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Power Doppler evaluation of joint effusions: investigation in a rabbit model

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Abstract *Objective.* To study the power Doppler findings of septic arthritis and noninfectious synovitis in an animal model.
Materials and methods. The right knees of 10 rabbits were inoculated with an aqueous suspension of *Staphylococcus aureus*. The right knees of 5 rabbits were injected with talc suspension. The right knees of 5 rabbits were injected with saline. All 20 left knees were injected with saline. Serial power Doppler images were obtained using constant-imaging parameters. Images were reviewed by blinded observers who assessed for increased power Doppler signal.
Results. All 10 knees inoculated with *S. aureus* developed septic arthritis. Each infected rabbit knee demonstrated increased signal on power Doppler on at least one examination, ranging from 1–6 days after inoculation. Only 23 of 45 examinations of infected knees were unequivocally positive by power Doppler on examinations performed 1 to 6 days after inoculation. No knee with talc synovitis demonstrated increased power Doppler signal. No control knee demonstrated increased power Doppler signal.
Conclusion. Increased power Doppler signal may be seen with septic arthritis; however, its intensity and timing may vary from subject to subject. A normal power Doppler examination does not exclude septic arthritis.

Introduction

Septic arthritis carries the potential for substantial morbidity and must be treated aggressively, usually with surgical incision and drainage in combination with intravenous antibiotics. In contrast, transient synovitis, the chief differential diagnosis for the irritable pediatric hip joint, is a self-limited process, requiring only symptomatic treatment and carrying little morbidity. While fever, elevated white-blood-cell count (WBC), elevated erythrocyte sedimentation rate (ESR) and generalized toxicity may suggest the diagnosis of septic arthritis, frequently the specific diagnosis is not clinically evident. The signs and symptoms of transient synovitis may overlap with mild or early septic arthritis.

Ultrasound has been used extensively to identify joint effusion in children presenting with hip pain [1, 2]. There are no gray-scale sonographic findings that reliably distinguish infected from noninfected fluid. Traditionally, the decision to aspirate an effusion has been

that of the referring service, relying on clinical factors and the presence of fluid. Some clinicians will request aspiration in all children with effusion; however, as transient synovitis is more common than septic arthritis, this policy leads to the aspiration of many noninfected hips [2]. While a relatively easy procedure to perform, hip joint aspirations can be an emotionally traumatic experience for young patients and their parents. The procedure can be painful. The procedure is not without risks, namely, the introduction of infection to a noninfected joint, bleeding, and the risks of sedation, if utilized. Any additional prospective information on the etiology of the effusion would be beneficial in the decision of whether to aspirate.

Power Doppler sonography is exquisitely sensitive to blood flow [3]. Early experience has shown that power Doppler may be useful in the diagnosis and follow-up of inflammatory and infectious processes. In particular, power Doppler has been used to evaluate the response of rheumatoid arthritis patients during treatment [4]. Anecdotal reports suggest that power Doppler may be useful in the assessment of infection [5]. In our practice, we have seen some children with septic arthritis with asymmetrically increased flow to the affected hip on power Doppler examination.

Several investigators have used the rabbit knee or hip as models to study infectious and noninfectious arthritis [6–9]. The rabbit is relatively inexpensive and the knee joint is readily accessible and of adequate size for sonographic evaluation. Injected staphylococcus of the proper strain will produce a clinical septic arthritis in 2 days [7]. As the etiology of transient synovitis is unclear, there is no ideal experimental model; however, injected talc will produce a clinically evident chemical synovitis in 14 days, lasting for weeks [8, 9].

Our hypothesis in undertaking this investigation was that power Doppler sonography allows the distinction of infectious from noninfectious joint effusions by showing differing degrees of blood flow to the joint capsule. To test our hypothesis we performed serial power Doppler examinations of rabbit knees: (a) injected with *Staphylococcus aureus*, (b) injected with sterile talc, and (c) injected with sterile saline.

Materials and methods

Rabbit model

Approval for the research protocol was obtained from the University Committee on Use and Care of Animals before starting the project. Adult white New Zealand rabbits weighing approximately 3 kg each were utilized. Specific pathogen-free rabbits were chosen to minimize the risk of respiratory infection over the course of multiple sedations. For the initial injection and each imaging session the rabbits were anesthetized with 35 mg/kg of ketamine and 5 mg/kg of xylazine intramuscularly. This provided approximately 30 min of adequate sedation. If additional sedation was required (only twice in 278 sedations), half of the original dose of ketamine and xylazine was administered. Before injection of each knee, the overlying hair was removed by clipping and the application of hair remover (Nair). If regrowth of hair interfered with subsequent study, the hair remover was later reapplied. Before injection, the rabbit knees were sterilized by washing with tincture of iodine. Injection was performed by palpating the patella and anterior/superior margin of the proximal tibia and passing a needle through patellar tendon into the joint (approximately 1 cm deep). On proper placement in the joint, fluid was easily injected with little resistance. Injection into joint was confirmed by gray-scale sonography identification of fluid in the suprapatellar pouch. Injection of bacteria and saline was done with 25-gauge needles. Injection of talc required an 18-gauge needle.

Staphylococcus injection

The right knees of 10 rabbits were injected with 1,000 colony-forming units of *S. aureus* suspended in 1 ml of sterile saline. The ATCC (American Type Culture Collection, Rockville, Md.) 27217 strain of *S. aureus* was utilized, as this has previously been shown to achieve a high rate of septic arthritis in the rabbit [6].

Talc injection

The right knees of 5 rabbits were injected with approximately 0.125 g of sterile surgical talc. This model was adapted from a similar study of the rabbit hip, previously described in the literature [8, 9]. Sterile surgical talc was obtained from the operating room pharmacy. As described in the literature, the talc was initially suspended in sterile saline to achieve a concentration of 0.25 g/l; however, at this concentration, the mixture could not be injected through a needle. By diluting the talc to 0.125 g/l, we were able to inject 1 ml of suspension through an 18-gauge needle.

Saline controls

The left knee of each of the 10 rabbits that were injected with *S. aureus* on the right and of each of the 5 rabbits that were injected with talc on the right was injected with 1 ml of sterile saline to serve as internal controls. To differentiate between a systemic response and a response to altered weight bearing (neither proved present) in the control left knees, we also studied 5 rabbits who received 1-ml injections of saline in both knees.

Imaging

All imaging was performed on a Spectra VST ultrasound scanner (Diasonics, Milipitas, Calif.) using a 10 MHz linear-array probe. Imaging examinations were conducted by the authors, radiologists with ultrasound expertise. Power Doppler images were obtained in a sagittal plane at four locations on every study: suprapatellar pouch and medially, midline, and laterally at the tibiofemoral joint. Each rabbit was imaged immediately before injection, immediately after injection, and serially thereafter. Rabbits were initially imaged daily, and then at 2- or 3-day intervals after the first 3 days. The rabbits were studied in groups of five. The dates of imaging varied slightly between groups, because of personnel and equipment availability. Imaging was discontinued when imaging findings stabilized or began to resolve in the *S. aureus* (5–6 days) and talc

(14–17 days) groups. Imaging was carried out to 10–11 days in the control rabbits. Hard-copy images were obtained for later review. All power Doppler images were obtained with standardized machine parameters (pulse repetition frequency, gain, and power Doppler window size). Settings were initially chosen based on the experience of one of the authors in a previous study (unpublished data). On adjusting settings in rabbits with septic arthritis and evidence of increased flow, we felt that these settings were appropriate.

Laboratory analysis

All rabbits were killed in a manner approved and recommended by the University Committee on Use and Care of Animals. Using sterile technique we aspirated each rabbit knee joint for culture. If no fluid could be obtained, the knee was lavaged with 1 ml of sterile saline, which was then cultured. All rabbits in the *S. aureus* and talc groups were submitted for pathologic examination. The rabbit knees were dissected, and, after decalcification, hematoxylin and eosin (H & E) stains were performed for microscopic analysis. Each knee was assessed for the degree of inflammatory changes histologically present.

Data analysis

Hard-copy images were coded and randomized. The four images of one knee obtained at the time of one examination were kept together. These images were then read by three of the authors who were blinded as to what was injected, the time since injection, and right versus left knee. Readers were asked whether power Doppler showed increased flow. Flow was considered increased if more periarticular power Doppler signal was seen than on baseline examinations. An examination was considered to be unequivocally positive, that is, demonstrating increased power Doppler signal, if all three readers agreed. An examination was considered equivocal if only two readers or one reader considered power Doppler signal to be increased. To assess intraobserver variability, all three readers repeated their readings after an interval of 1–2 months.

Statistics

Paired first readings of each combination of different observers were compared to calculate interobserver agreement, and repeated readings by the same observer were compared to calculate intraobserver agreement The sensitivity of an individual power Doppler examination was determined by the number of positive studies occurring in infected rabbit knees on day 1 after inoculation and subsequent days and the total number of such studies. The cumulative sensitivity of power Doppler over the course of imaging was determined by the number of infected knees with a positive study at some time over the course of imaging and the total number of infected knees studied. The specificity of power Doppler for septic arthritis was determined by the number of positive studies occurring in sterile knees.

Results

As early as the day following injection, rabbit knees injected with *S. aureus* were erythematous and swollen. Effusions persisted through imaging. Rabbit knees in-

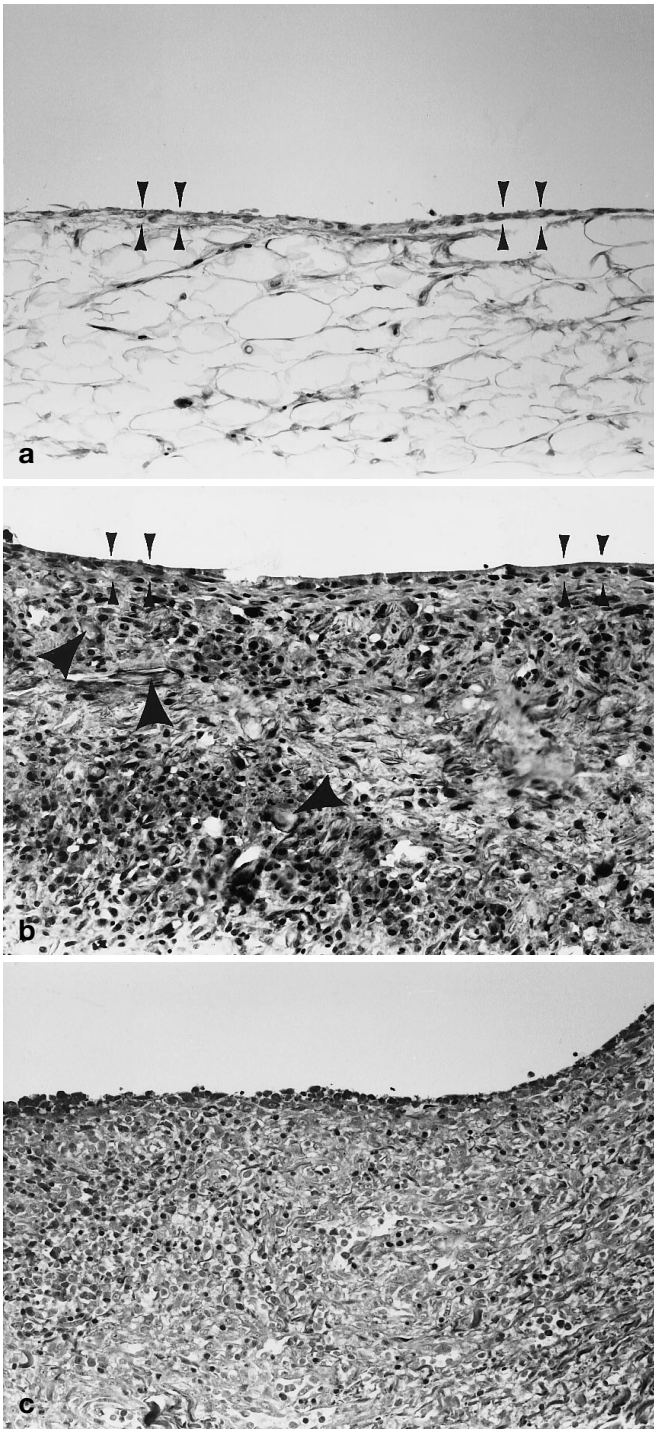

Fig. 1 **a** Histology of a saline-injected knee – no pathological changes are evident. The synovium is a well-defined thin layer (*arrowheads*). **b** Histology of a talc-injected knee – substantial inflammatory changes are present. A large number of mononuclear cells are present; however, the synovium is still intact (*small arrowheads*). Note talc crystals (*large arrowheads*). **c** Histology of *S. aureus*-infected knee – destructive changes are present. The synovium is destroyed and cannot be identified. A large number of inflammatory cells are present

Table 1 *S. aureus* – injected rabbit knees – daily results (*day 0 (pre)* immediately preceding injection, *day 0 (post)* immediately after injection, *day 1* 24 h after injection, *day 2* 48 h after injection, etc., ■ positive (3 of 3 readers), E_2 equivocal (positive by 2 of 3 readers), E_1 equivocal (positive by 1 of 3 readers), *n* normal, – not studied)

Rabbit	Day 0 (pre)	Day 0 (post)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	N	E_2	■	E_2	E_2	■	–	■
2	N	N	E_2	E_2	■	■	–	■
3	E_1	N	E_2	E_1	■	■	–	E_2
4	N	N	N	E_2	■	■	–	■
5	N	E_1	N	■	■	■	–	E_2
6	N	N	■	■	N	–	N	–
7	N	N	■	■	■	–	■	–
8	N	E_2	E_2	E_2	■	–	N	–
9	N	N	N	E_2	N	–	■	–
10	N	E_1	■	E_2	N	–	–	–

Table 2 Summary of power Doppler readings (*Day 0 (pre)* immediately preceding inoculation, *Day 0 (post)* immediately after inoculation, *Day 1* 24 h after inoculation)

Day of imaging	Day 0 (pre)		Day 0 (post)		Day 1 and subsequent		
Injected	Nothing	Staph	Talc	Saline	Staph	Talc	Saline
Positive (3 of 3 readers)	0	0	0	0	23	0	0
Equivocal (2 of 3 readers)	0	2	1	1	12	0	1
Equivocal (1 of 3 readers)	2	3	1	4	1	2	2
Normal	38	5	3	20	9	35	133
Total	40	10	5	25	45	37	136

jected with talc demonstrated lesser degrees of erythema and swelling. The effusions in talc-injected knees initially dissipated, but then recurred to a variable extent. Rabbit knees injected with saline demonstrated no clinical evidence of inflammation. Most effusions in saline-injected knees had resolved by the first day after injection, with a transient effusion seen on a single examination in two separate single saline-injected knees several days after injection.

Cultures confirmed septic arthritis in all 10 rabbit knees injected with *S. aureus*. No knee injected with either talc or saline produced a positive culture. On histologic examination, saline-injected knees were normal, demonstrating a normal thin, well-defined synovium in the joint (Fig. 1 a). Rabbit knees injected with talc showed diffuse mononuclear cell infiltrate (Fig. 1 b). The synovial lining was thickened but intact. Infected knees showed a marked degree of inflammation with a dense infiltration of neutrophils and destruction of the synovial lining, to the point that it was difficult to identify accurately (Fig. 1 c). Some infected knees showed evidence of extension of infection into adjacent soft tissue; however, on the sections obtained there was no evidence of osteomyelitis.

On power Doppler evaluation all 10 infected rabbit knees had at least one examination that was considered unequivocally positive (agreement of all three readers) (Table 1, Fig. 2). There were multiple examinations that were considered equivocal with either two readers (17 examinations) or one reader (15 examinations) calling increased signal on power Doppler (Table 2). The majority of equivocal studies with two readers calling increased power Doppler signal (12 of 17 such studies) occurred with infected knees on the first day after inoculation and on subsequent days. The timing and duration of positive studies varied from rabbit to rabbit (Table 1). Seven of ten infected knees had at least two positive studies. The earliest positive power Doppler study was obtained on day 1 following injection (four infected knees). It was not unusual to obtain negative or equivocal power Doppler results from knees that had been previously considered positive. There was some variance in the degree of power Doppler signal present in those studies called positive (Fig. 3). The suprapatellar site tended to show more power Doppler signal than the medial, midline, and lateral tibiofemoral sites, although in some knees increased flow was present at all four imaging locations (Fig. 4). There were no knees injected with either talc or saline that were considered positive on any examination by the consensus of the three readers. Some examinations of talc- or saline-injected knees were considered equivocal, having been called increased flow by two readers (three examinations) or one reader (nine examinations) (Table 2).

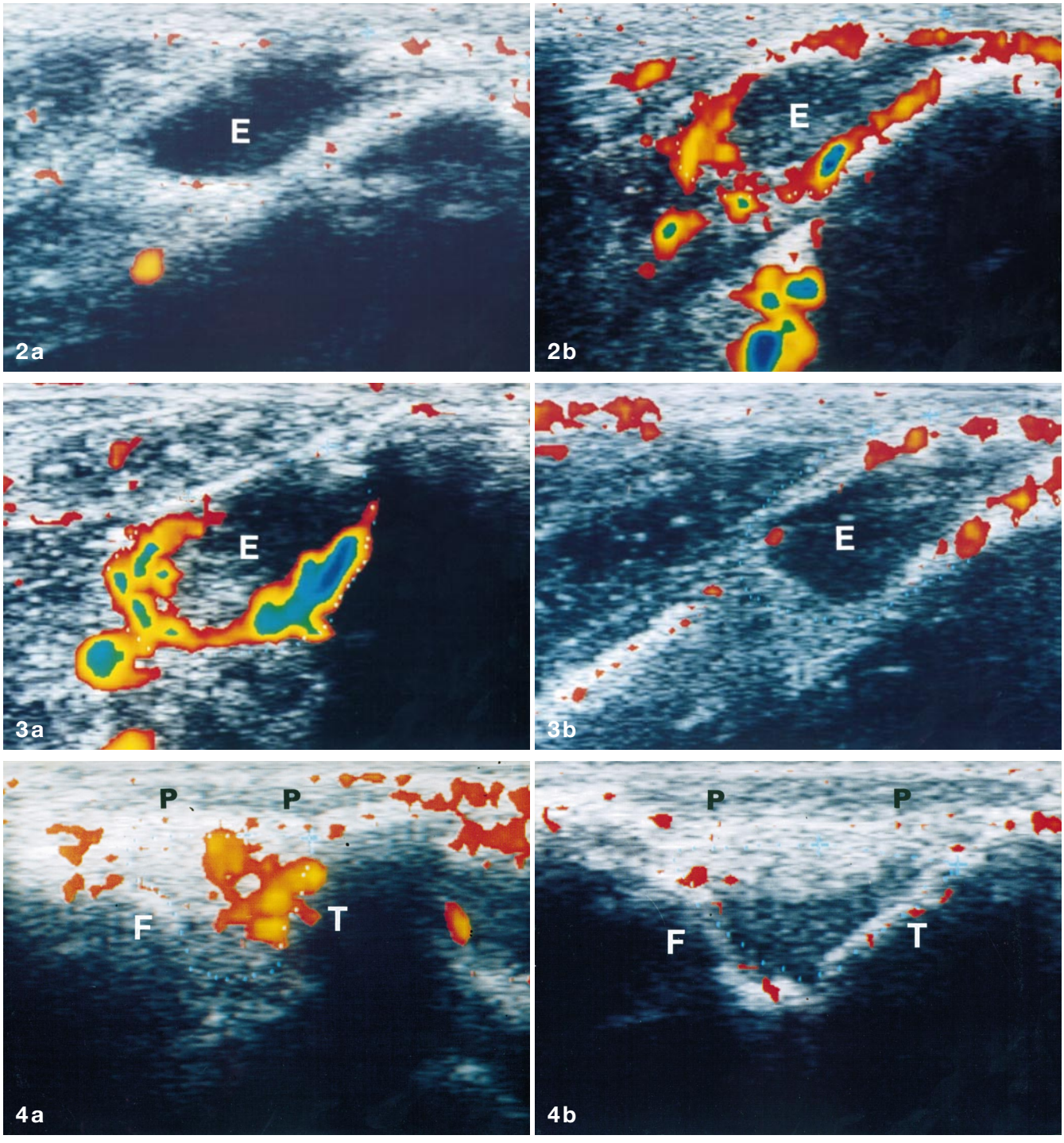

Fig. 2 **a** Sagittal view of the suprapatellar area of a rabbit knee immediately after the injection of 1 cc of saline. Minimal power Doppler signal is present (*E* effusion). **b** Sagittal view of the suprapatellar area of a rabbit knee 4 days after the injection with *S. aureus*. Diffuse increased power Doppler signal is present within soft tissues peripheral to the effusion (*E*) within the suprapatellar pouch

Fig. 3 Sagittal views of the suprapatellar area of two infected rabbit knees demonstrating a marked increase (**a**) and mild increase (**b**) in periarticular power Doppler signal (*E* effusion)

Fig. 4 Sagittal views of the tibiofemoral joint of two infected rabbit knees that both showed a marked increase in power Doppler signal in the suprapatellar region. Substantially increased power Doppler signal is evident in the tibiofemoral region in one knee (**a**), but not the other (**b**) (*F* femur, *T* tibia, *P* patellar tendon)

After we pooled the serial examinations performed on each rabbit, the cumulative sensitivity of power Doppler for septic arthritis over the course of imaging was 100 %, as all ten rabbits had at least one examination considered unequivocally positive. However, the sensitivity of an individual power Doppler examination to detect infection, when suspected, was only 51 % (23 of 45 examinations unequivocally positive on day 1 and subsequent days after injection). As no knee injected with either talc or saline had an unequivocally positive examination, the specificity of an examination with increased power Doppler signal for septic arthritis was 100 %. Overall intraobserver agreement on power Doppler readings was 96 %. Overall interobserver agreement (first readings) on power Doppler readings was 93 %.

Discussion

In this study we demonstrated the use of an animal model for studying septic arthritis and noninfectious arthritis. With this model we showed that power Doppler may be of some use in distinguishing infectious from noninfectious joint effusions.

In this study, increased periarticular power Doppler signal was specific for septic arthritis. This suggests that, if increased power Doppler signal is evident when examining an acute joint effusion of unknown etiology, septic arthritis should be suspected and an aspiration performed. Not all infected knees showed increased flow on every examination. A delay of 1–5 days before the knees showed increased power Doppler signal probably represents the time necessary for the development of the host response to the infection. Not all infected knees showed increased power Doppler signal at the same day, of the same duration, or of the same degree. Assuming that no errors were made in the size of the inoculum or during inoculation, this probably represents a variability of the host's response to the infection. In clinical practice, one would expect a much greater degree of variability caused by such factors as differing virulence of infecting bacteria, an unknown time since inoculation, varying inoculum size, and varying host response. As such, a negative power Doppler study does not exclude septic arthritis and should not preclude joint aspiration if there is clinical suspicion for infection.

In this study, it was often difficult to decide whether power Doppler signal was increased or not, as shown by the number of equivocal examinations and a 4 % intraobserver disagreement. Relatively high intraobserver and interobserver agreement reflects the high prevalence of negative examinations. In theory, one could perform follow-up examinations over subsequent days to determine whether power Doppler signal further increases or not. In clinical practice, this pathway of follow-up power Doppler studies would be limited to patients with no other clinical findings (i. e., elevated WBC, ESR, or temperature) to suggest infection. Improved power Doppler sensitivity and artifact reduction on newer ultrasound scanners may help. Additionally, quantitative estimates of flow, provided by fractional moving blood volume assessment, could aid in assessing borderline cases [10].

Several limitations of our study should be addressed. Our study was performed with an animal model, the rabbit. The applicability to human patients may be limited. As alluded to previously, the study was performed under controlled circumstances using a standard inoculum of a known bacteria and imaging with the same ultrasound scanner using the same settings for every examination. All of these factors, and others unmentioned, will vary in clinical practice and potentially affect the amount of power Doppler signal seen. Power Doppler signal will depend on the ultrasound machine used and the transducer selected. In addition, the timing of clinical presentation will have a bearing on the power Doppler findings. If patients present early in their course, possibly because of pain caused by joint distension, and before full establishment of the inflammatory response, then power Doppler may be of limited utility. This would be similar to studying the rabbit knees shortly after inoculation.

A potential for bias was introduced in selecting images to be saved and subsequently analyzed. This could potentially have been avoided by blinding the imagers as to the nature of the knee injection; however, this was not practical and probably would not have been successful, given the greater degree of joint swelling evident in the infected knees. A potential bias was also introduced by having the same authors obtain and read images. Coding and randomization of images before reading were performed to avert this bias. Although the talc model did produce a synovitis, it is unclear how accurately it mimics transient synovitis or other types of noninfectious synovitis. As with the talc model, it has been our experience that transient synovitis does not produce increased periarticular power Doppler signal. It is possible that a noninfectious synovitis could be produced that does cause increased power Doppler signal. There is some evidence that rheumatoid arthritis may produce increased power Doppler signal [4].

In conclusion, we have described the use of an animal model in studying septic arthritis and a noninfectious synovitis with power Doppler. This model suggests that increased power Doppler signal indicates a vigorous inflammatory response, as seen with septic arthritis. The lack of increased power Doppler signal in the presence of a joint effusion does not necessarily exclude infection and should not preclude aspiration if there is clinical suspicion for infection.

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