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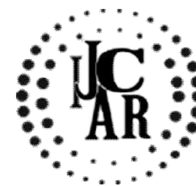

Research Article

# ANTINUCLEAR ANTIBODY SCREENING BY INDIRECT IMMUNOFLUORESCENCE TEST AND ENZYME- LINKED IMMUNOSORBENT ASSAY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: EXPERIENCE FROM MOROCCO

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**ABSTRACT**

Systemic lupus erythematosus (SLE) is an autoimmune disease produces autoantibodies against nuclear antigens (ANA), characterized by diverse clinical presentations. The presence of ANA is the most sensitive test for diagnosing SLE. The aim of this study was to compare the diagnostic performance for SLE of enzyme- linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA). Determination of out whether ANA patterns and ANA levels might reflect the level of disease activity was also investigated.

We tested the ANA in 77 patients with SLE and 16 healthy controls served as a control group. Clinical disease activity was scored according to Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. The moderate agreement between ELISA and IFA determined by the k statistic ($P < 0.001$) and the disparities between both ELISA-positive/IFA-negative samples and IFA-positive/ELISA negative were found. IFA (The positive likelihood ratio (+LR) = +infinity) performed better than the ELISA (+LR = 11.22) for the diagnosis of SLE at the manufacturer's cut off. IFA was also the best at distinguishing patients with active and inactive disease using manufacturer's cut off ($p = 0.036$). IFA was the best global test to screening ANA for both diagnosis and disease activity evaluation in SLE.

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## INTRODUCTION

Laboratory tests used for screening of antinuclear antibodies (ANA) are useful for diagnosis of SLE (Inês *et al*., 2015). The gold standard test for detecting ANA is indirect immunofluorescence assay (IFA) (Buchner *et al*., 2014). Nonetheless, its interpretation of results is subjective (Fenger *et al*., 2004; Jeong *et al*., 2018; Agmon-Levin *et al*., 2014). In an attempt to overcome this subjectivity, several commercial enzyme immunoassays (ELISA) have been developed to screening ANA (Tozzoli *et al*., 2013). Nevertheless, the heterogeneity regarding antigenic composition of ELISA exhibits highly variable sensitivity and specificity (Copple *et al*., 2011). The aim of this study was to compare the diagnostic performance for SLE of ELISA and IFA. Determination of out whether ANA patterns and ANA levels might reflect the disease activity was also investigated.

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### *Patients and methods*

### *Patients and controls*

The study included 77 patients with SLE, classified according to the American College of Rheumatology classification criteria for SLE (Hahn *et al*., 2012). Healthy controls comprised 16 kidney- and bone marrow-donors.The SLE patients were recruited from the Department of Nephrology, Rabat Ibn Sina University Hospital. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was used to assess disease activity (Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier *et al*., 1992). SLE patients with SLEDAI score $\geq 3$ were considered to have active disease (Postal *et al*., 2013; Yee *et al*., 2011).

The study protocol was approved by the ethical committee of the Rabat Medicine University, and informed consent was signed before blood collection. All the clinical data were assessed, at the time of blood collection, by a clinician.

Laboratory measurements

- Antinuclear antibodies by indirect immunofluorescence assay (IFA)

Indirect immunofluorescence on HEp-2 cells for IgG class ANA has been carried out according to the manufacturer's instructions (AESKU.DIAGNOSTICS). Slides were viewed for fluorescent patterns with a fluorescent microscope. Cut-off employed has been 1:160.

- Enzyme- linked immunosorbent assay (ELISA)

This assay is a solid phase enzyme immunoassay for the combined detection of IgG antibodies against HEp2 cells in human serum. Each well was coated with lysed HEp2 cells. Screening ANA has been carried out according to the manufacturer's instructions (AESKU.DIAGNOSTICS).Values ≥ 1.2 is considered positive.

***Statistical analysis***

Statistical analysis was performed using the SPSS (version 13.0) Statistical Software. The statistical significance of difference in the mean values between the two groups was calculated by the independent samples
Student T test.

The Pearson's correlation test was used to analyze the correlations between various laboratory measures and the SLEDAI score.

For each test, we determined the area under curve (AUC), sensitivity, specificity and likelihood ratio (LR) for the diagnosis of SLE using the manufacturer's cut off. The concordance between the different assays was determined by the k statistic. A value of $p < 0.05$ was accepted as statistically significant.

## RESULTS

- Diagnostic performance for SLE of the IFA

The clinical sensitivity of the IFA (using the threshold value of 1:160) was 66.23% for the diagnosis of SLE. The clinical specificity of the IFA was 100%. The positive LR for SLE diagnosis was +infinity (Table1).

**Table 1** Performance of the immunofluorescent and ELISA techniques for the diagnosis in 77 SLE patients, using a control group of 16 healthy subjects

| | AUC % | Cut off for positivity | Sensitivity % | Specificity % | LR+ | LR− |
|---|---|---|---|---|---|---|
| IFA | 72.04 | 1:160 | 66.23 | 100 | +infinity | 33.8% |
| ELISA | 74.19 | > 1.2 | 70.13 | 93.75 | 11.22 | 31.9% |

IFA: Indirect immunofluorescence assay.
ELISA: Enzyme- linkedimmunosorbentassay.
AUC: area under curve.
LR+: Positive likelihood ratio.
LR−: Negative likelihood ratio.

The most prevalent pattern (67%) in these patients was the speckled pattern, the homogenous pattern was found in 33%.

- Diagnostic performance for SLE of the ELISA (Table1).

The clinical sensitivity of the ELISA investigated in relation to SLE, using the manufacturer's cut off index value (≥ 1.2) was 70.13%. The clinical specificity of the ELISA was 93.75%.The positive LR for SLE diagnosis was 11.22.

- Relationship between IFA and ELISA

Levels of ANA tested by ELISA were significantly higher in patients tested positive by IFA (2.47 ± 1.03) compared to those who tested negative (1.02 ± 1.01) ($P < 0,001$).

Table 2 shows the comparison between IFA and ELISA in measuring the ANA among various groups of the study. Next, we have analyzed agreement (kappa index) between qualitative (positive or negative) results obtained with both assays (ELISA and IFA). For an IFA 1:160 titer the moderate agreement was obtained with ELISA (k=0.58, $P<0.001$).

**Table 2** Comparison between immunofluorescent and ELISA techniques in measuring the ANA among various groups of the study

| | | Positive ANA results | | Negative ANA results | |
|---|---|---|---|---|---|
| Study groups | | IFA | ELISA | IFA | ELISA |
| SLE cases | Count | 51 | 54 | 26 | 23 |
| N=77 | % within group | 66.23 | 70.13 | 33.77 | 29.87 |
| Healthycontrol | Count | 0 | 1 | 16 | 15 |
| N=16 | % within group | 0 | 6.25 | 100 | 93.75 |

SLE: Systemic lupus erythematosus.
IFA: Indirect immunofluorescence assay.
ELISA: Enzyme- linkedimmunosorbentassay.
N: number of individuals.

- SLE activity assessment performance of the IFA and ELISA

Patients with homogenous ANA pattern has markedly higher disease activity score (mean: 10.76 ± 8.04) than those of speckled pattern (mean: 6.53 ± 5.77, p = 0.036) (Table 3).
No correlation between the ELISA titer and SLEDAI score (r = 0.07, p = 0.55).

**Table 3** Comparison between the SLEDAI score and IFA ANA patterns for 51 SLE patients

| Parameters studied | Pattern | N | Mean | Std. deviation | p-value |
|---|---|---|---|---|---|
| SLEDAI score | Homogenous | 17 | 10.76 | 8.10 | 0.036 |
| | Speckled | 34 | 6.53 | 8.71 | |

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.
P: significance level; significant $p < 0.05$.
N: number of individuals.
Std. deviation: standard deviation.

## DISCUSSION

In this study, IFA was the best global test to screening ANA antibodies for diagnosis and homogenous patterns of ANA (using IFA) were significantly associated in SLE patients with active disease compared to those with inactive.

The current SLE classification criteria comprises clinical and laboratory criteria, one of which is the presence of ANA which is the most sensitive test for SLE (Inês *et al*., 2015).

The course of the disease in SLE patients is highly variable and difficult to predict suggests the need for highly sensitive and specific diagnostic tests (Lam and Petri, 2005).

Results from our study are similar with five studies of SLE (Brito *et al*., 2016; Copple *et al*., 2011; Divate *et al*., 2004; Emlen *et al*., 1997; González *et al*., 2002). These presented a higher sensitivity of ELISA than that of IFA, which was discordant to the overall results of other studies (Deng *et al*., 2012; Hira-Kaza *et al*., 2015; Jeong *et al*., 2017; Otten *et al*.,

2017). Study samples (number; disease), types of kits of ELISA and IFA used, might be the cause of these discordant results (Jeong *et al*., 2018).

When we compared ELISA to IFA, the results exhibited moderate agreement, with Cohen's kappa value of 0.58 ($P < 0.001$). The same results have been reported by Gonza´lez *et al*. (2002), Bayer *et al*. (1999), Bernardini *et al*.(2002) Paz *et al*.(2007) and Russell *et al*.(2003).

Indeed, the disparity between ELISA-positive/IFA-negative samples and IFA-positive/ELISA-negative *have been reported previously* (Bernardini *et al*., 2002; Olaussen *et al*., 1999).

The treating physicians need to have a test with a good specificity and a high positive LR to assess patients with a suspected diagnosis of SLE (Launay *et al*., 2010). At the manufacturer's cut off, the LR for SLE diagnosis was higher with a positive IFA result than a positive ELISA result. Altogether, these results suggest that ELISA can experience some difficulty in specifically distinguishing SLE patients from healthy subjects. However, these results should be assessed using a control group of patients with other autoimmune rheumatic diseases, and not only with healthy subjects.

Another issue is the performance of ANA antibody tests in the evaluation of SLE activity. We tried to compare ANA pattern (using IFA) with the disease activity using the SLEDAI score. SLE patients with homogenous ANA patterns had significantly higher SLEDAI score than patients with speckled ($p = 0.036$). This finding indicates that homogenous ANA could be considered a marker of disease activity. This result is in agreement with the results reported in previous studies (Farha *et al*., 2009; Riboldi *et al*., 2005). Whereas, there was an insignificant correlation between the ANA titers and the SLEDAI. A similar result has been demonstrated in several studies (Chen *et al*., 2003).

## CONCLUSION

It can be concluded that ANA detection by IFA (HEp-2 cells substrate) have a good specificity and a high positive LR to assess patients with a suspected diagnosis of SLE and that homogenous ANA patterns are associated with disease activity in SLE patients. Further studies will be needed in order to confirm the study by increasing the samples.

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**Declaration of interest**

The authors declare that they have no conflicts of interest concerning this article.

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