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● *Original Contribution*

VALIDATION OF MUSCULOSKELETAL ULTRASOUND IN THE ASSESSMENT OF EXPERIMENTAL GOUT SYNOVITIS

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Abstract—The objective of this study was to validate musculoskeletal ultrasound (US) in a rabbit model of acute gout. Acute gout was induced by intra-articular injection of monosodium urate (MSU) crystals in 10 rabbits; the 3 controls received vehicle. Rabbit knees were assessed by B-mode and power Doppler (PD) US 24 and 72 h after injections. After 72 h, all rabbits were euthanized. US discriminated between the MSU-injected and control groups with respect to the different inflammatory findings at both at 24 and 72 h and for MSU crystal-related findings after 24 h of injection. US synovial thickening, intra-synovial power Doppler signal and global joint distension significantly correlated with the synovial global histopathological score ($r = 0.47$, $p = 0.0188$), tissue vascularization measured by CD31 immunohistochemical-positive staining ($r = 0.46$, $p = 0.0172$) and tissue levels of interleukin-1β ($r = 0.53$, $p = 0.0078$), respectively. US is a valid method for assessment of synovial inflammation in experimental gouty arthritis in rabbits. (E-mail: gherrero@fjd.es)

Key Words: **Musculoskeletal ultrasound, Animal model, Experimental gout, Gout, Synovial inflammation, Synovial vascularization, Monosodium urate crystals.**

INTRODUCTION

Musculoskeletal ultrasound (MSKUS) has become an accurate imaging tool in rheumatic diseases (Keen et al. 2014). In gout, MSKUS is increasingly used as a diagnostic tool (Filippucci et al. 2009; Grassi et al. 2006; Naredo et al. 2014; Neogi et al. 2015; Ogdie et al. 2017; Ottaviani et al. 2012; Thiele and Schlesinger 2007; Wright et al. 2007), based on its proven capability to detect monosodium urate (MSU) crystal deposits in joints (*i.e.,* double-contour sign in the articular cartilage or intra- and periarticular tophi) compared with microscopic identification of MSU crystal in aspirated synovial fluid or tophi (Naredo et al. 2014; Perez-Ruiz et al. 2007). In addition, the presence of MSKUS-detected joint inflammation (*i.e.,* joint effusion, synovial thickening and synovial Doppler signal) has been widely described in human gout, both in chronic arthritis and in acute episodes of arthritis (Grassi et al. 2006; Stewart et al. 2017; Thiele and Schlesinger 2007).

Animal models provide essential information on the pathogenesis of joint diseases, as well as a research setting for testing potential therapeutic agents. Animal models of gout are induced mainly by MSU injection because of the difficulty of inducing chronic hyperuricemia associated with increased synthesis (Schett et al. 2015). Acute gout models have a rapid resolution, within days, hampering the analysis of gout pathophysiology and therapeutic response (Schett et al. 2015). A non-invasive technique such as MSKUS would more easily allow *in vivo* sequential analysis. Despite its potential for assessing synovial inflammation, the validity of MSKUS has been only scarcely investigated in animal models of joint inflammation, including gout (Bouta et al. 2015; Chen et al. 2016; Clavel et al. 2008; Pineda et al. 2015).

The objective of this experimental study was to determine whether MSKUS features can be used as an outcome measure in rabbit gouty arthritis for the evaluation of synovial inflammation. MSKUS features were compared with clinical, histologic and molecular standard measurements of tissue inflammation.

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Conflicts of Interest: All authors declare no competing interests with the topic.

METHODS

Experimental model

Thirteen white adult male New Zealand rabbits weighing 3 to 3.5 kg (Granja San Bernardo, Navarra, Spain) were used for the experimental procedures. Animal handling and experimentation were performed in accordance with national regulations and the *Guidelines for the Care and Use of Laboratory Animals* drawn up by the National Institutes of Health (Bethesda, MD, USA). The experimental protocol was approved by the institutional ethics committee.

The experimental model was induced as previously described with minor modifications (Miguélez et al. 1996). In brief, after 1 wk of adaptation to our facilities, 10 rabbits received an intra-articular injections of 1 mL of MSU crystals (50 mg/mL) into both knees (MSU group), and 3 rabbits received 1 mL of phosphate-buffered saline (as vehicle, control group). Ten rabbits (20 knees) were included in the MSU group, to diminish possible differences in the crystal injection response among different knees, based on recently published data in which ultrasound was found to have a great capacity to discriminate between MSU-injected and control knees with respect to features of MSU crystal deposition and synovial inflammation (Pineda et al. 2015). To minimize the number of animals employed, 3 rabbits (6 knees) were included to assess healthy rabbits, because of the more homogeneous features observed in these rabbits than in the MSU-injected rabbits. The degree of joint swelling was determined 24 and 72 h after injections by measuring knee perimeter with a digital caliper. All animals were euthanized 72 h after intra-articular injections with an overdose of pentobarbital (Braun Medical SA, Barcelona, Spain). Rabbit knees were dissected, and infrapatellar synovial membranes were cut into two pieces: one was fixed in 4% buffered paraformaldehyde, dehydrated and embedded in paraffin for histologic evaluation; the other was immediately frozen and employed for protein extraction studies (López-Armada et al. 2002).

MSKUS assessment

Rabbits underwent B-mode and power Doppler (PD) US assessment of both knees at two times, 24 and 72 h after injections, by a rheumatologist highly experienced in MSKUS who was blinded to the injection type and clinical data. All US examinations were carried out with a commercially available real-time scanner (LOGIQ e R7, GE Medical Systems, Jiangsu, China) equipped with a 22-MHz linear transducer.

B-Mode and PD machine settings were optimized before the study and standardized for the whole study as follows: B-mode gain of 47 dB, dynamic range of 72 dB, Doppler frequency of 14.3 MHz, Doppler gain of 28 dB, low-wall filters and pulse repetition frequency of 700 Hz.

After being anesthetized, rabbits were placed in the supine position with their knees flexed 10° to 20°. The US assessment consisted of systematic longitudinal and transverse B-mode and PD examination of the suprapatellar, lateral and medial recesses of the knee joint. Given its more superficial anatomic location, we selected the lateral recess for scoring MSKUS findings.

The MSKUS inflammatory findings investigated were B-mode recess global distension (GD), synovial fluid (SF) and synovial thickening (STh) and intra-synovial PD signal (PD). We used the Outcome Measures in Rheumatology (OMERACT) US definition for synovitis components in inflammatory arthritis (Wakefield et al. 2005) modified as follows. SF was defined as hypo-echoic or anechoic material within the synovial recess that was displaceable by compression with the US probe. STh was defined as abnormal hypo-echoic thickening of the tissue located between the outer recess boundary and the synovial space that was not displaceable and poorly compressible by compression with the US probe. GD, SF, STh and PD were scored semi-quantitatively on a scale of 0–3 (*i.e.,* 0 = absence, 1 = mild, 2 = moderate; 3 = marked) as described elsewhere (D'Agostino et al. 2017; Szkudlarek et al. 2003). A score ≥2 was considered pathologic for GD and SF, and a score ≥1 was considered pathologic for STh and PD. STh and PD scores were correlated with synovial histopathological damage and synovial tissue vascularization.

The following MSKUS findings previously described in acute gout (Grassi et al. 2006) were also investigated as features of MSU crystal deposition within the lateral recess of the rabbit knees: (i) iso-echoic sand-like material (SM) floating in the synovial fluid and displaceable when compressed with the US probe; (ii) hyper-echoic aggregates (AG) within the sand-like material; and (iii) bright foci (BF) within the sand-like material.

Intra-observer reliability of the MSKUS assessment was evaluated by recording representative images from the examinations of all rabbit knees at 24 and 72 h. The stored images were scored for inflammatory and MSU crystal findings under blinded conditions by the same ultrasonographer investigator a minimum of 3 mo later.

Histologic assessment

Synovial histopathology was evaluated in hematoxylin and eosin (HE)-stained sections by two blinded observers, according to the Krenn scale, as previously described (Alvarez-Soria et al. 2006; Krenn et al. 2002; Prieto-Potín et al. 2013). Briefly, lining hyperplasia, fibrovascular alterations at the interstitium and tissue cell infiltration were independently evaluated using 0- to 3-point subscales, where 0 = absent, 1 = mild, 2 = intermediate and 3 = strong. The total score was the sum of partial grades with a maximum total score of 9 (Krenn et al. 2002).