Thus, the choice of a rabbit model is suitable for research [23]. The lower price, suitable feeding, and molding were other factors influencing our experimental methods.

As stated in the RA chronic disease management guide, the true state of RA improvement includes not only the disappearance of clinical symptoms and the dissipation of inflammation but also the improvement of joint outcomes and functions, resulting in the improvement of quality of life [24]. Therefore, imaging is playing an increasingly important role in the diagnosis of RA and its follow-up assessment [25-28]. Ultrasound has the inherent advantage of being convenient and cost-effective. It can also be used for the assessment of multiple joints of the body simultaneously [29]. Compared to magnetic resonance imaging (MRI), ultrasound greatly reduces the time and cost of the examination. Hence, ultrasound has obvious advantages in the assessment of RA and should be recognized as a conventional means for diagnosis and follow-up [30].

Although both GSUS and PDI can show changes in the diseased region, they cannot reveal the most distant synovial tissues with microfluidic perfusion due to the limited resolution of GSUS and the limited sensitivity of blood flow illustration. With its harmonic effects and technology, CEUS has been widely used in various clinical fields, including microcirculation perfusion, tumor chemotherapy and ablation monitoring. Currently, evaluation of the application of CEUS in RA diagnosis and its efficacy has been very limited. Comparative studies have been mainly restricted to serological changes [31-34] and no comparative studies in a pathological context have been reported. Based on the results of our study, the role of CEUS in the early diagnosis and classification of RA is of demonstrable value. The results suggested that the synovial thickness measured...
by CEUS and GSUS was significantly different (P<0.05). Since
the synovial thickness measured by CEUS represents the mi-
crocirculation portion, the synovial components of necrotiz-
ing degeneration were excluded, so the data from CEUS mea-
surements were more representative of disease activity. The
results from different observers displayed good consistency,
including GSUS, PDI, and CEUS aspects.

Further, CEUS could determine the synovial blood flow perfu-
sion more sensitively (P<0.05) than PDI. In this study, we ob-
served that the synovial area measured by PDI was normally
larger than the contrast display area, likely because PDI can-
not distinguish whether the hypoechoic synovial tissue is fi-
brous or active villous pannus tissue. Therefore, the evalua-
tion by PDI is often more hypertrophied. Similarly, although
PDI can determine hypertrophied synovial tissue from the
blood flow filling region observed with PDI, it cannot achieve
the level of microcirculation display of CEUS, nor can it iden-
tify whether the distal nonblood flow signal filling area also
includes blood perfusion due to the limited blood flow signal
display of PDI. Hence, synovial inflammation determined by
PDI likely underestimates the condition, compared to CEUS.
In this study, we only drew conclusions based on the animal
model. Regarding its application for RA in humans, further clin-
cial studies are needed.

Our experimental study also found that GSUS assessment of
RA often overestimated the condition compared to CEUS, while
PDI demonstrated an underestimated evaluation. As such,
CEUS across the 2 methods could provide more accurate as-
sessments. Therefore, after completion of GSUS and PDI ex-
aminations, the RA status assessed by CEUS could be roughly
estimated, and the pathological analysis of RA could then be
determined. As a semiquantitative means of evaluation, CEUS
had high effectiveness. However, in terms of the time and cost,
GSUS and PDI have advantages over CEUS. Therefore, from a
practical point of view, except for in a few special cases, GSUS
and PDI have more practical value for extensive application.

Conversely, because the CEUS agent in the body subsides fast-
er than MRI agent and cannot assess multiple joints simultane-
ously, CEUS might not be aslogistically advantageous as MRI
in cases of multiple-diseased RA. The main obstacles to us-
ging US contrast media are high costs, technical limitations (for
instance in near fields), a relatively short time window of ex-
amination, and the need for optimally designed bubbles for
near field investigation at higher frequencies. The main spe-
cific limitations of CEUS in RA assessment are the small num-
ber of joints (usually 1) that can be examined at one time, the
blooming artifact after injection, and the lack of definition of
normal joint flows, so slightly increased flows are also unde-
fined. Thus, it is obvious that CEUS, although more accurate,
still has important practical limitations. Meanwhile, ultrasound
contrast agents also have potential negative effects and ad-
verse effects. When using ultrasound contrast agent, it is nec-
essary to understand its contraindications to avoid allergy to
the corresponding components in patients with a history of al-
lergy, but the possibility of allergic reaction is very small. The
adverse effects of ultrasound contrast agents are less and the
incidence is also very low. The main adverse effects are head-
ache, nausea, pain at the injection site, and abnormal sensa-
tion. These adverse reactions are temporary and mild. In ad-
in, CEUS in RA loses the ultrasound cost-effectiveness
advantage in terms of cost and prolongation of the examina-
tion and interpretation time.

In recent years, studies have shown that texture analysis and
high-frequency ultrasound are widely used [35-40]. In addi-
tion, they have lower cost and are less invasive. Texture anal-
ysis applies analysis analytic metrics to both the normals and
abnormals by extracting ultrasonic image features, and allows
the computer to learn from the schema. Textural analysis can
help identify abnormally thickened synovium and locate the
edge of the synovium clearly in RA so that synovium thickness
can be measured more accurately. Meanwhile, textural anal-
ysis can reduce the influence of acoustic shadowing on contour
measurement since we cannot assume that we will have a re-
liable contour in every case [41]. High-frequency ultrasound
(HRUS) is widely used in the early diagnosis of RA, particu-
larly in the introduction and application of high-frequency col-
or Doppler ultrasound (HCDU), which can monitor blood flow
change in the synovium and synovial thickening [42].

This study in and of itself also had some limitations. Due to
the lack of CEUS in the rabbit RA model, the 1-1.5 mL dose of
ultrasound contrast agent of SonoVue was used by us in re-
peated experiments. It is important to note that great differ-
ences exist between human and rabbit physiology, including
a much faster heart rate and the comparative dose required
by a human patient. Because the synovial membrane is close
to the cartilage and bone surface, there were many limita-
tions when implanting the needle to avoid injury to the car-
tilage and bone. At the same time, because of the different
depths of the needle, the synovial membrane tissue obtained
by puncture could not completely represent the full condition
of the synovial lesions, especially in cases of synovial fibrosis.
Even if the approximate range of the hypertrophied synovium
was determined beforehand by CEUS, some deviation in the
operation was still inherently inevitable, and the situation of
the synovium only represents one part of the whole joint pa-
thology and not all. It has been reported that immunohisto-
chemical analysis of the synovial membrane could be another
means to evaluate the activity of RA, and it is a complimen-
tary method to be considered in future studies [43-45]. This
method could largely avoid the errors caused by sampling and
more accurately reflects RA activity.
Conclusions

In summary, CEUS can accurately reflect the microcirculation status of RA synovium. It is good for the determination of synovial thickness and even better for the selection of ultrasound-guided synovial puncture biopsy sites. Although CEUS can more accurately determine the microcirculation and activity of RA synovial pathology, it will require refinement and optimization for large-scale application in routine and daily clinical applications. Nonetheless, this study elucidates the advantages of CEUS in a pathological context, encouraging future endeavors to expand our current understanding. The limitation of this study is that there was no ultrasonic observation of the knee joint in the model group and the control group at the 1st week and the 2nd week (ie, the 5th and the 6th week) after joint sensitization (ie, the 4th week). Therefore, we could not get the ultrasonic observation results of the model group and the control group during the period of reactive arthritis in which synovial pannus had not been formed. In the follow-up study, we will further improve the experiment, and rabbits with induced reactive arthritis will be included in the inflammatory control group. Ultrasound observation and pathological specimen collection will be selected in the inflammatory control group at the 1st week (ie, the 5th week) after joint sensitization (ie, the 4th week), while the time points for ultrasound observation and pathological specimen collection of the model group will be selected in the 7th and 8th weeks, respectively. The ultrasonographic and pathological results of the RA model group and the inflammatory control group will be compared to detect the difference between rheumatoid arthritis and reactive arthritis in the early stage by use of the above ultrasonic methods. In this study, we only drew conclusions based on the animal model. Regarding its application for RA in humans, further clinical studies are needed.

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Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interests

None.

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