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Gordon, Caroline

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DR. MAY Y CHOI (Orcid ID : 0000-0003-3760-2737)

DR. MICHELLE A PETRI (Orcid ID : 0000-0003-1441-5373)

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

Running Head: ANA-Negative SLE

[1]May Y. Choi MD; [1]Ann E. Clarke MD, MSc; [2]Yvan St. Pierre MSc; [3]John G. Hanly MD; [4]Murray B. Urowitz MD; [5]Juanita Romero-Diaz MD, MS; [6]Caroline Gordon MD; [7]Sang-Cheol Bae MD, PhD, MPH; [8]Sasha Bernatsky MD, PhD; [9]Daniel J Wallace MD; [10]Joan T. Merrill MD; [11]David A. Isenberg MD; [11]Anisur Rahman PhD; [12]Ellen M. Ginzler MD, MPH; [13]Michelle Petri MD, MPH; [14]Ian N. Bruce MD; [15]Mary A. Dooley MD, MPH;, Paul R. Fortin[16] MD, MPH; [4]Dafna D. Gladman MD; [17]Jorge Sanchez-Guerrero MD, MS; [18]Kristjan Steinsson MD, PhD; [19]Rosalind Ramsey-Goldman MD, DrPh; [20]Munther A. Khamashta MD, PhD; [21]Cynthia Aranow MD; [22]Graciela S. Alarcón MD, MPH; [23]Susan Manzi MD, MPH; [24]Ola Nived MD, PhD; [25]Asad A. Zoma MBChB; [26]Ronald F. van Vollenhoven MD, PhD; [27]Manuel Ramos-Casals MD, PhD; [28]Guillermo Ruiz-Irastorza MD, PhD; [29]S. Sam Lim MD, MPH; [30]Kenneth C. Kalunian MD; [31]Murat Inanc MD; [32]Diane L. Kamen MD, MSCR; [33]Christine A. Peschken MD, MSc; [34]Soren Jacobsen MD, DMSc; [35]Anca Askanase MD, MPH; [36]Thomas Stoll MD; [37]Jill Buyon MD; [38]Michael Mahler PhD, [1]Marvin J. Fritzler PhD, MD.

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[1]University of Calgary, Cumming School of Medicine, [2]Research Institute of the McGill University Health Centre; [3]Division of Rheumatology, Department of Medicine and Department of Pathology, Queen Elizabeth II Health Sciences Centre and Dalhousie University, Halifax, Nova Scotia, Canada; [4]Lupus Program, Centre for Prognosis Studies in The Rheumatic Disease and Krembil Research Institute, Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada; [5]Instituto Nacional de Ciencias Médicas y Nutrición, Mexico City, Mexico; [6]Rheumatology Research Group, Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; [7]Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, Korea; [8]Divisions of Rheumatology and Clinical Epidemiology, McGill University Health Centre; [9]Cedars-Sinai/David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [10]Department of Clinical Pharmacology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; [11]Centre for Rheumatology, Department of Medicine, University College London, UK; [12]Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA; [13]Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [14]Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester; and NIHR Manchester Biomedical Research Centre, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre; Manchester, UK; [15]Thurston Arthritis Research Center, University of North Carolina, Chapel Hill, NC, USA; [16]Division of Rheumatology, CHU de Québec - Université Laval, Québec City, Canada; [17]Mount Sinai Hospital and University Health Network, University of Toronto, Canada, [18]Center for Rheumatology Research, Landspitali University hospital, Reykjavik, Iceland; [19]Northwestern University and Feinberg School of Medicine, Chicago, IL, USA; [20]Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, King's College London School of Medicine, UK, London, UK; [21]Feinstein Institute for Medical Research, Manhasset, NY, USA; [22]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; [23]Allegheny Health Network, Pittsburgh Pennsylvania; [24]Department of Rheumatology, University Hospital Lund, Lund, Sweden; [25]Lanarkshire

Centre for Rheumatology, Hairmyres Hospital, East Kilbride, Scotland UK; [26]University of Amsterdam, Rheumatology & Immunology Center, Amsterdam, Noord-Holland, NL; [27]Josep Font Autoimmune Diseases Laboratory, IDIBAPS, Department of Autoimmune Diseases, Hospital Clínic, Barcelona, Spain; [28]Autoimmune Diseases Research Unit, Department of Internal Medicine, BioCruces Health Research Institute, Hospital Universitario Cruces, University of the Basque Country, Barakaldo, Spain; [29]Emory University School of Medicine, Division of Rheumatology, Atlanta, Georgia, USA; [30]UCSD School of Medicine, La Jolla, CA, USA; [31]Division of Rheumatology, Department of Internal Medicine, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; [32]Medical University of South Carolina, Charleston, South Carolina, USA; [33]University of Manitoba, Winnipeg, Manitoba, Canada; [34]Department of Rheumatology, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, 2100, Copenhagen, Denmark; [35]Hospital for Joint Diseases, NYU, Seligman Centre for Advanced Therapeutics, New York NY; [36]Department of Rheumatology, Kantousspital, Schaffhausen, Switzerland; [37]New York University School of Medicine, New York, US; [38]Inova Diagnostics Inc., San Diego, CA, USA.

Corresponding Author and Reprint Requests:

Ann E. Clarke MD, MSc

Division of Rheumatology, Cumming School of Medicine, University of Calgary

3330 Hospital Dr NW

Calgary, Alberta, CANADA T2N 4N1

Phone: +1 (403) 220-8737

Fax: 403-210-8165

Email: aeclarke@ucalgary.ca

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ABSTRACT

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

Methods: Anti-cellular antibodies were detected by IIF on HEp-2000 substrate utilizing the baseline serum. Three serological subsets were examined: 1) ANA-positive (presence of either nuclear or mixed nuclear/CMP staining), 2) anti-cellular antibody-negative (absence of any intracellular

staining), and 3) isolated CMP staining. The odds of being anti-cellular antibody-negative versus ANA or isolated CMP-positive was assessed by multivariable analysis.

Results: 1137 patients were included; 1049/1137 (92.3%) were ANA-positive, 71/1137 (6.2%) were anti-cellular antibody-negative, and 17/1137 (1.5%) had isolated CMP. The isolated CMP group did not differ from the ANA-positive or anti-cellular antibody-negative group in clinical, demographic or serologic features. Patients who were older (OR 1.02 [95% CI: 1.00, 1.04]), of Caucasian race/ethnicity (OR 3.53 [95% CI: 1.77, 7.03]), or on high dose glucocorticoids at or prior to enrolment (OR 2.39 [95% CI: 1.39, 4.12]) were more likely to be anti-cellular antibody-negative. Patients on immunosuppressants (OR 0.35 [95% CI: 0.19, 0.64]) or with anti-SSA/Ro60 (OR 0.41 [95% CI: 0.23, 0.74]) or anti-UI-RNP (OR 0.43 [95% CI: 0.20, 0.93]) were less likely to be anti-cellular antibody-negative.

Conclusions: In newly diagnosed SLE, 6.2% of patients were anti-cellular antibody-negative and 1.5% had isolated CMP. The prevalence of anti-cellular antibody-negative SLE will likely decrease as emerging nomenclature guidelines recommend that non-nuclear patterns should also be reported as a positive ANA.

Significance and Innovations

- This is the first study to examine the prevalence of anti-cellular antibody-negativity defined as the absence of any intracellular indirect immunofluorescence (IIF) staining in a large SLE cohort at inception.
- In newly diagnosed SLE, 6.2% (71/1137) of patients were anti-cellular antibody-negative and 1.5% (17/1137) had an isolated cytoplasmic and mitotic pattern (CMP). Therefore, among these

88 patients, 20% (17/88) would be "misclassified" as ANA negative under the traditional definition, when they in fact have antibodies directed against a variety of CMP targets.

- Anti-cellular antibody-negativity was more likely in those of older age, of Caucasian race/ethnicity, or on high dose glucocorticoids and less likely in those on immunosuppressants. Longitudinal data is needed to assess how anti-cellular antibody status is influenced by the disease course and therapy.

Autoantibodies directed against nuclear (anti-nuclear antibodies, ANA) and other intracellular autoantigens are a serological 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). ANA 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 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, are often 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 anti-cellular antibodies because CMPs are increasingly recognized as clinically relevant (6-8) and have implications for the diagnosis and classification of AARD (9). For instance, anti-ribosomal P proteins are highly specific for SLE and are associated with certain clinical and serological SLE features (10;11), but anti-ribosomal P antibodies may be reported as ANA-IIF negative because the prototypical staining pattern is localized to the cytoplasm (12). Therefore, ANA-IIF exhibits limited sensitivity for the detection of anti-ribosomal P antibodies (13). However, even after debate it was appreciated 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), it was concluded that the reclassification of ANA to include CMP 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 anti-cellular 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., absence of IIF staining localized only to the nucleus) has been reported to range from 1 to 28% (14-16) (reviewed in (17)). A recent systematic review and meta-analysis of 64 studies reported that an ANA of 1:80 was highly sensitive at 97.8% (95% confidence interval (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 these studies, there are several factors (laboratory performance, study design, and clinical) that could influence the ANA results. Laboratory performance factors could include 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/Ro60, Ro52/TRIM21, ribosomal P and other intracellular autoantigens. The prevalence of ANA-positivity is also likely impacted by whether it was measured cross-sectionally or longitudinally along the disease course. ANA status is also potentially influenced by 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 anti-cellular antibody-negative (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 SLE patients.

MATERIALS AND METHODS

Study Design and Setting

This study was conducted using data and patient sera collected by the 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.

Anti-cellular Antibody by Indirect Immunofluorescence assay (IIF)

The earliest available serum at enrolment from each patient was analyzed at Mitogen Advanced Diagnostic Laboratory (University of Calgary, Calgary, Alberta, Canada). Aliquots of the anonymized SLE sera obtained from the central SLICC biobank were stored at -80^{o}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, Sacramento, CA, USA) and fluorescein (FITC) conjugated to anti-human 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 has been transfected with the SSA/Ro60 cDNA, which is then

overexpressed in the cells, as an approach to intentionally increase the detection of anti-SSA/Ro60 autoantibodies, thereby increasing the sensitivity of this substrate (25;26). The results performed at a single center (Mitogen) were used for the ANA analysis in this study because the ANAs 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 hence were not comparable across sites. For the purposes of this study, patients were divided into three groups depending on their anti-cellular antibody IIF patterns: 1) ANA-positive (presence of nuclear IIF or mixed nuclear and CMP staining), 2) anti-cellular antibody-negative (no intracellular staining detected), and 3) isolated CMP staining.

Detection of Anti-dsDNA and Other Autoantibodies

All samples were also tested for the presence of anti-dsDNA antibodies by chemiluminescence immunoassay (CIA: QUANTA Flash, Inova Diagnostics Inc., San Diego, CA, USA) as previously described (27) using a cut-off 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 laboratory reference range (or >2-fold the reference range if tested by ELISA) (5).

Antibodies to PCNA, ribosomal P, recombinant Ro52/TRIM21, native SSA/Ro60, SSB/La, Sm, and U1-RNP were detected using the extractable nuclear antigen (ENA) FIDIS Connective Profile kit 13 addressable laser bead immunoassay (ALBIA: TheraDiag, Paris, France) on a Luminex 200 flow luminometer (Luminex, Austin, TX, USA) according to the manufacturer's instructions and read on a Luminex 200 system using the MLX-Booster software. Other autoantibodies such as IgG anti-cardiolipin, 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: http://www.anapatterns.org/index.php) (9).

Clinically Defined Samples

Demographic and clinical data were collected at enrollment and included age at diagnosis, sex, post-secondary education, disease duration, race/ethnicity, smoking, alcohol use, hypertension, nephritis at enrollment, proteinuria at enrollment (≥ 3g/day), ACR Classification Criteria fulfilled (total and individual), SLEDAI-2K (global score and organ system scores), and medication use (glucocorticoids, high dose glucocorticoids (any pulse steroid or prednisone ≥ 40mg/day), anti-malarials, and immunosuppressive agents including biologics) at or prior to cohort enrollment (Supplemental Table 1).

Statistical Evaluation

Statistical analysis was performed using Stata 14.1 (StataCorp, College Station, TX, USA). A three-way comparison between ANA-positive versus anti-cellular antibody-negative versus isolated CMP patients was performed. Univariable and multivariable logistic regression analyses were used to examine potential predictors of the odds of being anti-cellular antibody-negative versus being ANA-positive or having an isolated CMP. As a secondary analysis, three additional univariable and multivariable logistic regressions were performed: 1) anti-cellular antibody-negative versus ANA-positive, 2) isolated CMP versus ANA-positive, and 3) isolated CMP versus anti-cellular antibody-negative.

Potential univariable predictors included demographic, clinical, and serological 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 three serological groups: 1) ANA-positive, 2) anti-cellular antibody-negative, and 3) isolated CMP, are shown in Table 1. Overall, 1137 patients had sera available; their mean age at diagnosis was 35.1 years (standard deviation (SD) 13.5) (median 33 years), 89.9% were female, 66.7% (724/1085) had obtained post-secondary education, mean disease duration was 0.46 years (SD 0.35), and 45.2% (511/1130) were not of Caucasian race/ethnicity. Three hundred and twelve of 1084 (29%) of the cohort had lupus nephritis at enrollment, the mean global SLEDAI-2K was 5.3 (SD 5.3), and 80.3% (913/1137) had a history (either at or prior to enrollment) of glucocorticoid use, 73.6% (837/1137) of antimalarial use, and 42.7% (485/1137) of immunosuppressant use including four patients who had received biologics (rituximab only).

Nuclear and CMP anti-cellular antibody IIF patterns

The distribution of patients based on IIF staining patterns and specificities is shown in Supplemental Figures 1 and 2. 1049/1137 (92.3%) were ANA-positive which included 877 isolated nuclear (77.1%) and 172 mixed nuclear patterns and CMP (15.1%). 71/1137 (6.2%) were anti-cellular antibody-negative (i.e. no detectable IIF staining), and 17/1137 (1.5%) had an isolated CMP. Therefore 7.7%

were either anti-cellular antibody-negative or had isolated CMP. Isolated CMP and their related ICAP designations included 41.2% (7/17) cytoplasmic dense fine speckled (AC-19), 23.5% (4/17) cytoplasmic fine speckled (AC-20), 5.9% (1/17) cytoplasmic discrete dots (AC-18), 5.9% (1/17) mitotic chromosomal envelope (AC-28), and 23.5% (4/17) mixed CMP (ICAP does not have a pattern designation for mixed patterns at this time).

Comparison of isolated CMP with ANA-positive and anti-cellular antibody-negative and comparison of ANA-positive and anti-cellular antibody-negative

Patients with isolated CMPs were not clinically or serologically different from ANA-positive or anti-cellular antibody-negative patients for most variables (Table 1). In contrast, ANA-positive patients were markedly different from anti-cellular antibody-negative patients in terms of age at diagnosis (34.7 versus 40.9 years), race/ethnicity (higher proportion of Asians, African descendants, but fewer of Caucasian race/ethnicity, disease activity (SLEDAI-2K, 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 anti-cellular antibody IIF on HEp-2000 substrate, some SLE-related autoantibodies were still detected, notably anti-dsDNA by CIA (11.3%), and anti-Ro52/TRIM21 (21.1%), anti-SSA/Ro60 (22.5%), and anti-U1RNP by ALBIA (11.3%).

Multivariable analysis of anti-cellular antibody-negative patients versus ANA-positive patients combined with isolated CMP patients

As the isolated CMP group did not differ from the ANA-positive or anti-cellular antibody-negative for most variables, we chose to combine the isolated CMP with the ANA-positive for the primary multivariable analysis. In the primary multivariable analysis (Table 2), patients who were older (OR per year 1.02 [95% CI: 1.00, 1.04]), of Caucasian race/ethnicity (OR 3.53 [95% CI: 1.77, 7.03]), or were on high doses of glucocorticoids at or prior to enrollment (OR 2.39 [95% CI: 1.39, 4.12]) were

more likely to be anti-cellular antibody-negative. Patients who were on immunosuppressants at or prior to enrollment (OR 0.35 [95% CI: 0.19, 0.64]) or with anti-SSA/Ro60 (OR 0.41 [95% CI: 0.23, 0.74]) or anti-UI-RNP (OR 0.43 [95% CI: 0.20, 0.93]]) were less likely to be anti-cellular antibody-negative.

Multivariable analysis of anti-cellular antibody-negative versus ANA-positive, isolated CMP versus ANA-positive, and isolated CMP versus anti-cellular antibody-negative

In the secondary multivariable analysis comparing the odds of being anti-cellular antibody-negative versus being ANA-positive, the predictors were identical to those in the multivariable analysis of the anti-cellular antibody-negative patients versus the ANA-positive combined with the isolated CMP patients (Supplemental Table 2).

In secondary multivariable analyses comparing the odds of being isolated CMP versus ANA-positive or being isolated CMP versus anti-cellular antibody-negative, patients who had not attained post-secondary education or were hypertensive were more likely to be isolated CMP positive (Supplemental Tables 3 & 4).

DISCUSSION

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 anti-cellular antibody negative. Traditionally, ANA-negative referred only to the absence of any IIF staining localized to the nucleus. This is an important consideration, especially for AARD such as SLE where the ANA test has a central role in establishing the diagnosis. The need to clarify this issue is exigent as it 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 two reports: one that specifies nuclear staining patterns and titers and another that indicates if CMP staining is present. In the broader definition of ANA test results, the inclusive definition of ANA and CMP is more accurately referred to as anti-cellular antibody (1;9;29). However, because the ANA rubric is embedded in historical and scientific literature, the anti-cellular antibody terminology is held in abeyance until wider consensus and clinician education is achieved (3;9;29). The results of this study provide some insight into the potential diagnostic and clinical implications for SLE patients as a consequence of changing the definition of ANA to the wider anti-cellular 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% (88/1137). However, if isolated CMPs (1.5%, 17/1137) were subsequently excluded from the ANA-negative pool of 88 patients, the prevalence of anti-cellular antibody-negative SLE would decrease to 6.2% (71/1137). Accordingly, amongst these 88 ANA-negative patients, nearly one in five 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 pre-test probability of AARD may have a negative ANA test when in fact it should be regarded as positive if CMP staining is present.

In our study, SLE patients with isolated CMP could not be readily differentiated from ANA-positive and anti-cellular 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 isolated CMP. In contrast, there were many differences between the anti-cellular antibody-negative and ANA-positive patients, consistent with the current literature indicating 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 anti-cellular antibody-negative patients were older (40.9 versus 34.7 years) and a higher proportion were of Caucasian race/ethnicity (84.5% versus 52.4%). Further, anti-cellular 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 enrolment (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 non-Caucasian patients who tend to be ANA-positive, corroborating previous studies demonstrating higher disease activity in non-Caucasian SLE patients (32;33).

When the anti-cellular antibody-negative patients were compared to the isolated CMP combined with the ANA-positive patients, all the above observations regarding anti-cellular antibody-negative versus ANA-positive patients persisted in the univariable analysis. However, in the multivariable analysis, slight differences were observed. Older age and Caucasian race/ethnicity remained associated with a greater likelihood of being anti-cellular antibody-negative and high dose glucocorticoids now became associated with a greater likelihood of being anti-cellular antibody-negative; immunosuppressant medications (at or prior to enrolment) and certain autoantibodies remained associated with a lower likelihood of being anti-cellular antibody-negative. Our finding that high dose glucocorticoids are associated with a higher likelihood of anti-cellular antibody-negativity may be attributable to glucocorticoids influencing ANA status (34). However, this is merely speculation as we have no data on ANA status prior to the baseline assessment. Patients on other types of immunosuppressants (i.e. methotrexate, azathioprine, mycophenolate mofetil) were less likely to be anti-cellular 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 four patients treated with rituximab (data not shown) were anti-cellular 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 five of 164 (3%) patients had data on medications during ANA testing. This highlights the need to review concurrent medications and consider other known confounders such as proteinuria, as we have done.

The ANA of our cohort was tested on the HEp-2000 substrate, which has been engineered to intentionally increase the detection of anti-SSA/Ro60, 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/Ro60 (31). These findings are particularly relevant to the clinical subset of SLE that have 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 (reviewed in (20)) indicate a persistent gap in autoantibody detection by HEp-2 substrates, which in the present study included anti-SSA/Ro60 and even anti-dsDNA. For example, 22.5% of the anti-cellular antibody-negative SLE patients in our study still had anti-SSA/Ro60 antibodies using extractable nuclear antigen testing; 11.3% of our anti-cellular antibody-negative patients had anti-dsDNA by CIA. 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 have been reported (15;17;18;41); some of this variation relates to the performance of different HEp-2 assay kits (14). In this 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 67/71 of the available anti-cellular antibody-negative samples, we observed that 17/67 (25.4%) became clearly positive for nuclear and/or CMP staining (detailed data not shown). In a cross-sectional study, it was reported 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 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) and references therein). 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) and reviewed in (48)]. Therefore, longitudinal studies are needed to evaluate the serologic status of anti-cellular antibody-negative and isolated CMP patients over time and whether it varies with disease activity, damage accrual, therapeutic interventions, and/or specific substrate assays. Even among ANA-negative patients with lupus nephritis, it can take up to 10 years to seroconvert from ANA-negative to positive (17;40). Some patients may only have detectable positive serology when there is uncontrolled disease activity due to loss of self-tolerance from chronic auto-reactivity of T and B cells (17).

There are some limitations to our study. First, the similarities reported between CMP and ANA-positive patients are likely confounded by the high proportion of ANA-positive patients also expressing CMP (21.5%). Overall, approximately 17% of patients in the entire cohort expressed CMP (189/1137) but the majority (91.0%, 172/189) were seen in conjunction with nuclear IIF patterns. As

a result, the isolated CMP 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.

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Table 1. Baseline demographic, clinical and autoantibody profiles of ANA-positive (presence of any nuclear IIF pattern), anti-cellular antibody (ACA)-negative (no IIF pattern), and isolated cytoplasmic/mitotic (CMP) groups

	ANA+	ACA-	Isolated CMP#	Difference (95% CI)		
	n=1049	n=71	n=17	ANA+ and ACA-	ANA+ and CMP	ACA- and CMP
Demographics						
Age at diagnosis, year, 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)
Post-secondary education, % attended	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	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)
Caucasian	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)
# 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)
Immunological	1.6*	1.1*	1.1	0.5 (0.1, 0.9)	0.5 (-0.4, 1.3)	0 (-0.9, 0.8)
Hematological	0.1	0	0	0.1 (0, 0.1)	0.1 (-0.1, 0.3)	0 (-0.1, 0.1)

Medications, % ever used						
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 of 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)
Ro52/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/Ro60	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)
Anti-cardiolipin	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-ß2glycoprotein1	15	15.9	12.5	0.8 (-10.1, 8.5)	2.5 (-13.8, 18.9)	3.4 (-15.2, 21.9)

#Some predictors had a small portion of missing values - where these occurred, the observations were excluded from the relevant analysis. In particular, for the small group of patients with isolated CMP, the data included 1 missing value for education, smoking status, nephritis, proteinuria, #ACR criteria, anti-cardiolipin, anti-beta2glycoprotein 1, and 2 missing values for high alcohol use and lupus anticoagulant

†, *, or in combination: values with the same superscript are significantly different from each other, i.e. †* is different from† and *, but † and *are not.

Abbreviations: ACR, American College of Rheumatology; ANA, anti-nuclear antibody; dsDNA, double stranded DNA; IIF, indirect immunofluorescence; PCNA, proliferating cell nuclear antigen; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; Sm, Smith (U2-U6 RNP); SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B; TRIM 21, tripartite motif 21; yr, years.

Table 2. Univariable and multivariable analysis of demographic, clinical, and serologic profiles of anti-cellular antibody(ACA)-negative versus ANA-positive combined with isolated cytoplasmic/mitotic patterns (CMP)

	ACA- vs ANA+ or Isolated CMP	
	Univariate model Odds ratio 95% CI	**Multivariate model** Odds ratio 95% CI
Demographics		
Age at diagnosis, year	1.03 (1.01, 1.05)*	1.02 (1.00, 1.04)*
Female, %	1.03 (0.46, 2.31)	
Post-secondary educ., % attended	1.63 (0.92, 2.91)	
Disease duration, years	0.66 (0.32, 1.34)	
Race/ethnicity, %		
Asian	0.15 (0.05, 0.47)*	
African descendants	0.40 (0.16, 1.00)*	
Hispanic	0.85 (0.20, 3.60)	
Caucasian	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)
# ACR criteria, mean	0.89 (0.69, 1.14)
SLEDAI-2K score, mean	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)
Immunological	0.82 (0.69, 0.96)*
Hematological	0.41 (0.13, 1.27)
Medications, % ever using	

Glucocorticoids	0.71 (0.40, 1.23)	
High doses of 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)*	
PCNA	0.18 (0.02, 1.30)	
Ribosomal-P	0.31 (0.11, 0.87) *	
Ro52/TRIM21	0.48 (0.27, 0.86) *	
SSA/Ro60	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)	
Anti-cardiolipin	0.87 (0.39, 1.95)	
Anti-ß2glycoprotein1	1.07 (0.53, 2.15)	

*Denotes an odds ratio that is statistically significant

Abbreviations: ACR, American College of Rheumatology; ANA, anti-nuclear antibody; dsDNA, double stranded DNA; IIF, indirect immunofluorescence; PCNA, proliferating cell nuclear antigen; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; Sm, Smith (U2-U6 RNP); SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B; TRIM 21, tripartite motif 21; yr, years.