Letter to the Editor

The potential role of SLE-key test in identifying patients with Systemic Lupus Erythematosus: Results from a prospective, real-world experience

Sir,

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by extremely heterogeneous clinical manifestations [1].

The American College of Rheumatology (ACR) established a list of 11 criteria to classify SLE patients and these criteria are often used for diagnosis [2]. When looking at the laboratory criteria, the following antibody-specificities are considered: antinuclear antibodies (ANA), anti-double strand DNA (anti-dsDNA) antibodies, Sm nuclear antigens and antiphospholipid antibodies. ANA are found to be positive also in other autoimmune conditions and may occur in as many as 5–15% of otherwise healthy individuals [3]. On the other hand, anti-dsDNA and anti-Sm antibodies are more specific, but many SLE patients lack these antibodies [4].

Recently, SLE-key(®) rule-out test was developed and validated for a definitive rule-out of a diagnosis of SLE [5]. The test uses the proprietary iCHIP® micro-array technology platform [6] to identify discriminating patterns of circulating autoantibodies among SLE patients and was tested in comparison of self-declared healthy individuals.

The aim of our study was to prospectively investigate the clinical accuracy of SLE-key test in ruling out the diagnosis of SLE in a real life setting.

We prospectively enrolled during the period of 6 months 50 patients with all the following characteristics: 1) aged < 50 years old, 2) without any previously known autoimmune diseases (AIDs), 3) were referred to our center (the San Giovanni Bosco Hospital, Torino, IT) for a newly onset of persistent (> 3 months) arthralgia.

All patients were prospectively followed-up for the duration of 6 months and after clinical and laboratory evaluations a diagnosis was made.

Blood collection was performed during the first visit. More in detail, ANA were tested by indirect immunofluorescence on HEp-2 cell substrate (Euroimmun AG, Lübeck, Germany). Both anti-dsDNA and specific antibodies including those directed to extractable nuclear antigens (ENA) were detected using fluorenzyme EliA CTD screening immunoassay run on Phadia™2500 automated platform (ThermoFisher Scientific, Freiburg, Germany). The same method was adopted to assess IgG and IgM anti-cardiolipin and anti-β2 glycoprotein I (anti-β2GPI) antibodies. Serum samples were transported to Immunarray's CLIA-certified laboratory, Veracis (Richmond, VA, USA), for SLE-key Rule-Out testing and evaluation, as previously described [6]. Briefly, 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 [6]. Individuals with SLE-key scores of < 0.18 are considered "excluded" or "ruled-out" for SLE.

The significance of baseline differences was determined by the chi-squared test, Fisher's exact test or the unpaired *t*-test, as appropriate. Linear and logistic regressions were performed. A ROC curve analysis, categorical agreement and degree of linear association was performed. A two-sided *P*-value < .05 was statistically significant. All statistical analyses were performed using SPSS version 19.0 (IBM, Armonk, NY, USA).

After 6 months of follow-up, 8 patients (16%) were diagnosed with SLE, 2 patients (4%) were diagnosed with Sjogren's syndrome, 1 patient (2%) with rheumatoid arthritis and 3 (6%) patients with un-differentiated connective tissue disease (according to the respective classification criteria) [2,7–9]. The remaining 36 patients (72%) were not diagnosed with an AIDs based on imaging criteria or laboratory biomarkers and their arthralgia was considered attributable to mechanical and not-inflammatory diseases (e.g. arthrosis, fibromyalgia). Antibodies positivity, distributed according to diagnosis, are resumed in Table 1.

When considering SLE-key test results as continuous variable, patients with SLE diagnosis had statistically significant higher values of SLE-key score, when compared to patients with no AIDs (mean SLE-key score 0.55 ± 0.41 V.s. 0.18 ± 0.17, respectively, p < .0001). Similarly, SLE patients had higher SLE-key scores, also when compared to patients with other autoimmune diagnosis (mean SLE-key score 0.55 ± 0.41 V.s. 0.17 ± 0.16, respectively, p < .0001).

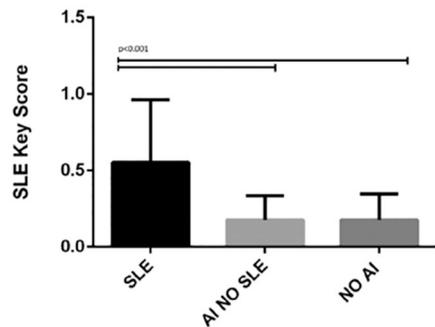When performing a ROC curve analysis considering patients with

Table 1
Antibodies positivity, distributed according to diagnosisand SLE key results.

	SLE (n=8)	Patients with AIDs other then SLE (n=6)	Patients with no AIDs (n=36)
ANA positive, n (%)	8 (100%)	5 (83%)	5 (14%)
anti-dsDNA positive, n (%)	2 (25%)	0	0
anti-Sm positive, n (%)	1 (12%)	0	0
aPL positive, n (%)	0	0	1 (3%)
SLE-key, SLE not ruled-out, n (%)	5 (62%)	2 (33%)	10 (28%)
SLE-key, Score, mean ±SD	0.55 ±0.41	0.17 ±0.16	0.18 ±0.17

	statistically significant when compared to patients with no AIDS
	statistically significant when compared to patients with other AIDs then SLE and no AD

SLE = Systemic Lupus Erythematosus; AIDs = autoimmune diseases; ANA = antinuclear antibodies; anti-dsDNA = anti-double strand DNA; aPL = antiphospholipid antibodies; SD = standard deviation.

SLE diagnosis, the area under the curve (AUC) was 0.726 when considering SLE key test as dichotomous and 0.759 when considering SLE key results as continuous.

Furthermore, a logistic regression showed that the odds ratio of having an SLE diagnosis when having a SLE-key score ≥ 0.31 was 8 compared to those with a SLE score of $\leq 0.12 (p < .05)$.

When considering SLE-key test results as dichotomous (SLE key score < 0.18 = SLE ruled-out; SLE-key score > 0.18 = SLE not ruled-out), 5 patients out of 8 with SLE diagnosis tested as "SLE not-ruled out". This observation was statistically significant when compared to the patients with no AIDs: 10 patients out of 36 tested "SLE not ruled-out" (chi square test 5.43; $p < .05$). Interestingly, when considering antibody-positivity, only ANA showed to be statistically more frequent in patients with SLE diagnosis when compared to patients with no AIDs [8 (100%) V.s. 5 (14%), respectively; $p < .0001$].

The nature of SLE of being a "multisystemic disease" is associated with a high degree of variability at onset, ranging from more specific symptoms such as anti-dsDNA, to non-specific findings such ANA positivity. Thus, especially at onset, the diagnosis of SLE can be challenging.

In this real-world prospective study, we observed statistically significant higher SLE-key scores in patients presenting with arthralgia who were diagnosed with SLE after a follow-up of 6 months, when compared to patients who were diagnosed with other autoimmune conditions and with patients with no AIDs.

Testing for autoantibodies in the clinical practice is becoming more and more relevant, not only of specialists, but also for general practitioners. In fact, autoantibodies testing is used for AIDs diagnosing but also, like in the case of SLE, for identifying different subsets of disease with different clinical manifestations, course and prognosis. Autoantibodies can potentially discriminate patients at the very early stage of disease and allow a tailored treatment and management. Recently, novel techniques for antibodies testing are emerging. From the one hand, specific pathogenic epitopes can be isolated, improving the specificity of antibodies testing. This is the case, for example, of anti-β2GPI-Domain I [10]. From the other hand, multiparametric autoimmune diagnostics, as multiplex and micro-array technology, can allow to simultaneously test for a panel of antibodies, which can prove to be cost-effective for diagnostics.

We acknowledge some limitations, especially considering the small sample size and the heterogeneity of the patients. However, the real-life prospective nature of our study should be considered a relevant strength of the study. SLE-key might in fact represent, mainly for practitioners not specialized in AIDs, an additional tool to identify SLE patients with a high degree of accuracy. In fact, especially in those patients with a SLE-key score < 0.31, in our study the likelihood of having a later SLE diagnosis was 8 times higher than the rest of the population.

In conclusion, the clinical utility of the SLE-key test for identifying patients with SLE seems promising and should be validated in larger prospective studies.

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Authors contributions

MR, SS, CI, and DRoccatello drafted the manuscript, figures and Tables and critically reviewed the manuscript.

MR, SS, IC, and GM participated in laboratory testing and critically reviewed the manuscript.

Declaration of Competing Interest

None.

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