methods are expected to be more accurate and sensitive than the clinical composite score, with numerous basic studies in this area [36, 37]. However, this method may have the disadvantages of high cost or being time consuming in practical use. Several research groups have produced unique US-based global scores that targeted limited joint sites to prove these disadvantages [38–44]. At present, there is no consensus as to which score should be used for clinical trials or in daily practice [45]. Additional and confirmatory trials are required to establish a US-based global score. Of note, Backhaus *et al.* produced a unique US-based composite score called the US7, which targets seven joints to reflect disease activity. They reported results of several trials and have steadily progressed in their study of this score [43, 44].

Treat to target (T2T) is a concept of ideal treatment of RA that has become widespread internationally [46]. It emphasizes that RA must be treated in the early phase and tightly controlled by appropriate measurement of disease activity so that patients will receive maximum therapeutic impact and achieve the goal of remission. The US-based global scores have the potential to be useful for the achievement of T2T because US can directly detect changes in synovitis. The various US-based composite scores mentioned here are now in the evaluation process, and additional detailed analyses are anticipated.

Change in SV for assessment of local joints in RA

In our investigation focusing on local joints, we reported that remaining SV at local joints increases the risk of structural deterioration, despite the fact that anti-rheumatoid therapy achieved low disease activity (LDA) clinically at 8 weeks [25, 47]. Interestingly, these joints tended to show improvement in clinical signs such as joint pain or swelling and thus clinical composite scores also showed improvement. Similar findings were reported by another group [48]. Subclinical synovitis and sonography were first reviewed by Bresnihan *et al.* [49], and Brown *et al.* [50, 51] reported that detailed sonographic observation detected subclinical synovitis in patients with long-term clinical remission. These joints with a poor prognosis were asymptomatic or mildly symptomatic but showed positive SV at the local level. Joints with remaining SV might be related to subclinical synovitis. Further longitudinal observation may clarify this relation.

We also reported that joints with a disappearance of SV with simultaneous overall disease improvement showed an improvement in joint prognosis [52]. Dougados *et al.* [53] reported a similar conclusion in their multicentre prospective trial. These results indicate that RA requires both an improvement in overall disease activity and the disappearance of local SV for remission and achievement of T2T. Remaining SV at a local joint indicates ongoing structural alteration. Recently the EULAR published recommendations for the use of joint imaging in RA [54] in which monitoring of inflammatory activity and prediction of response to treatment by imaging were discussed. Although PDS was considered useful for these purposes, more detailed data are needed for PDS to became an established examination tool. We have focused on the response of SV to treatment that may predict structural deterioration in local joints. Multicentre studies are necessary to establish the mechanism of response of SV to treatment.

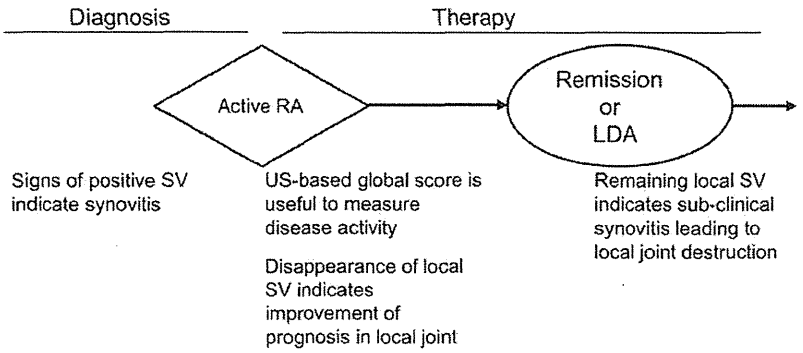
In RA, accumulation of inflammation leads to the progression of joint damage and, logically, time-integrated SV consequently relates to a change in structural alteration. Naredo *et al.* [55] showed that in the body overall, time-integrated joint counts with positive SV are related to the change in total Sharp score. We also reported that local joints showed poor prognosis when SV remained despite achievement of LDA clinically. However, changes in structural alteration of the joint are not related to time-integrated quantitative SV [52]. The reason for this unexpected result is unknown. We speculated that local synovitis might change to heterogeneous inflammation in a condition of LDA that is neither that of simple reduction of acute inflammation nor of prolonged recovery. Further studies are needed to confirm these results.

In daily clinical practice, joints with remaining SV are often detected by PDS, however, there are no definitive methods to treat them. Although these asymptomatic or mildly symptomatic joints with poor prognosis, namely those showing subclinical synovitis, need to be treated, it is unclear whether systemic intensive therapy or topical therapy are effective. Recently a research group called the Targeted Ultrasound Initiative started a multicentre international study called Targeted Ultrasound in RA to investigate the effect of corticosteroid injection in joints with remaining positive SV [56]. T2T emphasizes optimizing treatment by appropriate disease assessment, and clinical composite scores reflecting systemic disease activity are mostly used at present. The use of local assessment with US will help to achieve T2T.

Conclusion

Why should rheumatologists evaluate SV in RA? Early diagnosis and assessment of disease activity are at the heart of the T2T approach in RA. In the early diagnosis of RA, detection of SV to discover synovitis could be used as a screening test for entry into the ACR/EULAR classification algorithm. A US-based global score consisting of both the SV score and synovial hypertrophy score used to assess overall disease activity may be more sensitive and objective than the clinical composite score and thus may be useful as a guide for optimizing disease treatment. Also, changes in local SV have prognostic value for local joint destruction that may lead to meticulous control of inflammation. The evaluation of SV provides various important information and contributes to the clinical practice of RA (Fig. 2).

FIG. 2 Usefulness of SV in the clinical practice of RA.



Information obtained from SV at each clinical point is shown.

Rheumatology key messages

- Abnormal SV is strongly associated with synovitis of RA.
- Detection of SV is useful for proving the presence of synovitis and diagnosing RA.
- RA remission requires an improvement of overall disease activity and disappearance of local SV.

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Combination of MRI-detected bone marrow oedema with 2010 rheumatoid arthritis classification criteria improves the diagnostic probability of early rheumatoid arthritis

Efficient methods for distinguishing rheumatoid arthritis (RA) at an earlier phase from other diseases are strongly desired since early therapeutic intervention improves clinical and radiographic outcomes of RA.[1–4] The clinical 2010 RA classification criteria was established based upon the consensus that RA is an inflammatory disease that develops persistent and/or erosive arthritis.[2 3] Our series of studies as well as the article describing European League Against Rheumatism recommendations for the use of imaging for the clinical management of RA mention that MRI can be used to improve the certainty of a diagnosis of RA above clinical criteria.[5 6] The present study was undertaken to investigate whether MRI findings of wrist and finger joints improve the diagnostic performance of 2010 RA classification criteria. One hundred sixty-six patients with early arthritis, who do not fulfil the 1987 RA criteria or other international criteria for rheumatic disease at entry with disease duration less than 6 months (median disease duration at entry was 2 months), were consecutively enrolled from the Nagasaki Early Arthritis Cohort at our institution as previously described.[6] A total 166 patients are enrolled including 13 patients without obvious swollen joints and 2 patients with typical plain radiographic erosion. Each patient provided a signed consent form to participate in the study, which was approved by the Institutional Review Board of Nagasaki University.

All of the subjects underwent physical examination, blood tests and gadolinium diethylenetriamine penta-acetic acid-enhanced MRI (1.5T system, Sigma, GE Medical Systems, Milwaukee, Wisconsin, USA) of wrist and finger joints on the same day as described previously.[6–8] Reference standard RA of the present study was considered as reported previously[9 10] by the following two definitions: patients disease-modifying anti-rheumatic drugs (DMARDs) introduced within the 1st year or those who fulfil the 1987 RA criteria at 1 year (table 1). Figure 1 showed the classification of patients by 2010 RA classification criteria at entry or 1987 RA criteria at 1 year. We

Table 1 Classification of the 166 patients with early arthritis as reference standard RA by 2010 RA classification criteria or combination of 2010 RA classification criteria with MRI features

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Reference standard RA1*					
2010 RA classification criteria	61.9	82.6	83.3	60.6	70.5
Add-on MRI symmetrical synovitis	92.8	43.5	69.8	81.1	72.3
Add-on MRI bone marrow oedema	76.3	75.4	81.3	69.3	75.9
Add-on MRI erosion	72.2	73.9	79.5	65.4	72.9
Reference standard RA2†					
2010 RA classification criteria	61.1	77.6	76.4	62.8	68.7
Add-on MRI symmetrical synovitis	95.6	36.8	64.2	87.5	68.7
Add-on MRI bone marrow oedema	75.6	69.7	74.7	70.7	72.9
Add-on MRI erosion	71.1	68.4	72.7	66.7	69.9

The definition of synovitis, bone marrow oedema and bone erosion by MRI is made according to The Outcome Measures in Rheumatology (OMERACT) Rheumatoid Arthritis MRI Studies (RAMRIS). We have defined the patients as reference standard RA by the two methods in the present study.
*Reference standard RA1=patients introduced by any DMARDs within the 1st year.
†Reference standard RA2=patients fulfil the 1987 RA criteria at 1 year.
NPV, negative predictive value; PPV, positive predictive value; RA, rheumatoid arthritis.

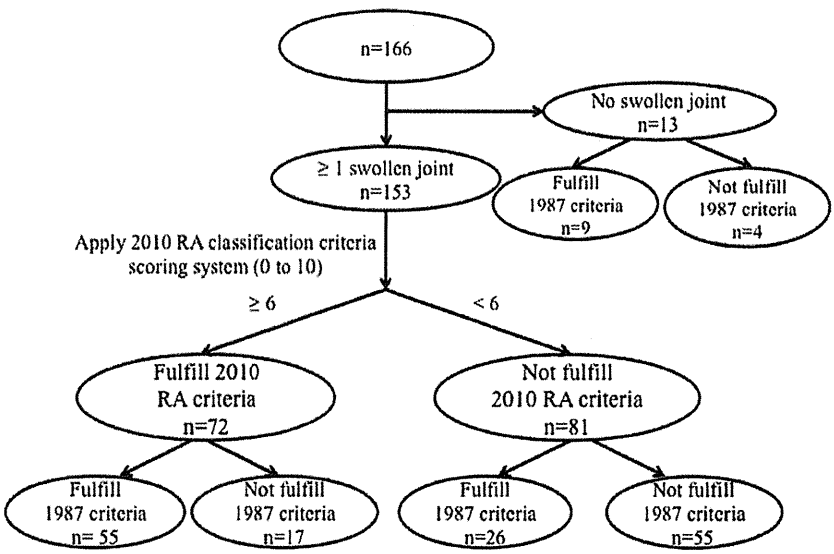

Figure 1 Classification of the patients at entry by 2010 rheumatoid arthritis (RA) classification criteria or at 1 year by 1987 RA criteria. 2010 RA classification criteria or 1987 RA criteria was applied towards 166 patients. The former was applied at entry whereas the latter at 1 year, respectively.

investigated the diagnostic performance of 2010 RA criteria with or without the finding of MRI-detected pathology. 2010 RA classification criteria classified RA with a sensitivity of 61.9%, a specificity of 82.6%, a positive predictive value of 83.3%, a negative predictive value of 60.6% and an accuracy of 70.5% if reference standard RA is considered as patients DMARDs introduced within the 1st year (table 1). The results were similar if RA is considered as the patients who fulfil the 1987 RA criteria at 1 year (table 1). As compared with symmetrical synovitis and bone erosion, bone marrow oedema was the most useful MRI finding since the positive predictive value of bone marrow oedema (84.9%) was higher than symmetrical synovitis (72.0%) or bone erosion (81.0%) in the reference standard patients with RA (DMARDs introduced within the 1st year). The results were similar if RA is considered as the patients who fulfil the 1987 RA criteria at 1 year (data not shown). We used a decision-tree algorithm that involves initially applying 2010 RA classification criteria, and if the patient does not fulfil these criteria, the MRI-detected bone marrow oedema rule is introduced. The tree algorithm has been shown to differentiate patients more efficiently than the 2010 RA classification criteria alone, exhibiting better sensitivity, negative predictive value and accuracy for the classification of reference standard RA (table 1). The present findings are the first evidence that the diagnostic probability of early RA using the 2010 RA classification criteria is improved by combining these criteria with MRI-detected bone marrow oedema of the wrist and finger joints. Our study may strengthen the statements of the European League Against Rheumatism recommendations for the use of imaging.

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Contributors Study design: MT, KaA, MU, KiA, KE, AK. Data acquisition: MT, JK, YN, TS, YH, AO, TK, S-yK, NI, KI, KaA, SY, HN, TO, MU, AF, KiA, KE, AK. Statistical analysis: MT, KaA, KiA, KE, AK. Discussion: MT, JK, YN, TS, YH, AO, TK, S-yK, NI, KI, KaA, SY, HN, TO, MU, AF, KiA, KE, AK. Paper preparation: MT, KaA, MU, KiA, KE, AK.

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EXTENDED REPORT

Cell cycle regulation therapy combined with cytokine blockade enhances antiarthritic effects without increasing immune suppression

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ABSTRACT

Objective Biological disease-modifying antirheumatic drugs (DMARDs) that inhibit aberrant immune reactions in rheumatoid arthritis (RA) cannot induce complete remission in all patients. Combination therapies using two biological DMARDs have failed to exert additive effects and increased serious infections. We have found that cell cycle inhibition of synovial fibroblasts with cyclin-dependent kinase (CDK) inhibitors ameliorated the disease in animal models of RA without attenuating acquired immunity. The objective of this study was to determine whether a clinically well-tolerated selective CDK 4/6 inhibitor (CDKI), palbociclib, is effective and whether combination with cytokine blockers acts additively without enhancing immune suppression.

Methods The effects of CDKI on haematopoiesis and physical and behavioural findings in mice were evaluated. Mice with collagen-induced arthritis (CIA) were treated with CDKI, etanercept or anti-interleukin (IL)-6 receptor antibody (MR16-1) alone or with a combination of CDKI with etanercept or MR16-1. Their clinical, histological and radiographic scores, serum anti-(type II collagen (CII)) antibody levels and proliferative responses of lymph node cells to CII were determined.

Results Although CDKI induced marginal myelosuppression, it was well tolerated and ameliorated CIA dose-dependently. The combinations of low-dose CDKI and either tumour necrosis factor-α or IL-6 blocker enhanced the antiarthritic effects additively. The addition of CDKI to either cytokine blocker did not affect the levels of anti-CII antibodies and proliferative responses of lymphocytes to CII.

Conclusions A clinically well-tolerated CDK4/6 inhibitor exerted antiarthritic effects in this mouse model. By combining therapeutic agents targeting immune reaction and synovial proliferation, we have demonstrated for the first time that two molecular targeting treatments act additively and may not increase immune suppression.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterised by synovial inflammation and hyperplasia. Previous studies have revealed that genetic and environmental factors contribute to the development of RA by activating innate immunity, generating aberrant autoreactive immune cells, and breaking the self-tolerance.[1 2] These responses induce production of various cytokines from activated lymphocytes and macrophages and their recruitment to the affected joints. These inflammatory processes provoke intense proliferation of synovial fibroblasts, which become activated to form a platform of inflammation, becoming another source of inflammatory cytokines and producing tissue-degrading proteinases. Thus, inflammatory and proliferative phases are two interacting processes in the pathogenesis of RA that form a vicious circle, resulting in joint dysfunction and destruction.[3 4]

Biological disease-modifying antirheumatic drugs (DMARDs) are designed to inhibit the effects of inflammatory cytokines or lymphocytes involved in RA.[5] They include tumour necrosis factor (TNF)-α- and interleukin (IL)-6-blocking agents and lymphocyte-targeting agents. These agents have been proved to be effective in management of RA and have resulted in disease remission in a proportion of patients with RA.[6] However, no biological DMARD has been shown to achieve more than 40% of American College of Rheumatology (ACR) 70 response in clinical trials.[7 8] Switching one biological DMARD for another results in ~10% of ACR 70 response.[9 10] Moreover, combination therapies using two biological DMARDs, such as etanercept with abatacept or etanercept with anakinra, failed to produce additive antirheumatic effects and were associated with an increase in serious infections.[11 12] These results indicate that excessive suppression of the inflammatory phase of RA could be harmful.

We have demonstrated that cell cycle regulation of the synovial fibroblasts may be a distinctive new therapeutic approach that inhibits the proliferative phase of RA. This approach focuses on cyclin-dependent kinase (CDK) and its inhibitors as primary cell cycle regulators. Inhibition of CDK4/6 activity by intra-articular transfer of endogenous CDK inhibitor genes or systemic administration of small-molecule CDK inhibitors ameliorated animal models of RA without attenuating acquired immune responses.[13–15] A large-scale genome-wide association study recently identified genes encoding CDK4/6 as RA risk genes, providing epidemiological support for the idea that inhibition of CDK4/6 activity may be a promising antirheumatic strategy.[16]

Palbociclib (formerly called PD0332991) is a highly selective CDK4/6 inhibitor (CDKI) under development as an anticancer agent.[17] Although its monotherapy was not effective in clinical trials,

combination therapy with letrozole extended progression-free survival of patients with advanced breast cancer over threefold compared with letrozole monotherapy.[18] Of note, it was clinically well tolerated, inducing transient myelosuppression in only a fraction of the treated patients.

To enhance the therapeutic effects without increasing the adverse effects, we assumed that blocking both inflammatory and proliferative phases would suppress arthritis synergistically. Generally, combination therapy is used to increase the therapeutic effect and reduce the toxicity of each agent.[19] The synergistic effect allows the required dose of each agent to be reduced, thereby decreasing adverse effects. The present study was conducted, using a mouse model of RA, to determine whether a clinically well-tolerated selective CDKI, palbociclib, exerts antiarthritic effects, and whether CDKI ameliorates arthritis by synergising with cytokine blockers without affecting the acquired immune responses.

MATERIALS AND METHODS

Mice

Six-week-old male DBA/1J mice were purchased from the Japan Charles River Breeding Laboratories (Kanagawa, Japan) and maintained in the animal facility at the Tokyo Medical and Dental University. All procedures in the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University.

Compounds and antibodies

Palbociclib was synthesised by Chemie Tek (Indianapolis, Indiana, USA) and dissolved in 0.5% methylcellulose. Etanercept was purchased from Takeda Pharmaceutical Co (Osaka, Japan). Rat anti-mouse IL-6 receptor IgG1 monoclonal antibody (MR16-1, rat IgG1)[20] and anti-dinitrophenol monoclonal antibody (KH-5) were provided by Chugai Pharmaceutical Co, (Tokyo, Japan).

Safety evaluation

Palbociclib or vehicle solution was administered orally to normal 9-week-old DBA/1J mice for 16 consecutive days. Physical and behavioural abnormalities were monitored during the treatment. Peripheral blood cell numbers were evaluated before and after 16 days of treatment.

Evaluation of collagen-induced arthritis (CIA)

CIA was induced as described previously.[21] Swelling in four paws was graded from 0 to 4: 0, no swelling; 1, detectable swelling in one joint; 2, non-severe swelling in two or more joints; 3, severe swelling in two or more joints; 4, severe swelling in two or more joints including digital swelling. The total score for the four paws was used as the arthritis score. Histological and radiological scores were evaluated in a blinded manner as described previously.[22 23]

Antigen-specific humoral and lymphocyte responses

Serum samples were collected 40 or 35 days after the initial immunisation for quantification of anti-(type II collagen (CII)) IgG antibodies with ELISA.[21] Inguinal lymph nodes (LNs) were isolated 40 days after the initial immunisation. Single-cell suspensions (5×10^5 cells per well in a round 96-well plate) derived from bilateral inguinal LNs were cultured in the presence or absence of 50 μg/mL denatured CII for 72 h. Cell proliferation was evaluated using [^{3}H]thymidine incorporation.[21]

Statistical analysis

The arthritis scores, histological scores and radiological scores were analysed statistically with the Mann–Whitney U test. Numbers of peripheral blood cells, levels of anti-CII antibody and relative percentage of [^{3}H]thymidine incorporation were compared with Student's paired t test.

RESULTS

Treatment of CIA with palbocilib (CDKI)

We first evaluated the toxicity of CDKI, palbociclib, which is a clinically well-tolerated CDK4/6 inhibitor, on DBA1/J mice. When mice were treated for 16 consecutive days with 20, 50 and 100 mg/kg CDKI or a vehicle control, no physical or behavioural abnormalities were observed. In clinical trials, CDKI treatment induced dose-dependent but manageable and reversible myelosuppression.[18 24 25] In the present study, treatment with 100 mg/kg CDKI decreased the numbers of white blood cells, red blood cells and platelets, while treatment with 50 mg/kg decreased platelet numbers (figure 1A). Although the mean number of neutrophils decreased in all of the treated groups, none of the mice developed severe neutropenia. Neutrophils might be more vulnerable than lymphocytes, which is consistent with clinical trials.[18 24]

To assess the antiarthritic effects of CDKI, we immunised DBA/1J mice with bovine CII and administered CDKI orally at doses of 20, 50 and 100 mg/kg. When CDKI administration was started before the onset of the disease, 21 days after the initial immunisation for 10 days, CDKI ameliorated the clinical manifestation of CIA dose-dependently (figure 1B). Mice treated with 100 mg/kg CDKI hardly developed arthritis. These results correspond well to a previous study, which used less specific CDK4/6 inhibitors.[15]

Treatment of CIA with CDKI and/or the TNF blocker, etanercept

Accumulated evidence indicates that TNF-α is a major contributor to the pathogenesis of RA.[26 27] Indeed, inhibiting TNF-α activity is an effective treatment for RA.[5] To study whether CDK4/6 and a TNF-α inhibitor would act synergistically in treating arthritis even after the onset of the disease, we treated CIA mice from 25 days until 39 days after the initial immunisation with 20 mg/kg CDKI or etanercept (3 or 10 mg/kg) or a combination of 20 mg/kg CDKI and 3 mg/kg etanercept, which reacts with murine TNF-α.[28]

CDKI and etanercept suppressed the disease similarly (figure 2A). Arthritis scores in mice treated with 3 and 10 mg/kg etanercept were almost equivalent, suggesting that the therapeutic effects of etanercept were saturated at 3 mg/kg. We found that the combination of CDKI and 3 mg/kg etanercept suppressed the progression of arthritis additively (figure 2A). The effect was immediate and complete.

Histological examination of the joints from the vehicle-treated mice revealed mononuclear cell infiltration, synovial hyperplasia, cartilage degeneration and bone erosion, which were all suppressed in the mice treated with either agent, and suppressed further in the mice treated with the combination of CDKI and etanercept (figure 2B, D). Radiological examination of the joints from the vehicle-treated mice revealed bone destruction, which was suppressed in the mice treated with etanercept alone and with the combination (figure 2C, E).

To evaluate the efficacy of combination therapy on severely established arthritis, we treated CIA mice from 30 days until 40 days after the initial immunisation with 20 mg/kg CDKI or 3 mg/kg etanercept or a combination of 20 mg/kg CDKI and 3 mg/

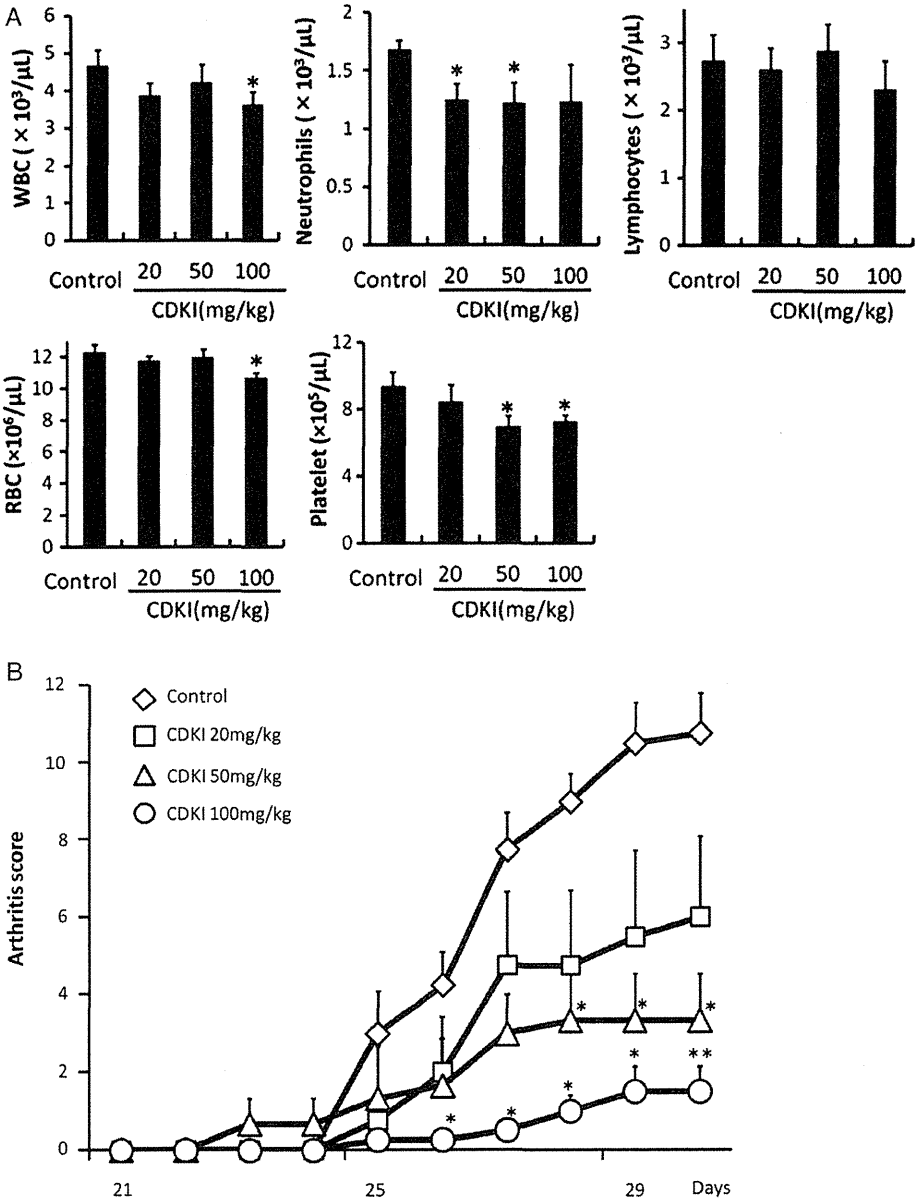

Figure 1 Evaluation of cyclin-dependent kinase inhibitor (CDKI) with regard to its safety and antiarthritic effects on collagen-induced arthritis (CIA). CDKI (20, 50 and 100 mg/kg/day) or vehicle was administered orally to normal 9-week-old DBA1/J mice for 16 consecutive days. Peripheral blood cell numbers were evaluated 16 days after the initial treatment (A). CIA mice were treated with oral administration of CDKI at the indicated doses or vehicle from 21 days until 30 days after the initial immunisation. Severity of arthritis was assessed as arthritis scores (B). Data represent the mean and SE. The statistical significance of differences between control group and each treated group was determined (*p<0.05; **p<0.01). WBC, white blood cell; RBC, red blood cell.

kg etanercept. TNF blockade and CDKI inhibited the increase in clinical scores, and, in addition, the combination therapy further ameliorated this severely developed arthritis (figure 2F).

Effects of treatment with CDKI and/or etanercept on antibody production and lymphocyte responses

We next assessed whether CDKI or etanercept affects acquired immune responses when administered individually or together. Serum anti-CII antibodies were quantified to assess antigen-specific humoral responses. No significant difference was observed between the treated and control mice (figure 3A). Cells were isolated from bilateral inguinal LNs and stimulated with 50 μg/mL CII for 72 h to assess CII-specific lymphocyte responses. No significant difference in proliferative responses was observed between the treated and control mice (figure 3B). Thus, the addition of CDKI did not induce suppression of CII-specific humoral and proliferative responses.

Treatment of CIA with CDKI and/or the IL-6 blocker, MR16-1

IL-6 plays a major role in the inflammation of RA and is an effective target for therapy, which has been demonstrated by the success of tocilizumab, anti IL-6 receptor monoclonal antibody[29 30] and MR16-1 (anti-mouse IL-6 receptor monoclonal antibody) on CIA.[20] It was reported that MR16-1 inhibited CIA

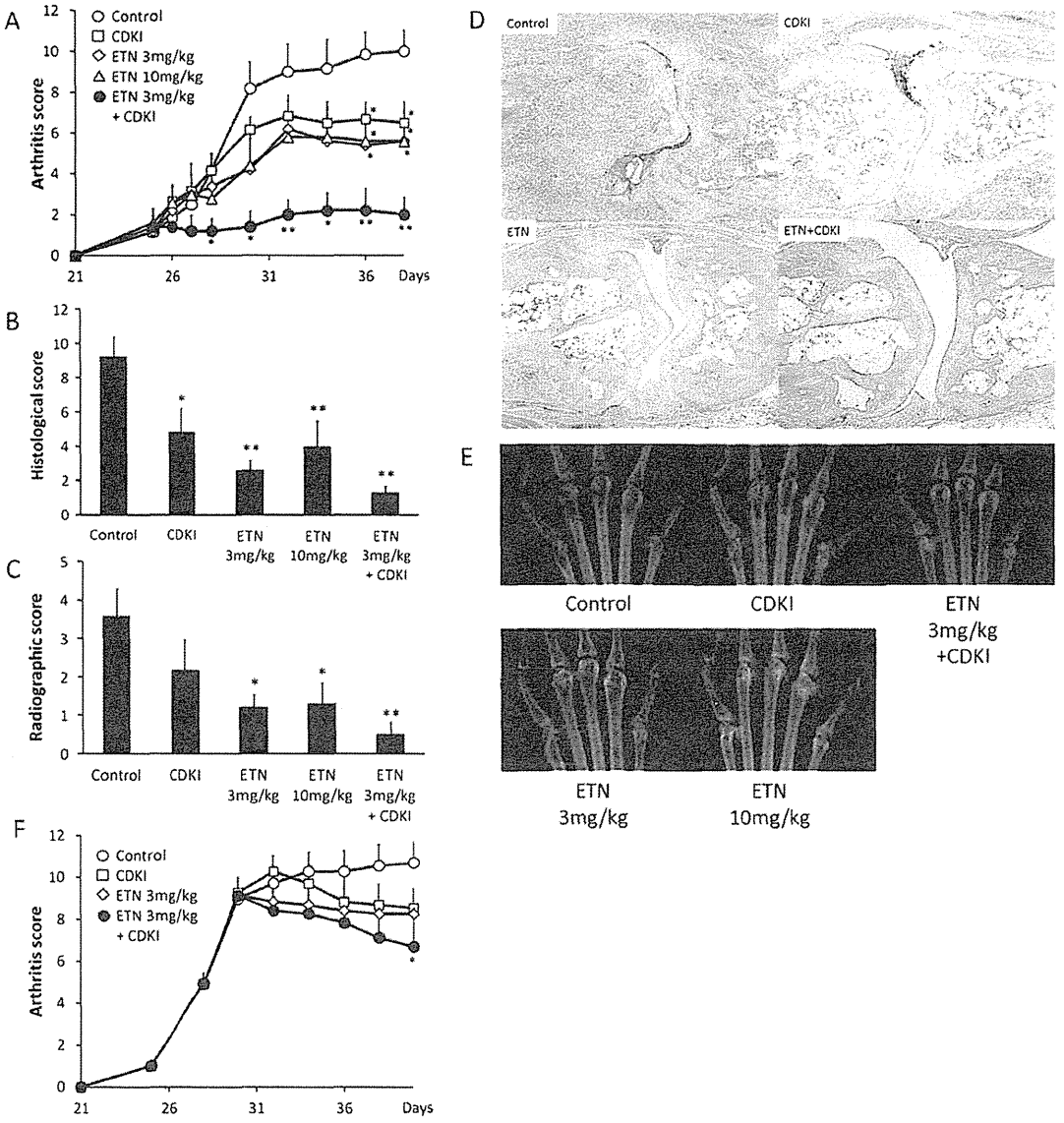

Figure 2 Antiarthritic effects of cyclin-dependent kinase inhibitor (CDKI) or etanercept (ETN) individually or together on collagen-induced arthritis (CIA). Mice immunised with type II collagen (CII) were divided into 5 groups (5 mice each) equating to the mean arthritis score of individual groups 25 days after the initial immunisation, and treated with 20 mg/kg CDKI or ETN (3 or 10 mg/kg), or in combination with 20 mg/kg CDKI and 3 mg/kg ETN from 25 days until 39 days after the initial immunisation (A–E). Data are representative of two independent experiments. The severity of arthritis was assessed using arthritis scores (A), histological scores (B) and radiographic scores (C). Representative histology (D) and radiology (E) images of CIA joints treated with ETN and/or CDKI are presented. Histological and radiographic findings of bilateral 2–4 metatarsophalangeal joints 40 days after the immunisation were assessed using haematoxylin and eosin staining of formalin-fixed paraffin-embedded sections or radiographic images. Similarly, mice immunised with CII were divided into 4 groups (7 mice each) equating to the mean arthritis score of individual groups, and were excluded if their arthritis score was >13 or <5 30 days after the initial immunisation. They were treated with 20 mg/kg CDKI or 3 mg/kg ETN, or in combination with 20 mg/kg CDKI and ETN from 30 days until 40 days after the initial immunisation (F). Data represent the mean and SE. The statistical significance of differences between control group and each treated group was determined (*p<0.05; **p<0.01).

only in the preventive protocol.[20] Its antiarthritic effect and immunosuppression depend on the dose (see online supplementary figure S1A–C): 2 mg/mouse of MR16-1 suppressed the development of arthritis less than 8 mg/mouse of MR16-1, and decreased the serum levels of anti-CII antibodies without suppressing the proliferative responses to CII. To evaluate the synergistic effects of the combination of inhibiting CDK4/6 activities and IL-6 signalling, we treated CIA mice with 20 mg/kg CDKI or 2 mg/mouse MR16-1, or in combination, or with a control monoclonal antibody (KH-5, anti-dinitrophenol monoclonal antibody). In accordance with the published report,[20] both monoclonal antibodies were administered intraperitoneally after the initial immunisation. Either 20 mg/kg CDKI or a vehicle control was administered orally from 21 days until 39 days after the initial immunisation. While CDKI and MR16-1 both decreased the arthritis scores, the combination of the two agents ameliorated the arthritis additively (figure 4A).

Histological findings on the joints were suppressed by CDKI and combination therapy (figure 4B). Inhibition of radiological bone destruction was not observed in mice treated with CDKI or MR16-1, but was apparent in mice treated with the combination (figure 4C).

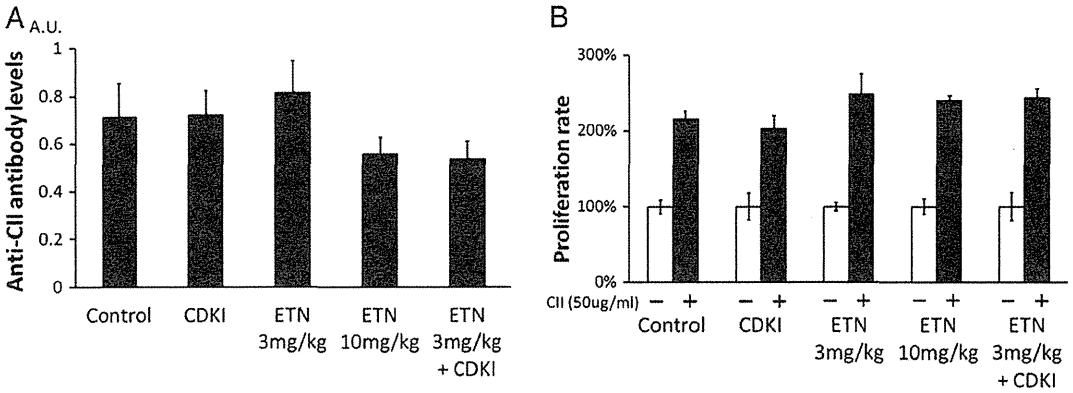

Figure 3 Effects of individual or combined treatment with cyclin-dependent kinase inhibitor (CDKI) and etanercept on anti-(type II collagen (CII)) antibody levels and lymphocyte proliferative responses to CII. Forty days after the initial immunisation, blood sample were collected and inguinal LNs were isolated. Serum anti-CII antibodies were quantified using a specific ELISA (A). A standard mixture of sera from control mice was defined arbitrarily as one unit. CII-specific proliferation of LN-derived cells stimulated with CII was quantified by the amount of [^{3}H]thymidine incorporation into DNA (B). Proliferation rates are shown as the relative percentage of the data for cultured cells without CII. Values represent the mean and SE.

Effects of CDKI and/or MR16-1 treatment on antibody production and lymphocyte responses

Because suppression of the antibody response by MR16-1 has been reported,[20 31] we investigated the effects of MR16-1 and CDKI on acquired immune responses when administered individually or together. Serum levels of anti-CII antibody were decreased equally in mice treated with MR16-1 alone or in combination with CDKI (figure 5A). When bilateral inguinal LN-derived cells were stimulated, no significant differences in proliferative responses were observed between the treated and control mice (figure 5B). Thus, the addition of CDKI did not lead to further suppression of CII-specific humoral responses and did not induce suppression of proliferative responses to CII.

DISCUSSION

We have demonstrated that a clinically well-tolerated selective CDK4/6 inhibitor, palbociclib, which targets the proliferative phase of RA, ameliorated the disease in an animal model of RA, and that the combination of palbociclib with cytokine blockers that targeting the inflammatory phase, which is the other pathological phase of RA, exerted antiarthritic effects additively without increasing suppression of acquired immune responses.

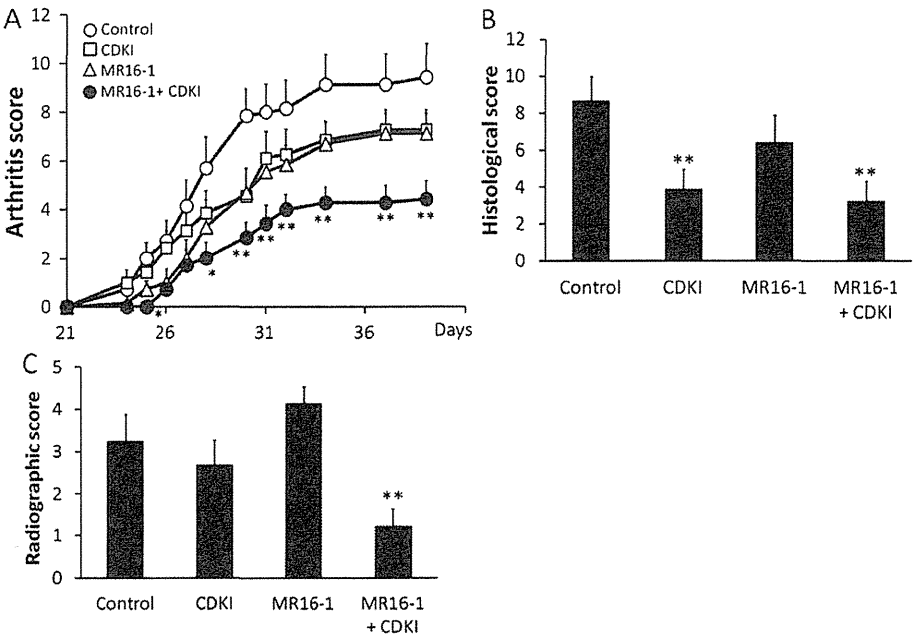

Figure 4 Antiarthritic effects of cyclin-dependent kinase inhibitor (CDKI) or anti-mouse interleukin-6 receptor monoclonal antibody (MR16-1) individually or together on collagen-induced arthritis. Mice in each group (7 mice per group) were treated with 20 mg/kg CDKI or 2 mg/mouse MR16-1, or in combination, or with control antibody. Both monoclonal antibodies were administered after the initial immunisation. CDKI or a vehicle control was administered from 21 days until 39 days after the initial immunisation. The severity of arthritis was assessed using arthritis scores (A), histological scores (B) and radiographic scores (C) 40 days after the immunisation. Data are representative of two independent experiments. Data represent the mean and SE. The statistical significance of differences between control group and each treated group was determined (*p<0.05; **p<0.01).

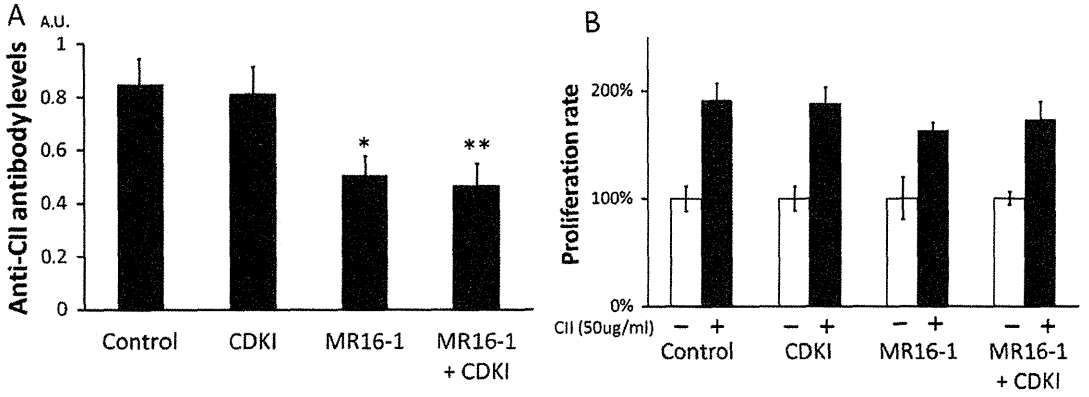

Figure 5 Effects of individual or combined treatment with cyclin-dependent kinase inhibitor (CDKI) and MR16-1 on anti-(type II collagen (CII)) antibody levels and lymphocyte proliferative responses to CII. Blood samples were collected 35 days after the initial immunisation. Serum anti-CII antibodies were quantified using a specific ELISA (A). Inguinal LNs were isolated 40 days after the initial immunisation. CII-specific proliferation of LN-derived cells stimulated with CII was quantified by the amount of [^{3}H]thymidine incorporation into DNA (B). Proliferation rates are shown as the relative percentage of the data for cultured cells without CII (B). Values represent the mean and SE. The statistical significance of differences between control group and each treated group was determined (*p<0.05; **p<0.01).

Although the use of biological DMARDs has improved the management of RA, there are still quite a few patients who respond inappropriately to anti-inflammatory DMARDs,[7 8] indicating the limitations of anti-inflammatory therapy. The addition of CDKI, which has a different therapeutic target, to biological DMARDs enhances the antirheumatic effects by a distinctive mode of action.

As biological DMARDs are used widely, their safety and cost effectiveness have been discussed with regard to appropriate dosage and indication.[30] The most serious adverse effect of biological DMARDs is immunosuppression. The results of several clinical trials on RA revealed that patients receiving higher doses of biological DMARDs are at a higher risk of serious infections.[32 33] Recent studies of etanercept have revealed that the standard dose is marginally superior in therapeutic efficacy to a half dose.[34 35] We expect that addition of CDKI will reduce the dose of biological DMARDs required, leading to a reduced risk of serious infections and increased cost effectiveness.

We have previously reported the antiarthritic effects of a pan-CDK inhibitor, alvocidib, and a moderately selective CDK4/6 inhibitor, compound A.[15 36 37] In the present study, palbociclib, a selective CDK4/6 inhibitor, exerted antiarthritic effects dose-dependently in a similar way to these two compounds. It has been reported that palbociclib had little or no inhibitory activity against a panel of 350 kinases that included a wide variety of serine/threonine or tyrosine kinases, while compound A inhibited 6 among 45 of these kinases.[15 38] Unlike palbociclib, compound A at high concentration inhibited growth of tumour cells that do not depend on excessive CDK4/6 activity and production of some cytokines and osteoclast formation from bone-marrow-derived macrophages (our unpublished observations). Palbociclib is the most selective CDK4/6 inhibitor available at the moment.

The 20 mg/kg dose of CDKI exerted an antiarthritic effect without apparent impairment of tissue turnover except for marginal neutropenia. More than 100 mg/kg palbociclib was required to inhibit tumour growth in a mouse model of breast cancer.[39] These results indicate that synovial fibroblasts may be more sensitive than other cells to cell cycle inhibition by CDKI. In clinical trials, patients with advanced breast cancer were treated with 125 mg/day (1.8 mg/kg) palbociclib.[18] The doses used in mice were a lot higher than those used in humans. However, drug doses used in animals can differ greatly from those used in humans based on the differences in drug absorption and metabolism. This dosage of palbociclib used for cancer induced mild neutropenia in 60% of patients, without any febrile events.[18] Although we cannot accurately predict adverse effects in humans on the basis of results from animal models, we expect that, if patients with RA respond to a lower dose of palbociclib than patients with cancers, adverse effects such as myelosuppression would be reduced in frequency and severity.

As cytokine blockers and CDKI differ in their modes of action, the difference might be reflected by the histological findings on synovial tissues from the treated mice. It has previously been reported that TNF inhibition can inhibit recruitment of arthrogenic inflammatory cells into the joints from the regional lymph nodes.[40] Our previous report demonstrated that compound A ameliorated CIA and decreased the number of phosphorylated retinoblastoma protein positive cells in the treated joints.[15] CDK4/6 specifically phosphorylates retinoblastoma protein, which is an essential process for cell cycle progression. The present study included side-by-side treatments with cytokine blockers and CDKI, and thus offered a preliminary chance to study the differences. Etanercept appeared to suppress inflammatory cell infiltration, while CDKI more clearly suppressed synovial hyperplasia. Both histological findings were suppressed by the combination therapy (figure 2D). Although cell cycle inhibition by CDKI should interfere with formation of the pannus, which is the platform of inflammation, further research will elucidate how the two treatments exert additive effects, which remains speculative at this moment.

CDK4/6 inhibition in the present study did not affect established autoreactivity to CII, indicating that it would not affect the acquired immune responses. However, it remains to be clarified whether CDK4/6 inhibition would affect beneficial acquired immune responses, such as vaccine responses. Further research on CDK4/6 inhibition should demonstrate its effects on other immunological reactions, which require cellular proliferation, and enable us to estimate the latent risk of infections.

Our studies indicate that CDK4/6 inhibitors may constitute a new class of antirheumatic drugs that act additively with biological therapies targeting immunity. Investigation of CDK4/6 in the treatment of rheumatic diseases will be facilitated by the fact that such drugs have already been tested in patients with cancer.

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Contributors Design of the study: TH, YY and HK. Acquisition of data: TH and YY. Interpretation of data: TH, HI, YY, KK, NM and HK. Manuscript preparation: TH, HI, KK, NM and HK. All authors approved the final version.

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ORIGINAL ARTICLE

Analysis of subclinical synovitis detected by ultrasonography and low-field magnetic resonance imaging in patients with rheumatoid arthritis

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Abstract

Objective To assess the utilities of ultrasonography (US) and low-field magnetic resonance imaging (compacTscan, cMRI) in the diagnosis of subclinical synovitis of hand joints of patients with rheumatoid arthritis (RA).

Methods A total of 1,540 joints of 77 RA patients were examined clinically, using US, using cMRI, and the baseline X-ray examination was performed. Clinical synovitis was defined as joint tenderness or swelling. Subclinical synovitis was diagnosed by US and by cMRI. The incidence of bone erosion and joint space narrowing was assessed by X-ray examination performed at approximately 40 weeks of follow-up.

Results Of the hand joints examined, 294 (19.1 %) were diagnosed with clinical synovitis, and 218 joints (14.1 %) were diagnosed with subclinical synovitis. The remaining 1,028 joints (66.8 %) were synovitis-free on clinical examination and imaging. For the diagnosis of subclinical synovitis, cMRI (11.4 %) was significantly more sensitive than power Doppler signals detected by US (US-PD; 6.8 %) ($P < 0.01$), and the combination of US-PD and cMRI was more useful (14.1 %) than US-PD or cMRI alone ($P < 0.05$). Follow-up X-ray examination of 600 joints showed a significantly higher incidence of bone erosion in joints with subclinical synovitis than in synovitis-free joints ($P < 0.05$).

Conclusion US-PD and cMRI are useful for detecting subclinical synovitis in patients with RA. Subclinical synovitis of the small joints of the hand can progress to bone destruction.

Keywords Bone erosion · Magnetic resonance imaging · Rheumatoid arthritis · Synovitis · Ultrasonography

Introduction

Rheumatoid arthritis (RA) is a common chronic inflammatory polyarticular disease associated with joint destruction and functional impairment. Early diagnosis and treatment of RA are therefore important for preventing joint destruction [1, 2]. In 2010, the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) recommended a new classification system [3] for the early diagnosis of RA patients to replace the ACR classification criteria recommended in 1987 [4]. Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis according to the 2010 ACR/EULAR criteria. Both MRI and US are useful for diagnosing early RA.

Conventional radiography is used to diagnose RA and monitor the progression of joint destruction. However, this technique visualizes only the late signs of preceding disease activity. Both magnetic resonance imaging (MRI) and ultrasonography (US) have improved the early diagnosis and monitoring of RA activity, and accumulating evidence suggests that MRI can be used to assess bone erosion, edema, and synovitis of RA joints, while the power Doppler (PD) signal on US (US-PD) can detect synovitis and intra-articular vascularity [5, 6]. Previous studies demonstrated that the presence of synovitis and edema on MRI and intra-articular hypervascularity on US-PD were

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high risk for bone erosion on X-ray radiography [6, 7]. Thus, early diagnosis of joint synovitis by MRI and US-PD as well as treatment can potentially prevent the progression of bone destruction. The present study examined the utility of low-field MRI (cMRI) and US-PD in the diagnosis of subclinical synovitis, and the clinical significance of subclinical synovitis in relation to future bone erosion and joint space narrowing (JSN).

Materials and methods

Patients and materials

We examined 1,540 hand joints of 77 patients with established RA who visited our hospital between February 2010 and April 2012. The patients fulfilled either the 1987 ACR criteria or 2010 ACR/EULAR criteria. Rheumatologists clinically examined 20 hand joints in each patient, including the first to fifth metacarpophalangeal (MP) joints, the second to fifth proximal interphalangeal (PIP) joints, and the wrist. The presence of tenderness and joint swelling was recorded separately for every joint. In some patients, the physical examination, US, and cMRI were performed on the same day; in the other patients, although they were not performed on the same day, the interval between them was kept as short as possible.

Ultrasonography

US examinations were performed on 154 hands of the 77 RA patients by five rheumatologists, using an Aplio^{TM} XG (Toshiba Medical Systems Corporation, Ohtawara, Japan) with a 7–14 MHz linear array transducer. The examiner observed the dorsal aspects of the 20 joints, the radial aspects of the first and second fingers, and the ulnar aspects of the first and fifth fingers. The palmar aspects were not examined. The ultrasonographic findings for the PIP and MP joints were scored according to a semiquantitative scoring system [grayscale (GS) from grade 0 to 3 and US-PD from grade 0 to 3] introduced in an earlier report [8]. Wrists were also scored semiquantitively; however, the grading was based on the subjective assessment of the examiner. The grades were assigned by consensus among the five rheumatologists. The US examiners were not completely blinded to the results of clinical examinations. The presence of US-PD grade $\geq$1 was recorded separately for every joint.

Magnetic resonance imaging

We invented a new low-field extremity MRI technique (compacTscan; cMRI, Cross Tech Corporation, Tokyo, Japan), with a 0.3 T static magnetic field, as reported previously [9]. The findings obtained by cMRI showed high agreement with those obtained by conventional 1.5 T MRI with contrast medium in RA patients [10]. Two rheumatologists separately operated the cMRI and reported the findings. These rheumatologists had been repeatedly trained together by a radiologist with expertise in musculoskeletal MRI. The cMRI examiners were not completely blinded to the results of the clinical examinations. Joint synovitis was defined as synovium showing a high-intensity signal on short tau inversion recovery (STIR) sequence in cMRI.

X-ray examination

The baseline X-ray examination was performed within two months of the physical examination, US, and cMRI. X-ray examinations of bilateral hands were performed using standard postero-anterior and oblique views. All X-rays were digitized and interpreted by the same rheumatologist. Locations in the hand that were used to assess erosion and JSN were the PIP of digits 2–5, the interphalangeal (IP) joint of the thumb and MP joints 1–5, carpometacarpal (CMC) joints 1–5, all carpal bones, the radiocarpal joint, and the styloid process of the ulna. Therefore, the locations used for erosion and JSN assessments in this study were different from those used in the Genant–modified Sharp scoring system [11] and the van der Heijde–Sharp scoring system [12]. The IP joint of the thumb was excluded from this study because it was not assessed by cMRI [9]; it is, however, included in the Genant–modified Sharp erosion scoring system, in the JSN scoring system, and in the van der Heijde–Sharp erosion scoring system. The locations used for erosion assessment in this study included CMC joints 2–5 and the capitate, hamate, and triquetral bones, which are not included in either scoring system. The trapezoid and lunate bones were assessed for bone erosion in this study, although these are not included in the Genant–modified Sharp scoring system. The JSN in CMC joints 1–2 and some of the spaces around carpal bones were assessed in this study; however, these are not included in either scoring system. Another X-ray was taken as follow-up X-ray and compared with the baseline X-ray. Any development and progression of bone erosion and JSN during this interval was noted.

Definition of subclinical synovitis

We defined an imaging abnormality as a US-PD grade of 1–3 and a high-intensity signal on STIR sequence of cMRI when imaging a neither tender nor swollen joint of a patient diagnosed with RA, based on the 1987 ACR classification or 2010 EULAR/ACR classification.

Statistical analysis and ethical issues

Fisher's exact test was used to compare the incidences of clinical and subclinical synovitis, bone erosion, and JSN during the follow-up. P values of <0.05 were considered statistically significant.

Results

Demographic and baseline characteristics

A total of 1,540 joints were evaluated in 77 patients. Table 1 details the patient profiles. The mean estimated RA duration was 7.2 ± 5.5 years. The mean tender joint count was 2.2 ± 2.5 per person and the mean swollen joint count was 4.2 ± 5.0 per person. Twenty-three patients were in remission, as indicated by a disease activity score 28-C-reactive protein (DAS28-CRP) ≤2.3; 4 presented low disease activity (LDA), with 2.3 < DAS28-CRP ≤ 2.7; 21 showed moderate disease activity (MDA), with 2.7 < DAS28-CRP ≤ 4.1; and 15 showed high disease activity (HDA), with DAS28-CRP >4.1. The DAS28-CRP was not calculated for 14 patients because the visual analog scale score was not recorded for the global health assessment of each of these patients. Fifty (64.9 %) patients were treated with prednisolone. Sixty (77.9 %) patients were treated with methotrexate at a mean dose of 7.6 ± 1.2 mg/week. Forty-four (57.1 %) patients were treated with biologics (infliximab, etanercept, adalimumab, tocilizumab, and abatacept).

For 13 patients, the physical examination, US, and cMRI were performed on the same day; for the other 64 patients, they were performed on different days but the interval between them was kept as short as possible. The mean interval between the physical examination and US was 9.2 ± 10.3 days, that between the physical examination and cMRI was 9.0 ± 10.5 days, and that between the US and cMRI was 5.9 ± 11.9 days.

Two hundred ninety-four joints (19.1 %) presented with tenderness and/or swelling: 70 joints presented with tenderness and swelling; 45 with tenderness only; and 179 with swelling only; 1,246 joints (80.9 %) showed neither tenderness nor swelling (Table 2).

Diagnosis of subclinical synovitis by US-PD and cMRI

Among the 294 joints with clinical synovitis, 123 joints were considered positive on US-PD, 133 joints showed high intensity on STIR sequence, and 170 joints were positive for synovitis on US-PD and/or cMRI. One hundred five joints (6.8 %) were positive for subclinical synovitis as detected by US-PD, 176 joints (11.4 %) were positive by based on high-intensity on STIR sequence, and 218 joints (14.2 %) were positive by according to US-PD and/or high-intensity STIR sequence (Fig. 1; Table 2). The incidence of cMRI subclinical synovitis (11.4 %) was significantly higher than that of US-PD subclinical syonovitis (6.8 %) ($P < 0.01$), and that of US-PD and/or cMRI subclinical synovitis (14.1 %) was higher than that of cMRI subclinical synovitis (11.4 %) ($P < 0.05$). The rates of positivity for clinical synovitis of the joints on US-PD, cMRI, and US-PD and/or cMRI were higher than the corresponding rate for joints that were neither tender nor swollen: 41.8 % > 8.4 % ($P < 0.01$), 45.2 % > 14.1 % ($P < 0.01$), 57.8 % > 17.5 % ($P < 0.01$), respectively.

US-PD subclinical synovitis was detected in 22 (3.6 %) of 616 PIP joints, 43 (5.6 %) of 770 MP joints, and 40 (26.0 %) of 154 wrists (Fig. 2a). cMRI subclinical synovitis was detected in 26 (4.2 %) PIP joints, 100 (13.0 %) MP joints, and 50 (32.5 %) wrists (Fig. 2b). US-PD and/or cMRI subclinical synovitis was detected in 40 (6.5 %) PIP joints, 118 (15.3 %) MP joints, and 60 (39.0 %) wrists (Fig. 2c). Another X-ray was taken as follow-up X-ray and compared with the baseline X-ray. The incidence of subclinical synovitis detected by cMRI, and US-PD and/or cMRI in MP joints was significantly higher than that in PIP joints ($P < 0.01$), and that detected by US-PD, cMRI, and US-PD and/or cMRI in wrists was higher than that in MP joints; however, the difference of that detected by US-PD between the MP joints and PIP joints was not significant.

Twenty-two PIP joints with US-PD subclinical synovitis were graded as follows: 3 (13.6 %) GS grade 2 (GS-2) and 19 (86.4 %) GS grade 3 (GS-3) (Fig. 2d). Forty-three MP joints with US-PD subclinical synovitis consisted of 7 (16.3 %) GS grade 1 (GS-1), 10 (23.2 %) GS-2, and 26 (60.5 %) GS-3. Forty wrists with US-PD subclinical synovitis consisted of 11 (27.5 %) GS-1, 21 (52.5 %) GS-2, and 8 (20.0 %) GS-3. Taken together, 105 joints with US-PD subclinical synovitis consisted of 18 (17.1 %) GS-1, 34 (32.4 %) GS-2, and 53 (50.5 %) GS-3.

The PIP joints with US-PD subclinical synovitis consisted of 11 (50.0 %) PD grade 1 (PD-1), 9 (40.9 %) PD grade 2 (PD-2), and 2 (9.1 %) PD grade 3 (PD-3) (Fig. 2e). The MP joints with US-PD subclinical synovitis consisted of 23 (53.5 %) PD-1, 19 (44.2 %) PD-2, and 1 (2.3 %) PD-3. Wrists with US-PD subclinical synovitis consisted of 23 (57.5 %) PD-1, 15 (37.5 %) PD-2, and 2 (5.0 %) PD-3. Taken together, the joints with US-PD subclinical synovitis consisted of 57 (54.3 %) PD-1, 43 (40.9 %) GS-2, and 5 (4.8 %) PD-3.

Based on the disease activity score 28 C-reactive protein (DAS28-CRP) findings, 1,260 joints of 63 patients were divided into four groups: remission ($n = 23$), LDA ($n = 4$), MDA ($n = 21$), and HDA ($n = 15$). The proportions of the joints with clinical synovitis with respect to

Table 1 Characteristics of patients with RA

Parameter	Value
Number of patients	77
Number of joints examined	1,540
Age (years)	50.6 ± 14.3
Sex (male: female)	13 : 64
Disease duration (years)	7.2 ± 5.5
Stage (I:II:III:IV:unknown)	26:26:13:8:4
Tender joint count (28 joints)	2.2 ± 2.5
Swollen joint count (28 joints)	4.2 ± 5.0
DAS 28-CRP (remission:LDA:MDA:HDA:unknown)	23:4:21:15:14
Rheumatoid factor (positive:negative)	60:17
Anti-CCP Ab (positive:negative:unknown)	49:9:4
Prednisolone (number, %)	50 (64.9 %)
Prednisolone (dose, mg/day)	5.1 ± 2.5
Methotrexate (number, %)	60 (77.9 %)
Methotrexate (dose, mg/w)	7.6 ± 1.2
History of treatment with biologics	44
Patients treated with IFX, ENT, ADA, TCZ, and ABT	20, 8, 4, 9, 3
Time between physical examination and US (days)	9.2 ± 10.3
Time between physical examination and cMRI (days)	9.0 ± 10.5
Time between US and cMRI (days)	5.9 ± 11.9

Data are the mean ± SD or the number of patients

DAS 28-CRP Disease Activity Score 28 C-reactive protein, *LDA* low disease activity, *MDA* moderate disease activity, *HAD* high disease activity, *anti-CCP Ab* anti-cyclic citrullinated peptide antibody, *IFX* infliximab, *ETN* etanercept, *ADA* adalimumab, *TCZ* tocilizumab, *ABT* abatacept, *US* ultrasonography, *cMRI* low-field MRI

Table 2 Incidence of joints with clinical synovitis, subclinical synovitis, and no synovitis in patients with RA

	TEN and/or SW	US-PD	cMRI	Number of joints (%)
Clinical synovitis	+	+	+	86 (5.6)
	+	+	−	37 (2.4)
	+	−	+	47 (3.0)
	+	−	−	124 (8.1)
Subtotal				294 (19.1)
Subclinical synovitis	−	+	+	63 (4.1)
	−	+	−	42 (2.7)
	−	−	+	113 (7.3)
Subtotal				218 (14.1)
No synovitis	−	−	−	1,028 (66.8)
Total				1,540 (100.0)

TEN tenderness, *SW* swelling, *US-PD* power Doppler signal detected by ultrasonography, *cMRI* high intensity on STIR sequence of low-field MRI without gadolinium enhancement

the total number of joints examined in the remission, LDA, MDA, and HDA groups, respectively, were 3.5, 23.8, 30.5, and 31.7 % (Fig. 3). The incidence of clinical synovitis in the remission group was significantly lower than the corresponding incidences in the LDA, MDA, and HAD groups ($P < 0.01$). The proportions of joints with subclinical synovitis detected by US and/or cMRI with respect to the total number of joints examined in the remission, LDA, MDA, and HDA groups, respectively, were 15.4, 16.3, 12.4, and 14.7 %, respectively. The incidences of subclinical synovitis were not significantly different among these four groups.

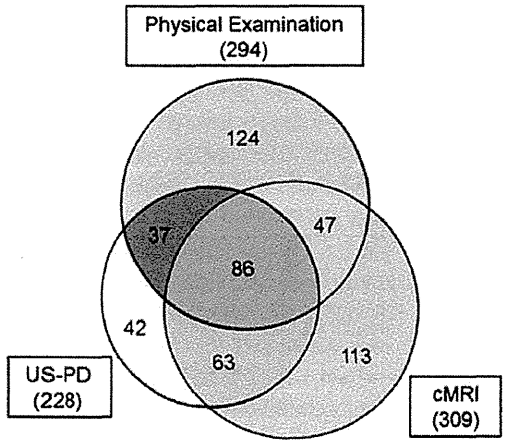

Fig. 1 Venn diagram of joint involvement. 1,540 joints were investigated via physical examination, ultrasonography, and low-field magnetic resonance imaging. More than one abnormality was observed in each of 512 joints by physical examination, ultrasonography, and/or low-field magnetic resonance imaging. No abnormality was detected in 1,028 joints. *US-PD* joints with a power Doppler signal detected by ultrasonography, *cMRI* joints with high intensity on short tau inversion recovery sequence of low-field MRI without gadolinium enhancement

Clinical significance of subclinical synovitis

Six hundred joints of 30 RA patients underwent follow-up X-ray examinations after the initial examination by US-PD and/or cMRI. One hundred twenty of those joints presented tenderness and/or swelling at baseline. Among the other 480 joints examined at baseline, subclinical synovitis was diagnosed in 30 joints by US-PD, in 54 joints by cMRI, and in 64 joints by US-PD and/or cMRI (Table 3). The mean interval between the two follow-up X-ray examinations performed was 282 ± 150 days.

Two (1.7 %) of the 120 joints with clinical synovitis at baseline had developed bone erosion at the follow-up X-ray examination, and the incidence of bone erosion in the joints with clinical synovitis was significantly higher than the corresponding incidence in the 416 joints that were free of tenderness and swelling and showed no imaging abnormalities (0.0 %) ($P < 0.05$) (Table 3). Six (5.0 %) of the joints with clinical synovitis developed JSN, and the incidence of JSN in the joints with clinical synovitis was significantly higher than that in the 416 joints with no

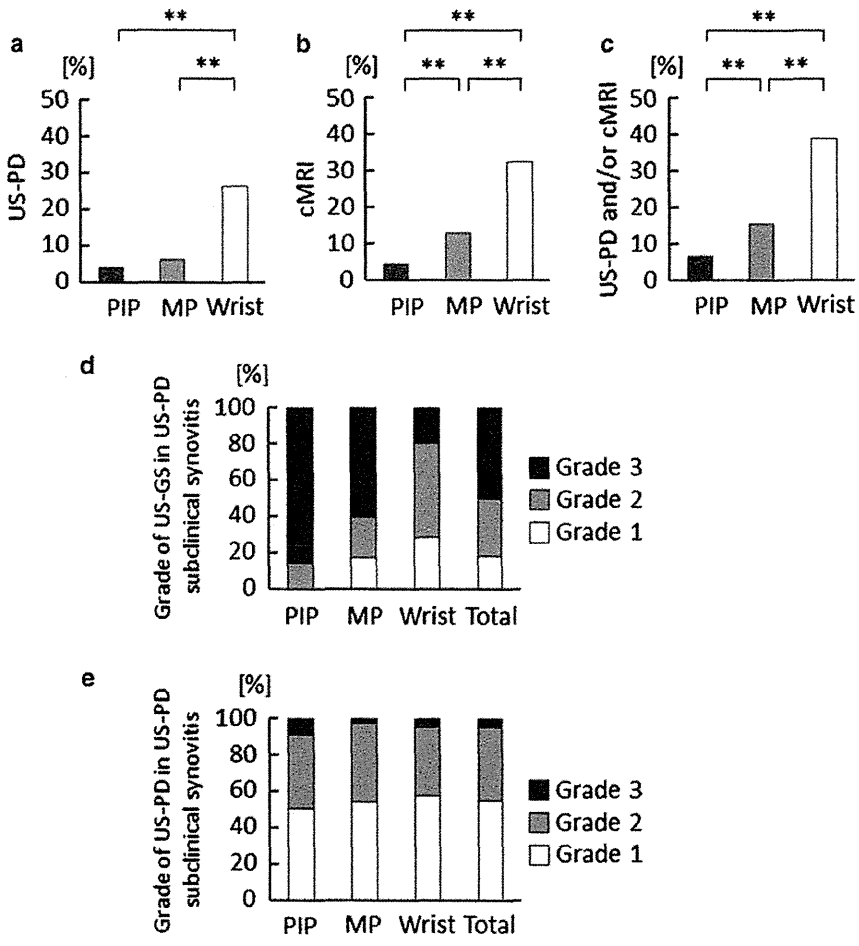

Fig. 2 The incidence of subclinical synovitis, as diagnosed by power Doppler ultrasonography and low-field MRI, in proximal interphalangeal joints, metacarpophalangeal joints, and wrist joints of patients with rheumatoid arthritis. The incidence of subclinical synovitis detected by ultrasonography (**a**), by short tau inversion recovery sequence of low-field MRI (**b**), and by ultrasonography and/or short tau inversion recovery sequence of low-field MRI (**c**). Proportions of the three US-GS grades (**d**) and the three US-PD grades (**e**) for each type of joint with subclinical synovitis. *PIP* proximal interphalangeal joint, *MP* metacarpophalangeal joint, *US-PD* joints with a power Doppler signal detected by ultrasonography, *MRI* joints with synovitis detected on short tau inversion recovery sequence by low-field MRI without contrast medium; $^{**}P < 0.01$

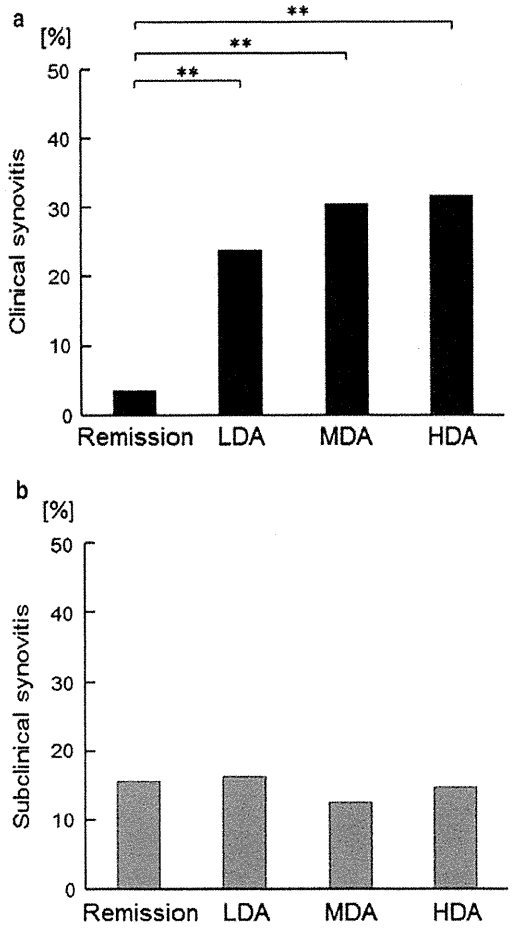

Fig. 3 a Proportion of the number of tender and/or swollen joints (i.e., those with clinical synovitis) with respect to the total number of joints examined in four groups categorized according to the DAS28-CRP score: remission ($n = 23$), LDA ($n = 4$), MDA ($n = 22$), and HDA ($n = 15$). **b** Proportion of the number of joints that were neither tender nor swollen, but showed imaging abnormality on power Doppler ultrasonography and/or short tau inversion recovery sequence of low-field MRI without contrast medium, with respect to the total number of joints examined in the remission, LDA, MDA, and HDA groups. *LDA* low disease activity, *MDA* moderate disease activity, *HDA* high disease activity, $^{**}P < 0.01$

tenderness, swelling, nor imaging abnormalities (0.5 %) ($P < 0.01$).

Two (3.1 %) of the 64 joints with US-PD and/or cMRI subclinical synovitis had developed bone erosion at the follow-up X-ray examination, and the incidence of bone erosion in the joints with US-PD and/or cMRI subclinical synovitis was significantly higher than that in 416 joints that were free of tenderness and swelling and showed no imaging abnormalities (0.0 %) ($P < 0.05$). The incidence of bone erosion (3.3 %) in the 30 joints with US-PD subclinical synovitis was not significantly higher than that in the 450 joints that were free of clinical symptoms and US-PD synovitis (0.2 %), while 2 (3.7 %) of the 54 joints with cMRI subclinical synovitis developed bone erosion, and the incidence of bone erosion in the joints with cMRI subclinical synovitis was significantly higher than that in the 426 joints that were free of clinical symptoms and cMRI syonovitis (0.0 %) ($P < 0.05$). One (5.0 %) of the 20 joints with US-PD and cMRI subclinical syonovitis developed bone erosion, and the incidence of bone erosion in the joints with US-PD and cMRI subclinical 309 synovitis was significantly higher than that in the joints that 310 were free of any type of synovitis (0.0 %) ($P < 0.05$). Two (3.1 %) of the 64 joints with US-PD and/or cMRI subclinical synovitis developed JSN; however, the incidence of JSN in the joints with US-PD and/or cMRI subclinical synovitis was not significantly higher than that in the 416 joints with no tenderness, swelling, nor imaging abnormalities (0.5 %).

There was no significant difference between the incidences of bone erosion (1.7 %) and JSN (5.0 %) in the 120 joints with clinical synovitis and the corresponding incidences in the 64 joints with subclinical synovitis (3.1 and 3.1 %, respectively). There was also no significant difference between the incidences of bone erosion (2.9 %) and JSN (8.8 %) in the tender and/or swollen 68 joints with imaging abnormalities confirmed by US-PD and/or cMRI and the corresponding incidences in the 64 joints with subclinical synovitis.

Discussion

Early diagnosis and treatment of RA is necessary to prevent the progression of bone destruction, including treatment with biologics such as anti-TNF-α, anti-IL-6R, CTLA-4 Ig, and anti-B cell antibodies [1, 2]. Although the ACR/EULAR classification criteria published in 2010 represent improved standard diagnostic criteria for early-stage RA, clinical arthritis is still defined as tender or swollen joints. Imaging studies such as US and MRI are therefore essential for detecting subclinical synovitis. Brown et al. [13] reported that 43.3 % of RA patients without painful, tender, and/or swollen joints showed high US-PD signals, and 96.2 % of those patients showed evidence of synovitis on enhanced MRI. In the present study, we evaluated the utilities of US and cMRI for the detection of subclinical synovitis in patients with RA, and found that the rates of subclinical synovitis were 11.4 and 6.8 % using cMRI and US-PD, respectively. Moreover, the combined use of cMRI and US-PD led to more sensitive detection of subclinical synovitis (rate of 14.2 %) compared with cMRI or US-PD alone. These findings support the notion that, in patients with RA, subclinical synovitis can be diagnosed by cMRI and/or US-PD, and confirm that these imaging

Table 3 Bone erosion and joint space narrowing identified on follow-up X-ray examination in joints diagnosed with clinical and subclinical synovitis by US-PD and cMRI

	T/S	US-PD	cMRI	Number of joints (%)	Bone erosion	Joint space narrowing
Clinical synovitis	+	+	+	31 (5.2)	1	2*
	+	+	−	14 (2.3)	1*	0
	+	−	+	23 (3.8)	0	4**
	+	−	−	52 (8.7)	0	0
Subtotal				120 (20.0)	2*	6**
Subclinical synovitis	−	+	+	20 (3.3)	1*	1
	−	+	−	10 (1.7)	0	0
	−	−	+	34 (5.7)	1	1
Subtotal				64 (10.7)	2*	2
No synovitis	−	−	−	416 (69.3)	0	2
Total				600 (100.0)	4	10

T/S tenderness and/or swelling, *US-PD* power Doppler signal detected by ultrasonography, *cMRI* high intensity on short tau inversion recovery sequence of low-field MRI without gadolinium enhancement

* $P < 0.05$ compared with the no-synovitis group; ** $P < 0.01$ compared with the no-synovitis group

modalities are valuable tools for detecting the residual inflammation that may cause the joint destruction.

Several studies [6, 7, 14–20] have reported the identification of synovitis [14, 15], bone edema [16–18], and bone erosion on MRI, as well as synovitis and hypervascularity [15, 17, 19, 20] on US-PD, predating the development of bone destruction on X-ray. The present study demonstrated that joints with subclinical synovitis detected by cMRI or US-PD are more likely to progress to show bone erosion on X-ray than joints free of any synovitis are. The frequencies of bone erosion and JSN on follow-up X-rays were similar for joints identified as having clinical and subclinical synovitis at baseline (Table 3). Together, these observations suggest that subclinical synovitis diagnosed by cMRI or US-PD can be used to predict bone erosion and JSN on follow-up X-ray examination. Hence, patients identified with subclinical synovitis should receive more intensive treatment to prevent the development of bone destruction.

Foltz et al. [21] showed that synovitis detected by low-field MRI (0.2 T) with gadolinium contrast did not predict structural disease progression. They considered that this was due to the high sensitivity of MRI, which could identify nonpathogenic synovitis. On the other hand, our study indicated that low-field plain MRI (0.3 T) can predict the progression of bone erosion. This discrepancy between Foltz's study and our study may be due to the different magnetic field strengths employed and the use of contrast medium in Foltz's study but not in ours.

An incompatibility between US-PD and plain cMRI was observed in this study. Incompatibilities have also been observed between PD-US and contrast enhanced MRI [22, 23] and GS/PD and plain cMRI [24] in previous reports. That may be due to differences between the targets of the examinations. High intensity on the STIR sequence of MRI indicates synovium and fluid collection. US-PD detects blood perfusion on inflamed synovium. Joints that show high intensity on the STIR sequence but no US-PD indicate fluid collection and synovium without blood perfusion. The cause of joints with US-PD but which do not show high intensity on the STIR sequence remains to be determined. The incidence of bone erosion in joints with subclinical synovitis detected by cMRI was higher than that in joints with neither clinical symptoms nor cMRI synovitis. The incidence of bone erosion in joints with subclinical synovitis detected by US-PD was not significantly different from that in joints with no clinical symptoms nor US-PD synovitis. However, it is reported that serial detection of US-PD synovitis predicted total joint destruction [6, 20, 21] and individual joint destruction [25]. We cannot conclude that US-PD is inferior to cMRI at this time.

Our results showed that wrists are more prone to developing subclinical synovitis than PIP and MP joints are (Fig. 2). This could be due to the difficulty involved in detecting tenderness and swelling of the wrist joint when compared with other sites, such as the PIP and MP joints.

The RA disease activity based on DAS28 also did not correlate with the incidence of subclinical synovitis, suggesting that confirmation of synovitis by imaging examination is necessary in order to prevent or lessen the progression to bone destruction, even in RA patients who are in remission (Fig. 3).

The results of this study permit only limited interpretation, for a number of reasons. First, progression of joint destruction was observed in only a small number of patients. Second, the patients in this study presented