Ultrasound-guided synovial biopsy of the wrist does not alter subsequent clinical or ultrasound disease activity assessments: a prospective study for incorporation of imaging in clinical trials

I. Lazarou[1], S. Kelly[2], F. Humby[1], M. Di Cicco[1], L. Zou[1], V. Rocher-Ros[1], R.E. Hands[1], N. Ng[1], A. Mahto[1], C. Pitzalis[1]

[1]Experimental Medicine and Rheumatology, Queen Mary University of London, William Harvey Research Institute, London, United Kingdom; [2]Rheumatology Department, Mile End Hospital, Barts Health NHS Trust, London, United Kingdom.

Abstract

Objective

Ultrasound-guided synovial biopsy (UGSB) is a minimally-invasive procedure capable of retrieving good quality tissue from small and large joints. The use of UGSB in prospective clinical trials poses a dilemma as to whether biopsied joints may be later included in core data sets for clinical or imagining response, as the procedure itself may alter disease activity assessment. In this study, we examine the impact of UGSB of the wrist on subsequent clinical and ultrasound (US) assessments in a cohort of rheumatoid arthritis (RA) patients prior to initiation of anti-TNF-alpha therapy.

Methods

Patients had active disease (DAS>5.1) involving their wrist. Both wrists were scanned and the most inflamed one underwent an UGSB. Ultrasonographic and clinical assessments were repeated at the patients' subsequent visit, without any changes in disease-modifying treatment between visits. US images were scored semi-quantitatively and quantitatively for synovial thickness (ST) and power Doppler (PD). Mixed-effects model and paired-Wilcoxon signed rank test were used to assess the effect of UGSB on these scores.

Results

Twenty-nine patients were enrolled. No significant difference in mean ST (p=0.32) or PD (p=0.21) was demonstrated pre- and post-biopsy (mean time 14.7 days). Similar results were obtained using quantitative measures. The DAS-28 and its components did not change significantly post-biopsy.

Conclusion

In this population, UGSB of the wrist did not significantly alter subsequent clinical or US assessments, indicating that a wrist joint, which has undergone UGSB, may be incorporated into an US dataset or clinical outcome assessment tools, such as the DAS-28, without prejudice.

Key words

ultrasonography, synovial biopsy, rheumatoid arthritis, disease activity

Ilias Lazarou, Stephen Kelly, Frances Humby, Maria DiCicco, Lu Zou,Vidalba Rocher-Ros, Rebecca Eve Hands, Nora Ng, Arti Mahto, Costantino Pitzalis.

Please address correspondence to: Dr Stephen Kelly, Rheumatology Department, Royal London Hospital (Mile End), 275 Bancroft Road, Barts Health NHS Trust, London E1 2DG, United Kingdom. E-mail: stephen.kelly@bartshealth.nhs.uk

Received on February 14, 2015; accepted in revised form on September 21, 2015.

Funding: I. Lazarou has received a scientific training bursary by the EULAR and is a clinical research fellow funded by the Swiss Society of Rheumatology training bursary.

Competing interests: none declared.

Introduction

Over the last two decades, synovial tissue analysis has attracted increasing interest for basic research on disease pathobiology and is increasingly valuable for early phase drug development studies (1-5). Historically, synovial tissue is obtained by blind-needle biopsy or arthroscopy. Arthroscopy is currently considered the optimum technique for synovial tissue retrieval, in the context of clinical trials, as much work has been done to standardise the procedure and tissue processing. With the advance of musculoskeletal ultrasound (US) as a reliable, safe, non-invasive imaging tool of rheumatic conditions, recent techniques of US-guided synovial biopsy (UGSB) have been developed (6). UGSB can be performed using a semi-automatic guillotine-type biopsy needle or with a portal-and-forceps approach (7-11). This approach to synovial tissue retrieval using a minimally invasive technique facilitates the retrieval of high quality tissue from both small and large joints and is well tolerated by patients (8). The reported safety profile appears to be good and the procedure can be repeated on a serial basis, an important consideration in prospective studies.

Ultrasound imaging is widely used in clinical practice and increasingly being included as an assessment tool in clinical trials. A consensus is beginning to emerge as to the optimal data set to be acquired. Proposed assessment data-sets include a 12-joint score (12), a 7-joint score (13) and the GLOSS (14), which has been recently validated in a prospective, randomised controlled clinical trial. All US scoring data sets include the wrist as a critical joint in such an assessment, which has previously been demonstrated in established RA to correlate well with overall disease activity. The ability, therefore, to retrieve synovial tissue from an affected wrist joint in the context of prospective clinical studies without inducing changes in subsequent ultrasonographic assessments or standard validated composite response criteria is an attractive proposition. To date, little is known on whether these interventional procedures alter classical disease activity measures or US parameters of the joint on which they are performed, irrespective of the technique employed. It is therefore questionable whether such joints can be satisfactorily incorporated into an assessment of disease activity following on from such an intervention in the context of prospective therapeutic clinical trials.

Thus, we undertook this study to examine whether an UGSB of the wrist, using a semi-automatic guillotine-type biopsy needle, in a DMARD-inadequate-responder RA population, induces significant aberration in the ultrasonographic or clinical parameters, which would, in turn, influence assessment of disease activity.

Materials and methods

Patients

Twenty-nine patients fulfilling the 1987 American College of Rheumatology criteria for RA were prospectively enrolled in an observational biopsy-based study, within the rheumatology clinic at Barts and The London NHS Trust. All patients were ≥18 years of age and met the National Institute for Health and Care Excellence (NICE) guidelines for commencement of tumour necrosis factor alpha (TNF-alpha) inhibitors therapy. The study was approved by the Trust's ethics committee (REC 10/H0801/47), and all subjects provided written informed consent prior to enrolment. All patients had been receiving methotrexate for at least four months at a stable dose not less than 15 mg per week for the last four weeks. Patients were permitted to be recruited if they were receiving the equivalent of 10 mg of oral prednisolone or less at a stable dose for at least four weeks prior to recruitment. Patients already receiving corticosteroids or non-steroidal anti-inflammatories were allowed to continue on the same dose and frequency between the two assessments. However, no alteration in corticosteroid, DMARD or non-steroid anti-inflammatories treatment was permitted between the synovial biopsy and follow up visit. Anti-TNF-alpha treatment was not initiated until the end of the follow-up assessment.

US evaluation and US-guided synovial biopsy

Ultrasonographic assessment was performed immediately prior to the biopsy and again on the patient's return to clinic prior to commencing anti-TNF-alpha therapy. Techniques of US examination, UGSB using a biopsy needle, tissue processing and histopathological assessment were carried out as we have previously described in detail (8). Briefly, US was performed using a General Electric Logiq 9® machine (GE Healthcare, Fairfield, Connecticut, USA) with a two-dimensional M12L transducer-grey-scale frequency 12 MHz. Power Doppler settings, depth and grain were optimised at the beginning of the study to the lowest achievable PRF and maximum gain without perceptual noise artefact (Frequency 7.5 MHz, Gain 45, PRF 1.4K Hz, WF 127 Hz, Depth 2.0 cm) and remained fixed during the study period. The wrist was assessed longitudinally on three dorsal sites (midline, radial, and ulnar) for synovial thickness (ST) and Power Doppler (PD) signal. Midline view represents imaging of the intercarpal and radio-carpal recesses. Radial view represents the distal radius, scaphoid and the second carpo-metacarpal joint. Ulnar view corresponds to the distal ulna, triquestral, and the fifth carpo-metacarpal joint.

Synovitis was defined according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) definitions (15-16). ST and PD signal were scored using a previously reported semi-quantitative score (12). Analysis was performed using both mean and maximum scores. Each view was scored on a scale from 0 to 3; the mean score for each wrist was the sum of the ST or PD on the three views described above, divided by three. The maximum score corresponds to the single assessment view of the wrist (midline, ulna or radial) with the greatest ST and PD scores. Quantitative measurements were analysed using the ImageJ software (17). Briefly, synovial thickness area is a count of the number of pixels within the reader-defined region of interest (ROI) in a standardised image of the joint. The power Doppler quantitative area is represented by the number of pixels with PD signal within the same ROI, uncorrected for intensity. Results are presented as the ratio of PD quantitative area over the ST area. Given the natural variation in RA disease activity over time, the interaction between the biopsied and non-biopsied wrists and its change over time was also assessed. The non-biopsied wrist is effectively an anchor and used to calibrate any change in US parameters in the biopsied arm against natural disease background activity.

Pre- and post-biopsy US examinations were performed by a single operator (MDC) and all images were scored independently by SK and IL, both blinded to patient clinical data. Excellent ICC values were obtained for semi-quantitative ST (0.85, CI 0.76–0.90) and PD (0.90, CI 0.84–0.94) measurements, as well as the quantitative ST area (0.89, CI 0.84–0.92) and PD over ST quantitative area (0.97, CI 0.96-0.98).

Minimally invasive UGSB was carried out as described (18-19) before using a 16G Quick-Core® Biopsy Needle (Cook medical, Limerick, Ireland) without an outer introducer. After choosing the most suitable wrist, and under sterile conditions, the skin, subcutaneous tissue, and finally the synovial space were anesthetised with lidocaine 1%. The US probe was covered in a sterile sheath and chlorhexidine (2%) cleaning fluid was used as a contact medium. The throw of the needle was positioned towards the region of synovial hypertrophy under US control and the samples were taken. In the present study 12 synovial tissue samples were taken with at least 6 demonstrating adequate lining layer for grading, in line with previous reports demonstrating that the examination of 4 biopsies at one time point provides a reliable sample mean in the very vast majority of samples (18).

Data collection

At baseline, the following data were recorded for each patient: age, sex, disease duration, current and previous DMARDs, corticosteroid use, rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody status, the components of the DAS-28 (ESR), the Health Assessment Questionnaire (HAQ), and inflammatory markers (C-reactive protein [CRP] level and erythrocyte sedimentation rate [ESR]). All patients underwent an US assessment of both wrists followed by an UGSB of the wrist with the highest ST and PD scores. Patients attended a second visit for anti-TNF-alpha initiation, during which US and the DAS-28 assessment were repeated. There was no change in medication between these two visits. Data was collected for adverse events related to the procedure.

Statistical analysis

Statistical analysis was performed with the R software v. 3.0.2, using both the mean and maximum scores for ST and PD for each wrist. Change in disease activity measures was tested using Wilcox signed rank test with continuity correction. Mixed effects model was used for semi-quantitative scores, taking into account the variation of two arms and time points within a patient, as well as the variation between patients. The paired Wilcoxon signed rank test was used to assess differences and changes of quantitative scores. Inter-reader reliability for the semi-quantitative and quantitative US measurements was assessed with intraclass correlation coefficient (ICC) with 95% confidence interval (CI) for each endpoint.

The minimal detectable difference was 1 for mean ST, 1.2 for mean PD and 0.95-1.2 for DAS28:

1. Difference of 0.95 on DAS28 before and after biopsy gives over 90% power at significance level of 0.05 in either biopsy or control arms.
2. Difference of 0.8-1 on ST mean before and after biopsy gives over 90% power at significance level of 0.05 in either biopsy or control arms.
3. Difference of 0.7-1.2 on PD mean before and after biopsy gives over 90% power at significance level of 0.05 in either biopsy or control arms.

Results

Patient demographics

Mean disease duration was 6.14 years, representing a cohort of patients with established RA, with 69% being anti-CCP positive. All patients had a high disease

activity score required to fulfil NICE criteria for the initiation of anti-TNF-alpha therapy in the UK and mean DAS-28 was 6.29. All patients were receiving methotrexate as described in the *Methods* section, with 34.5% being prescribed concomitant prednisolone at a stable dose not exceeding 10 mg /day. The mean duration between biopsy and subsequent assessment was 14.7 days. There were no drop-outs between the two time-points. Baseline characteristics of the study population are shown in Table I.

Safety and adverse events

No serious adverse events *(e.g.* bleeding, infection, neurological compromise, severe pain or thrombosis) related to the procedure were reported by patients in their subsequent follow up visit.

The US-guided synovial biopsy procedure did not cause significant differences between pre- and post-biopsy clinical, biochemical or US assessments

There were no significant differences in the clinical assessment of the patients' disease activity using the DAS-28(ESR) when assessed pre- and post- synovial biopsy. The inflammatory markers (ESR and CRP) did not significantly alter between visits nor did any of the components of the DAS-28 composite score including swollen joint, tender joint and patient global scores. (Table II). Similarly, there was no increase in the number of subjects reporting tenderness of the wrist before and after the procedure.

As joints with the highest US scores are selected for biopsy, the non-biopsied (contralateral) wrist had significantly ($p<0.001$, mixed effects model) lower semi-quantitative mean ST and PD scores at both time-points. The average difference in the mean ST score between wrists was 0.49 and 0.44 in the PD score. No significant change in either mean ST ($p=0.322$) or mean PD ($p=0.209$) scores of the biopsied joint was demonstrated prospectively. Similarly, no significant change in ST and PD values was demonstrated in the non-biopsied (contralateral) wrist before and after the procedure (Fig. 1A-B). Similar results were obtained when using the maximum ST and PD scores from each wrist, with no significant change detected before and after the procedure ($p=0.214$ and 0.279 respectively).

Tenderness of the wrist on clinical examination associated positively with the mean PD score (mixed effects model): its presence increased the mean PD score by 0.30 (SE=0.11, $p=0.010$).

In terms of quantitative synovial thickness area measurements, there was no significant change over time in biopsied ($p=0.096$) or contralateral ($p=0.983$) wrists (Fig. 1C). Similarly, there was no significant change over time for the power Doppler to synovial thickness quantitative area ratio for both biopsied and contralateral wrists ($p=0.069$ and 0.173 respectively), results comparable to the aforementioned semi-quantitative (Fig. 1D).

Table I. Baseline patient characteristics. All values are expressed in mean (SD) unless specified otherwise.

Gender, n (%)		
Male	6 (21)	
Female	23 (79)	
Age, years	57.24 (13.44)	
Age, median (IQR)	56 (53-66)	
Smoking status, n (%)		
Previous smoker	9 (31)	
Current smoker	5 (17)	
RF positive n (%)	15 (52)	
Anti-CCP positive n (%)	20 (69)	
Disease duration, years	6.14 (6.90)	
Disease duration (median, IQR)	3 (1-10)	
Number of DMARDs, median (range)	2 (1-3)	
Corticosteroid treatment* (%)	10 (34)	
	Mean (SD)	Median (IQR)
DAS-28 (range)	6.29 (50.87)	6.22 (5.60-8.00)
Tender joint count (range)	17.34 (7.55)	16 (10-24)
Swollen joint count (range)	8.79 (2.83)	9 (7-10)
Patient global for health, mm (range)	74.21 (18.46)	79 (70-85)
ESR, mm/h (range)	27.21 (18.57)	27 (12-40)
CRP, mg/l (range)	10.62 (012.95)	5 (0-14)
HAQ (range)	1.751 (0.63)	1.88 (1.38-2.10)
Biopsied wrist n (%)		
Right	17 (59)	
Left	12 (41)	
Time between assessments, days (median, IQR)	10 (9-23)	

CCP: cyclic citrullinated peptide; CRP: C-reactive protein; DAS-28: disease activity score; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire; RF, rheumatoid factor. *Corticosteroid dose did not exceed prednisolone 10 mg daily and was stable for at least four weeks prior to biopsy

Table II. Change in DAS-28 and its components before and after ultrasound-guided synovial biopsy of the wrist.

	Before				After				Paired Wilcox signed rank test with continuity correction
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	
DAS-28	6.29	0.87	6.22	5.60-6.90	6.27	0.68	6.08	5.86-6.76	$p=0.866$
TJC	17.34	7.55	16	10-24	18.64	7.33	20	9.75-25	$p=0.252$
SJC	8.79	2.83	9	7-10	8.25	3.54	7.50	6-9.25	$p=0.313$
PtGH	74.21	18.46	79	70-85	72.57	19.49	77.50	61-86.25	$p=0.509$
ESR	27.21	18.57	27	12-40	24.78	17.33	22	12-32.50	$p=0.151$
CRP	10.62	12.95	5	0-14	11.85	22.84	0	0-12	$p=0.209$

CRP: C-reactive protein (mg/l); DAS-28: disease activity score 28; ESR: erythrocyte sedimentation rate (mm/h); PtGH: patient global for health (0-100mm visual analogue scale); SJC: swollen joint count; TJC: tender joint count.

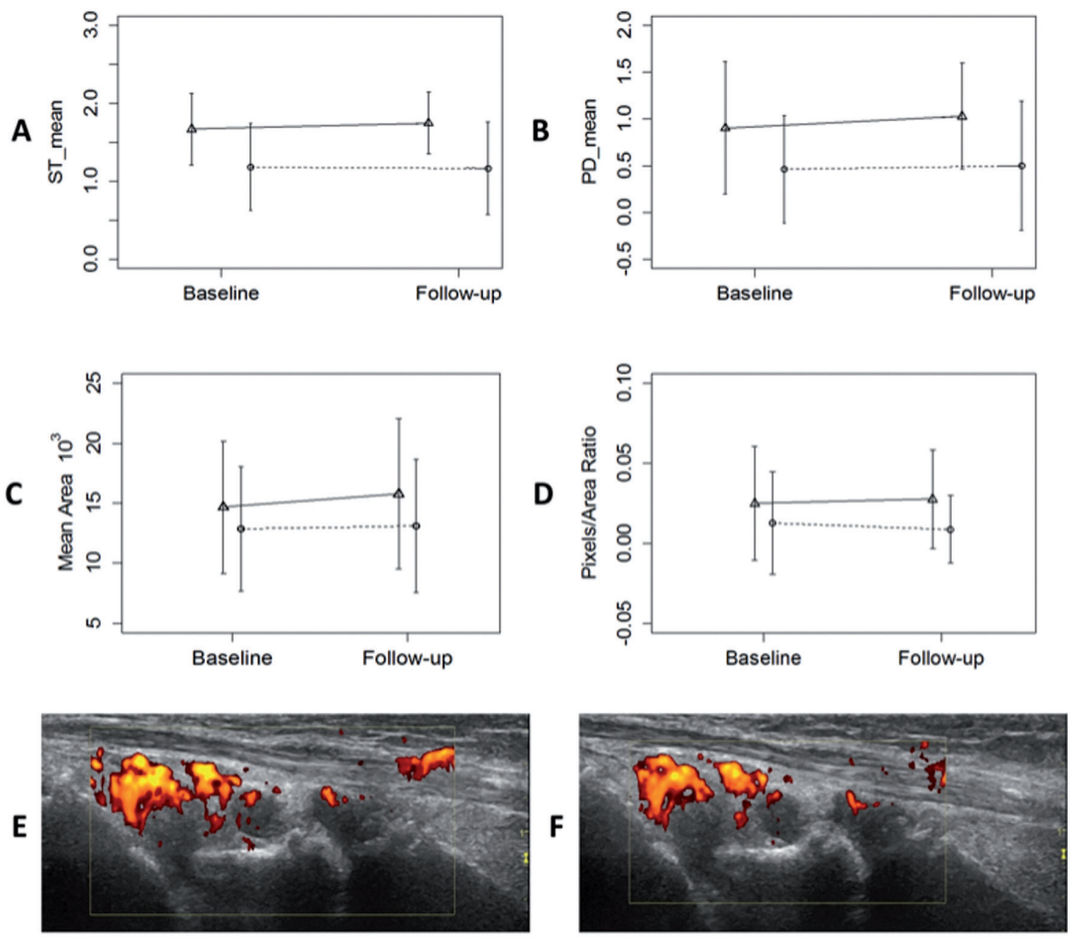

Fig. 1. Change over time in mean synovial thickness (**A**), Power Doppler signal (**B**), synovial area (**C**), and Power Doppler / Synovial Area ratio (**D**) for the biopsied (straight line) and contralateral (dashed line) wrists. Representative, single-patient images of the wrist (midline view) pre- (**E**) and post-biopsy (**F**): no significant difference in Power Doppler is detected.

Given the variation of the time of review from biopsy to repeat visit (range 8–28 days) we analysed a subgroup of patients who had all be assessed within a 14-day time frame form their initial synovial biopsy. Time between assessments was not a significant factor for change in either mean ST (p=0.939) or mean PD (p=0.337). Excluding subjects with follow-up time greater than 14 days did not change the results: no significant difference was detected between clinical, biochemical or imaging assessments pre- or post-biopsy.

Discussion

A synovial biopsy has the potential to affect joint symptoms positively, negatively or not at all. Joint lavage is employed during synovial biopsies with arthroscopy and may sometimes be required when using a US-guided portal & forceps approach. It is known that joint irrigation may help to remove pro-inflammatory cytokines from within the joint and subsequently improve symptoms and reduce disease activity in inflammatory arthritis (20-23). Conversely, any intervention into a joint has the potential to induce an inflammatory response as a result of trauma to the surrounding soft tissues or the joint itself. The technique descripted in this study does not require joint lavage and minimises soft tissue trauma with the use of a throw within the 16G needle to capture synovial tissue. In addition, the safety profile appears to be excellent with no patients reporting serious adverse events in this cohort. This is comparable to previously reported safety data for this procedure in both small and large joints (8). Whilst the wrist is a complex structure, the introduction of a 16G or 14G needle would appear to be relatively well tolerated and under US guidance significant structures such as vessels, nerves and tendons can be avoided.

Although US assessment of joint synovitis is known to be more sensitive than clinical examination (24), reassuringly it did not show substantial changes in ST and PD signal of the biopsied wrist following UGSB, or of the contralateral joint, thus suggesting no variation in US disease activity. In other words, the semi-quantitative scores for ST and PD of the biopsied joint did not change significantly over time. Similar results were obtained for the quantitative synovial thickness area, and ratio of PD signal to synovial thickness area. The clinical assessment of the patients' disease activity using inflammatory markers, the DAS-28 or any of its components, was not affected by the procedure. Furthermore, there was no increase in the number of subjects reporting tenderness of their wrist after the biopsy.

For this study, we investigated longitudinally a homogeneous population of RA patients prior to initiation of biologic therapy. We used a combination of grey-scale and PD multiplanar semi-quantitative assessment of the wrist and extended the analysis of the acquired

data to quantitative measurements, which are also sensitive to change (17). For both approaches, interobserver reliability was excellent and comparable to -if not higher than- previously reported (25). The small number of readers, the strict standardisation of the scanning technique, and adherence to the OMERACT definitions for ST and PD contributed to this result. The limited number of subjects should be mentioned amongst the limitations of our study. However, numbers are comparable to current literature with regards to biopsy-based mechanistic early-phase drug-development studies. In addition, the follow up period was variable and we cannot discount very early (less than 1 week) synovial changes, which could have regressed over time. However, typically, one would delay initiation of a new therapy by at least 1 week in practise following an invasive procedure such as a synovial biopsy. Finally, the results of this study can only be generalised to US-guided biopsies of the wrist using a semi-automatic guillotine-type biopsy needle technique. Thus, further studies should be carried out to examine change in clinical and US assessments following biopsies using different techniques.

Overall, in this biologic-naïve DMARD inadequate-responder RA population prior to anti-TNF-alpha therapy, minimally invasive UGSB of the wrist using a semi-automatic guillotine-type biopsy needle did not significantly alter subsequent clinical or US assessments, either at the individual joint level or using a validated, composite endpoint such as the DAS-28. This data indicates that such a biopsied wrist joint can be incorporated in an US dataset and in clinical outcome tools such as the DAS-28 or ACR response criteria without prejudice after a mean follow up period of 14 days. This is the first report of this nature and is particularly important in helping to inform clinical and imaging dataset assessment in the context of prospective clinical trials involving a synovial biopsy at baseline.

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