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What is This?
CONCISE REPORT

Changes in Farr radioimmunoassay and EliA fluorescence immunoassay anti-dsDNA in relation to exacerbation of SLE

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Measuring anti-dsDNA levels could support treatment adjustment during follow-up of patients with systemic lupus erythematosus (SLE). We investigated whether patients with exacerbations of SLE showed changes in anti-double-stranded DNA (anti-dsDNA) levels prior to the exacerbation using the Farr and EliA assay and examined which assay showed highest specificity and predictive value for exacerbations. Changes in anti-dsDNA of ≥25% prior to exacerbation were considered of clinical significance. Exacerbations were retrospectively abstracted from medical records. Eighteen of 48 patients showed one or more exacerbations. We found 22 exacerbations with complete lab work-up, all accompanied by ≥25% change in anti-dsDNA in one or both assays. Only 10 exacerbations showed concordant changes in anti-dsDNA in both assays. Changes in anti-dsDNA had a low predictive value for exacerbations of SLE, but the specificity of anti-dsDNA changes for patients with exacerbations was higher for EliA than Farr. We conclude that despite the limited relation between anti-dsDNA changes and exacerbations of SLE, anti-dsDNA testing could still support clinical decision making when used in the correct setting. We conclude that EliA is preferable over Farr for assaying anti-dsDNA during follow-up of patients with SLE because of higher specificity, less “hands-on” time and absence of radioactivity. Lupus (2013) 0, 1–5.

Key words: Anti-DNA antibodies; systemic lupus erythematosus; renal lupus; radioimmunoassay; disease activity; enzyme-linked immunosorbent assay

Introduction

Measuring levels of autoantibodies against double-stranded DNA (anti-dsDNA) is important for diagnosis, but might also be of importance for monitoring disease activity of systemic lupus erythematosus (SLE).1–2 Rising levels of anti-dsDNA have been reported prior to exacerbations. Therefore anti-dsDNA levels could direct prophylactic treatment of patients to prevent exacerbations.3–5 Evidence, however, is based on short-term studies using different test systems and remains controversial.5–8 The most frequently used test systems are radioimmunoassays (RIAs) based on immunoprecipitation (Farr assay), indirect immunofluorescence assays using the hemoflagellate Chritidia luciliae (CLIFT), and enzyme-linked immunosorbent assays (ELISA) or nowadays automated fluorescence immunoassays, like EliA.9 These assays show significant discrepancies concerning the antibody’s class, avidity, complement fixing ability, antigenic specificity and cross-reactive pattern.

As part of the process of re-evaluating our laboratory procedures for anti-dsDNA testing, we investigated whether patients with exacerbations of SLE showed changes in anti-dsDNA test results using Farr and EliA assay. We examined which assay showed highest specificity and predictive value for exacerbations. Such a comparison of performance of Farr and EliA anti-dsDNA tests in the follow-up of patients with SLE has not been published before.

Material and methods

Patients

Patients with SLE and lupus-like disease (LLD) treated in our hospital between 2003 and 2009
were included in the study. Patients with SLE fulfilled four or more updated American College of Rheumatology (ACR) classification criteria, whereas patients with LLD fulfilled two or three criteria (but may have fitted more criteria during the course of the study). Diagnostic samples of patients with SLE/LLD were excluded. Patients from whom less than three anti-dsDNA test results were obtained during follow-up, or from whom all anti-dsDNA test results were below both assays’ threshold, were also excluded. All samples in this retrospective study were obtained as part of routine work-up. According to local rules ethical approval or informed consent was not required for this study.

Anti-dsDNA

The RIA Farr assay has been the regular anti-dsDNA assay in our laboratory for several years and was executed according to the manufacturer’s protocol (Siemens Healthcare Diagnostics, the Hague, the Netherlands). Values <6 kU/l were considered negative. The EliA assay, an automated enzyme-labeled fluorescence immunoassay, was run on the Immunocap250 according to the manufacturer’s instructions (ThermoFisher Scientific, Nieuwegein, the Netherlands). Values <10 IU/l were considered negative. The EliA anti-dsDNA assay was run in a research-based setting; test results of EliA anti-dsDNA were not reported to physicians.

Exacerbations and changes in anti-dsDNA

Changes in disease activity were retrospectively abstracted from medical records. Regular follow-up of patients with stable disease involved one or two visits to a physician annually, but frequency increased with signs of disease activity. Exacerbations of disease were defined as changes in disease activity, new manifestations in affected organs, or increases in drug use and were scored using two global measures of disease activity: the SLE Disease Activity Index (SLEDAI) and the British Isles Lupus Assessment Group (BILAG) instrument. More than one exacerbation per patient could be observed during the course of the study. The SLEDAI (with Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) modification) can be used (retrospectively) to score mild/moderate or severe exacerbations based on the presence of 24 items in the last 10 days. The item involving anti-dsDNA levels was removed to avoid bias. The BILAG instrument consists of 86 questions, grouped over eight organ systems, for which presence, deterioration or improvement of complaints in the last four weeks can be scored (total scores from A to E), based on the principle of the physician’s intention to treat. Only A and B scores were analyzed further. The time of exacerbation was determined month 0, and anti-dsDNA test results collected in months −2 to +2 were considered “during exacerbation,” whereas anti-dsDNA test results collected in months −5 to −3 were considered “prior to exacerbation.” Changes in these anti-dsDNA test results of ≥25% were further analyzed. In a few cases when test results were not available “prior to exacerbation,” we used test results “after exacerbation” (months +3 to +5). Patients without exacerbations were also checked for presence of ≥25% change in anti-dsDNA test results using similar timeslots.

Data analyses

Characteristics of patients with and without exacerbations were compared using χ² tests and Mann-Whitney tests. Sensitivity, specificity and predictive value data were calculated from 2×2 contingency tables. The relation between anti-dsDNA levels, exacerbations and patients with exacerbations were explored using χ² tests and Cramer’s V significance using SPSS 15.0 (IBM, Chicago, IL, USA).

Results

Characteristics

We collected 560 anti-dsDNA test results from 102 patients with SLE/LLD. Fifty-four patients were excluded because of insufficient anti-dsDNA test results (n=35) or anti-dsDNA test results below threshold for both assays (n=19). Disease activity was investigated in the remaining 48 patients, whose characteristics are given in Table 1.

Eighteen (four originally diagnosed as LLD) of 48 patients with SLE had a total of 32 exacerbations, of which 16 were classified as severe, A, and 16 as mild/moderate, B. Two exacerbations obtained the most severe score by SLEDAI (severe), but not by BILAG (B), and vice versa. The clinical domains involved were: mucocutaneous (eight ×), musculoskeletal (six ×), renal (five ×), cardio-respiratory (five ×), neuropsychiatric (four ×), vasculitis (three ×) and hematological (one ×). Eight patients had multiple exacerbations and five patients died during the course of the study.
Exacerbations and changes in anti-dsDNA

Of 32 exacerbations, 22 exacerbations (14 patients) were explored for changes in anti-dsDNA test results. The remaining 10 exacerbations (four patients) had incomplete lab work-up. All 22 exacerbations were accompanied by changes in anti-dsDNA of ≥25% in one or both assays. Changes in Farr and EliA anti-dsDNA were concordant (e.g., Farr and EliA anti-dsDNA test results both increased ≥25%) for only 10 exacerbations (seven patients). The remaining 12 exacerbations (seven patients) were accompanied by discordant Farr and EliA anti-dsDNA test results. The relation between changes in anti-dsDNA test results and presence of exacerbations is depicted in Table 2. In three patients EliA test results were continuously below threshold, whereas Farr test results were not. Changes in Farr anti-dsDNA levels were frequently observed in patients with exacerbations, but also regularly in patients without exacerbations. Therefore, the relation between changes in anti-dsDNA levels and patients with exacerbations was stronger for EliA ($p = 0.08$) than for Farr ($p = 0.93$).

By increasing the threshold level for anti-dsDNA test results from 6 to 24 kIU/l for the Farr assay and from 10 to 40 kIU/l for the EliA assay, the relation between ≥25% changes in anti-dsDNA test results and patients with exacerbations became more profound (Farr: $p = 0.017$, Cramer’s $V$ 36%, EliA: $p = 0.002$, Cramer’s $V$ 45.8%). By increasing thresholds, more patients with evident exacerbations were, however, scored as having no change (<25%) in anti-dsDNA levels, thus sensitivity decreased at the cost of increased specificity.

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Patients with exacerbations (n = 18)</th>
<th>Patients without exacerbations (n = 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n female (F), n male (M))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at study onset (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAID (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMARDs (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxychloroquine (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other DMARDs (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$ samples, total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$ samples, per patient</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSAIDs: nonsteroidal anti-inflammatory drugs; DMARDs: disease-modifying antirheumatic drugs, including hydroxychloroquine, methotrexate, azathioprine, and cyclosporine; Anti-dsDNA: anti-double-stranded DNA. Presented data are medians (with ranges) or numbers (with %).

**Table 2** Relation between changes in anti-dsDNA levels by Farr or EliA assay and patients with exacerbations and without exacerbations

<table>
<thead>
<tr>
<th></th>
<th># patients with exacerbations</th>
<th></th>
<th># patients without exacerbations</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farr</td>
<td>EliA</td>
<td>Farr</td>
<td>EliA</td>
</tr>
<tr>
<td>Change anti-dsDNA present (≥25%)</td>
<td>12</td>
<td>10</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>$n$ samples, per patient</td>
<td>86</td>
<td>Sens 86</td>
<td>Sens 71</td>
</tr>
<tr>
<td>Change anti-dsDNA absent (&lt;25%)</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>$n$ samples, per patient</td>
<td>86</td>
<td>Sens 86</td>
<td>Sens 71</td>
</tr>
</tbody>
</table>

Anti-dsDNA: anti-double-stranded DNA; PPV: positive predictive value; NPV: negative predictive value; Sens: sensitivity; Spec: specificity.

**Discussion**

In this retrospective study we demonstrated that EliA is recommended over Farr to monitor exacerbations in patients with SLE. Although both tests had little positive predictive value (PPV) for exacerbation of disease, EliA had higher specificity than Farr in our test setting.

This study is unique for two reasons. First, sensitivity and specificity of Farr and EliA for monitoring exacerbations of SLE have not been examined before in the same population. Farr and EliA have, however, been compared for diagnosis of patients with SLE, which resulted in different findings. For example, it has been shown that EliA had slightly lower specificity (93% vs 96%), but higher sensitivity than Farr (71% vs 67%), resulting in a lower PPV (65% vs 80%) for diagnosing patients with active SLE vs antinuclear antibodies (ANA)-positive controls. In another study Farr was more sensitive than EliA (72% vs 44%) at
similar specificity (95%) for diagnosing patients with SLE vs controls (20% ANA positive).13

Second, we measured anti-dsDNA levels in patients with SLE by two test systems for seven years, a follow-up duration that has not been described before. Prolonged comparison of two assays in patients that infrequently experience exacerbations adds to the validity of this study.

Although regular anti-dsDNA testing is recommended by the European League Against Rheumatism (EULAR) and ACR,1,14 controversy still exists about the predictive value of anti-dsDNA levels for exacerbations. Few studies have been performed and led to different results,3–5,8,9 some of which may be explained by differences in study design, characteristics of patient and control groups, assay frequency and anti-dsDNA test systems. Approximately half of the exacerbations and patients in our study showed discordant changes in anti-dsDNA by Farr and EliA. These discordant responses may be explained by differences in type of exacerbation or antibodies involved. We observed a broad spectrum of exacerbations, but without a hint to a relation between type of exacerbation, severity and anti-dsDNA response. We also did not detect differences in patient characteristics accompanying concordant or discordant responses in anti-dsDNA. The relatively small sample size, however, excludes statistical support.

Our findings stand or fall with the validity of the 25% change criterion that we selected from the literature3,5 as well as the anti-dsDNA threshold level for a positive test result. We validated the 25% change criterion by calculating critical differences for both tests and found that these were smaller than the 25% change criterion. Manufacturers’ threshold levels for positive test results used throughout, but we found, similar to others,13,15 that by altering thresholds for positive test results specificity of the tests could be increased, at the costs of reduced sensitivity and logistic adaptations.

Retrospective monocenter studies are useful for testing exploratory hypotheses, but are known for inherent limitations. In this study such limitations include time of sampling, drug use and period of analysis. Anti-dsDNA testing was left to the discretion of the attending physician. This may have resulted in assaying more samples from patients with active illness than from those with stable SLE. The number of assayed samples was not, however, significantly different between patients with or without exacerbations (Table 1). We excluded patients with anti-dsDNA test results below thresholds for both assays. Our data are therefore of limited significance with respect to negative anti-dsDNA test results; however, an additional search through medical records of these patients showed no evidence of exacerbations. The majority of patients were prescribed drugs, and type and dosage differed between patients with and without exacerbations (Table 1). When assessing disease activity by SLEDAI and BILAG, we rejected phenomena that were clearly caused by drug use (e.g. low leukocytes following cyclophosphamide treatment), whereas we scored increases in drug dosage as deterioration of disease. Nevertheless it is clear that drug use may have blurred timing and number of exacerbations.

Changes in anti-dsDNA test results may also have been missed by using fixed time periods prior to and during exacerbation. The time periods were based on data from a prospective study in which changes in anti-dsDNA levels were observed between zero to seven months (median 2.1 months) before exacerbation.5 However, some controversy exists concerning this topic as it has been postulated that after initial rises anti-dsDNA levels quickly decrease before the exacerbation becomes evident, supposedly because of deposition in tissue.4

Despite Farr’s high specificity for diagnosis of SLE, the assay is relatively time consuming and involves radioactivity. Therefore one may consider using another specific, automated method (e.g. EliA) for regular monitoring of patients. Another approach might be to exploit a two-step set-up for anti-dsDNA testing in a laboratory: a specific test system for diagnosing clinically suspect patients (e.g. Farr), and a sensitive automated test system for monitoring patients with SLE (e.g. EliA). In any case, it is important that physicians are aware of the anti-dsDNA method used in their laboratory.

Despite the limited relation between anti-dsDNA test results and exacerbations of SLE and the discordance between assays, anti-dsDNA might still be helpful as a prognostic marker and support clinical decision making when used in the correct setting, where suspicion of deterioration of disease should be led by patient history and physical examination. We conclude that under our conditions the EliA assay is more specific than the Farr assay for detecting changes in anti-dsDNA levels corresponding to exacerbations of SLE. In addition, the automated EliA assay involves less “hands-on” time and does not involve radioactivity.
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Conflict of interest statement

The authors have no conflicts of interest to declare.

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