# Autoantibodies in Early Seropositive Rheumatoid Arthritis, Before and During Disease Modifying Antirheumatic Drug Treatment

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***ABSTRACT.*** ***Objective.*** Autoantibodies observed in patients with rheumatoid arthritis (RA) during clinical trials of immunomodulating agents may cause concern about possible induction of autoimmunity by the therapeutic intervention. We determined the frequency and variability of selected autoantibodies in patients with early rheumatoid factor (RF) positive RA during a prospective observational study.

***Method.*** The study cohort consisted of 276 patients with active RA and with RF ≥ 40 IU, who were enrolled between January 1, 1993, and April 1, 2000, before starting disease modifying antirheumatic drug (DMARD) therapy (average duration of symptoms, 7 mo). During an average of 3.5 years followup, a panel of autoantibodies was determined at entry, 6 months, 12 months, and yearly thereafter, in addition to routine clinical, radiographic, and laboratory assessments. After enrollment, patients were treated with DMARD at the discretion of their rheumatologists.

***Results.*** At entry before any DMARD therapy, antinuclear antibody (ANA; by HEp-2) values were negative in 31%, borderline (8 IU/ml) in 26%, and > 8 (mean 65.5 IU/ml) in 41%. Tender and swollen joint counts, Disease Activity Score, and RF values were significantly higher in those with ANA > 8. During followup 726 paired serial specimens were available; 12.5% changed from negative to positive ANA and 12.3% from positive to negative. Additional autoantibodies were present in specimens of 20% of the subjects; 8% had 2 and 1.4% had 3 other autoantibodies. Anti-dsDNA was detected in 13 (5.5%) patients; 4 changed from negative to positive and one from positive to negative. SSA IgG and SSB IgG autoantibodies were both present in one of these patients. Ribosomal P protein autoantibodies were noted in 2 other patients, but Sm (Smith) and uRNP/snRNP IgG autoantibodies were not present in any patient. No patient had a diagnosis of systemic lupus erythematosus. Antithyroid peroxidase (20 patients), parietal cell (15), smooth muscle (14), reticulin (9), mitochondrial (5), striational (2), SSB (2) and SCL-70 (1) autoantibodies were detected in some specimens. Seven patients were diagnosed with hypothyroidism, one with chronic thyroiditis, one with hepatitis C, and 9 with malignancies.

***Conclusion.*** In patients with early RF positive RA the frequent occurrence of autoantibodies before and during treatment with standard DMARD may make it difficult to attribute their presence to new therapies. (J Rheumatol 2002;29:2513–20)

*Key Indexing Terms:*
RHEUMATOID ARTHRITIS
ANTINUCLEAR ANTIBODY
AUTOANTIBODIES
ANTI-dsDNA ANTIBODY

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The clinical use of potent specific interventions in the immunoinflammatory process, such as interferon-α, interferon-γ, and inhibitors of the cytokine tumor necrosis factor-α (TNF-α), has been accompanied by occasional reports of lupus-like syndromes or lupus associated antibodies[1-6]. A defect in the production of TNF-α in the (NZB × NZW) F1 mouse model of systemic lupus erythematosus (SLE) and the association of low TNF-α production with SLE nephritis in patients[7-9] have led to concerns that these and other specifically targeted biologic products may induce manifestations of SLE or other autoimmune diseases. Alertness to such concerns prompted a recent US Food and Drug Administration "Dear Doctor" letter[10] regarding possible associations of TNF-α inhibition with neurologic symptoms

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and/or radiographic evidence of demyelinating disease[11-13] and hematologic manifestations such as agranulocytosis[10]. The possibility of routine anti-DNA antibody screening during treatment with TNF-α blockers has been discussed[7]. Although not yet considered to be indicated in clinical practice, serial routine monitoring of autoantibodies is likely to be included in clinical trials during the development of biological agents, and the newly noted presence of an autoantibody that was not detected at baseline may be considered evidence of its induction by the intervention.

Autoantibodies are well known to occur in patients with rheumatoid arthritis (RA), and sometimes are associated with autoimmune clinical manifestations, e.g., Sjögren's syndrome[14] and Felty's syndrome[15]. To assist in the assessment of autoantibodies found during routine screening of patients being treated with or participating in clinical trials of biological or other immunomodulating therapies, it would be useful to know the frequency and variability of positive finding in similar patients with RA who were followed during routine clinical care. We present the results of autoantibody panels that were repeated routinely every 6 to 12 months for 276 patients who participated in an observational study of early, rheumatoid factor (RF) positive active RA for an average of 3.5 years.

## MATERIALS AND METHODS

*Patients*. Patients who met diagnostic criteria for RA[16] and were within one year of symptom onset but had not yet been treated with a disease modifying antirheumatic drug (DMARD) could be entered in this longterm observational study if RF was positive (titer ≥ 1:80 or ≥ 40 IU) and the patient had ≥ 6 swollen joints and ≥ 9 tender joints. This report is based on 276 patients who were entered between January 1, 1993, and April 1, 2000, by 41 rheumatologists from 29 practices in the Western Region of the United States and Mexico who were participating in the Western Consortium of Practicing Rheumatologists. Four practices are in university medical centers; the remainder are community practices. Arthritis assessments were scheduled at entry, 6, 12 and 24 months, and yearly thereafter. Using standard methods[17] clinical assessments included all of the components of the American College of Rheumatology (ACR) core set of disease activity measures[18], as well as a detailed self-report questionnaire; grip strength; radiographs of hands/wrists and forefeet; and HLA susceptibility epitope genotyping. Westergren erythrocyte sedimentation rate (ESR) was done as clinically indicated in the rheumatologist's office or local laboratory.

Blood specimens were collected at each scheduled arthritis assessment. Plasma was separated from whole blood specimens anticoagulated with EDTA and placed in 2 provided screw-top plastic tubes, one of which contained futhan as a preservative. Blood also was collected in a Vacutainer containing SST gel and clot activator, spun down, and shipped to the laboratory, where serum was separated. The specimens were shipped by an overnight courier service at ambient temperature to Specialty Laboratories in Santa Monica, California. The autoantibody panel and plasma viscosity were determined within 24 h after the specimen arrived at the laboratory. Remaining specimens were aliquoted and frozen at –60°C.

The autoantibody assays were done by Specialty Laboratories using their proprietary ANA Lyzer® technique[19], which included the following panel: antinuclear antibodies (ANA; normal ≤ 7.5 international units/ml) by immunofluorescence on HEp-2 cells; ANA pattern by immunofluorescence (normal, not detected); dsDNA autoantibodies (Farr method; normal < 5 IU/ml); U.RNP/snRNP IgG autoantibodies (enzyme linked immunosorbent assay, EIA; normal, not detected); Sm (Smith) IgG autoantibodies (EIA; normal, not detected); SSA IgG autoantibodies (SSA/Ro; EIA, confirmed by immunoblot; normal, not detected); SSB IgG autoantibodies (SSB/La; EIA, confirmed by immunoblot; normal, not detected); SCL-70 IgG autoantibodies [SCL-70 (topoisomerase-I) autoantibodies; EIA, confirmed by immunoblot; normal, not detected]; thyroid peroxidase autoantibodies (immunochemiluminometric assay; normal < 35 IU/ml); C3 complement (nephelometry; normal 85–200 mg/dl); C4 complement (nephelometry; normal 14–53 mg/dl); RF IgM autoantibodies (nephelometry; normal < 20 IU/ml); ribosomal P protein autoantibodies (EIA; normal < 13 EIA units); myocardial autoantibodies [immunofluorescence assay (IFA); normal < 1:40 titer]; parietal cell total autoantibodies (IFA on mouse stomach frozen sections; normal < 1:40 titer); reticulin autoantibodies (IFA; normal < 1:40 titer); mitochondrial total autoantibodies (IFA; normal < 1:40 titer); smooth muscle total autoantibodies (IFA; normal < 1:40 titer); striational total autoantibodies (IFA on cryostat sections of skeletal muscle; normal < 1:40 titer). In this laboratory, "normal" values are set at the 95th to 97th percentile level of the values obtained when testing known healthy subjects or patients with nonautoimmune diseases. However, elderly healthy subjects are more likely to have ANA and 10% to 15% of persons older than 80 years may have positive values for ANA.

All available patient self-report and physician report documents in the Consortium files were examined for each of the 54 patients who had autoantibodies other than ANA or RF, to search for evidence of conditions that might be associated with specific autoantibodies, e.g., SLE, thyroid disease, Sjögren's syndrome, systemic sclerosis, pernicious anemia, atrophic gastritis, chronic active hepatitis, primary biliary cirrhosis, celiac disease, dermatitis herpetiformis, psychosis, myasthenia, thymoma, or other specific autoimmune diseases.

Patients entered the observational study as they became available. At the time of this analysis baseline data were available for 276 patients, 6 month data for 183, one year for 183, 2 year for 141, 3 year for 100, 4 year for 67, and 5 year data for 38 patients. The average duration of followup was 3.5 years. Patients could be treated with one or more DMARD at any time after the baseline evaluation. The following DMARD were started after completion of the baseline evaluation: methotrexate (MTX) by 47% of patients; hydroxychloroquine (HCQ) 21%; sulfasalazine (SSZ) 10.9%; MTX and HCQ 6.9%; step down bridge (MTX + HCQ + prednisone) 4.7%; injectable gold 4.0%; no DMARD [nonsteroidal antiinflammatory drug (NSAID) or prednisone only] 4.7%. Eight of the 13 patients initially taking no DMARD started MTX before the 6 month visit. By the 6th month, all except 5 of the patients had started DMARD. Changes were made as clinically indicated and at 6, 12, and 24 months MTX use ranged from 53% to 57% of patients, HCQ 30% to 31%, and SSZ 9% to 12%. Between 40% and 45% of patients were taking prednisone at the various assessment points, although some patients stopped prednisone and others started it. NSAID were used or changed as clinically indicated. Cumulative years of DMARD use, singly or in combination were: MTX 587 years, HCQ 308 years, SSZ 120 years, intramuscular gold 17 years, azathioprine 15 years, others 14 years.

## RESULTS

The average age of the 276 patients at study entry was 50 years (range 21–79); 78% were female; mean duration since onset of RA symptoms was 7 months. All had positive tests for RF (368 ± 496 IU/ml). At entry, before starting DMARD, patients had active RA, with tender joint count 22 ± 13; swollen joint count 19 ± 11; Health Assessment Questionnaire (HAQ) disability index 1.18 ± 0.72; ESR 40 ± 25mm/h; C-reactive protein 2.46 ± 3.22 mg/dl. Global assessments and patient pain were recorded on 0 to 100 visual analog scales as follows: physician global assessment 48 ± 21; patient global assessment 61 ± 26; patient pain 61

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± 27. The composite Disease Activity Score (DAS) was 4.58 ± 1.18. Rheumatoid nodules were noted by the rheumatologists in 13.4% of patients at entry; 22 patients (8.4%) had other extraarticular manifestations at baseline: keratoconjunctivitis sicca, 14 patients; pleural effusion 2; pleuritis 2; episcleritis one; scleromalacia one; cutaneous vasculitis one; digital vasculitis one; other vasculitis one; mononeuritis multiplex one; and pericarditis one. The rheumatoid epitope was present in 51%. Baseline total Sharp score for radiographs of hands/wrists and forefeet was 6.13 ± 7.75, with 49% of patients already having measurable erosive changes (erosion score ≥ 1) at study entry. Almost all patients were receiving NSAID and 53% were being treated with prednisone (average dose 3.5 ± 4.9 mg daily).

An ACR 20% response was achieved by 52.3% of patients at some time during followup; 35.5% attained ACR 50% responses. Mean total Sharp score progression rate per year was 0.911 ± 2.55; first year change in HAQ was –0.467 ± 0.617. During followup 12.9% developed new nodules and 7.38% developed new extraarticular manifestations.

*Antinuclear antibody.* ANA values of ≥ 8.0 IU/ml are considered positive. The distribution of ANA values over time is illustrated in the serial box plots in Figure 1. At study entry, 31% of patients were ANA negative, 26% had threshold values of 8.0, and 43% had positive values > 8.0 (mean 65.5 ± 107 IU/ml). Table 1 compares the characteristics of these 3 groups. Tender and swollen joint counts, RF values, and DAS scores are higher in the group with ANA >

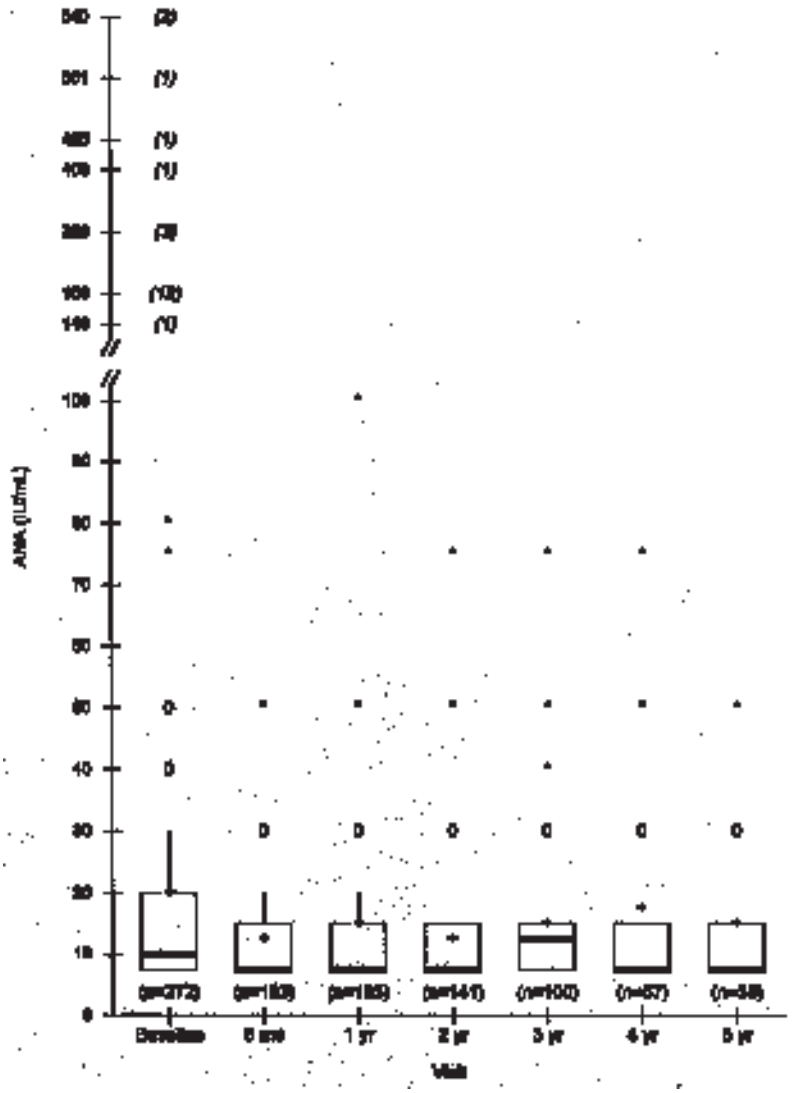

*Figure 1.* Boxplot showing the complete distribution of all ANA values during 5 years of observation. The double bar within each box indicates the median value at that time point; "+" is the mean value. The upper and lower edges of the box represent the 25th and 75th percentiles. "O" is a single outlying observation; "*" indicates multiple outlying observations. The number of subjects at each time point is indicated in parentheses.

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*Table 1*. Study variables for patients. Data are mean ± SD (number of subjects).

| Variable | ANA < 8 IU/ml (n = 85) | ANA 8 IU/ml (n = 72) | ANA > 8 IU/ml (n = 119) |
|---|---|---|---|
| Baseline demographics | | | |
| Age, yrs | 50.50 ± 14.05 (85) | 50.57 ± 13.96 (72) | 49.20 ± 12.68 (118) |
| Sex, % female | 77.65 (85) | 80.56 (72) | 76.47 (119) |
| Duration of RA, mo | 8.37 ± 8.84 (85) | 7.56 ± 4.96 (72) | 6.72 ± 6.07 (115) |
| Baseline clinical | | | |
| Grip strength, mm Hg | 146.19 ± 73.10 (79) | 136.53 ± 71.07 (62) | 141.35 ± 70.80 (103) |
| Tender joint count* | 20.84 ± 11.80 (82) | 19.75 ± 11.95 (68) | 24.91 ± 14.66 (107) |
| Swollen joint count* | 16.06 ± 9.35 (82) | 17.39 ± 9.64 (67) | 22.26 ± 12.04 (107) |
| HAQ Disability Index, 0–3 | 1.11 ± 0.71 (74) | 1.28 ± 0.73 (59) | 1.16 ± 0.71 (108) |
| Global, physician, 0–100 | 44.90 ± 19.07 (82) | 48.55 ± 21.19 (67) | 50.54 ± 21.85 (112) |
| Global, patient, 0–100 | 59.64 ± 25.97 (67) | 64.20 ± 28.79 (61) | 60.30 ± 23.55 (79) |
| Global, patient, 0–3 | 1.20 ± 0.73 (63) | 1.35 ± 0.71 (54) | 1.30 ± 0.69 (101) |
| Pain, VAS, 0–100 | 59.72 ± 25.39 (67) | 65.49 ± 28.36 (61) | 58.01 ± 26.25 (79) |
| Pain, from HAQ, 0–3 | 1.39 ± 0.76 (74) | 1.62 ± 0.75 (59) | 1.52 ± 0.71 (107) |
| Disease Activity Score | 4.13 ± 1.03 (77) | 4.47 ± 1.03 (63) | 4.86 ± 1.31 (102) |
| Nodules, % | 8.43 (83) | 16.42 (67) | 15.18 (112) |
| Other extraarticular manifestations, % | 9.64 (83) | 4.48 (67) | 9.91 (111) |
| Total Sharp score | 5.04 ± 5.40 (67) | 7.03 ± 11.38 (60) | 6.33 ± 6.26 (101) |
| Erosion score ≥ 1, % | 44.76 (67) | 50.00 (60) | 50.50 (101) |
| Prednisone use, % | 55.41 (74) | 53.33 (60) | 50.94 (106) |
| Baseline laboratory | | | |
| Hemoglobin, g/dl | 13.28 ± 1.40 (66) | 12.85 ± 1.20 (51) | 13.21 ± 1.35 (97) |
| Hematocrit, % | 39.58 ± 3.88 (67) | 38.42 ± 3.32 (51) | 39.39 ± 4.11 (91) |
| Platelets (× 100) | 325.55 ± 94.05 (67) | 341.20 ± 98.01 (51) | 340.49 ± 118.75 (96) |
| CRP, mg/dl | 2.10 ± 2.61 (85) | 3.15 ± 4.28 (72) | 2.28 ± 2.77 (119) |
| ESR, mm/h | 36.76 ± 21.16 (85) | 41.86 ± 28.82 (72) | 41.89 ± 25.06 (119) |
| Viscosity, m Pa.s | 1.79 ± 0.21 (85) | 1.83 ± 0.21 (72) | 1.81 ± 0.20 (119) |
| ANA, IU/ml† | 0 (85) | 8 ± 0 (72) | 65.51 ± 106.94 (119) |
| Albumin, g/dl | 4.05 ± 0.40 (51) | 3.98 ± 0.53 (37) | 3.95 ± 0.39 (83) |
| C3, mg/dl | 141.51 ± 27.33 (47) | 149.15 ± 29.29 (52) | 141.98 ± 28.19 (55) |
| C4, mg/dl | 32.72 ± 10.34 (47) | 33.73 ± 8.86 (52) | 33.45 ± 12.68 (55) |
| Rheumatoid factor, IU/ml‡ | 272.50 ± 308.83 (84) | 330.99 ± 585.38 (72) | 458.64 ± 531.22 (118) |
| Epitope +, % | 53.75 (80) | 50.75 (67) | 50.00 (106) |
| Initial DMARD | | | |
| MTX, % | 45.9 | 52.8 | 44.5 |
| HCQ, % | 24.7 | 13.9 | 22.7 |
| MTX + HCQ, % | 3.5 | 5.6 | 10.3 |
| SSZ, % | 7.1 | 16.7 | 10.1 |
| Step-down bridge, % | 7.1 | 2.8 | 4.3 |
| Gold, % | 3.5 | 4.2 | 4.3 |
| None or other, % | 8.3 | 4.2 | 4.2 |
| RA course during followup | | | |
| ACR 20% responders, % | 50.00 (41/82) | 53.73 (36/67) | 53.27 (57/107) |
| ACR 50% responders, % | 31.71 (26/82) | 31.34 (21/67) | 41.12 (44/107) |
| Sharp score progression rate per year | 0.68 ± 3.13 (54) | 1.12 ± 2.44 (47) | 0.86 ± 1.93 (74) |
| First year change in HAQ | −0.50 ± 0.59 (52) | −0.50 ± 0.79 (51) | −0.41 ± 0.54 (85) |
| New extraarticular manifestations, % | 6.02 (83) | 8.96 (67) | 7.21 (111) |
| New nodules, % | 8.43 (83) | 20.90 (67) | 11.61 (112) |

Nonparametric Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.001$, † $p < 0.0001$, ‡ $p < 0.005$.

8 IU/ml, but the other variables do not show any consistent trend. RF values correlated poorly with ANA values on the same specimens ($r = 0.08$, $p = 0.29$).

ANA pattern was homogeneous in 114 patients, speckled in 65, and nucleolar in 8. The variables shown in Table 1 were compared for these 3 groups (data not shown), but the only significant difference was for RF values (homogeneous pattern, RF 569 IU/ml; speckled pattern, RF 255 IU/ml; $p < 0.05$).

When categorized by ANA values (Table 1) at baseline there were no significant differences in the choice of initial DMARD or in the subsequent course of the patients' RA with respect to ACR 20% or 50% responses, total Sharp score progression rates, or changes in HAQ disability index

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*Table 2.* Patients with positive dsDNA values (IU/ml; normal < 5).

| Patient | Baseline | 6 Months | 1 Year | 2 Years | 3 Years | 4 Years | 5 Years | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 18 | 22 | | | Alopecia 1/95. SSZ, leflunomide, etanercept. Erosions |
| 2 | 5 | | | | | | | Male, rheumatoid nodules. Erosions |
| 3 | 14 | 6 | 0 | 0 | 0 | 0 | 0 | Rh nodules, sicca, alopecia, sun sensitivity, erosions. Etanercept |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | Dry mouth, SSZ 6 months, erosions, Pre-eclampsia 1979 |
| 5 | 7 | 6 | | | | | | Raynaud's '95, dry eyes. Breast cancer, radiation therapy. Erosions |
| 6 | 6 | 6 | | | | | | Male, Rh nodules. Erosions |
| 7 | 0 | 18 | 16 | 13 | | | | Raynaud's mouth sores, SSZ, erosions |
| 8 | 5 | 11 | | | | | | Pleurisy, Raynaud's, dry eyes, dry mouth Adenocarcinoma of endometrium. SSZ. Erosions |
| 9 | 7 | 7 | 14 | 12 | 9 | 13 | | Erosions |
| 10 | 13 | 25 | 25 | 33 | | | | Melanoma thigh 1988. Erosions |
| 11 | 686 | 31 | | | | | | Hypothyroid. Lost to followup. No joint radiographs |
| 12 | 19 | 93 | 12 | 220 | | | | Raynaud's, sun sensitivity, hair loss, mouth sores. No joint radiographs |
| 13 | 0 | 0 | 6 | | | | | Rh nodules, hypothyroid. Mouth sores, dry mouth, hair loss, sun sensitivity, SSZ, CD4 study, anti-TNF study. Erosions. |
| Median | 6 | 6.5 | 9 | 13 | 4.5 | 0 | 5 | — |
| Quartile 1/3 | 0/13 | 1.5/23 | 0/16 | 0/33 | 0/19 | 0/13 | 0/10 | — |
| Mean ± SD | 56.6 ± 188.6 | 16.9 ± 26 | 9.13 ± 9.2 | 42.3 ± 79.2 | 7.75 ± 10.4 | 4.33 ± 7.5 | 5 ± 7.1 | — |

scores during the year after entry. There were no significant differences in the presence of rheumatoid nodules at baseline or in the development of new extraarticular manifestations or new rheumatoid nodules during followup. However, among those who ever had nodules, 55 patients were ANA positive and 14 were ANA negative (p = 0.033).

*Changes in sequential ANA values.* During 3.5 years of observation (range 0.5–6 yrs) 994 ANA tests were done; 71% were positive, 29% were negative. Two or more measurements were available for 219 subjects. When 726 paired serial specimens were compared, 12.5% changed from negative to positive, and 12.3% changed from positive to negative.

Other autoantibodies were detected in the specimens of 54 of the 276 patients (20%), 51 of whom also had ANA. One additional autoantibody was found in 28 patients, 2 in 22 patients, and 3 were found in 4 patients. When the clinical and laboratory characteristics in Table 1 were compared for patients with one (RF), 2 (RF and ANA), 3 (RF, ANA, and one other autoantibody), and 4 or more autoantibodies, those with 3 or ≥ 4 autoantibodies had no evidence of significantly more severe RA.

*Anti-dsDNA antibodies.* Antibodies to dsDNA were assessed in 234 (85%) of the 270 patients, with 2 or more measurements in 69%, for a total of 469 assays. Thirteen patients (5.5%) with a total of 49 assays had 31 (range 1–6) specimens that were positive for anti-dsDNA antibodies (range 5–686 IU/ml) (Table 2). Nine were positive at baseline before DMARD. All 13 had positive tests for ANA (range 8–75 IU/ml). Two were male and 11 female, average age 45 years (range 22–71), with average disease duration 5.5 months at study entry. RF values on the same specimens ranged from 0 to 3090 IU/ml. All C3 and C4 values were within the normal range. Six of the 13 patients also had antibodies to thyroid peroxidase but only 2 had clinical diagnoses of hypothyroidism. One patient also had antibodies against SSA IgG in 4 of 4 evaluations and to SSB IgG in one of 4 evaluations. Four patients had rheumatoid nodules, 4 Raynaud's symptoms, 4 dry mouth, 3 dry eyes, 3 mouth sores, 4 hair loss, one history of pleurisy, 2 sun sensitivity, and one had a history of preeclampsia 20 years before the onset of RA. Three patients had a history of malignancy (breast, adenocarcinoma of endometrium, malignant melanoma of thigh). DMARD used included MTX in 11, antimalarials in 9, SSZ in 5, etanercept in 3, and cyclosporine, oral gold and leflunomide each in one patient. Four of the patients had negative anti-dsDNA tests at baseline that changed to positive on a subsequent specimen; 3 had been treated with SSZ before anti-dsDNA became positive; one became positive before starting SSZ. Each of 3 patients treated with etanercept had a positive anti-dsDNA value before starting etanercept. One changed from positive to negative. None had a clinical diagnosis of SLE.

*Other autoantibodies.* Antibody against thyroid peroxidase

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was detected in 56 of 88 specimens from 20 patients with values ranging from 41 to > 850 IU/ml; at baseline, 13 were positive and 7 negative. One patient had a diagnosis of chronic thyroiditis, 7 patients hypothyroidism; 6 were taking thyroid replacement therapy. SSA IgG, anti-dsDNA, and ANA autoantibodies were detected in all 4 specimens from one patient, who also had SSB IgG autoantibodies in the first of 4 specimens. The patient did not complain of dry eyes or dry mouth, but had episodes of pleuritic pain, hair loss, Raynaud's, and sun sensitivity, as well as modest pancytopenia. SSB IgG autoantibody was detected in the last of 6 samples of one additional patient who also had smooth muscle autoantibodies and ANA, but did not have dry eyes, dry mouth, or sicca syndrome. SCL-70 IgG autoantibody was detected in the third of 3 specimens of one patient, who did not have scleroderma or pulmonary fibrosis, but did complain of dry eyes, dry mouth, sun sensitivity, Raynaud's symptoms, and difficulty swallowing on one or more occasions.

Parietal cell total antibodies were detected in 41 of 61 specimens from 15 patients, only 4 of whom were positive at study entry; 86% also had ANA; 5 also had antibodies to thyroid peroxidase, one to dsDNA, and 2 had mitochondrial autoantibodies. None had pernicious anemia, atrophic gastritis, or hepatic disease. One had been diagnosed with optic neuritis and one with ocular myasthenia, both before the onset of RA.

Smooth muscle total autoantibodies were detected in 19 of 56 specimens from 14 patients, all of whom also had ANA. Three were positive at baseline. Seven also had other autoantibodies: 2 anti-dsDNA antibodies, 2 anti-thyroid peroxidase autoantibodies, and one each anti-SSB, anti-SCL-70, parietal cell, and reticulin autoantibodies. One patient had chronic hepatitis C, and one had a history of ulcerative colitis with a total colectomy 16 years before the onset of RA. One had primary malignancies of the lung and the breast, and one had cervical carcinoma. Six complained of dry eyes, but none had uveitis. Seven noted hair loss. None had chronic active hepatitis.

Reticulin autoantibodies were present in 16 of 26 specimens from 9 patients; 4 were positive at entry. All had ANA, 2 had thyroid peroxidase autoantibodies, and one smooth muscle autoantibodies. Two had malignancies, one breast cancer, and one adenocarcinoma of the bladder. None had celiac disease or dermatitis herpetiformis.

Mitochondrial total autoantibodies were reported in 6 of 24 specimens from 5 patients; 2 were positive at baseline. All were ANA+, but none had a nucleolar pattern. All had other autoantibodies, to thyroid peroxidase in 3, dsDNA in one, and parietal cells in 2. None had primary bilary cirrhosis or chronic active hepatitis, but 2 of the 5 had lung cancer.

Ribosomal P protein autoantibodies were detected in 2 of 9 specimens from 2 patients; both were negative at entry. Neither had lupus, depression, or psychosis. Striational autoantibodies were detected in 3 of 8 specimens from 2 patients; one was positive at entry. Both had ANA, one with a nucleolar pattern, but neither had myasthenia, thymoma, or a neoplasm. No specimen had detectable Sm (Smith) IgG autoantibodies, uRNP/snRNP IgG autoantibodies, or myocardial autoantibodies.

## DISCUSSION

The frequency of positivity and the specificity of tests for autoantibodies are generally considered to be method and laboratory related. Therefore, reports of studies of autoantibodies from different laboratories may not be directly comparable, and may not necessarily apply to the laboratories being used by individual physicians. Specifically, the findings reported here apply to routine testing done by Specialty Laboratories and to our cohort of patients and do not confirm or invalidate other published reports. In addition, during the sampling period of this report, anti-TNF-$\alpha$ treatment was rarely used in these patients with early RA. Our findings provide background information about our patients with early RA who were treated with standard therapies as part of routine clinical care.

Autoantibodies were frequently detected in our cohort of patients with early (< 1 year from symptom onset) RF positive RA. During an average of 3.5 years of observation, 71% had positive tests for ANA, almost all of whom were positive at study entry, before starting DMARD therapy. Changes from negative to positive (12.5%) and positive to negative (12.3%) during followup were almost equally balanced, suggesting that the changes may reflect technical variability in the assay process. The 43% with values > 8 had higher baseline tender and swollen joint counts, DAS scores, and RF values than those with negative or threshold values of 8 or less. The choice of initial DMARD and the subsequent course of the RA did not differ in the different ANA groups or patterns. Twenty percent of patients also had other autoantibodies, but the severity of RA was not greater in the 28 subjects with one or the 26 with 2 or 3 additional autoantibodies. Only 5.5% (13 patients) had antibodies to dsDNA; 69% of these were positive at baseline, before exposure to any DMARD. Three patients who converted from negative to positive anti-dsDNA antibodies had been treated with SSZ (for 6 mo, 9 mo, and 1 mo) during the year before anti-dsDNA antibody was first detected; SSZ has been suspected to induce these antibodies and occasionally to be associated with drug induced lupus[20]. The other patient converted before starting SSZ. None of our patients was diagnosed with lupus, although some had symptoms that may be associated with lupus, e.g., Raynaud's, dry mouth, dry eyes, mouth sores, hair loss, pleurisy, and sun sensitivity. Other lupus associated antibodies were not seen (Smith, anti-RNP) or were present inconsistently in only one or 2 patients (SSA, SSB IgG autoantibodies).

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Antibodies against thyroid peroxidase were noted at some time in 20 (7.2%) patients, but only 7 were diagnosed with hypothyroidism and one with chronic thyroiditis, and only 6 were being treated with thyroid replacement medication. Several of the untreated patients had documented normal thyroid function tests. Walter, *et al* found thyroid microsomal autoantibodies in 21% of 48 patients with RA from multicase families, but only 4 patients had clinical diagnoses of hyperthyroid or hypothyroid disease[21]. They also found gastric parietal cell autoantibodies in 6 patients, but only 3 had pernicious anemia.

Parietal cell, smooth muscle, and reticulin autoantibodies each were intermittently detected in 3% to 5% of patients, and 1% to 2% had a few specimens with mitochondrial, ribosomal P, or striational autoantibodies, but none had any of the diseases associated with these tests. Malignancies (lung 3; breast 3; bladder one; cervix one; endometrium one; melanoma one) occurred in 9 (16.6%) of the 54 patients with "other autoantibodies." One of these patients had 2 primary malignancies, of breast and of lung.

Reichlin and Harley report positive ANA tests in 50% to 75% of patients with RA when HEp-2 cells are used as the substrate[22]. Tan, *et al*, in a study of ANA in 15 international laboratories using HEp-2 cells as substrate, reported that 48.6% of 40 RA sera were positive at 1:40, 37.8% at 1:80, 13.5% at 1:160, and 2.7% at 1:320 dilutions[23], which was not much different than the frequencies of 31.7%, 13.3%, 5%, and 3.3%, respectively, for the same dilutions in 125 healthy individuals. Charles, *et al*[1] found that ANA (HEp-2 cell) was positive at a dilution ≥ 1:80 in 39% of 108 randomly selected RA patients, and in 29% of 193 pre-infliximab study patients. An additional 29% of the infliximab treated patients became positive during treatment, but 7 who were positive prestudy became negative. Badot, *et al*[6] found ANA antibodies > 1:80 titer in 28% of 28 patients with active erosive RA despite MTX treatment; during infliximab treatment, 47% had positive tests for ANA. One patient had anti-dsDNA at baseline; 7 had anti-dsDNA during infliximab treatment. None had clinical symptoms of lupus. Clegg, *et al*[24] found that 36% of patients with early RA had positive ANA (HEp-2 cell) at ≥ 1:32 dilution, and also found positive tests in 57% of undifferentiated polyarthritis and 59% of undifferentiated connective tissue disease patients. They also found antibodies to dsDNA in 2% and to SSA in 4% of their patients with early RA. Nishimura, *et al*[25] found positive ANA (HEp-2 cells) at a dilution ≥ 1:20 in 39% of 104 RA patients. The proportion was higher in patients with Stage III and IV RA, and ANA was present in 58% of the subgroup with disease duration of 2–5 years. Juby and Davis found that 51% of elderly patients with RA, 8% of healthy elderly, and 55% of frail elderly patients in a hospital or chronic care institution had positive ANA titers ≥ 1:40 on HEp-2 cells[26]. We did not note any difference in the age distribution of ANA positive and negative patients with early RA. Saraux, *et al* reported that ANA were more frequent in RA patients with nodules than in those without nodules (47% vs 31%)[27]. We found that rheumatoid nodules were more frequent in patients who were ANA positive than in those who were negative, although the difference was only weakly significant ($p = 0.033$). Darwin, *et al* reported that IgG antinuclear monoclonal antibodies isolated from autoimmune MRL-lpr/lpr mice have RF cross-reactivity and suggested that at least some ANA antibodies in RA may arise as components of the RF response[28].

Our patients with early seropositive RA were treated with the standard available DMARD and DMARD combinations. The selection of initial DMARD was not significantly different for the patients with ANA values < 8, 8, or > 8, nor are the total years of use of the various DMARD significantly different for the 3 groups. Except for 3 patients whose anti-dsDNA antibody test converted from negative to positive after treatment with SSZ, there did not appear to be any temporal relationship between the presence or absence of autoantibodies and specific DMARD treatments. In patients with ANA, anti-dsDNA, or anti-thyroid peroxidase antibodies, most of these antibodies were present at study entry before any DMARD exposure, and remained detectable in the majority of followup specimens. However, the other autoantibodies often were not present at entry, were only sporadically detected in a minority of the followup specimens, and were not associated with clinical diagnoses of autoimmune diseases. These findings suggest that routine screening with panels for autoantibodies in patients with early seropositive RA during treatment with DMARD may find many patients with one or more autoantibodies, but the relationship of the autoantibodies to the treatment is likely to be uncertain. Vigilance for clinical manifestations of other autoimmune diseases should be maintained, but the incidental presence of additional autoantibodies in the absence of specific signs and symptoms may not necessarily indicate a treatment related adverse event, and is probably insufficient evidence to prompt a change in the treatment regimen.

## APPENDIX

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