Machine Learning Identifies Clusters of Longitudinal Autoantibody Profiles Predictive of Systemic Lupus Erythematosus Disease Outcomes

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ABSTRACT

OBJECTIVES: A novel longitudinal clustering technique was applied to comprehensive autoantibody data from a large, well-characterized, multinational inception systemic lupus erythematosus (SLE) cohort to determine profiles predictive of clinical outcomes.

METHODS: Demographic, clinical, and serological data from 805 SLE patients obtained within 15 months of diagnosis and at three- and five-year follow-up were included. For each visit, sera were assessed for 29 ANA immunofluorescence patterns and 20 autoantibodies. K-means clustering on principal component analysis-transformed longitudinal autoantibody profiles identified discrete phenotypic clusters. One-way ANOVA compared cluster enrolment demographics and clinical outcomes at ten-year follow-up. Cox proportional hazards model estimated the hazards ratio (HR) for survival adjusting for age of disease onset.

RESULTS: Cluster 1 (n=137, high frequency of anti-Sm, anti-U1RNP, AC-5 (large nuclear speckled pattern), and high ANA titres) had the highest cumulative disease activity and immunosuppressants/biologics use at year ten. Cluster 2 (n=376, low anti-dsDNA and ANA titres) had the lowest disease activity, frequency of lupus nephritis, and immunosuppressants/biologics use. Cluster 3 (n=80, highest frequency of all five antiphospholipid antibodies) had the highest frequency of seizures and hypocomplementemia. Cluster 4 (n=212) also had high disease activity and was characterized by multiple autoantibody reactivity including to anti-histone, -dsDNA, -ribosomal P, -SSA/Ro60, -SSB/La, -Ro52/TRIM21, -PCNA, and -centromere B). Clusters 1 (adjusted HR 2.60 [95%CI: 1.12-6.05], p=0.03) and 3 (adjusted HR 2.87 [95%CI: 1.22-6.74], p=0.02) had lower survival compared to Cluster 2.

CONCLUSION: Four discrete SLE patient longitudinal autoantibody clusters were predictive of long-term disease activity, organ involvement, treatment requirements, and mortality risk.

Introduction

Systemic Lupus Erythematosus (SLE) is a complex disease that is challenging to diagnose, prognosticate, and effectively treat due to substantial disease heterogeneity. It can affect virtually any organ system at different time points during its course. To better understand SLE's underlying biology and how it relates to prognosis, attempts have been made to stratify SLE patients into endotypes including patient clusters based on common autoantibody profiles.[1-11] SLE pathogenesis is multifaceted, but certain autoantibodies are a hallmark of SLE and have proven useful as diagnostic and predictive biomarkers for disease manifestations and activity.

Past machine learning (ML) analyses to define SLE clusters were cross-sectional, studied patients seen at single centres and assessed relatively few SLE-related autoantibodies.[1-11] Over 200 different autoantibodies have been described in SLE, but only 10-20 are widely available through clinical diagnostic laboratories and utilized by clinicians and researchers.[12] Furthermore, there are no reports of ML approaches to study longitudinal autoantibody data in SLE to date. Previous evaluations of antinuclear antibodies (ANA) and SLE-related autoantibodies using traditional statistical methods suggest that a patient's antibody status can change from positive to within the normal range and vice versa during the disease course.[13-16] However, factors influencing changes in autoantibody status over time are also poorly understood.

ML is inherently flexible and can identify patterns and interactions in large datasets and distinguish multiple clinical factors and autoantibody status that would otherwise be challenging to ascertain within the modeling assumptions and restrictions of traditional statistical methods. For this reason, ML techniques have been applied to stratify SLE patients into distinct phenotypes associated with

206 different clinical outcomes including organ damage.[1-11, 17, 18] Here we apply a ML clustering
207 technique to a *longitudinal* comprehensive autoantibody panel to identify distinct subgroups of
208 SLE patients that are *predictive* of future clinical outcomes.

209

210 **Methods**

211 *Study Population*

212 Between 1999 and 2011, 1827 patients fulfilling the 1997 Updated ACR SLE Classification
213 Criteria for definite SLE [19] within 15 months of diagnosis from 31 medical centres in 11 countries
214 were enrolled into the Systemic Lupus International Collaborating Clinics (SLICC) inception
215 cohort (https://sliccgroup.org).[20] Sera, clinical and demographic data were collected at enrolment
216 and annually thereafter. Of the 1827 patients, 1432 (78.4%) were followed for ≥ four years; of
217 these 1432 patients, we included the 805 patients who provided an enrolment and two additional
218 serum samples within five years of enrolment, with the third sample being ≥ four years after
219 enrolment. Although we were not able to include the entire cohort, we demonstrated in a prior
220 study that the 805 patients were similar to the 627 patients who provided ≥ four years of data, but
221 did not have three available serial serum samples.[21] The study was approved by the Institutional
222 Review Board at each SLICC site. Permission from the SLICC Biological Material and Data
223 Utilization Committee was obtained to access the required data and biobanked serum samples.

224

225 *Clinically Defined Samples*

226 Demographic and clinical data at enrolment included patient age, sex, disease duration,
227 race/ethnicity, lupus nephritis (LN) (defined as fulfilling the ACR criterion for renal disease or if
228 a renal biopsy was performed prior to cohort entry), ACR Classification Criteria fulfilled (total

229 and individual), Systemic Lupus Erythematosus Disease Activity Index – 2000 (SLEDAI-2K)
230 (global score and organ system scores),[22] SLEDAI-2K adjusted mean score (AMS, measurement
231 of lupus disease activity over time or area under the curve of SLEDAI-2K over time by adding the
232 area of each of the blocks of visit interval divided by the length of time for the entire period)[23],
233 SLICC/ACR Damage Index (SDI),[24] medication use (current and ever use of glucocorticoids,
234 antimalarials, and immunosuppressive agents including biologics), and survival. Longitudinal data
235 on nephritis, SLEDAI-2K, SDI, and medication use at three, five, and ten years after enrolment
236 were also obtained. These demographic and clinical variables are described in greater detail in
237 **Supplemental Table 1**.
238
239 *ANA and Autoantibody Testing*
240 Aliquots of the 805 SLICC patient sera at 1) enrolment (sample #1); 2) two to four years after
241 enrolment (sample #2); and 3) four to ten years after enrolment (sample #3) were stored at -80°C
242 until required for immunoassays and analyzed at MitogenDx (Calgary, Canada). Hereafter,
243 samples #1 – 3 are referred to as enrolment, year three, and year five, respectively. Indirect
244 immunofluorescence assay (IFA) using HEp-2 substrate (NovaLite,Werfen, San Diego, USA),
245 was performed on all samples. In accord with the manufacturers' directions, a positive test was
246 defined as a titer of ≥1:80. IFA results (titres and patterns) were initially read by an automated
247 digital IFA microscope (NovaView, Werfen) and then visually validated by a technologist with
248 >15 years of experience. For any inconsistency or questionable patterns, a second individual (MJF)
249 with >40 years of experience reviewed and reached a consensus. ANA IFA patterns were classified
250 according to the most recently updated International Consensus on ANA Patterns
251 recommendations (http://www.anapatterns.org/index.php).[25] A quality assurance step was

252 performed by repeating all ANA that were within the normal range (titer <1:80) and a random
253 selection of the ANA-positive samples. The lab also participates in ICAP and College of American
254 Pathologists ANA survey for quality assurance.
255
256 Anti-dsDNA and titers were detected by chemiluminescence immunoassay test (CIA) (Werfen).
257 A cut-off of ≥27 IU/mL was utilized, where 27-35 IU/mL was indeterminate (borderline), and >35
258 IU/mL was positive. All samples were also tested for autoantibodies by an ALBIA (FIDIS
259 Connective13: TheraDiag, Paris, France) on a Luminex 200 flow luminometer (BioRad, Hercules,
260 CA USA) focusing on SLE-related analytes that included ribosomal P, Ro52/Tripartite Motif
261 Protein 21 (TRIM21), SSA/Ro60, SSB/La, Sm, U1-RNP, Jo-1, centromere B, PCNA, and
262 histones. The manufacturer's recommended cut-off of >40 median fluorescence units, which is >2
263 standard deviations above the mean of internal controls, was considered positive.
264
265 Anti-phospholipid antibodies (APLAs) IgG and IgM anti-cardiolipin and IgG and IgM anti–β2-
266 glycoprotein-1 (β2GP1) were measured using ELISA (Werfen). Using the revised Sapporo
267 antiphospholipid syndrome classification criteria,[26] a cut-off of >40 units for IgG/IgM anti-
268 cardiolipin was considered medium to high positive while a cut-off of ≥20 units (>99th percentile)
269 was considered positive for IgG/IgM anti–β2GP1. Non-criteria APLAs IgG and IgM anti-PS/PT
270 (phosphatidyl serine/prothrombin complex) and anti-β2GP1-Domain 1 were tested using ELISA
271 (QUANTA Lite, Werfen) and CIA (QUANTA Flash, Werfen) respectively. The cut-offs used were
272 as recommended by the manufacturer and sensitivity and specificity confirmed by internal quality
273 assurance and external quality assurance (EQA). All autoantibodies were measured at MitogenDx

except for lupus anticoagulant, which was measured at the Oklahoma Medical Foundation (Oklahoma City, OK), by previously reported methods.[27]

All samples were tested for the presence of anti-DFS70 (dense fine speckled 70/lens epithelium derived growth factor) antibodies by CIA (Werfen). The assay used purified full length human recombinant DFS70 coated onto paramagnetic beads. The established cut-off for anti-DFS70 antibodies was >20 chemiluminescent units (CU).

Statistical Analysis and Machine Learning

Principal component analysis (PCA) was applied to reduce the high dimensionality of longitudinal ANA and autoantibody profiles (results of 71 variables including positivity and titres of ANA and each autoantibody repeated over three visits: enrolment, year three, and year five). Cumulative variance explained was used to select the number of PCs, ensuring that the number of components chosen explains a significant proportion of the total variance, typically at least 70% to 80%, while avoiding overfitting. Then, K-means clustering algorithm on the PCA transformed ANA and autoantibody data was used. The optimal number of clusters was chosen using the elbow method.[28] To evaluate cluster robustness, the PCA transformation and K-means clustering were repeated five times with different random seeds. We compared cluster demographic and clinical outcomes, including longitudinal disease activity (total SLEDAI-2K and AMS), SDI and organ-specific domains, and SLE therapies at ten-years post-enrolment, using one-way ANOVA test and a Benjamini-Hochberg correction with false discovery rate alpha = 0.05. Chi-square pairwise comparisons were performed to study differences in outcomes between pairs of clusters (e.g., frequency of LN between clusters and its association with anti-dsDNA positivity). Results were

297 visualized using t-distributed stochastic neighbor embedding (t-SNE). Multivariable logistic
298 regression, adjusted for age of disease onset, was used to determine if clusters were predictive with
299 mortality at year ten. Survival curves were also constructed using Kaplan-Meier methods. Finally,
300 a multivariable Cox proportional hazards model was fitted to estimate the adjusted hazards for
301 survival, accounting for age of disease onset. For missing data (0.33% of the entire dataset), we
302 used multiple imputation, where for each missing feature in the longitudinal data, the missing value
303 was replaced by the mean of the other observed values for that time point. Python 3.7, scikit-learn,
304 R 4.1.1 and STATA 15.1 software were used.

305

306 **Results**

307 *Enrolment and Year Five Patient Clinical Characteristics*

308 The 805 patients included in the study had a mean age at diagnosis of 35.2 years (SD 13.6), 88.7%
309 (714/805) were female and 47.7% (384/805) were of race/ethnicity other than White
310 (**Supplemental Table 2**). At enrolment, the disease duration was 0.58 years (SD 0.49), the
311 frequency of nephritis was 28.9% and the mean total SLEDAI-2K score was 5.4 (SD 5.3). SLE
312 medications at enrolment included 70.1% on antimalarials, 69.6% on glucocorticoids, and 41.0%
313 on immunosuppressants. The changes in clinical characteristics of the patients from enrolment to
314 year five have been described previously.[16]

315

316 *Enrolment and Year Five Patient ANA and Autoantibody Profile*

317 The most common autoantibodies at enrolment were anti-SSA/Ro60 (42.5%) followed by anti-
318 Ro52/TRIM21 (37.5%), PS/PT IgG/IgM (36.3% either isotypes or 20.0% IgG, 26.6% IgM), anti-
319 dsDNA (34.2%), anti-histones (31.3%), anti-U1RNP (28.2%), anti-Ribosomal P (24.3%) and anti-

320 Sm (22.7%). The frequency of most SLE-related autoantibodies decreased at year five compared
321 to enrolment (**Table 1**). The most common ANA patterns were AC-4 representing nuclear fine
322 speckled (39.4%), AC-1 nuclear homogeneous (34.9%), AC-5 nuclear large speckled (34.4%),
323 AC-19 cytoplasmic dense fine speckled (13.8%), AC-20 cytoplasmic fine speckled (12.4%), and
324 AC-10 punctate nucleolar (7.2%). The frequency of ANA patterns at enrolment compared to year
325 five did not change significantly for most patterns.

326

327 *ANA and Autoantibody Clusters*

328 Four unique patient clusters (**Figure 1)** were identified using longitudinal trajectories of each
329 autoantibody **(Figure 2 and 95% confidence intervals shown in Supplementary Figure 1)** and
330 ANA pattern (**Figure 3**). These clusters were associated with clinical factors such as age of onset,
331 race/ethnicity, BMI, and predicted disease activity, organ involvement, and treatment course at ten
332 years of follow-up, and mortality (**Table 3 and Supplemental Table 3**). Of the 805 patients, date
333 of death or follow-up data up to ten-years were available for 581 patients, of whom 71 died
334 (12.2%). There were no significant differences in baseline demographic or clinical characteristics
335 between the subset of 581 patients used to examine ten-year clinical outcomes and the 224 patients
336 who did not provide ten-year clinical data (**Supplemental Table 4**).

337

338 Cluster 1 (n=137, 17.0%): These patients were characterized by a high frequency of anti-Sm and
339 anti-U1RNP antibodies (**Table 2 and Supplemental Table 5**). This group was the youngest at
340 disease onset (31.5 years [SD 10.8]), had the highest proportion of African (27.0%) ancestry and
341 lowest proportion of European ancestry (32.1%). At year ten, this cluster the highest cumulative
342 disease activity (AMS 4.2 [SD 2.7]), mean SDI score for the skin domain (0.25 [SD 0.53]) and

343 alopecia (0.14 [SD 0.35]), and frequency of immunosuppressant/biologic use (83.8% ever, 72.3%
344 currently), particularly azathioprine (55.2% ever, 33.3% current), mycophenolic acid (46.7% ever,
345 30.5% current), and belimumab (14.3% ever, 6.7% current). Patients in this cluster also had the
346 highest frequency of rituximab use (8.6% ever, 1.9% current), but this was not statistically
347 significant. A complete list of immunosuppressant/biologics is available in **Supplemental Table**
348 **3**.
349
350 Cluster 2 (n=376, 46.7%): This was the largest cluster and was characterized by low frequency of
351 anti-dsDNA and relatively high frequency of anti-DFS70. Patients in this cluster were oldest at
352 disease onset (36.9 years [SD 13.9]) and predominantly of European ancestry (61.7%). At year
353 ten, this cluster had the lowest proportion of patients with nephritis (32.1%), the lowest disease
354 activity (total SLEDAI-2K 2.3 [SD 2.9] and AMS 2.6 [SD 2.2]), lowest SLEDAI-2K score for
355 immunological subscale (0.90 [SD 1.31]) including low complement levels (0.50 [0.87]), and
356 lowest frequency of immunosuppressant/biologic use (63.8% ever, 45.5% currently), including
357 azathioprine (34.6% ever, 15.0% current), mycophenolic acid (27.6% ever, 14.2% current), and
358 belimumab (4.1% ever, 3.3% current).
359
360 Cluster 3 (n=80, 9.9%): This was the smallest cluster and had the highest frequency of both criteria
361 (anti-cardiolipin IgG/IgM, anti-β2GP1 IgG/IgM, lupus anticoagulant) and non-criteria APLAs
362 (PS/PT IgG/IgM and anti-β2GP1-Domain 1 IgG/IgM) over time. For most APLAs, titres were
363 highest at enrolment and then decreased over time (**Supplemental Table 6**). They had the highest
364 proportion of European ancestry (68.8%), lowest proportion of Asian (13.8%) and African (6.2%)
365 ancestry, and highest mean body mass index (26.1 kg/m^2 [SD 5.8]) at enrolment. At year ten, this

366 cluster had the highest SLEDAI-2K subscale scores for low complement levels (1.19 [SD 0.99])
367 and the highest mean SDI scores for neuropsychiatric involvement (0.37 [SD 0.86]) including
368 strokes (0.14 [SD 0.44]) and seizures (0.11 [SD 0.31]), however, only seizures were significantly
369 different between clusters after correcting for multiple comparisons. Of note, the association
370 between this cluster with neuropsychiatric involvement and strokes were statistically significant
371 at year five (data not shown).

372

373 Cluster 4 (n=212, 26.3%): This cluster was characterized by positivity to many autoantibodies
374 including histone, dsDNA, ribosomal P, SSB/La, Ro52/TRIM21, anti-SSA/Ro60, PCNA, and
375 centromere B. They had the highest proportion of patients of Asian ancestry (31.1%) and lowest
376 mean body mass index (24.1 kg/m^2 [SD 4.8]) at enrolment. At year ten, this cluster had the highest
377 total SLEDAI-2K score (3.5 [SD 3.4]), particularly for the immunological subscale (2.01 [SD
378 1.61]).

379 The ANA patterns corresponded to the autoantibody profile of each cluster (**Figure 3**). Cluster 1
380 had the highest ANA titres over time and while Cluster 2 had the lowest. Cluster 1 had the highest
381 mean maximum ANA titres for AC-5 (large speckled pattern, which is associated with anti-Sm
382 and anti-RNP). Cluster 4 had higher mean maximum ANA titres for AC-1 (homogeneous pattern
383 associated with anti-dsDNA, histones), AC-4 (fine speckled associated with anti-SSA/Ro60, anti-
384 SSB/La), and AC-19 (cytoplasmic dense fine speckled associated with anti-ribosomal P),
385 corresponding as well to its autoantibody profile. Remaining ANA patterns (AC-2, 3, 6-18, 20-29)
386 had mean titers <1:80 at all three visits for all cluster groups.

387

388 *Mortality*

389 Cluster 3 had the highest proportion of patients who died (7.9%) at year ten, followed by Cluster
390 1 (4.7%), 4 (3.7%), and 2 (3.2%). The odds of survival at 10 years were significantly lower in
391 patients in Cluster 3 compared to patients in Cluster 2 (adjusted odds ratio (OR) 0.28, 95%CI:
392 0.08-0.94). There were no statistical differences in odds of survival between the other clusters. The
393 Kaplan-Meier survival curves are shown in **Supplemental Figure 2**. Hazards of survival, adjusted
394 for age at disease onset in a multivariable Cox regression demonstrates that patients in Clusters 1
395 (adjusted hazards ratio (HR) 2.60 [95% CI: 1.12-6.05], p=0.03) and 3 (adjusted HR 2.87 [95% CI:
396 1.22-6.74], p=0.02) had lower survival compared to patients in Cluster 2.
397 Ten principal components were chosen to capture 75% of the cumulative explained variance of
398 the dataset (**Supplemental Figure 3**) prior to k-mean clustering. Cluster robustness was high as
399 the original four clusters presented above and the new clusters generated in the robustness
400 evaluation agreed, as indicated by a high average adjusted Rand index (ARI) (ARI 0.971, where
401 1.0 represents identical clustering and 0 represents exact opposite).
402
403 **Anti-dsDNA and LN**
404 A comparison of LN between pairs of clusters demonstrated that only cluster 2 (lowest frequency
405 of anti-dsDNA positivity) had significantly lower frequency of LN compared to cluster 1, 3, and
406 4 at year five (p =0.01, p=0.01, p=0.007, respectively) and year ten (p<0.001, p=0.01, p=0.004,
407 respectively). There was no difference in LN frequency when clusters 1, 3 and 4 were compared
408 to each other (data not shown). LN and anti-dsDNA frequency were strongly associated with each
409 other by current anti-dsDNA positivity (p<0.0001), mean titre (p=0.0002), and ever positive
410 (p<0.0001).
411

412 **Discussion**

413 SLE is a heterogeneous disease with respect to manifestations, progression, and treatment
414 responses, but the presence of circulating autoantibodies points to a fundamental underlying
415 mechanism of immune dysregulation and disease pathogenesis. Therefore, grouping SLE patients
416 into autoantibody subsets to reconcile disease heterogeneity may elucidate this complex disease
417 and identify more personalized monitoring and treatment plans, as well as distinguish those
418 patients at higher risk for disease progression and organ damage. This is the first study to identify
419 endotypes of SLE patients based on ML analysis using longitudinal autoantibody profiles (20
420 autoantibodies and 29 ANA pattern interpretations) over the first five years of disease from a large
421 international, multicenter inception cohort. Four distinct serologic clusters were associated with
422 clinical features such as age of onset, race/ethnicity, BMI, and predictive of long-term disease
423 activity, organ involvement, treatment course, and mortality.

424

425 While similar clusters have been described, prior studies were based on smaller cohorts, single-
426 centres, and/or cross-sectional analysis of only a limited set of autoantibodies, thereby limiting the
427 generalizability of the results.[1-11] This current study fulfilled a need for a in-depth analysis of more
428 diverse SLE patients who were well characterized both at inception and in long-term follow-up,
429 providing a comprehensive analysis of autoantibodies and ANA patterns, especially when over
430 200 SLE-related autoantibodies have been described.[12] This study also analyzed several novel
431 autoantibodies that have important clinical implications, including anti-DFS70,[29] anti-PS/PT
432 IgG/IgM,[30] and anti-β2GP1-Domain1 IgG/IgM[31]. Unlike prior cross-sectional studies that
433 examined associations, we used prospectively collected data in a protocolized fashion that
434 demonstrated that clustering based on biomarker data within the first five years can

435 add *predictive* value for clinically relevant outcomes at year ten and beyond, including the risk of
436 mortality. All ANAs and other autoantibodies were tested in one accredited, central laboratory,
437 thereby avoiding interlaboratory variation as a factor in ANA and autoantibody fluctuations over
438 time. We also used assays that were CE marked, Health Canada and/or U.S. Food and Drug
439 Administration approved, setting us apart from some studies that have used research use only or
440 laboratory developed tests. Using this robust approach, we demonstrated that ANA titres and most
441 autoantibodies decreased in frequency over the first five years of follow-up. To examine multiple
442 autoantibody profiles and their potential evolution over time, considering their linkages and
443 interactions, we leveraged ML to identify meaningful patterns and relationships with disease
444 outcomes. We believe these are crucial strengths of this study that adds novel information to the
445 current understanding of SLE heterogeneity.
446
447 While there is promise in incorporating these clusters into future personalized models of health
448 care for patients, this study purposefully conducted extended longitudinal autoantibody profiling
449 that may not be available at all centres. However, this detailed autoantibody testing approach
450 allowed us to achieve a better understanding of SLE heterogeneity and disease pathogenesis. Two
451 high risk clusters (1 and 4) characterized by multiple autoantibody reactivities, high disease
452 activity and immunosuppressant/biologic use, were found more commonly among non-White
453 races/ethnicities known to have more severe SLE.[32] This is further evidence that genetic factors
454 may be underpinning differences in immune dysregulation susceptibility that lead to increased
455 autoantibody production, immune complex formation, inflammation, and eventual organ damage.
456

457 Epitope spreading in genetically susceptible individuals may also explain why these two high risk
458 clusters (1 and 4) had distinct patterns of autoantibody reactivities.[33, 34] For instance,
459 autoantibodies to Sm and U1RNP, which were frequently observed in Cluster 1, are directed
460 against distinct components of related macromolecular complexes. For example, U1RNP is one of
461 several small nuclear ribonucleoprotein particles (snRNP), each consisting of a unique small
462 nuclear RNA (U1-U6 RNAs), specific associated proteins, and common core Smith (Sm) proteins.
463 An antibody response beginning with one particular epitope can then be followed by a spread of
464 the immune response to other epitopes in the same polypeptide (intramolecular) and/or other
465 distinct but structurally similar molecules (intermolecular). [35] Therefore, autoantibodies can exist
466 in "linked" sets, a well described phenomena that helps explain co-prevalence of many
467 autoantibodies.[33, 34]
468
469 Another high-risk profile cluster was cluster 3, which had multiple elevated APLAs and severe
470 disease outcomes including seizures and mortality. In a prior SLICC study of lupus anticoagulant,
471 anti-cardiolipin, and anti-ß2GP1 tested at baseline, only an association between lupus
472 anticoagulant and increased risk of cerebrovascular disease ($p= 0.04$) could be detected.[29] As
473 APLAs are known to fluctuate over the disease course,[30] serial measurements on all five APLAs
474 were analyzed in this current study. We showed that when all five APLAs were persistently
475 positive, this was *predictive* of the future occurrence of several severe SLE-related outcomes such
476 as seizures and mortality. There was also an association between APLAs with strokes at year five
477 and year ten, although it was not significant at year ten after adjustment for multiple comparisons.
478 This may be related to APLAs titres declining over time and a higher frequency of strokes in other
479 clusters, but due to non-SLE related atherosclerosis. Our study is also the first to examine

480 longitudinal profiles of less commonly reported non-criteria APLAs. Both anti-PS/PT IgG/IgM
481 and anti-β2GP1-Domain1 were identified in cluster 3. Recent studies have shown that aPS/PT
482 antibodies are predictive of cardiovascular disease events in SLE including strokes, irrespective of
483 a history of antiphospholipid syndrome.[31] aPS/PT antibodies can additionally identify patients that
484 are negative for the criteria aPLAs, thereby closing the seronegative gap, and are associated with
485 increased risk of thrombosis that is additive to other criteria aPLAs.[32] In our study, the frequency of
486 aPS/PT antibodies IgG/IgM over the five years (36.3-26.0% for the presence of either isotype,
487 16.0-20.2% IgG only, 16.6-26.6% IgM only) was consistently higher than the frequency of the
488 other APLAs, which is in keeping with other studies and suggesting they may be important
489 biomarkers for SLE patients.[33]
490
491 The absence of specific autoantibodies in SLE or the presence of others may represent patients
492 who are at lower risk of severe SLE. We demonstrated that SLE patients belonging to cluster 2
493 had a milder disease course characterized by low titre ANAs and lack of autoantibody reactivity,
494 including anti-dsDNA. Accordingly, Cluster 2 also had the lowest frequency of LN at years 5 and
495 10, which is not surprising as we also demonstrated that the frequency of anti-dsDNA was strongly
496 associated with LN in a univariate analysis. This group also had a relatively higher frequency of
497 anti-DFS70 antibodies over time. In an earlier SLICC study, monospecific anti-DFS70 (no other
498 detectable autoantibodies) at *disease inception* has been shown to be uncommon in SLE (1.1%).[34]
499 In this longitudinal study, the results of anti-DFS70 in cluster 2 suggest that it may be a good
500 prognostic biomarker among those with established disease.
501

502 We acknowledge some important limitations of this study. First, although this is the first to report
503 longitudinal clusters, the duration of follow-up is rather short, which may explain why there were
504 no differences observed for many comparisons. One previous single centre study showed reduced
505 survival among SLE patients with APLAs after 20 years of follow-up.[6] Future studies with longer
506 follow-up data are underway which will allow examination of disease damage and survival.
507 Second, as the enrolment visit could occur up to 15 months after diagnosis (although mean disease
508 duration at enrolment was 0.58 years), most patients (>96%) had already been exposed to at least
509 one immunomodulatory medication by enrolment, potentially influencing ANA and autoantibody
510 results. We showed that although the frequency of most autoantibodies fluctuated over time, the
511 autoantibody profiles of the clusters themselves remained stable. The clinical applicability of the
512 results (i.e., value of monitoring an extended autoantibody profile over time) is also limited as
513 most centres will not be able to perform serial measurements of all 20 autoantibodies included in
514 the cluster analysis. Future studies to build and validate a panel of the key autoantibodies that can
515 stratify patients into these clusters are needed, taking into consideration test availability and costs.
516
517 In summary, our ML analysis of comprehensive and longitudinal ANA and autoantibody
518 signatures has identified four unique endotypes of SLE patients associated with important SLE
519 outcomes. This suggests that early characterization of autoantibody profiles may be helpful in
520 reconciling disease heterogeneity and understanding disease pathogenesis, which may guide
521 clinical prognostication to identify those with more aggressive disease phenotypes and inform
522 design of personalized diagnostic and treatment strategies. Future studies are required to determine
523 whether other 'omics' biomarkers (exposome, epigenome, genome, transcriptome, microbiome,
524 metabolome, proteome) with the aid of ML approaches can add value to the predictive power of

525 autoantibodies demonstrated in this study. We anticipate that these clusters will become a
526 benchmark to study other SLE-related outcomes, including potential use as a stratification factor
527 for heterogeneous patient populations in clinical trials and for evaluating differential burden of
528 health care resource utilization. Further validation studies may also inform clinical follow-up and
529 therapeutic approaches after diagnosis.
530
531
532 **Data Sharing Statement:**
533
534 All data relevant to the study are included in the article or uploaded as supplementary information.
535
536
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541

KEY MESSAGES

What is already known on this topic

- To better understand systemic lupus erythematosus' (SLE) underlying biology and disease heterogeneity, attempts have been made to stratify patients into endotypes based on common autoantibody profiles and using machine learning (ML).
- However, past studies were cross-sectional, studied patients seen at single centres and assessed relatively few SLE-related autoantibodies.

What this study adds

- A comprehensive panel of autoantibodies (20 autoantibodies and 29 antinuclear pattern interpretations) was evaluated in a large, well-characterized cohort of SLE patients using a longitudinal and machine learning approach.
- We demonstrated that there were four distinct serologic clusters in the first five years of disease associated with clinical features such as age of onset, race/ethnicity, BMI, and predictive of disease activity, organ involvement, and treatment course at ten years of follow up, and mortality.

How this study might affect research, practice or policy

- Early characterization of autoantibody profiles may be helpful in reconciling disease heterogeneity and understanding disease pathogenesis.
- This may guide clinical prognostication and inform design of personalized diagnostic and treatment strategies.

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566

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667

668 TABLES AND FIGURES

669

670 **Table 1. Patient ANA and autoantibody profile at enrolment and year five (n=805)**

	Enrolment	Year 5	Difference[1] (95% CI)
ANA ICAP Pattern	%	%	
AC-0 (No staining)	1.7	1.1	-0.6 (-1.9, 06)
AC-1	34.9	30.1	**-4.8 (-8.4, -1.3)**
AC-2	1.1	1.2	0.1 (-0.8, 1.1)
AC-3	1.1	1.4	0.2 (-0.6, 1.1)
AC-4	39.4	47.5	**8.1 (3.5, 12.6)**
AC-5 (includes nuclear matrix pattern)	34.4	36.1	1.7 (-2.2, 5.6)
AC-6	0.6	0.5	-0.1 (-1.0, 0.7)
AC-7	4.0	5.7	1.7 (-0.2, 3.7)
AC-8	2.6	3.2	0.6 (-1.1, 2.3)
AC-9	0.5	1.2	0.7 (-0.2, 1.7)
AC-10	7.2	8.4	1.2 (-1.3, 3.8)
AC-11	0.1	0.4	0.2 (-0.3, 0.9)
AC-12	1.5	1.1	-0.4 (-1.6, 0.9)
AC-13	0.2	0.2	0 (-0.6, 0.6)
AC-14	0	0	0 (-0.1, 0.1)
AC-15	0.2	0	-0.2 (-0.7, 0.2)
AC-16	0.2	0.4	0.1 (-0.5, 0.7)
AC-17	0.2	0.2	0 (-0.5, 0.5)
AC-18	1.5	1.1	-0.4 (-1.5, 0.7)
AC-19	13.8	17.5	**3.7 (0.6, 6.9)**
AC-20	12.4	12.2	-0.2 (-3.5, 3.0)
AC-21	5.8	4.7	-1.1 (-3.0, 0.8)
AC-22	0.2	0.1	-0.1 (-0.7, 0.4)
AC-23	0.1	0.2	0.1 (-0.4, 0.7)
AC-24	2.0	3.6	**1.6 (0.1, 3.1)**
AC-25	0.1	0.1	0 (-0.1, 0.1)
AC-26	0.4	0.6	0.2 (-0.6, 1.1)
AC-27	1.5	0.9	-0.6 (-1.7, 0.4)
AC-28	0.2	0.2	0 (-0.6, 0.6)
AC-29	0.1	0	-0.1 (-0.5, 0.2)
Autoantibodies, %			
dsDNA[2]	34.2	29.1	**-5.1 (-8.7, -1.6)**
Ribosomal P	24.3	20	**-4.3 (-7.8, -0.9)**
Ro52/TRIM21	37.5	37.4	-0.1 (-3.4, 3.2)
SSA/Ro60	42.5	42.0	-0.5 (-3.7, 2.7)
SSB/La	20.7	16.3	**-4.5 (-7.5, -1.5)**
Sm	22.7	14.7	**-8.1 (-11.1, -5.0)**
U1RNP	28.2	23.0	**-5.2 (-8.5, -2.0)**
Histones	31.3	22.7	**-8.6 (-12.1, -5.0)**
Jo-1	1.5	3.7	**2.2 (0.7, 3.7)**

Centromere B	2.7	5.5	**2.7 (0.9, 4.5)**
PCNA	15.8	18.4	2.6 (-0.9, 6.1)
DFS70	6.1	6.0	-0.1 (-1.3, 1.1)
Cardiolipin IgG/IgM[3]	20.5	16.4	**-4.0 (-7.5, -0.4)**
β2GP1 IgG/IgM[3]	19.9	12.9	**-7.0(-9.9, -4.1)**
Lupus anticoagulant[4]	19.5	14.2	**-5.3 (-8.3, -2.2)**
PS/PT IgG/IgM	36.3	26.0	**-10.3 (-13.9, -6.7)**
β2GP1-Domain 1	10.3	7.8	**-2.5 (-4.8, -0.2)**

Abbreviations: AC, anti-cellular pattern according to ICAP nomenclature; ACR, American College of Rheumatology; ANA, anti-nuclear antibodies; β2GP1, β2-glycoprotein-1; CI, confidence interval; DFS, dense fine speckled; dx, diagnosis; dsDNA, double-stranded DNA; ICAP, International Consensus on ANA Patterns; IgG/M, immunoglobulin G/immunoglobulin M; Jo-1, histidyl tRNA synthetase; PCNA, proliferating cell nuclear antigen; PS/PT, phosphatidyl serine-prothrombin complex; RNP, ribonucleoprotein; SD, standard deviation; SLEDAI-2K, systemic lupus erythematosus disease activity index-2000; SDI, SLICC Damage index; Sm, Smith antigen (U2-U6 RNP) ; SSA, Sjögren syndrome antigen A or Ro60: SSB, Sjögren syndrome antigen B or La: TRIM21, Tripartite Motif Protein (TRIM) 21; yrs, years.

1. Difference between enrolment and year 5 visit
2. Complete data available for n=798 patients
3. Complete data available for n= 800
4. Complete data available for n=282

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672

Table 2. Frequency of Autoantibodies in Each Cluster Over Time

	Cluster 1 (n=137)			Cluster 2 (n=376)			Cluster 3 (n=80)			Cluster 4 (n=212)		
Antibody	Enrolment	Y3	Y5	Enrolment	Y3	Y5	Enrolment	Y3	Y5	Enrolment	Y3	Y5
Sm	78.8	69.3	50.4	5.3	1.3	3.2	11.3	6.3	6.3	21.7	10.4	15.1
U1RNP	95.6	90.5	74.5	8.8	6.6	6.6	12.5	10.0	12.5	25.0	19.3	22.6
DFS70	1.5	1.5	2.2	8.8	9.8	9.6	5.0	5.0	3.8	4.7	4.8	2.8
β2GP1 IgG	4.4	4.4	3.7	4.8	4.3	3.7	46.3	40.0	40.0	7.1	5.2	9.0
β2GP1 IgM	9.5	9.5	2.2	9.6	10.9	3.5	46.3	46.3	31.3	10.4	13.2	3.3
Cardiolipin IgG	13.1	6.6	12.4	8.0	3.7	6.4	55.0	37.5	37.5	20.3	7.1	13.7
Cardiolipin IgM	1.5	1.5	2.2	5.1	2.9	4.8	33.8	22.5	23.8	2.4	4.2	3.3
β2GP1-Domain 1	6.6	4.4	3.7	6.4	4.0	2.7	45.0	50.1	47.5	6.6	4.7	4.7
Lupus anticoagulant	8.6[1]	10.7[2]	8.6[1]	16.0[3]	12.4[4]	8.3[3]	64.8[5]	70.2[6]	60.6[5]	15.0[7]	14.5[8]	10.6[7]
PS/PT IgG	15.3	19.0	10.9	9.8	8.8	5.9	71.3	63.8	61.3	21.7	25.0	20.3
PS/PT IgM	19.0	16.8	13.9	17.6	16.5	8.2	78.8	77.5	62.5	27.8	20.3	16.0
dsDNA	35.0	38.7	33.6	18.9	11.2	9.3	36.3	41.3	40.0	59.0	56.1	56.1
Histone	29.2	21.2	21.2	15.7	6.9	10.9	33.8	21.3	26.3	59.4	46.7	43.4
PCNA	16.8	10.2	13.9	7.4	4.8	12.8	17.5	6.3	18.8	29.2	18.4	31.1
Ribosomal P	33.6	28.5	26.3	11.4	6.1	8.5	23.8	15.0	18.8	41.5	36.3	36.8
Ro52/TRIM21	39.4	29.9	37.2	22.3	18.1	21.3	16.3	17.5	25.0	71.2	69.8	70.8
SSA/Ro60	48.9	42.3	46.0	25.5	22.3	26.3	21.3	21.3	25.0	76.4	79.7	73.6
SSB/La	15.3	7.3	8.0	8.8	6.9	9.0	12.5	8.8	13.8	48.6	39.6	35.4
Centromere B	2.9	0.7	4.4	0.8	1.1	2.1	3.8	3.8	6.3	5.7	6.6	11.8
Jo-1	0.0	0.7	0.7	1.1	1.3	2.4	1.3	0.0	7.5	3.3	0.9	6.6

Darker red shading indicates higher frequency, lighter shading indicates lower frequency of autoantibody
[1]n=116 complete data, [2]n=84, [3]n=337, [4]n=237, [5]n=71, [6]n=47, [7]n=180, [8]n=117

Table 3. Demographic and clinical characteristics that were statistically significant[1] at enrolment and ten-year follow-up between the four SLE longitudinal autoantibody clusters

	Group 1 (n=137)	Group 2 (n=376)	Group 3 (n=80)	Group 4 (n=212)	p-value	FDR
Enrolment Demographics						
Mean Age of Diagnosis (SD), yrs	31.5 (10.8)	36.5 (13.9)	32.5 (13.9)	34.4 (14.1)	**<0.001**	**0.014**
% Ethnicity						
White	32.1	61.7	68.8	42.5	**<0.001**	**<0.001**
Asian	30.7	20.5	13.8	31.1	**<0.001**	**0.014**
African	27.0	9.6	6.2	14.6	**<0.001**	**<0.001**
Mean BMI (SD), kg/m^2	24.3 (4.9)	25.6 (6.1)	26.1 (5.8)	24.1 (4.8)	**0.003**	**0.029**
Clinical Characteristics at Year 10 Follow-Up						
% Nephritis[2]	56.2	32.1	50.9	46.9	**<0.001**	**0.001**
Mean SLEDAI-2K Score (SD)						
Total Score[3]	3.2 (3.2)	2.3 (2.9)	3.0 (2.1)	3.5 (3.4)	**0.002**	**0.020**
Adjusted Mean Score[4]	4.2 (2.7)	2.6 (2.2)	3.5 (1.7)	3.9 (2.2)	**<0.001**	**<0.001**
Immunological Subscale	1.64 (1.60)	0.90 (1.31)	1.96 (1.33)	2.01 (1.61)	**<0.001**	**<0.001**
Low Complement	0.85 (0.99)	0.50 (0.87)	1.19 (0.99)	0.97 (1.00)	**<0.001**	**<0.001**
Mean SLICC Damage Index (SD)						
Seizures	0.01 (0.10)	0.02 (0.16)	0.11 (0.31)	0.01 (0.8)	**<0.001**	**0.004**
Skin Domain	0.25 (0.53)	0.08 (0.29)	0.07 (0.32)	0.07 (0.28)	**<0.001**	**0.002**
Alopecia	0.14 (0.35)	0.04 (0.19)	0.04 (0.19)	0.04 (0.19)	**<0.001**	**0.004**
Medications Ever						
% Immunosuppressives/ Biologics	83.8	63.8	68.4	73.8	**0.002**	**0.014**
% Azathioprine (Imuran)	55.2	34.6	45.6	43.1	**0.003**	**0.025**
% Mycophenolic Acid	46.7	27.6	28.1	33.8	**0.005**	**0.031**
% Belimumab	14.3	4.1	5.3	6.3	**0.005**	**0.031**
Medication Current						
% Immunosuppressives/ Biologics	72.3	45.5	47.4	53.8	**<0.001**	**0.001**
% Azathioprine (Imuran)	33.3	15.0	15.8	13.1	**<0.001**	**0.001**
% Mycophenolic Acid	30.5	14.2	19.3	22.5	**0.005**	**0.031**

1. Comparison between cluster groups using one-way ANOVA test (null hypothesis that there is no difference between the means of the groups) and a Benjamini-Hochberg correction with false discovery rate (FDR) alpha = 0.05
2. LN was diagnosed by renal biopsy or fulfillment of the renal item on the ACR classification criteria.
3. The total score of SLEDAI-2K is the sum of all 24 descriptor scores. The total SLEDAI-2K score falls between 0 and 105, with higher scores representing higher disease activity.
4. A measurement of lupus disease activity over time determined by the calculation of the area under the curve of SLEDAI-2K over time by adding the area of each of the blocks of visit interval and then dividing by the length of time for the whole period.

Abbreviations: BMI, body mass index; SD, standard deviation; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC, Systemic Lupus International Collaborating Clinics; yrs, years.

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679 **Figure 1. Four autoantibody cluster groups identified among 805 SLE patients followed**
680 **from enrolment through years 3 and 5.** Latent space visualized using a t-distributed stochastic
681 neighbor embedding (t-SNE) with colors based on cluster labels.
682
683
684 **Figure 2. Autoantibody profile of 805 SLE patients in order of most prevalent**
685 **autoantibodies in A) Cluster 1, B) Cluster 2, C) Cluster 3, D) Cluster 4.** Standard deviation
686 bars have been removed to make graphs easier to visualize.
687
688 **Figure 3. ANA titres and patterns for each cluster. A**) Mean maximum ANA titers slightly
689 decreased over time. Cluster 1 (anti-Sm/RNP) highest mean maximum ANA titer. Cluster 2
690 (low anti-dsDNA) lowest mean maximum ANA titer. **B**) AC-1 (homogeneous pattern associated
691 with anti-dsDNA, histones), AC-4 (fine speckled associated with anti-SSA/Ro60, anti-SSB/La),
692 and AC- 19 (cytoplasmic dense fine speckled associated with anti-ribosomal P) correspond to the
693 autoantibody profile observed in cluster 4. High titres of AC-5 (large specked associated with
694 anti-Sm and anti-U1RNP antibodies) correspond to cluster 1 antibody profile. Remaining AC
695 patterns had mean titers <1:80 at all three visits for all cluster groups.