A "new" technique for the diagnosis of chondrocalcinosis of the knee: sensitivity and specificity of high-frequency ultrasonography

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single-nucleotide polymorphisms (SNPs) in the MTHFR coding region suggest possible links between other specific genotypes and MTX response.[2]

We recently identified an association between the 14 bp deletion/insertion polymorphism in exon 8 at the 3′ untranslated region (UTR) of the HLA-G gene and the clinical response to MTX treatment in rheumatoid arthritis.[3] The HLA-G antigens are non-classic MHC class Ib molecules with limited allelic polymorphisms,[4] restricted tissue distribution,[5–7] and alternative splicing mechanisms for mRNA that allow the production of both membrane-bound and soluble isoforms.[8] HLA-G molecules are associated with the development or persistence of several autoimmune diseases because of their tolerogenic capacity against innate and adaptive responses.[7 9] The 14 bp insertion/deletion polymorphism in the HLA-G gene influences mRNA stability and quantitative protein production. Furthermore, the 14 bp insertion allele (+14 bp) destabilises mRNA and decreases soluble HLA-G (sHLA-G) protein production.[10]

In this study, we showed that MTX can induce the in vitro production of circulating HLA-G molecules by peripheral blood monocyte cells from healthy subjects and patients with rheumatoid arthritis, with interindividual differences. Moreover, the highest quantitative in vitro production of sHLA-G molecules was associated with the presence of the deletion (−14/−14 bp) genotype.

To have in vivo confirmation of the pharmacogenetic role of the 14 bp polymorphism in MTX response, we also performed a retrospective study of the genotype distribution in 156 patients with rheumatoid arthritis, who were subdivided into two cohorts on the basis of their clinical response to MTX. The data obtained indicated a significantly higher frequency of the −14/−14 bp genotype in ''responder'' patients compared with ''non-responders'' ($\chi^2 = 6.12$; df = 1; $p = 0.02$ (χ^2 test); odds ratio 2.46, 95% CI 1.26 to 4.84; $p<0.009$).

These results suggest a pharmacogenetic role for the HLA-G 14 bp polymorphism, and that there is clinical advantage of the −14/−14 bp genotype in response to MTX. The HLA-G 14 bp polymorphism should be investigated in association with other MTHFR SNPs that predominantly show a role in MTX toxicity.[1] The combined analysis of such MTHFR SNPs and the HLA-G 14 bp polymorphism could help in assessing the likelihood that patients will experience MTX-related toxicity or benefits.

In conclusion, in rheumatoid arthritis it seems to be important to consider the concurrence of different genetic polymorphisms to help predict the clinical response to MTX treatment.

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A ''new'' technique for the diagnosis of chondrocalcinosis of the knee: sensitivity and specificity of high-frequency ultrasonography

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According to the criteria proposed by Ryan and McCarty,[1] the diagnosis of calcium pyrophosphate dihydrate (CPPD) deposition disease has been based on radiological evidence of the characteristic calcifications and on verification of the synovial liquid of CPPD crystals.

Joint ultrasonography is an innocuous diagnostic technique that is well tolerated by patients, and is the elected method for observing calcified deposits in soft tissues.[2]

Abbreviation: CPPD, calcium pyrophosphate dehydrate

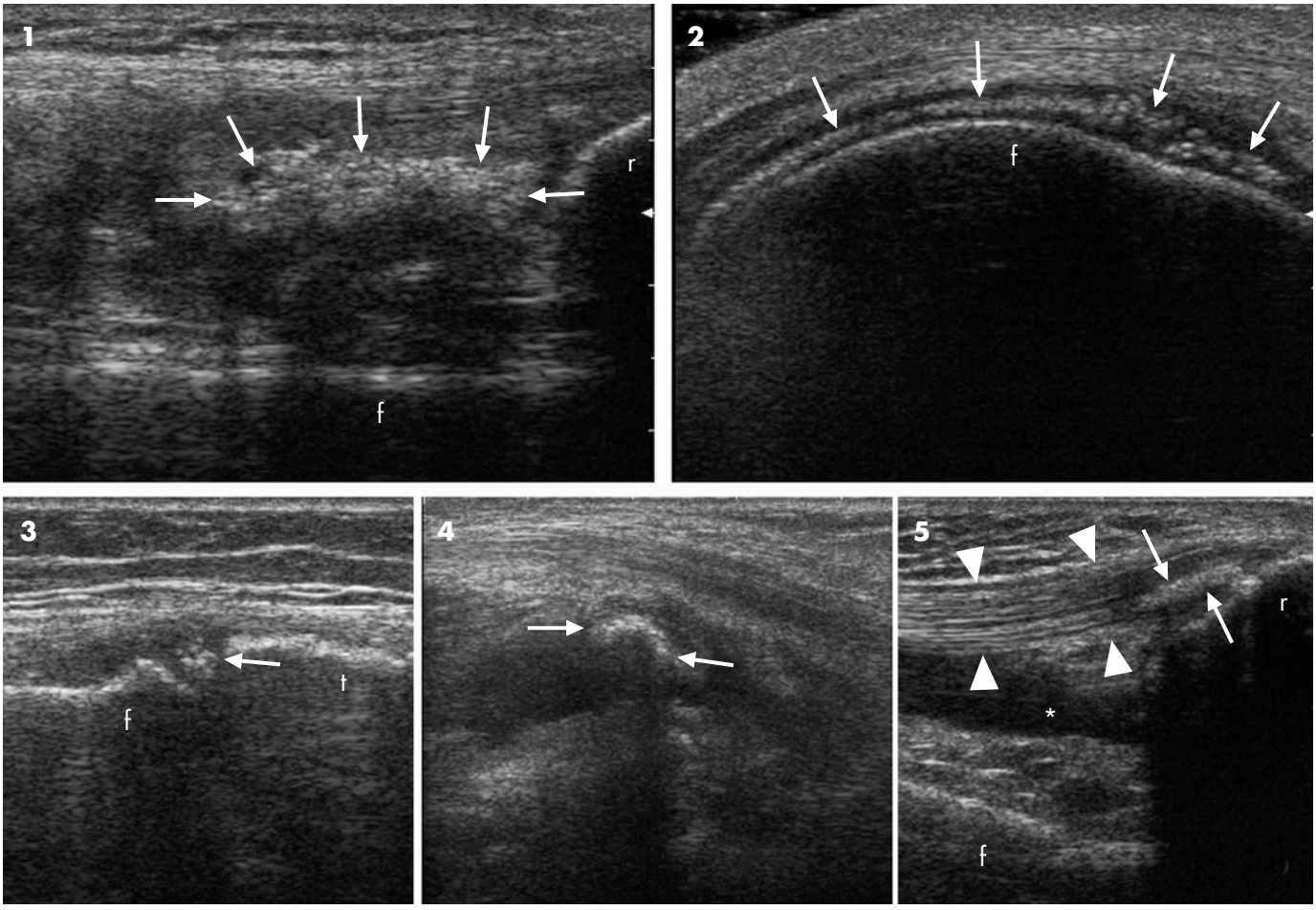

Figure 1 Hyperechoic deposits. Deposits (arrows) are shown that are compatible with calcium pyrophosphate dihydrate (CPPD) calcifications in the context of the synovial membrane (1), hyaline cartilage of the femur (2), meniscus (3), in the sinovial fluid of a Baker's cyst (4) and in the insertional tract of quadricipital tendon (5); arrowheads: quadricipital tendon (purposely appearing hypoechoic at insertion—anisotropic artefact—to better highlight the CPPD deposits). Patterns 1, 4 and 5 are rare and generally occur when a large number of CPPD crystals are present in synovial fluid. In all cases, the deposits do not create an evident posterior shadow unless they reach moderate thickness. In such cases, a partial posterior shadow may be observed (photos 1 and 4). f, femur; r, rotula; t, tibia; *, joint effusion.

We carried out a longitudinal study, enrolling patients affected with ultrasonographic chondrocalcinosis according to previously proposed criteria[3] from a sample of consecutive patients that came to our joint ultrasonography department for gonalgia (fig. 1). A total of 47 patients were identified, of which 14 had joint effusion.

The control group was made up of 29 patients who did not present CPPD calcifications in joint tissues affected by osteoarthritis, but who presented joint effusion. All subjects underwent synovial fluid aspiration. The two groups were similar in age.

Ultrasonography examination was performed by an experienced sonographer, blind to previous diagnosis, on an Esaote Technos MP with a 7.5–13 Mhz dedicated linear transducer by the method described elsewhere.[2 3] Synovial fluid analysis was performed on wet preparations, within 1 hour after aspiration, by an expert biologist blind to ultrasonographic findings. Each slide was observed under transmitted light microscopy and by compensated polarised microscopy. Crystals with a parallelelipedic or rhomboid shape and weak birefringence with positive elongation were considered to be CPPD crystals.[4] We used synovial fluid analysis as the "gold standard" for diagnosis.

Table 1 Characteristics of patients and results of the study

	n	Average age, years (range)	CPPD crystals in synovial fluid	Sensitivity	Specificity	Localisation		
						Meniscus	Cartilage	Other*
Patients classified as affected with CC	14	68 (40–92)	13	86.7%	96.4%	14	4	3
Controls	29	69 (55–84)	2			–	–	–

*Mobile deposits in synovial liquid, intratendinous or intrasynovial.

Of the 14 patients classified by ultrasonography as affected with chondrocalcinosis, 13 presented with CPPD crystals in synovial liquid, whereas 2 of the 29 patients of the control group were positive for CPPD crystals at microscopic analysis (table 1). Therefore, ultrasonography demonstrated a high specificity (equal to 96.4%) and good sensitivity (equal to 86.7%), with a positive predictive value of 92% and a negative predictive value of 93%.

Considering that CPPD crystals could be found in synovial fluid even when characteristic calcifications are not found in joint tissues by traditional radiology,[5 6] a sensitivity of 86.7% must be an excellent result. The important data of this study is, however, the high specificity of ultrasonography (96%) in identifying CPPD calcifications.

Other studies have been carried out on the utility of ultrasonography in knee chondrocalcinosis,[7 8] but this is the only study that has used the presence of CPPD crystals in synovial liquid as a gold standard. The objective of this study was to evaluate the capacity of ultrasonography to offer a diagnosis, by identifying CPPD calcifications. For this purpose, microscopic synovial fluid analysis, the most widely used examination for the diagnosis of CPPD crystal deposition disease, was used as the gold standard.

Therefore, ultrasonography, as opposed to traditional radiology (ionizing radiation) and to magnetic resonance imaging (still conflicting data),[9 10] is the only innocuous examination currently available to the physician that is able to identify CPPD crystal deposits. Considering that CPPD crystal deposition disease frequently has a subclinical course and the patient does not always reach the surgery in an acute stage, it is important for the physician to have a tool at his disposal that permits a diagnosis even in the absence of joint effusion. Moreover, the possibility that ultrasonography can be carried out rapidly during a rheumatology examination places it as a prime tool of diagnostic practice when there is suspected CPPD arthropathy.

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CORRECTION

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There was an error in table 1 of the article by Micheloud D, Calderón M, Caparros M, D'Cruz D P. Intravenous immunoglobulin therapy in severe lupus myocarditis: good outcome in three patients. *Ann Rheum Dis* 2007;**66**:986–7. The units IU/ml and ug/l should refer to CK and troponin levels, respectively. There should also be an extra line in the table. The corrected table is available on our website at http://ard.bmj.com/Supplemental.