RHEUMATOLOGY

Original article

doi:10.1093/rheumatology/key149

The SLE-key test serological signature: new insights into the course of lupus

Chaim Putterman[1], David S. Pisetsky[2], Michelle Petri[3], Roberto Caricchio[4], Alan H. B. Wu[5], Ignacio Sanz[6], Jim C. Oates[7], Steve Wallace[8], Rachel Sorek[9], Robert Gerwien[10], Pennina Safer[9], Keren Jakobi-Brook[9] and Irun R. Cohen[11]

Abstract

Objective. We previously described the multiplex autoantibody SLE-key Rule-Out test, which detects a signature of autoantibody reactivity that distinguishes healthy subjects from SLE patients with 94% sensitivity, 75% specificity and 93% negative predictive value; thus, an individual manifesting a positive Rule-Out test score is unlikely to have SLE (e.g. lupus is excluded). The objective of this current study was to evaluate the stability of the lupus-associated signature over time.

Methods. We used banked serum samples from healthy subjects ($n = 51$) and lupus patients ($n = 50$ individual samples and $n = 181$ paired samples, for a total of $n = 412$ serum samples). The samples were drawn at different times after diagnosis to analyse the impact on the SLE-key Rule-Out test of time elapsed since diagnosis and any changes in disease activity (as reflected by the SLEDAI score).

Results. The SLE signature remains stable for the first 10 years after diagnosis; in this time frame, <10% of patients manifested a positive Rule-Out score and the SLE-key Rule-Out score was independent of the underlying disease activity as reflected by the SLEDAI score. After ⩾10 years, ~30% of lupus subjects scored as SLE Ruled-Out; the proportion of patients manifesting this status was even greater in the subset of individuals with a SLEDAI score of 0.

Conclusion. These findings raise the possibility that a significant number of SLE patients manifest a change in their serological signature over time, and that such a signature change may signify an evolution in the immunological features of their disease relevant to patient management.

Key words: autoantibodies; diagnosis; iCHIP; SLE-key; microarray; systemic lupus erythematosus; natural history; multivariate classifier

CLINICAL SCIENCE

Rheumatology key messages

- The SLE-key multiplex autoantibody test detects an SLE serological signature not found in health.
- The SLE-key signature can normalize after 10 years, suggesting a fundamental change in disease in certain lupus patients.
- These dynamic changes suggest that repeated SLE-key testing might be useful in managing selected patients.

[1]The Division of Rheumatology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, [2]Durham VA Medical Center, Duke University Medical Center and Medical Research Service, Durham, NC, [3]Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD, [4]Temple Lupus Clinic, Temple University, Philadelphia, PA, [5]Department of Laboratory Medicine, San Francisco General Hospital, San Francisco, CA, [6]Division of Rheumatology, Emory University School of Medicine, Atlanta, GA, [7]Division of Rheumatology & Immunology, Medical University of South Carolina, Charleston, SC, [8]ImmunArray Inc., Richmond, VA, USA, [9]ImmunArray Ltd, Rehovot, Israel, [10]Gerwien Statistical Consulting, Newington, CT, USA and [11]Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

Submitted 13 December 2017; revised version accepted 24 April 2018

Correspondence to: Chaim Putterman, Division of Rheumatology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, USA.
E-mail: chaim.putterman@einstein.yu.edu

Introduction

SLE is a chronic systemic autoimmune disease that causes inflammation and injury in multiple organs, and leads to significant morbidity, mortality and societal costs [1, 2]. Primarily a disease of women, SLE usually begins in young adulthood and can affect the skin, kidneys, joints, blood elements and nervous system among other organs. SLE can be highly variable clinically, and is often characterized by recurrent episodes of flares and intensification of disease activity. Similar to most autoimmune diseases, the aetiology of lupus is complex and likely involves both environmental and genetic factors [3–9]. At present, the therapeutic paradigm in SLE involves a choice among multiple anti-inflammatory and

© The Author(s) 2018. Published by Oxford University Press on behalf of the British Society for Rheumatology. All rights reserved. For permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

immunosuppressive agents to reduce disease activity and limit acute and cumulative organ damage. Therapy, however, is often unsatisfactory, and can lead to undesirable side effects [7, 10].

Among the many immunological disturbances observed in lupus, the production of autoantibodies to components of the cell nucleus is the serological hallmark. These autoantibodies (ANA) can target DNA, RNA and proteins as well as complexes of proteins and nucleic acids. Because of the clinical heterogeneity and variable symptomatology of SLE, diagnosis can be difficult and may require several years and serial evaluations before a definitive diagnosis is made [3, 8]. The ACR established a list of 11 criteria to classify SLE patients and these criteria are often used for diagnosis; a classification of SLE is made when four or more clinical and laboratory findings from this list of 11 criteria are fulfilled [7, 10]. ANA positivity is one of these criteria; an immunological disorder, including autoantibodies to DNA, Sm nuclear antigens or phospholipid antigens, is another criterion [11, 12]. ANA positivity, which is very common in SLE patients, also occurs in as many as 5–15% of otherwise healthy individuals; this significantly limits the use of the ANA test for screening, especially when the probability of SLE is low [13, 14]. Anti-DNA and anti-Sm antibodies are more specific than the ANA test, but many SLE patients lack these antibodies. Moreover, levels of antibodies to DNA can vary widely over time and may become undetectable [15, 16].

To facilitate the laboratory evaluation of SLE, we have developed a microarray device and informatics platform called iCHIP to analyse a multiplex profile of autoantibodies that distinguish SLE patients from healthy control subjects. The iCHIP programme began as a laboratory study [17, 18], but has now advanced technically and informatically to become a robust and reliable diagnostic assay [19, 20] The first clinical application of this iCHIP platform has been the SLE-key Rule-Out test, which is based on detection of serum antibodies to an array of self-antigens followed by analysis of the resulting pattern of reactivity, or signature, using a linear discriminant analysis algorithm whose product, or score, is a value between 0 and 1. Individuals with SLE-key Rule-Out test values of <0.18 are considered excluded, or 'Ruled-Out', for SLE. In other words, a low score indicates a positive SLE-key Rule-Out test and excludes the disease. Development and validation of the test was based on the study of hundreds of SLE patients and healthy controls, as previously described [19].

The SLE-key Rule-Out test distinguishes healthy subjects from SLE patients with 94% sensitivity, 75% specificity and 93% negative predictive value [19–21]. Thus, an individual manifesting a positive, or 'Ruled-Out' serology, is highly unlikely to have SLE. In the present study, we explored the stability of the SLE-key test signature in lupus patients as a function of time following disease diagnosis. We asked three questions: Is the autoimmune signature affected by the duration of disease after diagnosis? Is this signature associated with the patient's SLEDAI [22]? Do SLE subjects manifest changes in their SLE signature profiles over time?

Our results indicate that the SLE immunological signature may be affected by the duration of the disease. Accordingly, changes in the SLE-key score may be useful for tracking the course of disease and for aiding key decisions regarding patient management.

Methods

SLE patient samples

All SLE patients ($n=412$ tests) fulfilled criteria for classification as SLE as defined by standard ACR and/or SLICC criteria [8]. Table 1 shows clinical and demographic data of the SLE subjects.

Individual SLE samples

We analysed SLE-key Rule-Out test results in samples drawn once from 50 SLE patients within 3 years of diagnosis. SLE serum samples and clinical information were obtained from the repositories of four independent, major lupus centres in the USA, in studies approved by each Institutional Review Board: Albert Einstein College of Medicine ($n=15$), Johns Hopkins University ($n=15$), Medical University of South Carolina ($n=16$) and Emory University ($n=4$). SLEDAI scores at the time of blood draw ranged from 0 to 21.

Paired SLE samples

We analysed SLE-key Rule-Out test results at two time points in 181 SLE patients (362 samples); the time interval between the first time point (T1) and the second time point (T2) ranged from several weeks to 12 years [mean = 1.54 (2.31) years]. SLE serum sample pairs and clinical information were obtained from the repositories of four independent, major lupus centres in the USA, in studies approved by each Institutional Review Board: Albert Einstein College of Medicine ($n=55$), Johns Hopkins University ($n=30$), Medical University of South Carolina ($n=68$) and Temple University ($n=28$).

We studied two subgroups of the 181 paired samples; in 84 patients, both samples were obtained at $\leqslant 10$ years after diagnosis [the mean time after diagnosis for the T1 sample was 3.92 (2.86) years; group 1]. In the remaining 97 patients, the mean time after diagnosis for the T1 sample was 18.52 (8.34) years: in 81/97 cases, both samples were obtained at >10 years after diagnosis (group 3), and in the remaining 16/97 patients, T1 was obtained at $\leqslant 10$ and T2 at >10 years (group 2) (Table 1).

SLEDAI scores at the time of blood draw in the paired sample cohort ranged from 0 to 22 points; differences in the SLEDAI scores between T1 and T2 ranged from 2 to 20 points. 65.2% of the paired samples ($n=118$) manifested a decrease in SLEDAI score at T2 relative to T1, and 34.3% ($n=62$) showed an increase.

Healthy subjects

Sera ($n=51$) were collected from self-declared healthy subjects who had no history of immunologically active disease or steroid use within 3 months of sample

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

TABLE 1 Clinical and demographic data

Sample demographics	Healthy controls	SLE patients		
		Individual samples	Paired samples	
	$n=51$	$n=50$	$n=84$	$n=97$
Time after diagnosis, years	—	<3 years	T1, T2 ⩽ 10	T1 ⩽ 10, T2 > 10 $n=16$; T1, T2 > 10 $n=81$
Time after diagnosis for T1 sample, mean (S.D.), years	—	1 (0.96)	3.92 (2.86)	18.52 (8.34)
Age[a], mean (S.D.), years	37.8 (11.2)	36.6 (11.8)	37.2 (13.7)[b]	47.8 (12.3)[b]
Gender				
Female, %	100	100	93	97.9
Male, %			7	2.1
Ethnic category, n (%)				
Afro-American	21 (41.2)	23 (46.0)	28 (50)	30 (52.6)
White non-Hispanic	15 (29.4)	15 (30.0)	3 (5.4)	15 (26.3)
Indian/Asian/Middle Eastern	6 (11.8)	1 (2)	2 (3.6)	2 (3.5)
White Hispanic	8 (15.7)	9 (18.0)	23 (41.1)	10 (17.5)
Other	1 (2.0)	2 (4.0)	28 unknown	40 unknown
SLEDAI				
SLEDAI = 0, n	—	15	49	46
SLEDAI > 0[c], n	—	32	35	50
SLEDAI > 0 range	—	0–21	0–19	0–22

[a]Age at date of sampling. [b]Based on T1 time point. [c]Missing SLEDAI for four patients.

collection, and no first-degree relatives with SLE. Samples were obtained from five sites: Baylor College of Medicine ($n=19$), CTI Clinical Research Center ($n=5$), Medical University of South Carolina ($n=17$), Veracis Laboratory (Richmond, VA) ($n=5$) and San Francisco Medical Center ($n=5$), and were collected in a Health Insurance Portability and Accountability Act compliant manner and with appropriate informed consent. Table 1 shows the clinical and demographic data of the healthy subjects.

SLE-key Rule-Out testing

Serum samples were obtained and transported to Immunarray's CLIA-certified laboratory, Veracis (Richmond, VA, USA), for SLE-key Rule-Out testing and evaluation, as described [19].

The slides were scanned using an Agilent SureScan Microarray scanner (Agilent Technologies, Santa Clara, CA, USA) with laser settings at two wavelengths (532 nm for IgG and 633 nm for IgM), and the data were recorded and analysed as described previously [19].

Statistical analysis

Patient characteristics were summarized using descriptive statistics (percentages, means and S.D.). Analyses comparing scores between groups used the Wilcoxon rank sum test with $\alpha=0.05$; both were done using MATLAB R2017b. Analyses comparing fractions of Ruled-Out (lupus-excluded) patients used Pearson's chi-squared test with the simulated P-values using R version 3.1.2 (31 October 2014) (R Foundation for Statistical Computing, Vienna, Austria).

Results

The samples obtained from the SLE patients and the healthy subjects were tested for antibody binding to the arrayed antigens and analysed as described above. Table 1 summarizes clinical and demographic data. Table 2 shows the prevalence of high anti-dsDNA antibodies, low serum C3 and low serum C4 in the different groups, all defined as values outside the normal range in each institution at the time of the blood draw. Information regarding usage of immunosuppressant medications, corticosteroids and anti-malarial drugs is provided in Table 3.

Persistence of the SLE-key signature over time

To determine whether the SLE-key signature of SLE patients varied with the time elapsed since diagnosis, we stratified the cohort of SLE patients into those tested within 3 years of diagnosis ($n=116$); those tested between 3 and 10 years of diagnosis ($n=117$); and those tested after 10 years or more after diagnosis ($n=178$), and determined the percentage of subjects Ruled-Out in each group. Figure 1 shows that within 3 years of diagnosis, only 8.6% of the SLE patients were designated as Ruled-Out; this result is similar to that observed with the original SLE-key Rule-Out test validation cohort [19]. The percentage of patients Ruled-Out increased slightly in patients from 3–10 years after diagnosis (10.3%),

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

TABLE 2 Serological results

	High anti-dsDNA	Low C3	Low C4
Group 1: T1 and T2 ⩽ 10 years			
N	47	74	73
Positive	40	22	32
Negative	45	57	44
Group 2: T1 < 10; T2 > 10			
N	12	16	16
Positive	**8***	13	19
Negative	75	69	63
Group 3: T1 and T2 > 10 years			
N	38	66	66
Positive	**16***	12	**15****
Negative	68	74	76
Group 2 + 3 (combined)			
N	50	82	82
Positive	**14****	12	**16****
Negative	70	73	73

Values are a percentage unless otherwise stated. *N*: number of pairs with data available at both time points. Positive: patients who were positive at both time points. Negative: patients who were negative at both time points. All comparisons are *vs* group 1 using Fisher's exact test; significant comparisons are shown in bold. $^{*}P < 0.05$; $^{**}P < 0.01$.

TABLE 3 Medication use

Group 1: T1 and T2 ⩽ 10 years	
N	82
Immunosuppressants	26 (32)
Corticosteroids	52 (63)
Anti-malarials	21 (26)
Group 2: T1 < 10; T2 > 10	
N	**16**
Immunosuppressants	4 (25)
Corticosteroids	**4 (25)****
Anti-malarials	1 (6)
Group 3: T1 and T2 > 10 years	
N	**77**
Immunosuppressants	27 (35)
Corticosteroids	**31 (40)****
Anti-malarials	24 (31)
Group 2 + 3 (combined)	
N	**93**
Immunosuppressants	31 (33)
Corticosteroids	**35 (38)*****
Anti-malarials	25 (27)

Values are *n* (%) unless otherwise stated. *N*: total number of pairs in the group. Values are for patient pairs receiving medication at T1 and T2. Immunosuppressants: CYC, AZA, ciclosporin, tacrolimus, MTX, rituximab. Corticosteroids: prednisone or methylprednisolone. Anti-malarials: HCQ or quinacrine. All comparisons are *vs* group 1 using Fisher's exact test; significant comparisons are shown in bold. $^{**}P < 0.01$; $^{***}P < 0.001$.

although the increase was not statistically significant ($P = 0.91$) (Fig. 1).

In contrast to the results of the SLE-key Rule-Out test until 10 years after diagnosis, at ⩾10 years after diagnosis, we saw a significant increase to 30.9% ($P = 2.3 \times 10^{-7}$) in the number of SLE patients manifesting an Ruled-Out status (Fig. 1). This group also includes subjects with an initially high (e.g. disease not excluded) SLE-key Rule-Out test score.

The change in the frequency of a Ruled-Out designation among SLE patients could not be attributed to time of serum storage [4.04 (3.46), 5.61 (4.05) and 6.07 (3.44) years, respectively, for the three groups]. The ages of patients at diagnosis were also similar [35 (14), 32 (14) and 29 (12) years, respectively] indicating that the decrease in SLE-key score could not be explained by a late onset of disease. Moreover, there were no significant differences in ethnicity among the groups ($P = 0.47$). A site effect, however, could not be excluded ($P = 0.032$): for samples that were drawn ⩽10 years after diagnosis, no more than 33% of the samples came from any one of the five clinical sites. However, the samples that were drawn >10 years after diagnosis came from only four of the five sites, and almost 70% of these samples came from two clinical sites.

The SLE-key test is based on an integrated analysis of IgG and IgM antibodies binding to a set of classifier antigens [19]: ssDNA (IgG), U1snRNP (IgG and IgM), histone 3S (IgM), Sm (IgG) and a proprietary synthetic oligonucleotide (IgM). The decrease of the SLE-key Rule-Out score after 10 years could not be attributed to a change in reactivity to any individual antigen among the six classifier antigens (data not shown); this finding highlights the importance of the integrated multiplex signature that takes into consideration the reactivities against all six antigens to determine the Ruled-Out and the not-Ruled-Out status.

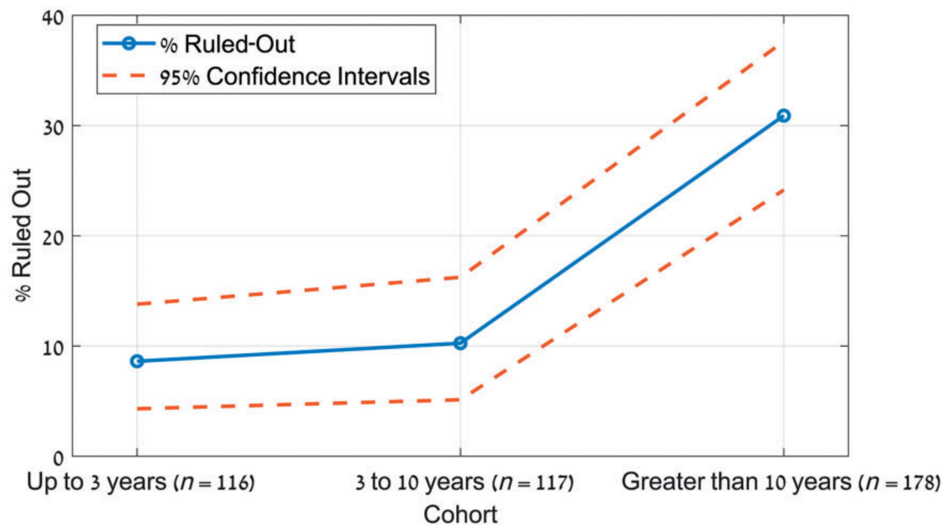
Disease activity does not account for differences in the SLE-key signature

SLE disease activity at the time of serum sampling could not explain a significant portion of the observed decrease in SLE-key Rule-Out tests over time. During the first 10 years following diagnosis, a time when the SLE-key Rule-Out test identifies >90% of SLE patients as not Ruled-Out (Fig. 1), the patients exhibited a wide range of SLEDAI scores—between 0 and 19. Likewise, the range of SLEDAI scores was between 1 and 18 in patients who remained not Ruled-Out at ⩾10 years after diagnosis.

Figure 2 shows the percentage of clinically asymptomatic patients (SLEDAI score 0 at the time of serum draw) who were ruled out by the SLE-key test. Despite SLEDAI scores of 0, only ~10% of the patients with samples obtained within 3 years of diagnosis, or between 3 and 10 years, exhibited positive SLE-key Ruled-Out test designations. Similar to the results shown in Fig. 1, the percentage of subjects with designations of SLE Ruled-Out increased to about 35% after ⩾10 years.

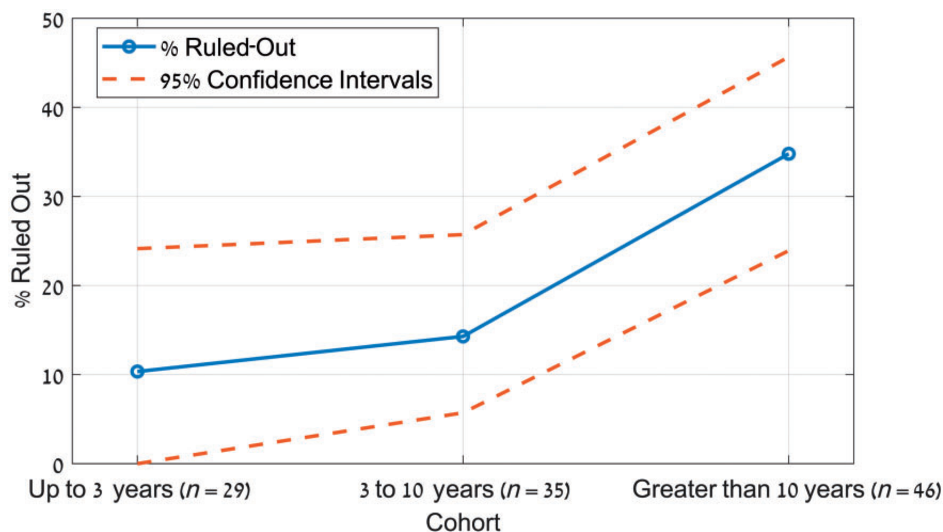
Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

FIG. 1 SLE-key Rule-Out test results over time



Results of the SLE-key Rule-Out test on serum samples from patients obtained at three time points after diagnosis: up to 3 years; from 3 to 10 years; and >10 years. The solid line indicates percentage Ruled-Out. The dashed lines indicate the 95% CI.

FIG. 2 SLE-key Rule-Out test results over time in asymptomatic lupus patients



The results of the SLE-key Rule-Out test over time in serum samples from clinically asymptomatic patients (SLEDAI = 0). The solid line indicates percentage Ruled-Out. The dashed lines indicate the 95% CI.

The poor correlation between the SLE-key signature and the SLEDAI scores suggests that the six autoantibody reactivities measured in the SLE-key test are not directly involved in the pathogenesis of target tissue inflammation/damage. Rather, the SLE-key test signature is more likely to reflect an underlying autoantibody profile that distinguishes the immune systems of SLE subjects from those of healthy individuals.

There were several patients in our study with discrepant SLE-key Rule-Out and SLEDAI scores deserving of further study. We focused our analysis on those patients who had a positive Rule-Out score at any time point but that concurrently had active disease as defined by a SLEDAI > 6. However, we found no significant differences between these patients and the rest of the cohort in the time elapsed since blood draw, age at sampling, years since

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

diagnosis, use of prednisone or immune suppressants, or frequency of serological abnormalities (data not shown).

The SLE-key test score wanes late in disease

We found that after ⩾10 years there was an increase in the frequency with which previously diagnosed SLE patients achieved an SLE-key designation of SLE Ruled-Out (Figs 1 and 2). Figure 3A shows the shift in numerical SLE-key signature scores in the patient subsets categorized according to the time since SLE diagnosis. The median numerical scores of 0.89 [interquartile range (IQR) = 0.51] and 0.83 (IQR 0.5) in disease <3 years and 3–10 years respectively, fell to a median of <0.44 (IQR = 0.78) at ⩾10 years after diagnosis ($P = 1.3 \times 10^{-9}$). Thus, there is both an increase in the number of subjects developing an SLE-key Ruled-Out designation and a general decrease in the mean SLE-key test scores after ⩾10 years.

To dissociate the change in immune profile from potential variations in disease activity, we separately examined patients with low disease activity. Figure 3B shows a waning of the SLE-key Rule-Out test scores in asymptomatic subjects manifesting SLEDAI scores of 0; after 10 years the mean numerical score of asymptomatic SLE patients approached that of healthy individuals.

Consistent with our observation that the immunological profile of lupus patients can change 10 years after the original diagnosis of the disease, we found that patients in group 1 (where both samples in the longitudinal study were obtained within 10 years of diagnosis) manifested a significantly higher prevalence of abnormal anti-dsDNA antibodies and serum C4 complement levels (Table 2). We further analysed a possible relationship between medication use and the SLE-key score. We did not observe an increased incidence of a positive Rule-Out score (an excluded lupus diagnosis) with higher usage of immunosuppression or immunomodulation. Indeed, the opposite was observed. In patient pairs in which at least one of the samples was obtained >10 years after the diagnosis (groups 2 and 3), the prevalence of corticosteroid use was significantly decreased (Table 3); these patients apparently could be managed with less corticosteroids.

Taken together, these results indicate that an autoimmune signature characteristic of SLE may evolve in some patients over time to a signature score closer to that observed in healthy individuals.

Discussion

We developed the SLE-key test to identify an autoantibody profile generally shared by SLE patients and absent from most healthy subjects [19–21]. In the present study, we found that this autoantibody profile marks SLE patients irrespective of the severity of their disease manifestations or clinical state at the time of diagnosis and early in the disease course; hence, we hypothesize that the SLE-key not-Ruled-Out designation denotes an antibody profile that reflects an underlying autoimmune aberration that separates SLE patients from healthy subjects. In addition, we found that this SLE-key signature may dissipate in about 30% of patients over time and approach more closely the scores and the SLE-key Ruled-Out designations of healthy individuals. These findings suggest that certain fundamental immunological features of SLE may change or resolve over time and treatment in a significant number of patients.

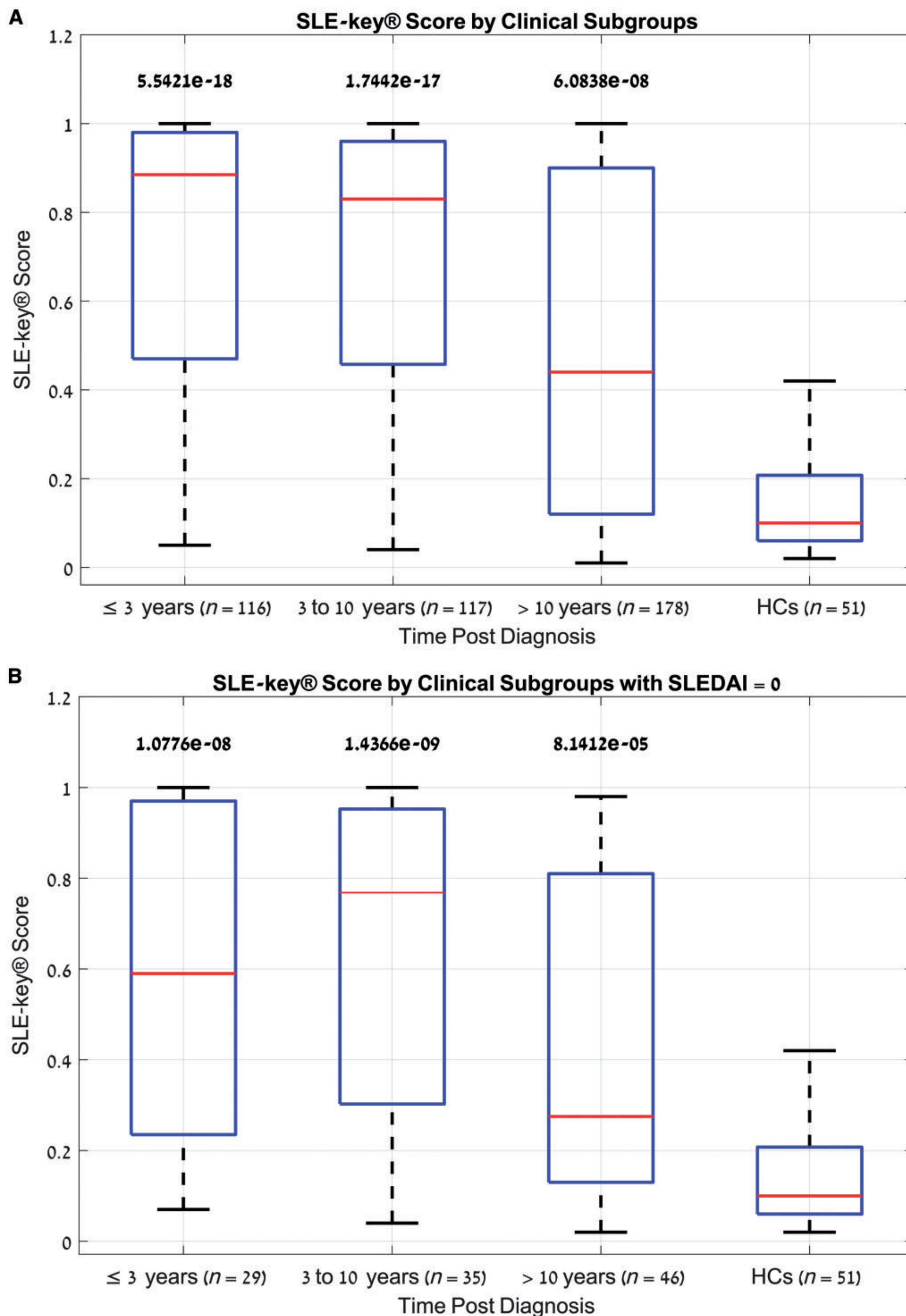
In view of the importance of biomarkers for SLE, multiple studies have performed cross-sectional analyses of various immunological and clinical features to determine a relationship to disease activity. Many of these studies, however, have not included longitudinal analysis, especially for durations as long as ⩾10 years. Among the various ANAs, anti-DNA is the only antibody that is routinely assayed in clinical practice to assess disease activity although many patients do not produce antibodies of this specificity, limiting its use as a biomarker. Moreover, evaluating the information provided by anti-DNA testing is often difficult since there are many assay platforms that differ significantly in the frequency of positivity among patients with lupus. Anti-DNA assays that are more specific are also less sensitive, and specificity may sometimes be considered more valuable.

Among studies of serological changes over time, Faria and associates [23] reported that antibodies to RNA-binding proteins such as Sm, RNP, Ro, La and dsDNA show little variation in levels of expression during follow-up of ~10 years. The stability of these antibody reactivities may reflect the role of different B cell populations in autoantibody production; anti-RNP antibodies might be produced by relatively long-lived plasma cells. Similarly, another study by Agarwal and associates showed no correlation between anti-ENA antibodies and disease activity for the population as a whole or for individual subjects over a 2-year period [24]. In view of these findings, the authors concluded that repeated quantitative measurements of anti-ENA antibodies do not provide useful information in assessing the SLE disease state [24].

We did not find a significant correlation between the SLE-key signature and the SLEDAI scores. This finding would suggest that the particular antibody specificities measured in this test are not instrumental in the pathogenesis of target organ involvement in lupus, or at least in those reflected by the SLEDAI-score (e.g. renal and skin disease). A lack of correlation with disease activity does not, however, detract from the clinical utility of the test; indeed, of more than 100 autoantibodies associated with SLE [25], only a handful (e.g. IgG anti-dsDNA, IgG anti-Ro, IgG anti-cardiolipin) appear to be directly pathogenic [26–28]. Anti-Sm antibodies, while highly specific for SLE and routinely used in clinical practice, do not reliably fluctuate with disease activity and their contribution to pathogenesis remains uncertain [29]. Other autoantibody specificities also do not track disease activity yet are helpful in the serological diagnosis of patients with possible lupus. Based on our previous results and clinical experience to date [21], the SLE-key Rule-Out test provides important information in the workup of patients with suspected SLE, and, as we show here, may also have a potential role and diagnostic value in following established patients.

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

FIG. 3 SLE-key Rule-Out score distribution in individual lupus patients



(**A**) SLE-key Rule-Out score distribution of individual samples, grouped by the time after diagnosis, relative to healthy controls (HCs). (**B**) SLE-key Rule-Out score distribution of SLEDAI = 0 patients, grouped by the time after diagnosis, relative to the HCs.

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

We did identify a limited number of patients who exhibited a positive SLE-key Rule-Out designation despite concurrent active lupus as determined by their SLEDAI score. We could not identify any distinctive clinical features in this group, possibly because this discrepancy was only present in a small number of patients (4%). Nevertheless, analysis of more individuals displaying divergent SLEDAI and SLE-key scores in a future study would be important and may provide interesting insights. It further remains to be determined if relatively lower Rule-Out test scores may, over time, predict improved responses to therapy.

As we now show, multiplex profiling of autoantibodies appears to be more revealing of immunological changes in SLE, perhaps because the relationships between the reactivities, detected by informatics, is more meaningful than any isolated change in the individual reactivities. It may be argued that the classical ANA also measures multiple antibodies binding to nuclei by immunofluorescence on a fixed cell line presenting a large array of different molecules. In the ANA assay, however, binding to even a single antigen can produce a positive result; moreover, the immunofluorescence assay cannot discern particular reactivities and their interrelationships. The iCHIP platform, in contrast, readily analyses data from a panel of autoantibody responses using an algorithm that integrates the findings into a single score.

The results presented here indicate that important immunological shifts can occur during the course of SLE. These shifts may have a counterpart in classical ANA testing where the occurrence of ANA negativity during the course of SLE is now recognized [15]. The clinical importance of the loss of serological reactivity can be seen in the development of belimumab, a monoclonal antibody to B cell activating factor/B lymphocyte stimulator, where a reanalysis of the phase II clinical data indicated that those patients who were serologically negative (ANA negative) did not respond to therapy. The subsequent phase 3 enrolled only those patients who were seropositive and was successful. In our current study, there was some improvement in serological activity and a decrease in corticosteroid use during the longitudinal follow-up. It is possible that these findings reflect attenuation of the autoimmune process over time, which is also reflected in the increased incidence of a positive SLE-key Rule-Out test. These changes may not have been reflected in the SLEDAI score because serological changes often precede changes in clinical manifestations in lupus; moreover, the SLEDAI score may lack sufficient sensitivity.

Thus, it appears that our findings support the notion that important shifts in autoreactivity can occur during the course of SLE, and that these shifts may be relevant in assessing the state of the disease and determining clinical management.

The SLE-key Rule-Out test was developed to identify a serological signature that distinguishes SLE patients from healthy controls. A serological test that differentiates between patients with autoimmune disease *vs* other conditions, or between different types of autoimmune disease, would also be valuable in the differential diagnosis of SLE. While these issues are outside of the scope of the current study, we are currently developing an iCHIP application to further improve the diagnosis and management of patients with autoimmune rheumatic conditions other than lupus [30].

The present findings raise important questions that will be the subject of future studies. Does a change in signature reflect a fundamental difference in disease pathogenesis, clinical manifestations or prognosis in such SLE patients compared with the SLE patients who do not manifest a shift in test status? Do shifts in SLE-key signature persist? Can the SLE-key score identify phases in SLE and should this be reflected in disease management? There has been great interest in detecting immune reactivities that precede the onset of clinical SLE—'pre-autoimmunity' [3, 31]; few studies have addressed the subsequent immunological phases of disease—what can be termed 'post-autoimmunity'. We believe that the SLE-key test will be a valuable marker in making critical assessments throughout the course of disease.

Acknowledgements

The authors wish to acknowledge the invaluable contributions of Yakov Blumenstein, Ilana Gilkaite, Miriam Lerner, Ornit Cohen-Gindi, Olga Tarnapolski, Adrian Javaherian, Annmarie Goldstein, Vanessa Cabra-Hodge, Justin Pitts, Maggie Barton and Emileigh Wong

The Hopkins Lupus Cohort is supported by the National Institute of Arthritis and Musculoskeletal Diseases at the National Institutes of Health [grant numbers R01 AR 43727 and 69572]. The MUSC cohort and authors were funded by the National Center for Research Resources at the National Institutes of Health [grant number UL1 RR029882], MUSC General and Clinical Research Center [grant number M01 RR001070], South Carolina Clinical & Translational Research Institute [grant number UL1 RR029882], the Multidisciplinary Clinical Research Center at MUSC [grant number P60 AR062755] and the Core Center for Clinical Research [grant number P30 AR072582].

Funding: This study was funded by ImmunArray.

Disclosure statement: C.P., D.S.P., M.P. R.C., A.H.B.W., I.S., J.C.O., R.G. and I.R.C. are consultants to ImmunArray. S.W., R.S., P.S., K.J.B. are employees of ImmunArray. All other authors have declared no conflicts of interest.

References

1 Turchetti G, Yazdany J, Palla I, Yelin E, Mosca M. Systemic lupus erythematosus and the economic perspective: a systematic literature review and points to consider. Clin Exp Rheumatol 2012;30:S116–22.

2 Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus 2006;15:308–18.

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018

3 Arbuckle MR, McClain MT, Rubertone MV *et al*. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33.

4 Schwartz N, Goilav B, Putterman C. The pathogenesis, diagnosis and treatment of lupus nephritis. Curr Opin Rheumatol 2014;26:502–9.

5 Mohan C, Putterman C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. Nat Rev Nephrol 2015;11:329–41.

6 Li QZ, Zhou J, Wandstrat AE *et al*. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol 2007;147:60–70.

7 Lateef A, Petri M. Unmet medical needs in systemic lupus erythematosus. Arthritis Res Ther 2012;14 (Suppl 4):S4.

8 Olsen NJ, Li QZ, Quan J *et al*. Autoantibody profiling to follow evolution of lupus syndromes. Arthritis Res Ther 2012;14:R174.

9 Agmon-Levin N, Mosca M, Petri M, Shoenfeld Y. Systemic lupus erythematosus: one disease or many? Autoimmun Rev 2012;11:593–5.

10 Petri M, Orbai AM, Alarcon GS *et al*. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86.

11 Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. Arch Pathol Lab Med 2000;24:71–81.

12 Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010;69:1420–2.

13 Abeles AM, Gomez-Ramirez M, Abeles M, Honiden S. Antinuclear antibody testing: discordance between commercial laboratories. Clin Rheumatol 2016;35:1713–8.

14 Mariz HA, Sato EI, Barbosa SH *et al*. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011;63:191–200.

15 Pisetsky DS, Rovin BH, Lipsky PE. Biomarkers as entry criteria for clinical trials of new therapies for systemic lupus erythematosus: the example of antinuclear antibodies and anti-DNA. Arthritis Rheumatol 2017;69:487–93.

16 Pisetsky DS. Anti-DNA antibodies—quintessential biomarkers of SLE. Nat Rev Rheumatol 2016;12:102–10.

17 Fattal I, Shental N, Mevorach D *et al*. An antibody profile of systemic lupus erythematosus detected by antigen microarray. Immunology 2010;30:337–43.

18 Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR. Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. J Clin Invest 2007;117:712–8.

19 Putterman C, Wu A, Reiner-Benaim A *et al*. SLE-key® rule-out serologic test for excluding the diagnosis of systemic lupus erythematosus: developing the ImmunArray iCHIP. J Immunol Methods 2016;429:1–6.

20 Cohen IR. Antigen-microarray profiling of antibodies in SLE: a personal view of translation from basic science to the clinic. Lupus Open Access 2016;1:118.

21 Massenburg D, Oldenbrug J, Sell A, Krause T, Wells AF. Using the SLE-key Rule-Out test in clinical practice. Lupus Open Access 2017;2:126.

22 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. Arthritis Rheum 1992;35:630–40.

23 Faria AC, Barcellos KS, Andrade LE. Longitudinal fluctuation of antibodies to extractable nuclear antigens in systemic lupus erythematosus. J Rheumatol 2005;32:1267–72.

24 Agarwal S, Harper J, Kiely PD. Concentration of antibodies to extractable nuclear antigens and disease activity in systemic lupus erythematosus. Lupus 2009;18:407–12.

25 Yaniv G, Twig G, Shor DB *et al*. A volcanic explosion of autoantibodies in systemic lupus erythematosus: a diversity of 180 different antibodies found in SLE patients. Autoimmun Rev 2015;14:75–9.

26 Putterman C. New approaches to the renal pathogenicity of anti-DNA antibodies in systemic lupus erythematosus. Autoimmun Rev 2004;3:7–11.

27 Wen J, Stock AD, Chalmers SA, Putterman C. The role of B cells and autoantibodies in neuropsychiatric lupus. Autoimmun Rev 2016;15:890–5.

28 Vasquez-Canizares N, Wahezi D, Putterman C. Diagnostic and prognostic tests in systemic lupus erythematosus. Best Pract Res Clin Rheumatol 2017;31:351–63.

29 Arroyo-Avila M, Santiago-Casas Y, McGwin G Jr *et al*. Clinical associations of anti-Smith antibodies in PROFILE: a multi-ethnic lupus cohort. Clin Rheumatol 2015;34:1217–23.

30 Putterman C, Balbir-Gurman A, Safer P *et al*. The autoimmune discovery iCHIP distinguishes healthy individuals from those with SLE, rheumatoid arthritis (RA), scleroderma (SSc), Sjogren's syndrome (SS), and the antiphospholipid syndrome (APS). Arthritis Rheumatol 2017; 69 (suppl 10): abstract 1023.

31 Munroe ME, Young KA, Kamen DL *et al*. Discerning risk of disease transition in relatives of systemic lupus erythematosus patients utilizing soluble mediators and clinical features. Arthritis Rheumatol 2017;69:630–42.

Downloaded from https://academic.oup.com/rheumatology/advance-article-abstract/doi/10.1093/rheumatology/key149/5032989
by Albert Einstein College of Medicine user
on 05 June 2018