A prospective, randomised, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue


Published in:
Annals of the Rheumatic Diseases

DOI:
10.1136/ard.2008.091389

Citation for published version (APA):
A prospective, randomised, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue

A W R van Kuijk,1 D M Gerlag,1 K Vos,1,2 G Wolbink,2 M de Groot,3 M A de Rie,3 A H Zwinderman,4 B A C Dijkmans,2,5 P P Tak1

ABSTRACT
Objective: To determine which of the changes in synovial tissue correlates best with clinical response associated with effective therapy (adalimumab) to facilitate the planning of future studies with therapeutic agents for psoriatic arthritis (PsA).

Methods: A total of 24 patients with active PsA were randomised to receive adalimumab (n = 12) or placebo (n = 12) for 4 weeks. Synovial biopsies were obtained before and after 4 weeks of treatment. Immunohistochemical analysis was performed to characterise the cell infiltrate, expression of cytokines and matrix metalloproteinases (MMPs) and vascularity. Sections were analysed by digital image analysis. Statistical analysis was performed using covariance analysis.

Results: The mean Disease Activity Score in 28 joints (DAS28) after 4 weeks was 1.92 units lower (95% confidence interval (CI) 1.07 to 2.77) after adalimumab therapy compared with placebo. Paired pretreatment and post-treatment synovial samples were available from 19 patients. Many cell types were reduced after adalimumab treatment compared to placebo. After applying a ranked analysis of covariance (ANCOVA) model to correct for baseline imbalances, a significant effect of treatment was observed on CD3-positive cells: there was a median reduction of 248 cells/mm² after adalimumab versus placebo treatment (p = 0.035). In addition, the expression of MMP13 was significantly reduced after active treatment: the integrated optical density (IOD)/mm² was 18 190 lower after adalimumab treatment as compared to placebo (p = 0.033).

Conclusion: Adalimumab therapy in PsA is associated with a marked reduction in T cell infiltration and MMP13 expression in synovial tissue, suggesting that these parameters could be used as biomarkers that are sensitive to change after active treatment in small proof of concept studies in PsA.

The increase in the development of a variety of new, targeted therapies clearly raises the need for sensitive biomarkers, which could be used for selection purposes during the development process. In RA a decrease in synovial macrophages has been shown to correlate with clinical improvement.6–10 No synovial changes were detectable with analysis of serial synovial samples from patients with RA who received either placebo or ineffective treatment.9–13 This suggests that analysis of serial biopsies could be used as a screening method to test new compounds requiring relatively small numbers of subjects. The absence of changes in the ST after treatment would suggest that the therapy is probably not effective.

To date, only a few studies have been conducted in PsA evaluating synovial changes after therapy. These studies were not placebo controlled, with biopsies taken at different time points and in part with variable results.6–18 Based on the limited data available it was hypothesised that an early decrease in macrophages (or macrophage subsets), combined with decrease in vascular markers and/or adhesion molecules, which were observed in some of these studies, would best predict clinical response in PsA.

The primary objective of this study was, therefore, to investigate the early changes in the ST alongside clinical response, by using a known clinically effective therapy (ie, adalimumab 40 mg subcutaneously every other week),19 to identify sensitive biomarkers that may facilitate the planning of future studies with novel agents to treat PsA.

PATIENTS AND METHODS
Patients
Patients with PsA fulfilling the CAS泗fication of Psoriatic ARthritis (CASPAR) criteria for PsA,20–21 aged 18–80 years, were included into the study after written informed consent was obtained. Patients had to have active disease at time of entry, defined by the presence of at least 2 tender and 2 swollen joints out of the 68 joints for tenderness and 66 joints for swelling assessed. One of the swollen joints had to be a knee, ankle or wrist joint that was accessible for arthroscopy.

Patients were allowed to use concomitant methotrexate, which had to be stable for at least 28 days. They were not allowed to use any other disease-modifying antirheumatic drugs (DMARDs)
1 month prior to baseline. For leflunomide, a 2-month washout period was required. Use of non-steroidal anti-inflammatory drugs was allowed, provided that the dose had been stable for at least 28 days. Parenteral, intra-articular or oral use of corticosteroids within 28 days before enrolment into the study was not allowed. Topical treatments for psoriasis were not allowed 14 days prior to baseline, with the exception of low potency (class I) topical steroids to be used on scalp, palms, groin and/or soles of feet only. Other exclusion criteria were the use of any biological agent or investigational drug within the previous 6 months and having a history of tuberculosis or a malignancy in the past 10 years. Infection with HIV, hepatitis B or C virus was excluded via serological testing. Patients with another serious infection within 4 weeks before baseline, or a significant history of cardiac, renal, neurological or metabolic disease were excluded from the study. Female patients who were pregnant or breastfeeding were not allowed to enter the study.

**Study protocol**

This was a randomised, double-blind, placebo-controlled, single centre study performed at the Academic Medical Center of the University of Amsterdam. The study protocol was approved by the Medical Ethics Committee of the institute and all patients signed informed consent before the start of the study. They had a complete medical history taken and underwent a full physical examination, including joint assessment by a rheumatologist and skin assessment by a dermatologist. Routine clinical chemistry, haematology and urinalysis assessments were performed, as well as a chest x-ray and purified protein derivative (PPD) skin test prior to baseline to exclude patients with (latent) tuberculosis. Eligible patients were included into the study within 2 weeks after screening, and were seen at baseline, week 4 and week 12.

Clinical assessment included a 68 joint count for tenderness and 66 joint count for swelling, a psoriasis area and severity index (PASI), a subject’s visual analogue scale (VAS) score for pain, ranging from 0 mm (no pain) to 100 mm (the worst possible pain), a subject’s and investigator’s global disease assessment and a Health Assessment Questionnaire (HAQ) for functional (dis)ability. Furthermore, C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were determined. A blinded, independent assessor performed the clinical evaluation. The 28-joint Disease Activity Score (DAS28), which has been shown to discriminate between active drug and placebo in clinical trials in PsA, was chosen to monitor changes in disease activity after study medication. The 28-joint Disease Activity Score (DAS28), which has been shown to discriminate between active drug and placebo in clinical trials in PsA, was chosen to monitor changes in disease activity after therapy. In addition, the American College of Rheumatology (ACR) criteria for improvement were assessed.

**Treatment**

Patients were randomised to receive subcutaneous injections with either adalimumab 40 mg or matching placebo at baseline (day 1) and day 15 in a 1:1 ratio. After the second arthroscopy all patients received adalimumab 40 mg every other week.

**Arthroscopy**

A needle arthroscopy of an actively inflamed joint (knee, ankle, or wrist) was performed under local anaesthesia in all patients before treatment and 28 days after initiation of study medication from the same joint. The procedures for needle arthroscopy and tissue processing were performed as described previously in detail. In summary, to minimise sampling error biopsies were taken from six or more sites of the joint during each procedure. ST biopsy specimens were immediately embedded in TissueTek OCT (Miles Diagnostics, Elkhart, Indiana, USA), snap frozen by immersion in methylbutane (−80°C) and stored in liquid nitrogen until further processing. Before staining, coded ST samples were cut with a cryostat (5 μm), fixed with acetone and endogenous protease activity was blocked with 0.5% hydrogen peroxide.

**Immunohistochemical analysis**

Serial sections were stained with the following antibodies: anti-CD3 (SK7; BD Biosciences, San Jose, California, USA), anti-CD4 (SK3; BD Biosciences), anti-CD8 (C8/114B; Dako, Glostrup, Denmark), anti-CD15 (CD5-1; Dako), anti-CD22 (CLB-B-ly/1,6B11; Sanquin, Amsterdam, The Netherlands), anti-CD58 (HB-7; BD Biosciences), anti-CD55 (clone 67; Serotech, Oxford, UK), anti-CD68 (EMB11; Dako) and anti-CD165 (5C6 FAT; Bachem Peninsula Laboratories, San Carlos, California, USA) to analyse the cell infiltrate. Antibodies against myeloid-related protein (MRP)3 (B-5C2; BMA Biomedicals, Augst, Switzerland) and MRP14 (S86.48; BMA Biomedicals) were used to detect infiltrating monocytes/macrophages in an early stage of differentiation.

For immunohistochemical analysis of cytokine expression, interleukin (IL)1β (2D8; Immunokontakt, Stockholm, Sweden) and IL6 (B-E8; Invitrogen, Breda, The Netherlands) were stained. Furthermore, von Willebrand factor (vWF) (F8/86; Dako) was used as a marker to detect blood vessels. Additionally, the expression of matrix metalloproteinase (MMP)3 (MAB1359; Bio-Connect, Huissen, The Netherlands) and MMP13 (VIII12; Oncogene, Cambridge, Massachusetts, USA) was determined.

Sections with non-assessable tissue, defined by the absence of an intimal lining layer, were excluded from analysis. Staining for cellular markers was performed using a three-step immunoperoxidase method as previously described. For the determination of cytokine expression, biotinylated tyramine (BT) was used for amplification after incubation with the secondary antibody and incubation with streptavidin-horseradish peroxidase (HRP) conjugate was followed by detection with aminomethylcarbazole (AEC), as previously described.

Control sections, the primary antibodies were omitted or irrelevant antibodies were applied.

**Digital image analysis**

After immunohistochemical staining, all sections were coded and randomly analysed by computer-assisted digital image analysis (DIA). For all markers, 18 high-power fields were analysed. CD68 and CD165 expression (macrophage markers) were analysed separately in the intimal lining layer and the synovial sublining. The images of the high-power fields were analysed using the Qwin analysis system (Leica, Cambridge, UK), as described in detail previously.

**Statistical analysis**

SPSS for Windows (V 12.0.2; SPSS, Chicago, Illinois, USA) was used for statistical analysis. Baseline characteristics between the two groups were compared using a Student t test for normal distributed data and a Mann–Whitney U test for variables with a very skewed distribution. Correlations of changes in clinical parameters and immunohistochemical markers were analysed with Spearman rank correlation. Additionally, each of the end points was analysed using an analysis of covariance model (ANCOVA) after rank transformation to correct for baseline
The model included terms for treatment as a fixed effect and the baseline measurement as a covariate. The aim was to assess the treatment difference.

RESULTS

Demographic features
A total of 24 patients (15 men, 9 women) were included in the trial, 12 patients were randomised to receive adalimumab and 12 placebo treatment. The mean age was 42.8 (range 21–61) years for the patients in the adalimumab group and 47.2 (25–78) years in the placebo group (table 1). The mean PsA disease duration was comparable in both groups. Concomitant methotrexate was used by seven patients in the adalimumab group and five patients in the placebo group in a comparable dose (mean 18.2 mg/week and 19.0 mg/week, respectively). None of the patients had used tumour necrosis factor (TNF) inhibitors in the past. Most patients (n = 16) had polyarticular involvement according to the Moll and Wright classification, a minority had an oligoarticular phenotype (n = 7) or predominantly distal interphalangeal involvement (n = 1). Two of the patients with polyarticular disease also had axial involvement. The PsA phenotypes were not equally distributed between the 2 groups, with the placebo group harbouring more patients with polyarticular disease than the adalimumab group (10 versus 6). Despite this difference, the patient groups were comparable with regard to disease activity markers, such as CRP and DAS28.

All patients received the two injections with study medication according to the protocol. Overall study medication was well tolerated. No infections other than common cold were reported in the 4-week study period. One patient in the placebo group complained of more pain in her ankle after the first arthroscopy, but could undergo the second arthroscopy.

Clinical response
As anticipated, a markedly positive effect of adalimumab treatment was seen on the DAS28, which was 1.92 units lower compared with placebo after 4 weeks of treatment (95% confidence interval (CI) 1.07 to 2.77, p = 0.001) (fig 1). The mean PASI score after 4 weeks of adalimumab treatment was 2.61 points lower compared to placebo (95% CI −0.08 to 5.50, p = 0.056). The mean PASI decreased from 5.89 (4.25) to 4.01 (2.49) in the adalimumab group, whereas there was a slight increase in the placebo group from 4.72 (2.55) to 5.45 (4.65).

At 4 weeks the ESR and CRP levels were respectively 58% (95% CI 30% to 86%, p = 0.001) and 57% (95% CI 29% to 84%, p < 0.001) lower after adalimumab treatment than after placebo treatment. The mean ESR was reduced from 24.2 (21.7) mm/h to 8.1 (7.5) mm/h after adalimumab treatment, and the mean CRP from 19.9 (25.7) mg/litre to 2.1 (1.4) mg/litre, whereas after placebo treatment there was no clear cut change: ESR from 22.4 (18.7) to 19.6 (11.9) mm/h and CRP from 9.9 (9.1) to 9.2 (10.0) mg/litre, respectively.

Table 1 Demographic and clinical features of the 24 patients with psoriatic arthritis (PsA) enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Adalimumab (n = 12)</th>
<th>Placebo (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>42.8 (21–61)</td>
<td>47.2 (25–78)</td>
</tr>
<tr>
<td>No. men/women</td>
<td>9/3</td>
<td>6/6</td>
</tr>
<tr>
<td>No. (%) currently receiving MTX</td>
<td>7 (58)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Dose MTX, mg/week</td>
<td>18.2 (10–25)</td>
<td>19.0 (15–25)</td>
</tr>
<tr>
<td>PsA disease duration, years</td>
<td>5.5 (4.0–14.1)</td>
<td>8.4 (1.9–18.2)</td>
</tr>
<tr>
<td>No. (%) RF positive</td>
<td>2 (17)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>No. (%) anti-CCP positive</td>
<td>1 (8)</td>
<td>0</td>
</tr>
<tr>
<td>No. (%) erosive</td>
<td>7 (58)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>24.2 (4–66)</td>
<td>22.4 (3–66)</td>
</tr>
<tr>
<td>CRP, mg/litre</td>
<td>19.9 (2.3–81.6)</td>
<td>9.9 (1.3–26.7)</td>
</tr>
<tr>
<td>DAS28 score</td>
<td>4.67 (3.0–5.78)</td>
<td>5.07 (2.21–6.83)</td>
</tr>
<tr>
<td>Patient global assessment of disease activity (VAS 0–100 mm)</td>
<td>73 (45–94)</td>
<td>62.8 (18–92)</td>
</tr>
<tr>
<td>Patient assessment of pain (VAS 0–100 mm)</td>
<td>72.8 (55–91)</td>
<td>67.4 (11–89)</td>
</tr>
<tr>
<td>PASI</td>
<td>5.89 (0–14.0)</td>
<td>4.72 (0–7.0)</td>
</tr>
</tbody>
</table>

All values are mean (range) except where indicated otherwise.

CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; MTX, methotrexate; PASI, psoriasis area and severity index; RF, rheumatoid factor; VAS, visual analogue scale.

Immunohistochemical analysis

Paired pretreatment and post-treatment synovial samples from 19 patients were available for analysis. The pretreatment ST biopsies of three patients did not contain an intimal lining layer and therefore did not pass the quality control tests. Due to an unfortunate freezer accident ST biopsies of two other patients were lost. The remaining 19 paired ST samples were analysed, 10 were in the adalimumab group and 9 in the placebo group. The results of this analysis are shown in table 2.

![Figure 1](https://example.com/figure1.png)
Despite randomisation, there were clear differences at baseline for several synovial markers between adalimumab and placebo groups, as shown in table 2 (adalimumab and placebo before). Most of these baseline differences were not statistically significant except for the number of CD8-positive cells, CD165-positive cells and MMP3 expression, which were significantly higher in the adalimumab group. A marked reduction was observed in most cell types after active treatment. The effect after adalimumab was significant for CD3-positive T cells: there was a median decrease of 248 cells/mm$^2$ after adalimumab as compared to placebo. The effect of treatment remained significant (p = 0.035 for CD3-positive cells and MMP13 expression) the expression of MMP13 was decreased with 9188 (8653) IOD/mm$^2$ after placebo as compared to placebo. When ANCOVA was applied to correct for the imbalances at baseline, there was no statistically significant effect on change in any parameter tested, but for reduction in the number of CD3-positive cells (p = 0.039) and MMP13 expression (p = 0.33)*.

There was a trend towards reduction in the expression of the vascular marker vWF and the proinflammatory cytokines IL1 and IL6 after treatment with adalimumab as compared to placebo, but the differences were not statistically significant. There was considerable reduction of expression of MMP5 and MMP13 in the adalimumab group as compared to placebo. The expression of MMP13 was decreased with 9188 (8653) IOD/mm$^2$ (median (SE)) after adalimumab treatment as compared to an increase of 9002 (5469) IOD/mm$^2$ after placebo. When ANCOVA was applied to correct for the imbalances at baseline, it turned out that the baseline measurement had the strongest effect on change in any parameter tested, but for reduction in the number of CD3-positive cells and MMP13 expression the effect of treatment remained significant (p = 0.035 for CD3-positive cells and p = 0.038 for expression of MMP13).

**Correlation between clinical improvement and changes in synovial biomarkers**

For the cellular markers clinical improvement was strongly correlated with a decrease in CD3-positive T cells (Spearman $\rho = 0.644$, p = 0.003), CD4-positive cells (rho = 0.649, p = 0.003) and MRP8-positive macrophages (rho = 0.651, p = 0.012) (fig 3), but there was no statistically significant correlation with changes in other cell types. There was also a strong correlation between clinical improvement and the reduction of MMP13 (rho = 0.619, p = 0.005) (fig 3) and MMP$\beta$ (rho = 0.560, p = 0.013).

**DISCUSSION**

This placebo-controlled study with adalimumab was conducted to address the question which features in PsA ST samples could be used as a biomarker for clinical efficacy on the group level in relatively small studies of short duration. The results presented
**Figure 2** Representative photograph showing CD3+ T cells (red–brown) in psoriatic arthritis synovial tissue before and after treatment with adalimumab (upper panel left and right) or placebo (lower panel left and right). Magnification ×200. A marked reduction of the number of CD3+ T cells was observed after 4 weeks of adalimumab treatment.

**Figure 3** Scatter plots showing individual data points for the correlation between change in 28-joint Disease Activity Score (ΔDAS28, a positive value represents clinical improvement) on the y axis and change in biomarker (a positive value represents a reduction of the expression) on the x axis after 4 weeks for CD3-positive cells (rho = 0.644, p = 0.003), CD4-positive cells (rho = 0.649, p = 0.003), expression of myeloid-related protein (MRP)8 (rho = 0.561, p = 0.012) and matrix metalloproteinase (MMP)13 (rho = 0.619, p = 0.005).
here show that clinically effective adalimumab therapy is particularly associated with a marked reduction in CD3-positive T cell infiltration and expression of MMP13 in the ST of patients with PsA 4 weeks after initiation of treatment.

Previous work on synovial biomarkers in patients with PsA has been mainly limited to open studies, mostly of longer duration. Open label treatment of 10 patients with PsA with methotrexate resulted in a decrease in T cells and macrophages as well as reduced expression of IL8, E-selectin, intercellular adhesion molecule 1 (ICAM-1) and MMP3 after 6 to 12 months of treatment. In another study 52 patients with peripheral arthritis due to spondyloarthritis were included; 16 of these had PsA. The patients underwent synovial biopsy at baseline and 12 weeks following different treatment regimens (infliximab, etanercept, sulfasalazine or no DMARD). Clinical improvement correlated with a decrease in CD16S-positive macrophages, polymorphonuclear cells (PMNs) and MMP3 expression. A study on the effects of TNF blockade showed in 11 patients with PsA, who were treated with infliximab (5 mg/kg) combined with stable methotrexate treatment, a significant reduction of ICAM-1, vWF and αvβ5 expression. There was also a trend towards a reduction of T cells and macrophages. Similarly, another study demonstrated a decrease in macrophage numbers and vascular markers in the synovium of nine patients with PsA 8 weeks after initiation of infliximab (5 mg/kg) treatment. A study on the effects of alefacept revealed a significant decrease in T cell numbers after 4 weeks of treatment, and a significant reduction of T cells and macrophages after 12 weeks of treatment in 11 patients with PsA. Finally, experimental treatment with IL10 subcutaneously for 4 weeks resulted in decreased T cell and macrophage infiltration in the synovium.

The present study was designed to determine which synovial biomarkers are associated on the group level with active treatment, identical to the approach that we have previously described in patients with RA. In RA macrophage numbers were previously identified as synovial biomarkers associated with active treatment. These results have been confirmed and validated in various studies. Of interest, although we did observe clear trends towards decreased numbers of macrophages and macrophage subsets after active treatment, analysis of covariance indicates that in PsA especially the number of CD3-positive T cells and the expression of MMP13 could be used to screen for potentially active drugs in small proof of concept studies of short duration. The results presented here underscore the important role of T cells in the pathogenesis of PsA, consistent with the observation that specific targeting of T cells may result in clinical benefit in this disease.

A potential drawback of this study is the fact that there were baseline differences—in clinical and synovial variables—between the adalimumab and placebo group despite randomisation, which is probably related to the relatively small number of patients. The ANCOVA model, however, includes the baseline value for each marker as a covariate, thereby correcting for these baseline imbalances. Using this model to describe the relationship between clinical improvement and changes in ST, the effect of treatment was sustained for a reduction of T cells and MMP13 expression. Another potential limitation of this exploratory study is related to the high number of synovial parameters tested and the chance of erroneously reporting statistical significance in the context of multiple comparisons. The limited power of our study did not allow a conservative Bonferroni correction. Of importance, other investigators have very recently independently confirmed the specific decrease in CD3+ T cells in the synovium after initiation of TNF blockade in patients with PsA. Thus, it appears unlikely that the identification of CD3+ T cells as a key synovial biomarker associated with active treatment can merely be explained by chance.

The reduction of synovial inflammation associated with clinical improvement of the joint and skin after adalimumab treatment is also consistent with previous reports showing a decrease in leukocyte numbers (including T cells and macrophages), as well as reduced vascularity and expression of proinflammatory cytokines and MMPs in the synovium of patients with PsA after infliximab treatment. The cellular changes might be explained in part by changes in cell migration due to reduced neoangiogenesis and reduced expression of adhesion molecules and chemokines, as suggested previously.

Analysis of molecular markers in synovial tissue is increasingly used in clinical trials on targeted therapies. With this approach, tissue specificity is not a problem and examination of serial biopsy samples can be used to monitor the response in individual patients and screen for interesting biological effects at the site of inflammation. This study clearly shows that changes in T cell numbers and the expression of MMP13 in the synovium of patients with PsA may be used as biomarkers to screen for effective therapies during early drug development. It can be anticipated that future developments will include the use of more extensive markers of synovial inflammation and joint degradation as well as the use of panels of biomarkers in serum and synovial tissue samples.

Acknowledgements: The authors wish to thank Ilse Burgman, Marjolein Vinkenoog and Dr Tom Smeets for their expert assistance with immunohistochemistry and digital image analysis.

Funding: This clinical study was supported by Abbott BV, The Netherlands. DMG was supported by the Dutch Arthritis Association ("Reumafonds"). This research was also supported by the European Community’s FP6 funding ("Auto cure").

Competing interests: This publication reflects only the author’s views; the European Community is not liable for any use that may be made of the information herein. This research is integrated in the work of the OMERACT Special Interest Group on Synovial Analysis in Clinical Trials.

Ethics approval: The study protocol was approved by the medical ethics committee of the institute and all patients signed informed consent before start of the study.

REFERENCES


A prospective, randomised, placebo-controlled study to identify biomarkers associated with active treatment in psoriatic arthritis: effects of adalimumab treatment on synovial tissue

A W R van Kuijk, D M Gerlag, K Vos, et al.

doi: 10.1136/ard.2008.091389
Articles on similar topics can be found in the following collections

- **Unlocked** (638 articles)
- **Immunology (including allergy)** (45086 articles)
- **Pathology** (450 articles)
- **Radiology** (16199 articles)
- **Degenerative joint disease** (9755 articles)
- **Musculoskeletal syndromes** (17295 articles)
- **Surgical diagnostic tests** (6279 articles)
- **Clinical diagnostic tests** (19823 articles)

To request permissions go to:  
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:  
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:  
http://group.bmj.com/subscribe/