Title

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Journal

Arthritis Care & Research, 71(7)

ISSN

2151-464X

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Publication Date

2019-07-01

DOI

10.1002/acr.23712

Peer reviewed

HHS Public Access

Author manuscript

Arthritis Care Res (Hoboken). Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

Arthritis Care Res (Hoboken). 2019 July ; 71(7): 893–902. doi:10.1002/acr.23712.

Antinuclear Antibody–Negative Systemic Lupus Erythematosus in an International Inception Cohort

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Author Contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Clarke had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design

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Acquisition of data

Choi, Clarke, Hanly, Urowitz, Romero-Diaz, Gordon, Bae, Bernatsky, Wallace, Merrill, Isenberg, Rahman, Ginzler, Petri, Bruce, Dooley, Fortin, Gladman, Sanchez-Guerrero, Steinsson, Ramsey-Goldman, Khamashta, Aranow, Alarcón, Manzi, Nived, van Vollenhoven, Ramos-Casals, Ruiz-Irastorza, Lim, Kalunian, Inanc, Kamen, Peschken, Jacobsen, Askanase, Stoll, Buyon, Fritzler.

Analysis and interpretation of data

Choi, Clarke, St. Pierre, Hanly, Romero-Diaz, Gordon, Bernatsky, Wallace, Petri, Bruce, Dooley, Sanchez-Guerrero, Steinsson, Khamashta, Aranow, Zoma, van Vollenhoven, Lim, Kalunian, Inanc, Kamen, Buyon, Mahler, Fritzler.

The views expressed herein are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health, UK.

Additional Disclosure

Michael Mahler is an employee of Inova Diagnostics Inc.

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Abstract

Objective—The spectrum of antinuclear antibodies (ANAs) is changing to include both nuclear staining as well as cytoplasmic and mitotic cell patterns (CMPs) and accordingly a change is occurring in terminology to anticellular antibodies. This study examined the prevalence of indirect immunofluorescence (IIF) anticellular antibody staining using the Systemic Lupus International Collaborating Clinics inception cohort.

Methods—Anticellular antibodies were detected by IIF on HEp-2000 substrate using the baseline serum. Three serologic subsets were examined: ANA positive (presence of either nuclear or mixed nuclear/CMP staining), anticellular antibody negative (absence of any intracellular staining), and isolated CMP staining. The odds of being anticellular antibody negative versus ANA or isolated CMP positive was assessed by multivariable analysis.

Results—A total of 1,137 patients were included; 1,049 (92.3%) were ANA positive, 71 (6.2%) were anticellular antibody negative, and 17 (1.5%) had an isolated CMP. The isolated CMP–positive group did not differ from the ANA-positive or anticellular antibody–negative groups in clinical, demographic, or serologic features. Patients who were older (odds ratio [OR] 1.02 [95% confidence interval (95% CI) 1.00, 1.04]), of white race/ethnicity (OR 3.53 [95% CI 1.77, 7.03]), or receiving high-dose glucocorticoids at or prior to enrollment (OR 2.39 [95% CI 1.39, 4.12]) were more likely to be anticellular antibody negative. Patients on immunosuppressants (OR 0.35 [95% CI 0.19, 0.64]) or with anti-SSA/Ro 60 (OR 0.41 [95% CI 0.23, 0.74]) or anti–U1 RNP (OR 0.43 [95% CI 0.20, 0.93]) were less likely to be anticellular antibody negative.

Conclusion—In newly diagnosed systemic lupus erythematosus, 6.2% of patients were anticellular antibody negative, and 1.5% had an isolated CMP. The prevalence of anticellular antibody–negative systemic lupus erythematosus will likely decrease as emerging nomenclature guidelines recommend that non-nuclear patterns should also be reported as a positive ANA.

INTRODUCTION

Autoantibodies directed against nuclear autoantigens (antinuclear antibodies [ANAs]) and other intracellular autoantigens are a serologic hallmark of systemic lupus erythematosus (SLE) and other ANA-associated rheumatic diseases (AARD), such as systemic sclerosis, mixed connective tissue disease, and Sjögren's syndrome (1–3). ANAs are widely regarded as an important classification criterion of SLE, as officially recognized by both the American College of Rheumatology (ACR) (4) and the Systemic Lupus International Collaborating Clinics (SLICC) (5). ANA positivity is traditionally defined as the presence of an indirect immunofluorescence (IIF) staining pattern localized to the nucleus, while isolated cytoplasmic and mitotic cell patterns (CMPs), although staining positive by IIF, often are not reported or classified as ANA-positive and are not included in the ANA test reports by some laboratories. The International Consensus on ANA Patterns (ICAP) Committee has debated a suggestion that CMPs should be included in ANA result reports and that there should be a change in terminology to anticellular antibodies, because CMPs are increasingly recognized as clinically relevant (6–8) and have implications for the diagnosis and classification of AARDs (9). For instance, antiribosomal P proteins are highly specific for SLE and are associated with certain clinical and serologic SLE features (10, 11), but antiribosomal P antibodies may be reported as ANA IIF negative, because their prototypical staining pattern is localized to the cytoplasm (12). Therefore, ANA IIF exhibits limited sensitivity for the detection of antiribosomal P antibodies (13). After debate, however, the ICAP recognized that current disease classification criteria are predicated on a more traditional definition of ANA and that jurisdictional precedents (i.e., reimbursement fee structures) only allow reporting of classical ANA results, so the ICAP concluded that the reclassification of ANA to include CMPs should be delayed (9).

Inclusion of these additional CMPs in the ANA test results would likely help minimize misclassification of SLE patients, and the prevalence of anticellular antibody–negative SLE (i.e., the complete absence of any intracellular IIF staining patterns) will accordingly be decreased (12). The exact prevalence of ANA-negative SLE using the traditional definition (i.e., the absence of IIF staining localized only to the nucleus) has been reported to range from 1% to 28% (14–17). A recent systematic review and meta-analysis of 64 studies showed that an ANA of 1:80 was highly sensitive at 97.8% (95% confidence interval [95% CI] 96.8, 98.5), but not specific (74.7% [95% CI 66.7, 81.3]) for SLE (18). Pisetsky et al (14) compared different commercial ANA assays, including the HEp-2000 substrate, in an established SLE cohort and demonstrated significant variation in frequencies of ANA positivity that ranged from 77.7% to 95.1%. In studies to date, there are several factors (laboratory performance, study design, and clinical factors) that could influence the ANA results. Laboratory performance factors could include the ANA kit selected, the definition of an ANA (i.e., whether it includes isolated CMPs), the ANA IIF screening dilution chosen, and technical errors such as variable substrate sensitivity and specificity for the detection of autoantibodies directed against DNA, SSA/Ro 60, Ro 52/tripartite motif 21 (TRIM21), ribosomal P, and other intracellular autoantigens. The prevalence of ANA positivity is also likely impacted by whether it is measured cross-sectionally or longitudinally along the disease course. ANA status is also potentially influenced by the level of disease activity,

concurrent treatment with glucocorticoids and other immune-modulating drugs, and persistent proteinuria leading to renal immunoglobulin loss (2, 9, 15, 19, 20).

The purpose of this study was to examine the prevalence of anticellular antibody negativity (no intracellular IIF pattern) in a large international SLE inception cohort and to assess demographic, clinical, or other autoantibody characteristics associated with these redefined subgroups of patients with SLE.

MATERIALS AND METHODS

Study design and setting

This study was conducted using data and patient sera collected by SLICC, a network of 53 investigators in 43 academic medical centers in 16 countries (21–23). Between 1999 and 2011, SLICC investigators enrolled patients fulfilling the ACR classification criteria for definite SLE 4 within 15 months of diagnosis. The study was approved by the institutional review board at each participating site and complied with the Helsinki Declaration.

Anticellular antibody by IIF assay

The earliest available serum at enrollment from each patient was analyzed at the Mitogen Advanced Diagnostic Laboratory (University of Calgary). Aliquots of the anonymized SLE sera obtained from the central SLICC biobank were stored at −80° C until required for immunoassays. The IIF immunoassay was initially performed at a screening dilution of 1:160 24 using HEp-2000 cell substrate (ImmunoConcepts) and fluorescein isothiocyanate conjugated to antihuman IgG (H + L) according to the manufacturer's instructions. IIF results were read by technologists with >10 years of experience at Mitogen Advanced Diagnostics, as previously described (25). The HEp-2000 substrate had been transfected with the SSA/Ro 60 complementary DNA, which was then overexpressed in the cells, as an approach to intentionally increase the detection of anti-SSA/Ro 60 autoantibodies and thereby increasing the sensitivity of this substrate (25, 26). The results obtained at a single center (Mitogen) were used for the ANA analysis in this study, because the ANA analyses performed at each regional site had a wide variation in testing parameters (date of test performance, serum screening dilutions, test kits and protocols, microscopes, readers, etc.) and thus were not comparable across sites. For the purposes of this study, patients were divided into 3 groups depending on their anticellular antibody IIF patterns: ANA positive (the presence of nuclear IIF or mixed nuclear and CMP staining), anticellular antibody negative (no intracellular staining detected), and isolated CMP staining.

Detection of anti–double-stranded DNA (anti-dsDNA) and other autoantibodies

All samples were also tested for the presence of anti-dsDNA antibodies by chemiluminescence immunoassay (QUANTA Flash, Inova Diagnostics) as previously described (27) using a cutoff of 70 IU/ml, established in accord with the SLICC classification criterion for anti-dsDNA positivity, which requires that the cutoff for the anti-dsDNA antibody level be above the laboratory reference range (or >2-fold the reference range if tested by enzyme-linked immunosorbent assay) (5).

Antibodies to proliferating cell nuclear antigen, ribosomal P, recombinant Ro 52/TRIM21, native SSA/Ro 60, SSB/La, Sm, and U1 RNP were detected using the extractable nuclear antigen FIDIS Connective Profile, kit 13 addressable laser bead immunoassay (TheraDiag) on a Luminex 200 flow luminometer, according to the manufacturer's instructions, and using MLX-Booster software. Other autoantibodies, such as IgG anticardiolipin, IgG anti–β2-glycoprotein 1, and lupus anticoagulant, were measured in a central laboratory as previously described (28). ANA IIF patterns were classified according to the new ICAP standards (http://www.anapatterns.org/index.php) (9).

Clinically defined samples

Demographic and clinical data were collected at enrollment and included the age at diagnosis, sex, postsecondary education, disease duration, race/ethnicity, smoking status, alcohol use, hypertension, nephritis at enrollment, proteinuria at enrollment (≥3 grams/day), ACR classification criteria fulfilled (total and individual), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) global score and organ system scores, and medication use (glucocorticoids, high-dose glucocorticoids [any pulse steroid or prednisone ≥40 mg/day], antimalarials, and immunosuppressive agents, including biologics) at or prior to cohort enrollment (see Supplementary Table 1, available on the *Arthritis Care & Research* web site at http://onlinelibrary.wiley.com/doi/10.1002/acr.23712/abstract).

Statistical analysis

Statistical analysis was performed using Stata software, version 14.1. A 3-way comparison was performed between patients who were ANA positive versus anticellular antibody negative versus having an isolated CMP. Univariable and multivariable logistic regression analyses were used to examine potential predictors of the odds of being anticellular antibody negative or ANA positive or having an isolated CMP. As a secondary analysis, 3 additional univariable and multivariable logistic regressions were performed: anticellular antibody negative versus ANA positive, isolated CMP positive versus ANA positive, and isolated CMP positive versus anticellular antibody negative.

Potential univariable predictors included the demographic, clinical, and serologic data listed above. For the most informative multivariable model, only statistically significant predictors at the 95% CI were included, after eliminating all other potential predictors individually, starting with the least likely to be associated with the outcome.

RESULTS

Cohort demographic, clinical, and serologic characteristics

The baseline demographic, clinical, and serologic characteristics of the 3 serologic groups (ANA positive, anticellular antibody negative, and isolated CMP positive) are shown in Table 1. Overall, 1,137 patients had sera available; their mean ± SD age at diagnosis was 35.1 ± 13.5 years (median 33 years), 89.9% were female, 66.7% (724 of 1,085) had obtained postsecondary education, the mean ± SD disease duration was 0.46 ± 0.35 years, and 45.2% (511 of 1,130) were not of white race/ethnicity. A total of 312 of 1,084 (29%) of the cohort had lupus nephritis at enrollment, the mean ± SD global SLEDAI-2K score was 5.3 ± 5.3,

and 80.3% (913 of 1,137) had a history (either at or prior to enrollment) of glucocorticoid use, 73.6% (837 of 1,137) of antimalarial use, and 42.7% (485 of 1,137) of immunosuppressant use, including 4 patients who had received biologics (rituximab only).

Nuclear and CMP anticellular antibody IIF patterns

The distribution of patients based on IIF staining patterns and specificities is shown in Supplementary Figures 1 and 2, available on the *Arthritis Care & Research* web site at http://onlinelibrary.wiley.com/doi/10.1002/acr.23712/abstract. Overall, 1,049 of 1,137 patients (92.3%) were ANA positive, which included 877 isolated nuclear (77.1%) and 172 mixed nuclear patterns and CMPs (15.1%). A total 71 of 1,137 patients (6.2%) were anticellular antibody negative (i.e., with no detectable IIF staining), and 17 of 1,137 patients (1.5%) had an isolated CMP. Therefore, 7.7% of patients were either anticellular antibody negative or had an isolated CMP. Isolated CMPs and their related ICAP designations included 41.2% (7 of 17) cytoplasmic dense fine speckled (ANA pattern AC-19), 23.5% (4 of 17) cytoplasmic fine speckled (AC-20), 5.9% (1 of 17) cytoplasmic discrete dots (AC-18), 5.9% (1 of 17) mitotic chromosomal envelope (AC-28), and 23.5% (4 of 17) mixed CMP (ICAP does not have a pattern designation for mixed patterns at this time).

Comparison of isolated CMP positive with ANA positive and anticellular antibody negative and comparison of ANA positive and anticellular antibody negative

Patients with isolated CMPs were not clinically or serologically different from ANA-positive or anticellular antibody–negative patients for most variables (Table 1). In contrast, ANA-positive patients were markedly different from anticellular antibody–negative patients in terms of age at diagnosis (34.7 versus 40.9 years), race/ethnicity (a higher proportion of Asians and African descendants, but fewer patients of white race/ethnicity), disease activity (SLEDAI-2K score 5.4 versus 4.1), use of immunosuppressants at or prior to enrollment (43.7% versus 23.9%), and frequency of SLE-related autoantibodies. Interestingly, despite a negative anticellular antibody IIF on HEp-2000 substrate, some SLE-related autoantibodies were still detected, notably anti-dsDNA by chemiluminescence immunoassay (11.3%), and anti–Ro 52/TRIM21 (21.1%), anti-SSA/Ro 60 (22.5%), and anti–U1 RNP by addressable laser bead immunoassay (11.3%).

Multivariable analysis of anticellular antibody–negative patients versus ANA-positive patients combined with isolated CMP–positive patients

Because the isolated CMP–positive group did not differ from the ANA-positive or anticellular antibody–negative groups for most variables, we chose to combine the isolated CMP–positive with the ANA-positive groups for the primary multivariable analysis. In that analysis (Table 2), patients who were older (odds ratio [OR] per year 1.02 [95% CI 1.00, 1.04]), of white race/ethnicity (OR 3.53 [95% CI 1.77, 7.03]), or receiving high doses of glucocorticoids at or prior to enrollment (OR 2.39 [95% CI 1.39, 4.12]) were more likely to be anticellular antibody negative. Patients who were receiving immunosuppressants at or prior to enrollment (OR 0.35 [95% CI 0.19, 0.64]) or who had anti-SSA/Ro 60 (OR 0.41 [95% CI 0.23, 0.74]) or anti–U1 RNP (OR 0.43 [95% CI 0.20, 0.93]) were less likely to be anticellular antibody negative.

Multivariable analysis of anticellular antibody negative versus ANA positive, isolated CMP positive versus ANA positive, and isolated CMP positive versus anticellular antibody negative

In the secondary multivariable analysis comparing the odds of being anticellular antibody negative versus being ANA positive, the predictors were identical to those in the multivariable analysis of the anticellular antibody–negative patients versus the ANA-positive patients combined with the isolated CMP–positive patients (see Supplementary Table 2, available on the *Arthritis Care & Research* web site at http://onlinelibrary.wiley.com.ezproxy.galter.northwestern.edu/doi/10.1002/acr.23712/abstract).

In secondary multivariable analyses comparing the odds of being isolated CMP positive versus ANA positive or being isolated CMP positive versus anticellular antibody negative, patients who had not attained postsecondary education or who were hypertensive were more likely to be isolated CMP positive (see Supplementary Tables 3 and 4, available on the *Arthritis Care & Research* web site at http://onlinelibrary.wiley.com.ezproxy.galter.northwestern.edu/doi/10.1002/acr.23712/abstract).

DISCUSSION

To our knowledge, this is the first study of ANA IIF in a large SLE inception cohort redefining negative ANA as the absence of any intracellular IIF staining, which we referred to as anticellular antibody negative. Traditionally, ANA negative referred only to the absence of any IIF staining localized to the nucleus. This definition is an important consideration, especially for AARDs such as SLE, where the ANA test has a central role in establishing the diagnosis. The need to clarify this issue is exigent, because the topic is currently under international review (9), and the state of nomenclature uncertainty is the source of variability in ANA definitions and related clinical reports by different laboratories. Some laboratories do not report CMP staining, whereas others provide 2 reports: one that specifies nuclear staining patterns and titers and another that indicates whether CMP staining is present. In the broader definition of ANA test results, the inclusive definition of ANA and CMP together is more accurately referred to as anticellular antibody (1, 9, 29). However, because the ANA rubric is embedded in historical and scientific literature, the anticellular antibody terminology is held in abeyance until wider consensus and clinician education is achieved (3, 9, 29). The results of the current study provide some insight into the potential diagnostic and clinical implications for patients with SLE as a consequence of changing the definition of ANA to the wider anticellular antibody paradigm.

In our analysis of patients enrolled in the SLICC inception cohort, we demonstrated that the prevalence of ANA-negative SLE by routine IIF on a HEp-2000 substrate at a serum dilution of 1:160 was 7.7% (88 of 1,137). However, if isolated CMPs (17 of 1,137 [1.5%]) were subsequently excluded from the ANA-negative pool of 88 patients, the prevalence of anticellular antibody–negative SLE would decrease to 6.2% (71 of 1,137). Accordingly, among these 88 ANA-negative patients, nearly 1 in 5 is misclassified as ANA-negative, when they in fact have antibodies directed against a variety of CMP targets (8). Therefore, clinicians should be aware of which approach their laboratory employs for routine ANA IIF testing, because some patients with a high pretest probability of an AARD may have a

negative ANA test, when in fact the test should be regarded as positive if CMP staining is present.

In our study, SLE patients with an isolated CMP could not be readily differentiated from ANA-positive and anticellular antibody–negative patients based on clinical or conventional serologic features. These results must be interpreted cautiously, however, given the small sample size (n = 17) of patients with an isolated CMP. In contrast, there were many differences between the anticellular antibody–negative and ANA-positive patients, consistent with the current literature indicating that ANA-negative SLE follows a more benign clinical course characterized by photosensitive skin rashes and arthritis (19, 30, 31). We demonstrated in the SLICC cohort that anticellular antibody–negative patients were older (age 40.9 versus 34.7 years) and that a higher proportion were of white race/ethnicity (84.5% versus 52.4%). Further, anticellular antibody–negative patients compared to ANA-positive patients had a lower global SLEDAI-2K score (4.1 versus 5.4), less frequent use of immunosuppressants at or prior to enrollment (23.9% versus 43.7%), and a decreased likelihood of having multiple SLE-associated autoantibodies, including anti-dsDNA (11.3% versus 28.4%). These observations likely relate to earlier onset of more aggressive, severe disease in nonwhite patients, who tend to be ANA-positive, corroborating previous studies demonstrating higher disease activity in nonwhite patients with SLE (32, 33).

When the anticellular antibody–negative patients were compared to the isolated CMP–positive combined with the ANA-positive patients, all the above observations regarding anticellular antibody–negative versus ANA-positive patients persisted in the univariable analysis. However, in the multivariable analysis, slight differences were observed. Older age and white race/ethnicity remained associated with a greater likelihood of being anticellular antibody negative, and high-dose glucocorticoids now became associated with a greater likelihood of being anticellular antibody negative; immunosuppressant medications (at or prior to enrollment) and certain autoantibodies remained associated with a lower likelihood of being anticellular antibody negative. Our finding that high-dose glucocorticoids are associated with a higher likelihood of anticellular antibody negativity may be attributable to glucocorticoids influencing ANA status (34). However, this possibility is merely speculation, because we have no data on ANA status prior to the baseline assessment. Patients taking other types of immunosuppressants (i.e., methotrexate, azathioprine, mycophenolate mofetil) were less likely to be anticellular antibody negative, perhaps due to a different effect on B cell responses (35, 36). Further, immunosuppressants are potentially a proxy for elements of disease activity that are not measured through the other clinical variables included in the regression. Interestingly, in univariable analysis, all 4 patients treated with rituximab (data not shown) were anticellular antibody negative (OR 11.54 [95% CI 2.00, 66.74]). As suggested in a review by Cross et al (15), previous literature on ANA-negative SLE has been poor at documenting concurrent therapies. In that review and commentary, only 5 of 164 patients (3%) had data on medications during ANA testing. This lack of documentation highlights the need to review concurrent medications and consider other known confounders, such as proteinuria, as we have done.

The ANA status of our cohort was tested on the HEp-2000 substrate, which has been engineered to intentionally increase the detection of anti-SSA/Ro 60, thereby lowering the

prevalence of ANA-negative SLE (25, 26, 37, 38). Up to two-thirds of patients with mild SLE and persistently negative ANA tested on rodent liver substrate have been serologically linked to SLE due to precipitating autoantibodies to SSA/Ro 60 (31). These findings are particularly relevant to the clinical subset of SLE that has subacute cutaneous SLE and/or features of secondary Sjögren's syndrome (39). However, even with the technical improvements, such as HEp-2000 substrates, our study and others 20 indicate a persistent gap in autoantibody detection by HEp-2 substrates, which in the current study included anti-SSA/Ro 60 and even anti-dsDNA. For example, 22.5% of the anticellular antibody–negative SLE patients in our study still had anti-SSA/Ro 60 antibodies using extractable nuclear antigen testing; 11.3% of our anticellular antibody–negative patients had anti-dsDNA by chemiluminescence immunoassay. Our observations are consistent with a recent study showing that there is significant lack of agreement between positive results using a conventional multiplex array technology and the IIF on HEp-2 cells (40).

Significant variation in the frequencies of positive ANA in well-characterized SLE patients has been reported (15, 17, 18, 41); some of this variation relates to the performance of different HEp-2 assay kits (14). In the current study, we used a serum dilution of 1:160 to maximize specificity of the test at the possible expense of sensitivity (24). When the IIF test was repeated at a serum dilution of 1:80 on 67 of 71 of the available anticellular antibody–negative samples, we observed that 17 of 67 (25.4%) became clearly positive for nuclear and/or CMP staining (detailed data not shown). A cross-sectional study showed that only 76% of unselected SLE sera had a positive ANA, but a relatively high serum dilution of 1:200 was used (16). Taken together, this finding suggests that newer multiplexed autoantigen array technologies might be considered in the future as a replacement for the ANA IIF.

The presence of anti-dsDNA in ANA-negative SLE patients has been reported by others (42, 43). These patients were reported to have more severe complications, including nephritis (44), dystrophic calcification (45), or severe autoimmune neutropenia (46). Thus, the detection of anti-dsDNA antibodies even in ANA-negative cases is still important and may aid in risk assessment for clinical complications. Furthermore, the anti-dsDNA repertoire is diverse, such that there is no current anti-dsDNA assay that is able to detect all of the subpopulations of anti-dsDNA autoantibodies (47). Overall, the reports of anti-dsDNA–positive/ANA-negative sera found in the literature provide evidence that not all anti-dsDNA antibodies are detected on conventional HEp-2 substrates and that unique dsDNA epitopes may be missed by HEp-2 IIF screening tests.

Biomarkers such as autoantibodies and a variety of immune-related and inflammation-related molecules can appear years prior to clinical symptoms and/or the diagnosis of SLE and can accrue over time (40, 48). Therefore, longitudinal studies are needed to evaluate the serologic status of anticellular antibody–negative and isolated CMP–positive patients over time and to evaluate whether the status varies with disease activity, damage accrual, therapeutic interventions, and/or specific substrate assays. Even among ANA-negative patients with lupus nephritis, the patient can take up to 10 years to seroconvert from ANA negative to positive (17, 40). Some patients may only have detectable positive serologic

results when there is uncontrolled disease activity due to loss of self-tolerance from chronic autoreactivity of T and B cells (17).

There are some limitations to our study. First, the similarities reported between CMP-positive and ANA-positive patients are likely confounded by the high proportion of ANA-positive patients also expressing a CMP (21.5%). Overall, approximately 17% of patients in the entire cohort expressed a CMP (189 of 1,137), but the majority (172 of 189 [91.0%]) were seen in conjunction with nuclear IIF patterns. As a result, the isolated CMP–positive group size (n = 17) was small, limiting the statistical power of our analysis. We also did not perform statistical correction for multiple comparisons, which is consistent with the exploratory and hypothesis-generating aspect of our study. Additionally, we evaluated ANA status only at disease inception, but we have the capacity with this inception cohort, where data and sera are collected longitudinally, to evaluate ANA status and factors influencing it over the disease course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Ms. Haiyan Hou and Meifeng Zhang (Mitogen Advanced Diagnostics, University of Calgary) for technical assistance.

The Montreal General Hospital Lupus Clinic is supported by the Singer Family Fund for Lupus Research. The Hopkins Lupus Cohort is supported by the NIH (grants AR-043727 and AR-069572). Dr. Hanly's work was supported by the Canadian Institutes of Health Research (grant MOP-88526). Dr. Gordon's work was supported by Lupus UK, Sandwell and West Birmingham Hospitals NHS Trust, and the NIHR/Wellcome Trust Clinical Research Facility in Birmingham. Dr. Bae's work was supported by the Korea Healthcare technology R&D project, Ministry for Health and Welfare (A120404). Dr. Isenberg's and Dr. Rahman's work was supported by the NIHR University College London Hospitals Biomedical Research Centre. Dr. Bruce's work was supported by Arthritis Research UK, the NIHR Manchester Biomedical Research Centre, and the NIHR/Wellcome Trust Manchester Clinical Research Facility. Dr. Dooley's work was supported by the NIH (grant RR-00046). Dr. Ramsey-Goldman's work was supported by the NIH (grants 1U54-TR-001353 [formerly 8UL1-TR-000150], UL-1RR-025741, K24-AR-02318,and P60AR064464 [formerly P60-AR-48098]). Dr. Ruiz-Irastorza's work was supported by the Department of Education, Universities, and Research of the Basque Government.

Dr. Clarke has received consulting fees from AstraZeneca/MedImmune, Bristol-Myers Squibb, and Exagen Diagnostics (less than 10,000 each). Dr. Gordon has received consulting fees, speaking fees, and/or honoraria from Eli Lilly, UCB, GlaxoSmithKline, Merck Serono, and Bristol-Myers Squibb (less than $10,000 each). Dr. Bruce has received consulting fees, speaking fees, and/or honoraria from Eli Lilly, UCB, Roche, Merck Serono, and MedImmune (less than $10,000 each) and grants from UCB, Genzyme Sanofi, and GlaxoSmithKline. Dr. Gladman has received consulting fees, speaking fees, and/or honoraria from GlaxoSmithKline (less than $10,000). Dr. Kalunian has received grants from UCB, Human Genome Sciences/GlaxoSmithKline, Takeda, Ablynx, Bristol-Myers Squibb, Pfizer, and Kyowa Hakko Kirin, and has received consulting fees from Exagen Diagnostics, Genentech, Eli Lilly, Bristol-Myers Squibb, and Anthera (less than $10,000 each). Dr. Fritzler has received consulting fees from Inova Diagnostics, Werfen International, and Alexion Canada (less than $10,000 each). No other disclosures relevant to this article were reported.

REFERENCES

1. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014;73:17–23. [PubMed: 24126457]

2. Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. J Immunol Res 2014;2014:315179. [PubMed: 24868563]

3. Pisetsky DS. Antinuclear antibody testing: misunderstood or misbegotten? Nat Rev Rheumatol 2017;13:495–502. [PubMed: 28541299]

4. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.

5. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86. [PubMed: 22553077]

6. Rattner JB, Mack G, Fritzler MJ. Autoantibodies to components of the mitotic spindle apparatus. Mol Biol Rep 1998;25:143–55. [PubMed: 9700050]

7. Mack GJ, Rees J, Sandblom O, Balczon R, Fritzler MJ, Rattner JB. Autoantibodies to a group of centrosomal proteins in human autoimmune sera reactive with the centrosome. Arthritis Rheum 1998;41:551–8. [PubMed: 9506584]

8. Stinton LM, Eystathioy T, Selak S, Chan EK, Fritzler MJ. Autoantibodies to protein transport and messenger RNA processing pathways: endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes and GW bodies. Clin Immunol 2004;110:30–44. [PubMed: 14962794]

9. Damoiseaux J, von Muhlen CA, Garcia-de la Torre I, Carballo OG, de Melo CW, Francescantonio PL, et al. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. Auto Immun Highlights 2016;7:1. [PubMed: 26831867]

10. Mahler M, Kessenbrock K, Szmyrka M, Takasaki Y, Garcia-De La Torre I, Shoenfeld Y, et al. International multicenter evaluation of autoantibodies to ribosomal P proteins. Clin Vaccine Immunol 2006;13:77–83. [PubMed: 16426003]

11. Shi ZR, Cao CX, Tan GZ, Wang L. The association of serum anti-ribosomal P antibody with clinical and serological disorders in systemic lupus erythematosus: a systematic review and meta-analysis. Lupus 2015;24:588–96. [PubMed: 25406488]

12. Mahler M, Kessenbrock K, Raats J, Fritzler MJ. Technical and clinical evaluation of anti-ribosomal P protein immunoassays. J Clin Lab Anal 2004;18:215–23. [PubMed: 15202113]

13. Mahler M, Ngo J, Schulte-Pelkum J, Luettich T, Fritzler MJ. Limited reliability of the indirect immunofluorescence technique for the detection of anti-Rib-P antibodies. Arthritis Res Ther 2008;10:R131. [PubMed: 19000323]

14. Pisetsky DS, Spencer DM, Lipsky PE, Rovin BH. Assay variation in the detection of antinuclear antibodies in the sera of patients with established SLE. Ann Rheum Dis 2018;77:911–3. [PubMed: 29440000]

15. Cross LS, Aslam A, Misbah SA. Antinuclear-negative lupus as a distinct diagnostic entity: does it no longer exist? QJM 2004;97: 303–8. [PubMed: 15100425]

16. Sjowall C, Sturm M, Dahle C, Bengtsson AA, Jonsen A, Sturfelt G, et al. Abnormal antinuclear antibody titers are less common than generally assumed in established cases of systemic lupus erythematosus. J Rheumatol 2008;35:1994–2000. [PubMed: 18785312]

17. Simmons SC, Smith ML, Chang-Miller A, Keddis MT. Antinuclear antibody-negative Lupus nephritis with full house nephropathy: a case report and review of the literature. Am J Nephrol 2015;42:451–9. [PubMed: 26812129]

18. Leuchten N, Hoyer A, Brinks R, Schoels M, Schneider M, Smolen J, et al. Performance of antinuclear antibodies for classifying systemic lupus erythematosus: a systematic literature review and meta-regression of diagnostic data. Arthritis Care Res (Hoboken) 2018;70:428–38. [PubMed: 28544593]

19. Reichlin M ANA negative systemic lupus erythematosus sera revisited serologically. Lupus 2000;9:116–9. [PubMed: 10787008]

20. Fritzler MJ. Choosing wisely: review and commentary on anti-nuclear antibody (ANA) testing. Autoimmun Rev 2016;15:272–80. [PubMed: 26687321]

21. Urowitz MB, Gladman DD, Ibanez D, Sanchez-Guerrero J, Romero-Diaz J, Gordon C, et al. American College of Rheumatology criteria at inception, and accrual over 5 years in the SLICC inception cohort. J Rheumatol 2014;41:875–80. [PubMed: 24692526]

22. Hanly JG, O'Keeffe AG, Su L, Urowitz MB, Romero-Diaz J, Gordon C, et al. The frequency and outcome of lupus nephritis: results from an international inception cohort study. Rheumatology (Oxford) 2016;55:252–62. [PubMed: 26342222]
23. Isenberg DA, Ramsey-Goldman R, Gladman D, Hanly JG. The Systemic Lupus International Collaborating Clinics (SLICC) group: it was 20 years ago today. Lupus 2011;20:1426–32. [PubMed: 22095889]
24. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 1997;40:1601–11. [PubMed: 9324014]
25. Fritzler MJ, Hanson C, Miller J, Eystathioy T. Specificity of autoantibodies to SS-A/Ro on a transfected and overexpressed human 60 kDa Ro autoantigen substrate. J Clin Lab Anal 2002;16:103–8. [PubMed: 11948800]
26. Keech CL, Howarth S, Coates T, Rischmueller M, McCluskey J, Gordon TP. Rapid and sensitive detection of anti-Ro (SS-A) antibodies by indirect immunofluorescence of 60 kDa Ro HEp-2 transfectants. Pathology 1996;28:54–7. [PubMed: 8714273]
27. Choi MY, Clarke AE, St Pierre Y, Hanly JG, Urowitz MB, Romero-Diaz J, et al. The prevalence and determinants of anti-DFS70 autoantibodies in an international inception cohort of systemic lupus erythematosus patients. Lupus 2017;26:1051–9. [PubMed: 28420054]
28. Hanly JG, Urowitz MB, Siannis F, Farewell V, Gordon C, Bae SC, et al. Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. Arthritis Rheum 2008;58:843–53. [PubMed: 18311802]
29. Chan EK, Damoiseaux J, de Melo CW, Carballo OG, Conrad K, Francescantonio PL, et al. Report on the second International Consensus on ANA Pattern (ICAP) workshop in Dresden 2015. Lupus 2016;25:797–804. [PubMed: 27252255]
30. Gladman DD, Chalmers A, Urowitz MB. Systemic lupus erythematosus with negative LE cells and antinuclear factor. J Rheumatol 1978;5:142–7. [PubMed: 78982]
31. Maddison PJ, Provost TT, Reichlin M. Serological findings in patients with "ANA-negative" systemic lupus erythematosus. Medicine (Baltimore) 1981;60:87–94. [PubMed: 6971388]
32. Fernández M, Alarcón GS, Calvo-Alén J, Andrade R, McGwin G Jr, Vilá LM, et al. A multiethnic, multicenter cohort of patients with systemic lupus erythematosus (SLE) as a model for the study of ethnic disparities in SLE. Arthritis Rheum 2007;57: 576–84. [PubMed: 17471524]
33. Feldman CH, Hiraki LT, Liu J, Fischer MA, Solomon DH, Alarcón GS, et al. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000–2004. Arthritis Rheum 2013;65:753–63. [PubMed: 23203603]
34. Barnett EV, North AF Jr, Condemi JJ, Jacox RF, Vaughan JH. Antinuclear factors in systemic lupus erythematosus and rheumatoid arthritis. Ann Intern Med 1965;63:100–8. [PubMed: 14305956]
35. Weisbart RH, Colburn K. Effect of corticosteroids on serum antinuclear antibodies in man. Immunopharmacology 1984;8:97–101. [PubMed: 6334672]
36. Lindholm C, Borjesson-Asp K, Zendjanchi K, Sundqvist AC, Tarkowski A, Bokarewa M. Longterm clinical and immunological effects of anti-CD20 treatment in patients with refractory systemic lupus erythematosus. J Rheumatol 2008;35:826–33. [PubMed: 18398943]
37. Bossuyt X, Meurs L, Mewis A, Marien G, Blanckaert N. Screening for autoantibodies to SS-A/Ro by indirect immunofluorescence using HEp-2000TM cells. Ann Clin Biochem 2000;37:216–9. [PubMed: 10735367]
38. Peene I, Van Ael W, Vandenbossche M, Vervaet T, Veys E, De Keyser F. Sensitivity of the HEp-2000 substrate for the detection of anti-SSA/Ro60 antibodies. Clin Rheumatol 2000;19:291–5. [PubMed: 10941811]
39. Sontheimer RD, Maddison PJ, Reichlin M, Jordon RE, Stastny P, Gilliam JN. Serologic and HLA associations in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. Ann Intern Med 1982;97:664–71. [PubMed: 6982644]
40. Perez D, Gilburd B, Cabrera-Marante O, Martinez-Flores JA, Serrano M, Naranjo L, et al. Predictive autoimmunity using autoantibodies: screening for anti-nuclear antibodies. Clin Chem Lab Med 2018;56:1771–7. [PubMed: 28628475]

41. Worrall JG, Snaith ML, Batchelor JR, Isenberg DA. SLE: a rheumatological view. Analysis of the clinical features, serology and immunogenetics of 100 SLE patients during long-term follow-up. Q J Med 1990;74:319–30. [PubMed: 2385739]

42. Compagno M, Jacobsen S, Rekvig OP, Truedsson L, Heegaard NH, Nossent J, et al. Low diagnostic and predictive value of anti-dsDNA antibodies in unselected patients with recent onset of rheumatic symptoms: results from a long-term follow-up Scandinavian multicentre study. Scand J Rheumatol 2013;42:311–6. [PubMed: 23496224]

43. Baronaite R, Engelhart M, Mork HT, Thamsborg G, Slott JH, Stender S, et al. A comparison of anti-nuclear antibody quantification using automated enzyme immunoassays and immunofluorescence assays. Autoimmune Dis 2014;2014:534759. [PubMed: 24592328]

44. Lindstedt G, Lundberg PA, Westberg G, Kaijser B. S.L.E. nephritis with positive tests for antibodies against native D.N.A. but negative tests for antinuclear antibodies [letter]. Lancet 1977;2:135. [PubMed: 69212]

45. Morris CN, Calobrisi SD, Matteson EL. Antinuclear antibody negative lupus associated with dystrophic calcification. J Rheumatol 1998;25:825–6. [PubMed: 9558201]

46. Zhao M ANA-negative presentation of SLE in man with severe autoimmune neutropenia. Case Rep Med 2016;2016:6853936. [PubMed: 28077945]

47. Compagno M, Rekvig OP, Bengtsson AA, Sturfelt G, Heegaard NH, Jonsen A, et al. Clinical phenotype associations with various types of anti-dsDNA antibodies in patients with recent onset of rheumatic symptoms: results from a multicentre observational study. Lupus Sci Med 2014;1:e000007. [PubMed: 25396058]

48. Choi MY, Barber MR, Barber CE, Clarke AE, Fritzler MJ. Preventing the development of SLE: identifying risk factors and proposing pathways for clinical care. Lupus 2016;25:838–49. [PubMed: 27252260]

SIGNIFICANCE & INNOVATIONS

- This is the first study to examine the prevalence of anticellular antibody negativity defined as the absence of any intracellular indirect immunofluorescence staining in a large systemic lupus erythematosus (SLE) cohort at inception.
- In 1,137 patients newly diagnosed with SLE, 6.2% (71) were anticellular antibody negative and 1.5% (17) had an isolated cytoplasmic and mitotic pattern (CMP). Therefore, among these 88 patients, 20% (17) would be misclassified as antinuclear antibody negative under the traditional definition, when in fact they have antibodies directed against a variety of CMP targets.
- Anticellular antibody negativity was more likely in patients who were older, were of white race/ethnicity, or were receiving high-dose glucocorticoids, and it was less likely in those patients using immunosuppressants. Longitudinal data are needed to assess how anticellular antibody status is influenced by the disease course and therapy.

Table 1.

Baseline demographic, clinical, and autoantibody profiles of antinuclear antibody (ANA)–positive (presence of any nuclear indirect immunofluorescence [IIF] pattern), anticellular antibody (ACA)–negative (no IIF pattern), and isolated cytoplasmic/mitotic (CMP) groups

	ANA+ (n = 1,049)	ACA− (n = 71)	Isolated CMP (n = 17)†	ANA+ and ACA−	ANA+ and CMP	ACA− and CMP
Demographics						
Age at diagnosis, years, mean	34.7‡	40.9‡	35.8	−6.2 (−9.4, −2.9)	−1 (−7.5, 5.4)	5.1 (−2.4, 12.7)
Female, %	89.7	90.1	100	−0.4 (−7.6, 6.7)	−10.3 (−24.8, 4.2)	−9.9 (−24.2, 4.5)
Postsecondary education, %	66.7‡	76.1§	31.3†*	−9.5 (−20.1, 1.2)	35.4 (12.5, 58.3)	44.9 (20, 69.8)
Disease duration, years, mean	0.47	0.42	0.35	0.05 (−0.03, 0.14)	0.12 (−0.05, 0.29)	0.07 (−0.12, 0.25)
Race/ethnicity, %						
Asian	23.2‡	4.2‡	11.8	19 (13.7, 24.3)	11.5 (−4.1, 27)	−7.5 (−23.6, 8.5)
African descendant	16.2‡	7.0‡	5.9	9.2 (2.8, 15.5)	10.3 (−1.1, 21.7)	1.2 (−11.5, 13.8)
Hispanic	3.4	2.8	0	0.5 (−3.5, 4.5)	3.4 (−5.2, 11.9)	2.8 (−1, 6.7)
White	52.4†*	84.5‡	76.5§	−32.1 (−41.1, −23.2)	−24.1 (−44.5, −3.7)	8 (−13.8, 29.9)
Other	4.8	1.4	5.9	3.4 (−1.6, 8.4)	−1.1 (−12.3, 10.2)	−4.5 (−16, 7)
Smoking status, %						
Current smoker	15.1	21.9	18.8	−6.8 (−17.1, 3.6)	−3.7 (−22.9, 15.6)	3.1 (−18.5, 24.8)
Former smoker	21.1	26.6	25	−5.5 (−16.6, 5.6)	−3.9 (−25.3, 17.5)	1.6 (−22.3, 25.4)
High alcohol use, %	1.5	1.5	0	0 (−3, 3)	1.5 (−4.6, 7.5)	1.5 (−4.7, 7.6)
Hypertension, %	32.6‡	29.6§	58.8†*	3 (−8, 14)	−26.2 (−49.8, −2.7)	−29.2 (−54.9, −3.6)
Nephritis at enrollment, %	28.7	26.6	50	2.1 (−9, 13.3)	−21.3 (−46, 3.4)	−23.4 (−50.2, 3.3)
Proteinuria at enrollment, %	4.5	3.3	12.5	1.2 (−3.4, 5.9)	−8 (−24.3, 8.3)	−9.2 (−26, 7.6)
No. of ACR criteria, mean	4.8	4.7	4.7	0.1 (−0.1, 0.4)	0.1 (−0.4, 0.6)	0 (−0.5, 0.5)
SLEDAI-2K score, mean	5.4‡	4.1‡	5.4	1.3 (0, 2.6)	0 (−2.7, 2.6)	−1.3 (−3.8, 1.1)
Neurological	0.3	0.3	0	−0.1 (−0.5, 0.3)	0.3 (−0.5, 1)	0.3 (−0.5, 1.2)
Mucocutaneous	1.1	1	1.3	0.1 (−0.4, 0.5)	−0.1 (−1.1, 0.8)	−0.2 (−1, 0.6)
Musculoskeletal	0.8	0.7	1.3	0.1 (−0.3, 0.5)	−0.4 (−1.3, 0.4)	−0.5 (−1.4, 0.4)
Renal	1.4	0.7	1.8	0.7 (−0.1, 1.5)	−0.4 (−2, 1.2)	−1.1 (−2.5, 0.4)
Serositis	0.1	0.1	0	0 (−0.1, 0.1)	0.1 (−0.1, 0.3)	0.1 (−0.2, 0.4)
Constitutional	0	0	0	0 (0, 0.1)	0 (−0.1, 0.1)	0 (0, 0.1)
Immunologic	1.6‡	1.1‡	1.1	0.5 (0.1, 0.9)	0.5 (−0.4, 1.3)	0 (−0.9, 0.8)
Hematologic	0.1	0	0	0.1 (0, 0.1)	0.1 (−0.1, 0.3)	0 (−0.1, 0.1)
Medications, % ever used						

	ANA+ (n = 1,049)	ACA− (n = 71)	Isolated CMP (n = 17)†	ANA+ and ACA−	ANA+ and CMP	ACA− and CMP
Glucocorticoids	80.6	74.6	82.4	6 (−4.4, 16.4)	−1.7 (−20, 16.6)	−7.7 (−28.5, 13.1)
High-dose glucocorticoids	42.3	46.5	58.8	−4.2 (−16.1, 7.8)	−16.5 (−40.1, 7.1)	−12.3 (−38.5, 13.8)
Antimalarials	74.3	69	52.9	5.2 (−5.8, 16.3)	21.3 (−2.6, 45.2)	16.1 (−10, 42.1)
Immunosuppressants	43.7‡	23.9†*	58.8§	19.7 (9.3, 30.1)	−15.2 (−38.7, 8.4)	−34.9 (−60.3, −9.5)
Autoantibodies, %						
dsDNA	28.4‡	11.3‡	17.7	17.2 (9.3, 25)	10.8 (−7.5, 29.1)	−6.4 (−25.9, 13.2)
PCNA	7.3	1.4	11.8	5.9 (−0.2, 12)	−4.4 (−19.8, 11)	−10.4 (−25.9, 5.2)
Ribosomal P	16.1‡	5.6‡	11.8	10.5 (4.7, 16.3)	4.3 (−11.1, 19.8)	−6.1 (−22.4, 10.1)
Ro 52/TRIM21	35.9‡	21.1‡	23.5	14.8 (4.9, 24.7)	12.4 (−8, 32.8)	−2.4 (−24.7, 19.9)
SSA/Ro 60	47.3‡	22.5‡	29.4	24.7 (14.6, 34.9)	17.9 (−4, 39.7)	−6.9 (−30.6, 16.9)
SSB/La	15.9‡	5.6‡	11.8	10.3 (4.5, 16.1)	4.2 (−11.3, 19.6)	−6.1 (−22.4, 10.1)
Sm	24.7‡	5.7‡	11.8	19 (12.9, 25)	12.9 (−2.6, 28.5)	−6.1 (−22.3, 10.2)
U1 RNP	32.4‡	11.3‡	11.8	21.1 (13.3, 29)	20.6 (−1.7, 43)	−0.5 (−17.5, 16.5)
Lupus anticoagulant	20.8	20.6	6.7	0.1 (−10.2, 10.5)	14.1 (−6.5, 34.7)	14 (−2.1, 30.1)
Anticardiolipin	12.6	11.1	12.5	1.5 (−6.6, 9.5)	0.1 (−16.3, 16.4)	−1.4 (−19.4, 16.6)
Anti–β2-	15	15.9	12.5	0.8 (−10.1, 8.5)	2.5 (−13.8, 18.9)	3.4 (−15.2, 21.9)

*Values are the difference (95% confidence interval) unless indicated otherwise. ACR = American College of Rheumatology; SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index 2000; dsDNA = double-stranded DNA; PCNA = proliferating cell nuclear antigen; TRIM21 = tripartite motif 21; SSA = Sjögren's syndrome antigen A; SSB = Sjögren's syndrome antigen B; RNP = ribonucleoprotein.

†Some predictors had a small number of missing values. When these occurred, the observations were excluded from the relevant analysis. In particular, for the small group of patients with an isolated CMP, the data included 1 missing value for education, smoking status, nephritis, proteinuria, no. of ACR criteria, anticardiolipin, and anti–β2-glycoprotein 1, and 2 missing values for high alcohol use and lupus anticoagulant.

‡Values with the same footnote symbol are significantly different from each other.

§Values with the same footnote symbol are significantly different from each other.

†*Values are significantly different from ‡ and §, but ‡ and § are not different from each other.

Table 2.

Univariable and multivariable analysis of demographic, clinical, and serologic profiles of anticellular antibody–negative versus antinuclear antibody–positive combined with isolated cytoplasmic/mitotic patterns*

	Univariate	Multivariate
Demographics		
Age at diagnosis	1.03 (1.01, 1.05) †	1.02 (1.00, 1.04) †
Female	1.03 (0.46, 2.31)	–
Postsecondary education	1.63 (0.92, 2.91)	–
Disease duration	0.66 (0.32, 1.34)	–
Race/ethnicity		
Asian	0.15 (0.05, 0.47) †	–
African descendant	0.40 (0.16, 1.00) †	–
Hispanic	0.85 (0.20, 3.60)	–
White	4.88 (2.54, 9.38) †	3.53 (1.77, 7.03) †
Other	0.28 (0.38, 2.07)	–
Smoking status		
Current smoker	1.57 (0.85, 2.90)	–
Former smoker	1.35 (0.76, 2.39)	–
High alcohol use	1.04 (0.14, 8.00)	–
Hypertension	0.85 (0.50, 1.44)	–
Nephritis at enrollment	0.88 (0.50, 1.56)	–
Proteinuria at enrollment	0.70 (0.17, 2.95)	–
No. of ACR criteria	0.89 (0.69, 1.14)	–
SLEDAI-2K score	0.94 (0.89, 1.00) †	–
Neurological	1.03 (0.90, 1.18)	–
Mucocutaneous	0.98 (0.85, 1.12)	–
Musculoskeletal	0.97 (0.84, 1.14)	–
Renal	0.91 (0.81, 1.01)	–
Serositis	1.12 (0.72, 1.75)	–
Constitutional	0.37 (0.05, 2.73)	–
Immunologic	0.82 (0.69, 0.96) †	–
Hematologic	0.41 (0.13, 1.27)	–
Medications, ever using		
Glucocorticoids	0.71 (0.40, 1.23)	–
High-dose glucocorticoids	1.17 (0.72, 1.90)	2.39 (1.39, 4.12) †
Antimalarials	0.79 (0.47, 1.32)	–
Immunosuppressants/biologics	0.40 (0.23, 0.70) †	0.35 (0.19, 0.64) †
Autoantibodies		
dsDNA	0.32 (0.15, 0.68) †	–

	Univariate	Multivariate
PCNA	0.18 (0.02, 1.30) †	–
Ribosomal P	0.31 (0.11, 0.87) †	–
Ro52/TRIM21	0.48 (0.27, 0.86) †	–
SSA/Ro 60	0.33 (0.19, 0.58) †	0.41 (0.23, 0.74) †
SSB/La	0.32 (0.11, 0.88) †	–
Sm	0.19 (0.07, 0.52) †	–
U1 RNP	0.27 (0.13, 0.57) †	0.43 (0.20, 0.93) †
Lupus anticoagulant	1.00 (0.54, 1.88)	–
Anticardiolipin	0.87 (0.39, 1.95)	–
Anti–β2-glycoprotein 1	1.07 (0.53, 2.15)	–

*Values are the odds ratio (95% confidence interval). ACR = American College of Rheumatology; SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index 2000; dsDNA = double-stranded DNA; PCNA = proliferating cell nuclear antigen; TRIM21 = tripartite motif 21; SSA = Sjögren's syndrome antigen A; SSB = Sjögren's syndrome antigen B; RNP = ribonucleoprotein.

†Statistically significant.