RHEUMATOLOGY

Concise report

Subclinical remodelling of draining lymph node structure in early and established rheumatoid arthritis assessed by power Doppler ultrasonography

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Abstract

Objective. To investigate the suitability of power Doppler ultrasonography (PD-US) for the assessment of lymph node (LN) status in RA, evaluating the existence of structural and dynamic modifications in well-characterized stages of the disease.

Methods. Ten patients with active disease and five patients in clinical remission underwent complete clinical and PD-US examination of hands, wrists, axillary and cervical LNs on the same day. Synovitis and PD were graded 0–3. LN assessment included maximum short axis, cortical hypertrophy (CH) and PD signal distribution. All patients with active disease were re-evaluated prospectively 3 months after initiation of therapy.

Results. PD-US signs of axillary LN remodelling were observed in 7 out of 10 patients with active disease despite the absence of clinical lymphoadenopathy. Subclinical alterations were detected in both early untreated RA and in established disease. Characteristic structural changes consisted of hypertrophy of the LN cortex and PD signal amplification in cortical and hilar regions. Cervical LNs in active disease and axillary LNs in clinical remission were unaffected. LN PD amplification returned to normal ranges in patients with baseline alterations re-evaluated 3 months after therapy with TNF-α blocking agents and/or MTX.

Conclusion. Draining LNs in RA are subjected to subclinical intra-parenchymal changes and vascular flow modulation detectable by PD-US. Sonographic signs of LN involvement associate with disease activity and are reversible upon treatment. These data point at LN reactivity as a dynamic component of RA inflammatory cascade and an attractive platform to be explored in prognostic and response to therapy evaluations.

Key words: Rheumatoid arthritis, Lymph nodes, Lymphadenopathy, Doppler ultrasonography, Biological therapy.

Introduction

RA is a chronic inflammatory disease of unknown aetiology. While the SM is the main target of the inflammatory process, pathological alterations can be detected both systemically [1] and juxta-articularly [2]. In this perspective, a relevant but still poorly investigated district is the draining lymph node (LN), an anatomical structure potentially involved in multiple pathological aspects of the disease such as the control of cell efflux from the joint and the generation of local immunological responses [3].

Prompted by recent elegant studies in animal models, this issue is now the object of growing attention. Changes in LN drainage capacity have indeed been recognized in TNF transgenic mice, showing a highly predictive value for arthritis progression in the knee [4]. Draining LNs are essential for early induction of autoantibodies in K/BxN arthritic mice [5]. This process has been proved by loss of function experiments to be dependent on LN follicular

dendritic cells [6], a cell subset whose integrity is disrupted by anti-TNF therapy in RA [7]. The possible applicability of these findings to human pathology is supported by the well-known occurrence of lymphadenopathy in the course of the disease and by histological studies reporting follicular hyperplasia, increased plasmacytosis and RF deposition in peripheral LNs [8].

Despite these observations, the actual prevalence, the patho-physiological role and the dynamics of draining LN involvement in the course of human arthritis are still largely unclear due to the ethical limitations in performing serial assessments through biopsy and invasive imaging procedures. The introduction of non-invasive techniques allowing LN structural analysis in large and unbiased cohorts through serial assessments is, therefore, a key step to allow translation of current knowledge from mice to humans.

Power Doppler ultrasonography (PD-US) is an accurate and sensitive tool for the characterization of superficial LNs in humans, adopted in the pre-operative work-up of cancers, and in follow-up studies aimed at assessing the efficacy of chemotherapy or radiotherapy [9]. In this study, we have explored the suitability of PD-US for the assessment of axillary LN status in RA, evaluating the existence of structural and dynamic modifications in well-characterized stages of the disease.

Methods

Study population

Fifteen patients fulfilling the 1987 ACR revised criteria for the classification of RA [10] were selected within three disease categories according to stringent clinical parameters: (i) early untreated RA (DMARD and CS naïve, disease duration $<$6 months, 44-joint DAS $>$ 2.4). This group included two females and three males with mean (S.D.) age of 54.4 (17.7) years, disease duration of 4.4 (1.4) months and DAS 3.64 (0.45). All patients were evaluated at baseline and at 3 months after therapy with MTX 15 mg/week; (ii) established, DMARD-refractory RA (disease duration $>$12 months, candidate for anti-TNF therapy, DAS $>$ 2.4). This group included four females and one male with mean (S.D.) age of 60.4 (6.1) years, disease duration of 110.4 (142.3) months and DAS 3.84 (0.49). All patients were evaluated at baseline and at 3 months after additional therapy with etanercept 50 mg/week; and (iii) established RA in clinical remission [disease duration $>$12 months, previous history of active disease (DAS $>$ 2.4), DMARD-induced stable remission (DAS $<$ 1.6) in the previous 6–9 months]. This latter group included four females and one male with mean (S.D.) age of 55 (16.6) years, disease duration of 28.2 (10.5) months and DAS 1.09 (0.53). Five healthy volunteers (four females and one male, aged 35–68 years) were recruited as controls. Exclusion criteria: (i) clinical history of neoplastic, autoimmune and chronic infectious diseases; and (ii) vaccinations or acute infections in the 6 months before recruitment. Informed consent was obtained in all cases according to the local ethical committee guidelines. The study was approved by IRCCS Policlinico San Matteo Foundation Ethics Committee (code no. 08004598/b).

Joint and LN PD-US

Axillary and cervical LNs were examined by two independent radiologists (S.A. and F.C.) with experience in LN PD-US. A GE LogiqE9 (General Electric Medical Systems) with a high-frequency linear-array transducer (6–15 MHz) was used. The same operators blinded to clinical data performed B mode and PD examinations assessing the following structural parameters: LN maximum short axis, the degree of cortical hypertrophy (CH), vascular architecture and distribution by PD [9, 11]. Axillary LN CH and PD were rated according to semi-quantitative (CH) or qualitative (PD) three-point scores adapted from previously published methods [9, 12]. For CH: 0 = thin (hypoechoic cortex $<$ 3 mm); 1 = hypertrophic (hypoechoic cortex thicker than 3 mm); 2 = highly hypertrophic (hypoechoic cortex thicker than 6 mm) [12]; and for vascularity: 0 = no vessels or only hilar; 1 = hilar and cortical; 2 = predominantly subcapsular/cortical [9]. Exact agreement between investigators with regard to the presence of CH and PD alterations was found in 37 (92.5%) out of 40 and 36 (90%) out of 40 axillary stations analysed with weighted κ values of 0.793 and 0.813 for CH and PD scores, respectively. Minor discrepancies were resolved by mutual agreement. Synovitis was assessed using a GE LogiqE9 (General Electric Medical Systems) with a high-frequency linear-array transducer (9–15 MHz) on MCP and wrist joints as dorsal transversal and longitudinal scanning. Each joint was assessed for grey scale (GS) and PD signal with a semi-quantitative scale (0–3/joint, total score: 0–18) as previously described [13].

Serum autoantibody measurement

Immunoglobulin (Ig)M RF and IgG anti-citrullinated peptide autoantibodies (ACPAs) were determined by nephelometry (Immage 800; Beckman Coulter) and a second-generation fluorescence enzyme immunoassay (Immunocap; Phadia), respectively, according to the manufacturer's recommendations.

Results

Ten patients with active disease underwent clinical examination and PD-US assessment of axillary-cervical LNs and of ipsilateral wrist–MCP joints on the same day. At recruitment, none but one of the patients showed clinically detectable lymphadenopathy, despite evidence of arthritis in all cases (mean swollen joints/arm: 4.1, range: 0–8; mean tender joints/arm: 3.8, range: 0–9). US joint examination confirmed the presence of PD-positive signal in nine patients (median PD score/arm: 1, range: 0–9) and GS alterations in 10 out of 10 patients (median GS score/arm: 3, range: 1–7).

Despite negative clinical examination, US could reveal clear-cut changes in axillary LN structure in 7 out of 10 patients (5 out of 5 with established disease and 2 out of 5 with early RA). Blood flow alterations (score $\geqslant$1)

were detected in all seven patients, bilaterally in six out of seven cases. Compared with controls, the most distinguishable trait was PD signal amplification in LN hilus associated with its extension through the cortex, sometimes reaching the subcapsular rim (Fig. 1). Intra-parenchymal changes were detected in six out of seven patients, bilaterally in four cases and occurred as an enlargement of the hypoechoic cortical region (score 1 in all cases) associated with LN short axis increase (mean 0.8 *vs* 0.56 cm, in patients with normal cortex; *P* = 0.03 by *t*-test) (Fig. 1). Despite cortical enlargement, no signs of focal lobulation or other major morphological abnormalities [12] were observed in our study population.

LN PD-US alterations were observed in both ACPA/RF seropositive and seronegative disease. No significant differences were observed in ACPA IgG or RF IgM titres in patients with or without axillary LN modifications (data not shown). Conversely, LN status appeared related to the degree of arthritis in drained joints, as indicated by the systematic detection of normal parameters in cervical LNs during active disease and by the association between axillary LN PD-US alterations and joint PD-US scores in ipsilateral arms (Fig. 1).

In keeping with this concept, no signs of LN reactivity were observed in five out of five patients with previous history of active disease and in stable remission (Fig. 2), who showed LN PD-US features comparable with normal controls. To confirm the plasticity of LN structural changes, all patients with active disease were re-evaluated after 3 months of therapy. Eight out of 10 patients showed good (*n* = 6) or moderate (*n* = 2) EULAR response. Of note, despite persistence of LN cortical widening at this time point, PD amplification reversal was observed in six out of six patients with baseline axillary LN alterations who showed clinical response (Fig. 2), whereas no changes were observed in LNs featuring score 0 at baseline. LN PD alterations persisted after treatment in the only non-responder patient who had a positive baseline PD signal.

Discussion

We show that LNs draining affected joints in RA are subjected to intra-parenchymal structural changes and vascular flow modulation, independently of clinically

Fig. 1 Ultrasonographic appearance of axillary LNs in RA. (**A**) Representative images of an LN with thin cortex (white ↓, left panel) and rare PD signal (right panel). A PD-US image of a representative ipsilateral MCP joint is shown in (**B**). (**C**) Representative images of an LN with hypertrophic cortex (white ↓ in left panel) and PD signal amplification (right panel). A PD-US image of an ipsilateral MCP joint is shown in (**D**). In (**E**), PD and GS total scores per arm (wrist and I–V MCP joints) are plotted according to the presence (LN⁺) or absence (LN⁻) of LN alterations in ipsilateral axillae (*n* = 20). The horizontal line represents the median. *P*-value was calculated by Mann–Whitney U-test.
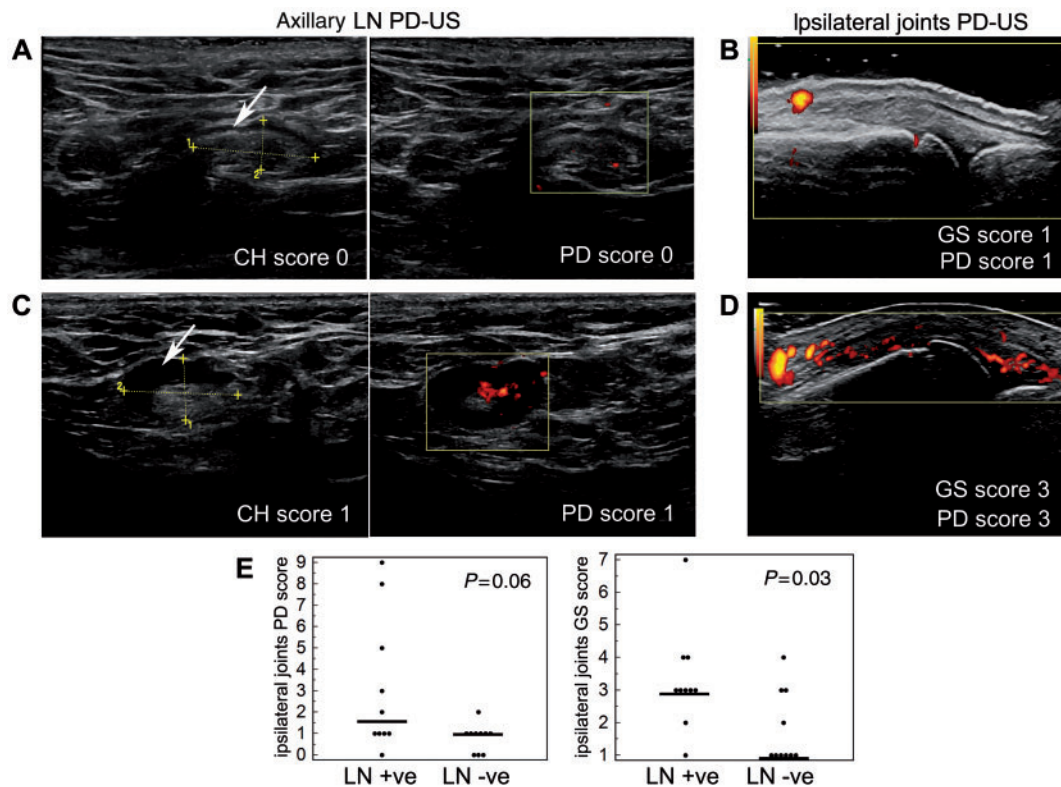
Fig. 2 Ultrasonographic appearance of axillary LNs following treatment and in remission. Representative images of LN PD-US in a patient with good EULAR response is shown in (**A**) (baseline) and (**B**) (3 months follow-up). Note the reversal of PD alterations (**B**, middle panel) despite persistency of CH (**B**, upper panel). Graphs in (**A**) and (**B**) show the mean (dark grey columns) and s.d. (error bars) of the DAS value at baseline (**A**) and at 3 months (**B**) in the eight patients with good/moderate EULAR response. Reference scale: left side of the graphs. The light grey columns show the percentage of axillary sites with LN CH and PD alterations out of 16 sites analysed. Reference scale: right side of the graphs. (**C**) A representative PD-US image of an axillary LN from a patient in clinical remission with no PD or CH alterations. The graph in (**C**) shows the mean DAS value and the percentage of axillary LN alterations in the five patients in remission.
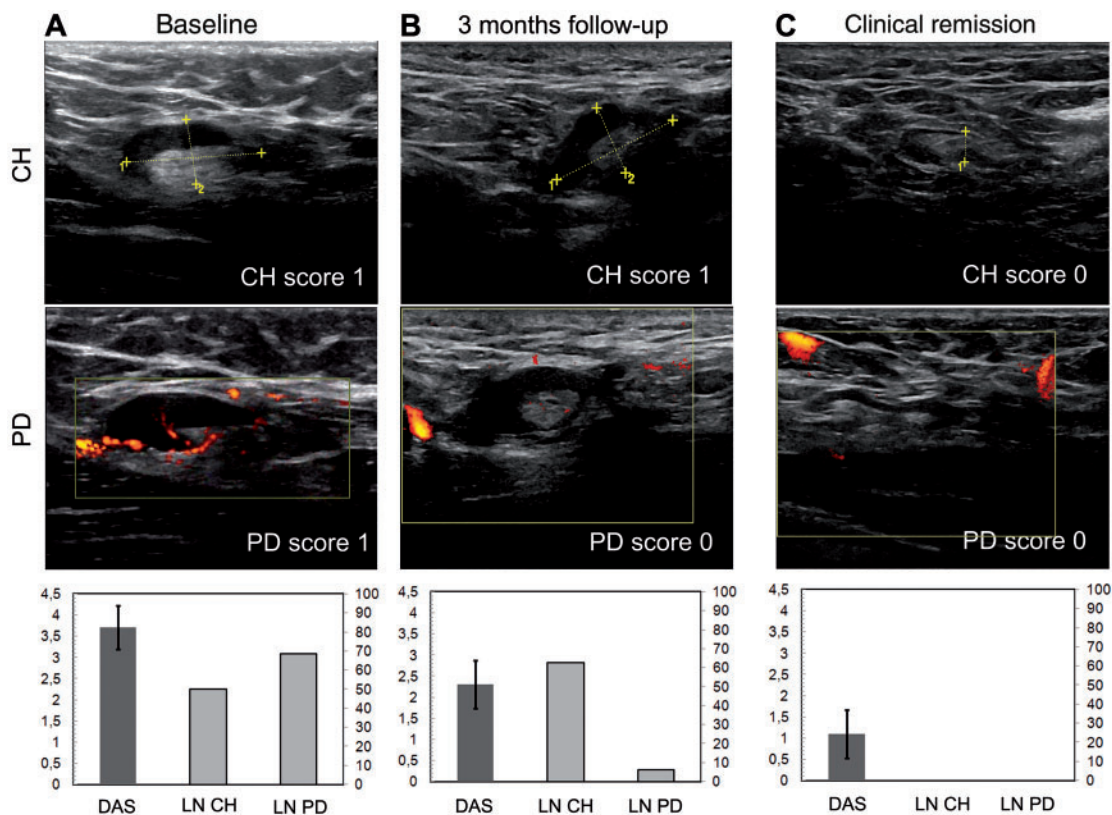
detectable lymphadenopathy. Subclinical LN involvement associates with disease activity, is reversible upon treatment and can be monitored by non-invasive techniques such as PD-US.

It has been proposed that synovitis severity and LN drainage are functionally inter-dependent in animal models, pointing at the relevance of the joint–LN complex as a pathological unit [4]. Data presented herein corroborate and extend these observations, demonstrating that involvement of LNs draining hands and wrists (the primary target of RA) can integrate joint pathology in humans both in early and established disease. This concept is relevant not only for its physio-pathological implications, but also in light of recent response to treatment studies revealing that pivotal modifications of the afferent lymphatic system and secondary lymphoid organ function are induced by effective biological agents such as anti-TNF, anti-CD20 and CTLA-4-Ig [4, 7, 14–16].

In this perspective, an important finding of our work is that PD-US signs of LN involvement in RA are not limited to dimensional increase, but include intra-nodal modifications indicative of biological changes. It has been shown that the hypoechoic cortex on sonograms relates to the follicular and paracortical areas of human LNs, the latter enriched in lymphatic sinuses and T cells [12]. The observed hypertrophy of RA LN cortical region is thus likely to be determined by specific expansion of these compartments, a phenomenon that is inducible by experimental immunization of normal mice [17] and that occurs spontaneously in TNF transgenics and K/BxN strains [4, 18]. LN reactivity in these systems associates with amplification of local blood flow [18, 19], a process that we could clearly detect by PD-US in RA.

Ultrasonographic signs of LN remodelling were detected in both ACPA/RF seropositive and seronegative RA. High ACPA/RF titres were maintained without ongoing axillary LN reactivity, indicating that LN involvement is not an intrinsic feature of the ACPA/RF+ disease subset. Rather, our cross-sectional and prospective analyses point at LN involvement as a dynamic phenomenon,

partly related to the degree of disease activity at drained sites and reversible upon anti-inflammatory treatment. As the effect of biologic therapies is not restricted to the synovium [7, 15, 16] and clinical response cannot be entirely predicted by synovial changes [20], it would therefore be relevant to investigate LN reactivity at earlier time points in larger prospective studies to dissect the relationship between LN and joint changes according to clinical outcomes. Further studies are also warranted to characterize the LN US picture in its histological and molecular aspects, in order to ascertain whether US alterations are determined by specific adaptive immunological responses or by non-specific adaptations to inflammation.

In conclusion, despite the small number of patients studied, our data suggest that the draining LN participates in the pathological cascade of the disease and that assessment of its structural changes can be explored for the definition of dynamic and clinically significant biomarkers in RA. Hypertrophy of the hypoechoic cortical region and alterations of vascular flow can be monitored prospectively through non-invasive, cost-effective imaging approaches, indicating the feasibility of their serial assessment in human research.

Rheumatology key messages

- PD-US identifies subclinical involvement of draining LNs in RA.
- Typical LN changes recognized by PD-US include CH and vascular flow amplification.
- PD-US signs of LN involvement associate with disease activity and are reversible upon treatment.

Acknowledgements

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