Anti-nucleosome antibodies outperform traditional biomarkers as longitudinal indicators of disease activity in systemic lupus erythematosus

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Abstract

Objective. The aim of this study was to determine whether anti-nucleosome antibodies function as activity-specific biomarkers in SLE.

Methods. Fifty-one patients were recruited and followed prospectively with periodic clinical and biochemical assessments over a 14-month period. Disease activity was determined by the SLEDAI-2K. Anti-nucleosome antibody levels were measured by an ELISA and its utility as an activity-specific biomarker as compared with that of anti-dsDNA antibodies and C3 was assessed both at baseline and in longitudinal analysis.

Results. Anti-nucleosome antibodies were significantly elevated in SLE patients vs controls and showed a moderate positive correlation with disease activity. The utility of anti-nucleosome antibodies in identifying patients with active disease in a cross-sectional analysis was comparable to that of anti-dsDNA antibodies and C3. Analysis of variance demonstrated that the level of anti-nucleosome antibodies and C3 varied significantly with changes in disease activity over time. Changes in clinical state were not mirrored by changes in anti-dsDNA antibodies. In time-dependent analysis, anti-nucleosome antibodies showed a better fit over time than anti-dsDNA antibodies and C3. In pairwise comparisons, C3 and anti-nucleosome antibodies outperformed other models, including the conventional pairing of C3 and anti-dsDNA antibodies, however, no biomarker alone or as a group accurately predicted impending remissions or exacerbations.

Conclusion. Anti-nucleosome antibodies demonstrate greater fidelity as a biomarker for changes in SLE disease activity than traditional biomarkers, supporting the routine monitoring of this antibody in clinical practice.

Key words: systemic lupus erythematosus, anti-nucleosome antibodies, biomarkers, disease activity.

Introduction

SLE is a chronic autoimmune condition targeting multiple organs including joints, skin and kidneys. The clinical presentation and course of SLE is heterogeneous, hampering early definitive diagnosis and monitoring of disease progression. Although clinical assessment is the cornerstone of SLE patient management, these evaluations are limited and require additional instruments to confirm diagnosis and determine disease activity. At present, effective SLE patient care relies on serological biomarkers (e.g. anti-dsDNA antibodies and complement) to inform clinical decisions. Levels of anti-dsDNA antibodies serve as a diagnostic marker and when coupled to complement, fluctuations in these immunological parameters serve to
monitor disease activity [1]. Although these biomarkers are criticized for their poor performance as indicators of disease activity in discordant patients [2, 3] and for their failure to predict impending disease flares [4], they remain the benchmark by which the clinical utility of novel biomarkers is assessed.

SLE is characterized by the production of autoantibodies directed against nuclear antigens such as chromatin. The structural unit of chromatin is the nucleosome, which consists of a segment of dsDNA coiled around a histone core. Anti-chromatin autoantibodies are segregated into two groups: antibodies directed against the individual components of chromatin such as DNA and antibodies with a higher affinity to the intact nucleosome. Studies have demonstrated that the nucleosome is the primary antigen in SLE [5] and that anti-dsDNA antibodies represent a subset of this antibody population. These observations suggest that although anti-dsDNA antibodies are an accepted serological marker for the diagnosis and monitoring of SLE [1], anti-nucleosome antibodies may provide a better reflection of disease activity. Multiple studies have demonstrated that anti-nucleosome antibodies are increased in SLE patients, with antibody positivity correlating with global disease activity [6, 7]. Further, a specific association between anti-nucleosome antibodies and LN has been reported [7, 9], although this remains a contentious issue, as a meta-analysis failed to confirm this relationship [8]. On this basis, anti-nucleosome antibodies have been proposed as potential activity-specific biomarkers. Accordingly, we have examined the clinical utility of anti-nucleosome antibodies across multiple clinically relevant situations, including the diagnosis of SLE, the identification of patients with active disease and the longitudinal monitoring of disease activity.

Patients and methods

Subjects and data collection

Fifty-one patients satisfying the revised 1997 ACR classification criteria for SLE [1] were recruited from the University of Toronto Lupus Clinic. All patients had naturally occurring SLE with no history of drug-induced disease. Patients were followed longitudinally for an average of 28 months (range 8–48) with at least five clinical (mean 9.5, range 5–16, total 484) and three biochemical (mean 5.4, range 3–9, total 274) assessments for which anti-nucleosome antibodies were measured. Where possible, clinical data extending 6 months past the last biochemical assessment were obtained (n=48). Healthy controls (n=49) were also recruited. The demographics for the patient and control groups are summarized in Table 1. At inception, 41 patients were receiving prednisone, 41 antimalarials and 35 DMARDs (Table 1). At the conclusion, 43 patients were receiving prednisone [mean dose 13.4 mg/day (s.d. 12.1)], 48 antimalarials and 41 DMARDs [AZA (12), MMF (21), MTX (6) and CYC (2)]. Several patients were recruited at the onset of disease flare, accounting for the increase in DMARD use during

| TABLE 1 Demographic and clinical variables for SLE patients |
|-----------------|-----------------|-----------------|
| Demographic variable | Controls (n = 49) | SLE (n = 51)* |
| Age               | 22–58           | 18–65           |
| Range, years      | 35.2 (9.8)      | 30.9 (11.3)     |
| Mean (s.d.) (median), years | 42 (85.7) | 47 (92.2) |
| Gender, female, n (%) | NA             | 47 (92.2) |
| Clinical features, n (%) | NA             | 47 (92.2) |
| Rash              | 14 (27.5)       | 29 (56.9)       |
| Mucocutaneous     | 9 (17.6)        | 5 (9.8)         |
| Alopecia          | 9 (17.6)        | 2 (3.8)         |
| Arthritis         | 11 (21.6)       | 5 (9.8)         |
| Serositis         | 8 (15.7)        | 2 (3.8)         |
| Haematological    | 5 (9.8)         | 29 (56.9)       |
| Fever             | 2 (3.8)         | 2 (3.8)         |
| Nephritis (one or more S-2K criteria) | 5 (9.8) | 29 (56.9) |
| Vasculitis        | 5 (9.8)         | 2 (3.8)         |
| Medication*       | 41 (80.4)       | 41 (80.4)       |
| Prednisone, n, mean (s.d.) (median) | 41 (80.4) | 17.1 (15.2) (13.8) |
| Antimalarials, n (%) | 35 (68.6) | 12 (23.5)         |
| Immunosuppressants, n (%) | 12 (23.5) | 35 (68.6)         |
| AZA, n (%)        | 18 (34.6)       | 35 (68.6)       |
| MMF, n (%)        | 5 (11.9)        | 12 (23.5)       |
| MTX, n (%)        | 1 (2.0)         | 5 (11.9)        |
| CYC, n (%)        | NA              | 1 (2.0)         |

*There was one more patient in the longitudinal analysis than in the cross-sectional analysis. P = 0.02. P = ns. *Clinical variables and treatment at time of recruitment. NA: not applicable; S-2K: SLEDAI-2K.
the study. Disease activity at each visit was assessed utilizing the SLEDAI-2K [11]. Modified SLEDAI-2K (mS-2K) scores were calculated by subtracting the contribution of hypocomplementaemia and anti-dsDNA positivity from the total score. Patients with an mS-2K score $>0$ were considered to have active disease. The study was approved by the University Health Network research ethics board and participants signed informed consent.

**Human IgG anti-nucleosome ELISA**

H1-stripped chromatin was isolated from MOLT4 human acute lymphoblastic leukaemia cells utilizing an established protocol [12] that generates mostly mono- and dinitucleosomes. Serum was diluted at 1:1000 and tested in triplicate for binding to immobilized chromatin by ELISA. Bound IgG was detected with anti-human IgG alkaline-phosphatase conjugate (1:1000) and absorbance read at 405nm. Human sera with known anti-nucleosome activity were utilized as positive and negative controls and to allow for interplate standardization. A threshold for anti-nucleosome positivity was defined as $>3$ s.d. above the mean for healthy controls [10].

**Testing of clinical serological parameters**

Anti-dsDNA antibodies were determined by the Farr assay with normal defined as $\leq 7$. Complement levels were measured by nephelometry, with the normal range for C3 being $\geq 0.9$.

**Statistical analysis**

Antibody levels and mS-2K scores were collected from multiple visits for each patient. Concordance between disease activity and antibody levels was assessed using Spearman’s correlation. The Mann-Whitney non-parametric test was used to compare patient groups (where $P < 0.05$ indicates a statistically significant difference). Youden’s index analysis was performed to establish the optimal discriminatory threshold to identify patients with active disease for anti-dsDNA antibodies, complement (C3) or anti-nucleosome antibodies utilizing data from the inception visit. Linear modelling with Akaike information criterion (AIC) was performed to determine the relative contribution of each immunological marker to mS-2K. Variation between visits was examined as follows: a change in mS-2K score of $\pm 4$ between two consecutive visits was deemed to be a clinically significant event. This definition was based on the ACR recommendation that a gain of 8 points defines a clinically meaningful change [13]. In our study, the contribution of the immunological parameters was subtracted, yielding a meaningful change score of 4. Event classification was compared with analyte levels using a one-way analysis of variance (ANOVA) followed by visualization. The ability of analyte levels to forecast disease activity was evaluated using a leave-one-out cross-validation k nearest neighbours (knn) analysis using the class package (version 7.3-7) for R (R Project for Statistical Computing, Vienna, Austria).

**Results**

**Anti-nucleosome antibodies and disease activity in SLE**

To examine the relationship between selected immunological parameters and disease activity, anti-dsDNA antibody, complement (C3) and anti-nucleosome antibody levels were determined in 50 patients and 49 healthy controls. Although a number of anti-dsDNA antibody methods are available, the Farr assay was selected because it shows greater fidelity with disease activity than other antibody detection methods [14] and is a requirement for the calculation of disease activity using the SLEDAI-2K [11]. Cross-sectional analysis demonstrated that anti-nucleosome antibody levels were significantly elevated in SLE patients when compared with controls ($P < 0.05$; Fig 1A). Antibody positivity was common, with 39 (78%) SLE patients having anti-dsDNA and 44 (88%) having anti-nucleosome antibodies. Consistent with previous reports suggesting that the immunological requirements for production of anti-nucleosome antibodies are less stringent than those for anti-dsDNA [15], six patients (12%) were anti-nucleosome antibody positive and anti-dsDNA negative, whereas only two patients were anti-dsDNA (4%) positive and anti-nucleosome negative. Anti-nucleosome antibody levels were highly correlated with other immunological parameters showing a statistically significant positive correlation with anti-dsDNA antibodies (Spearman’s $r = 0.71$, $P = 1.08 \times 10^{-4}$; Fig. 1B) and a negative correlation with complement levels ($r = -0.56$, $P = 2.39 \times 10^{-5}$; Fig. 1C). Given that anti-dsDNA positivity and complement levels contribute to the calculation of the SLEDAI-2K and that they correlate strongly with anti-nucleosome antibodies, the relationship between all three immunological parameters and disease activity was determined utilizing the mS-2K to limit bias. Traditional biomarkers (anti-dsDNA antibodies and C3) showed strong correlations in opposing directions ($r = 0.54$ and $-0.58$, $P = 5.58 \times 10^{-5}$ and $9.96 \times 10^{-6}$; Fig. 1D and E, respectively) with global disease activity as defined by the mS-2K [9.3 (s.d. 6.3)]. A moderate positive correlation ($r = 0.43$, $P = 1.63 \times 10^{-3}$; Fig. 1F) was found between anti-nucleosome antibodies and disease activity. At enrolment, 44 patients were defined as active (mS-2K $> 0$), of which 29 (65.9%) had active LN, with the remainder having a range of clinical manifestations (Table 1). No significant difference in anti-nucleosome antibodies was noted between the groups. Taken together, these results suggest that anti-nucleosome antibody levels might serve as an activity-specific biomarker.

**Anti-nucleosome antibodies effectively discriminate between active and inactive SLE patients**

SLE exacerbations requiring the introduction or intensification of therapy are diagnosed based on clinical symptoms and concordant biomarker abnormalities. This clinical context argues for the selection of activity-specific biomarkers with high specificity to limit the misidentification of quiescent patients as active and reduce
unnecessary treatment. To examine the capacity of anti-nucleosome antibodies to fulfill this clinical mandate, Youden’s index analysis was performed to establish a suitable discriminatory threshold. An anti-nucleosome antibody level \(>0.84\) was found to most effectively discriminate active (mS-2K \(>0, n = 44\)) from inactive disease. This metric was used to determine the specificity, sensitivity and positive and negative predictive values (PPV and NPV) of anti-nucleosome antibodies, while these values were calculated using traditional thresholds for anti-dsDNA antibody levels and C3. Anti-nucleosome antibodies outperformed traditional biomarkers, correctly identifying 80% of patients with inactive disease (sensitivity = 0.55, specificity = 0.83). In contrast, anti-dsDNA antibodies and C3 identified only 67% and 50% of inactive patients, respectively (Table 2). These results suggest that anti-nucleosome antibodies may serve as a better discriminator between active and inactive disease.

Table 2 Performance characteristics of biomarkers utilizing reference and calculated thresholds

<table>
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<th>Biomarkers</th>
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<th>Calculated threshold</th>
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<tr>
<td></td>
<td>Sn</td>
<td>Sp</td>
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<td>C3</td>
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Results are for biomarker association, individually or in combination, with disease activity. Comparison of sensitivity, specificity, PPV and NPV of anti-dsDNA antibodies, C3 and anti-nucleosome antibodies calculated utilizing reference and calculated (optimal) thresholds. Optimal thresholds were determined as the cut-off that generated the largest Youden’s index value. Calculations were based on an mS-2K score \(>0\) to define disease activity. Sp: specificity; Sn: sensitivity; PPV: positive predictive value; NPV: negative predictive value; Ab: antibody; anti-Nu: anti-nucleosome.
activity than traditional biomarkers, arguing for its use as an activity-specific biomarker.

Determination of context-specific thresholds optimizes the performance of traditional SLE biomarkers

A potential limitation of this analysis is that reference thresholds, which are commonly established by testing an index group reflective of the general population and defining abnormal values as exceeding an arbitrary percentile threshold, may not be suitable to effectively discriminate between SLE patients with active and inactive disease. To address this issue, Youden’s index analysis was used to establish the optimal discriminatory threshold for each biomarker [16] and their performance individually and/or in pairs was evaluated. Calculated thresholds were <0.7 and >18 for C3 and anti-dsDNA antibodies, respectively. Using these thresholds, a modest improvement in the operator characteristics of anti-dsDNA antibodies was noted (shown by increased sensitivity and specificity, as well as improved predictive values), whereas use of a more stringent C3 threshold greatly enhanced its ability to discriminate between active and inactive disease with no patients falsely labelled as active (Table 2). In contrast to our initial analysis in which the C3 and anti-dsDNA antibody pairing performed more poorly than either in isolation, application of the optimized threshold improved the performance characteristics of combinations including either one (C3 or anti-dsDNA antibodies) or both (C3 and anti-dsDNA) serological parameters (Table 2). In particular, the discriminatory capacity of the classic clinical pairing of C3 and anti-dsDNA antibodies identified patients with active disease while effectively limiting the misidentification of inactive patients (sensitivity = 0.62, specificity = 1 using these calculated thresholds; sensitivity = 0.85, specificity = 0.6 for traditional thresholds). Taken together, these results illustrate the need to establish context-specific thresholds for biomarkers to maximize their clinical utility.

Given that anti-nucleosome antibodies were comparable to C3 and anti-dsDNA antibodies, we examined whether their inclusion would improve upon the discriminatory capacity of traditional biomarkers. The performance of anti-nucleosome antibodies in permissive combinations, defined as having two or more biomarkers in which only one was positive, or in strict combinations, requiring all biomarkers to be positive, was examined. Overall, the inclusion of anti-nucleosome antibodies did not significantly improve on the performance characteristics of traditional biomarkers. None of the permissive combinations outperformed the traditional biomarker component of the pairing. For example, the combination of anti-nucleosome antibodies and C3 had a lower specificity (0.86) than C3 alone (1.00) (Table 2). Overall the incorporation of anti-nucleosome antibodies did not improve the performance of the traditional biomarker pairing of anti-dsDNA antibodies and C3.

Anti-nucleosome antibodies outperform traditional biomarkers in longitudinal monitoring of SLE disease activity

Changes in biomarker levels over time, rather than absolute values, may be more indicative of disease activity. To determine whether changes in immunological parameters are reflective of alterations in disease status, we compared the relationship between meaningful fluctuations in disease status between consecutive visits (indicated by $|\Delta \text{mS-2K}| \geq 4$) and biomarker levels using one-way ANOVA (Fig. 2). Changes in disease state were not significantly reflected by changes in anti-dsDNA antibody level ($P = 0.10$). In contrast, levels of C3 and anti-nucleosome antibodies

![Fig. 2 Relationship of marker levels to mS-2K](image)

Association between change in mS-2K ($\Delta \text{SLEDAI}$) and change in biomarker levels (expressed as the log2-fold change). Clinically relevant change in disease activity was defined as a change in mS-2K score of ±4 (indicating an event). The number of events was: C3, 435 total (99, ≤−4; 278, −3 to 3; 58, >4); anti-dsDNA antibody, 437 total (100, ≤−4; 279, −3 to 3; 58, >4); anti-nucleosome antibody, 155 total (32, ≤−4; 102, −3 to 3; 21, >4). (A) shows all data including outliers. In (B) the scale has been enlarged to better demonstrate the differences observed. One-way ANOVA indicated that changes in C3 ($P = 3.49E-03$) and anti-nucleosome antibody ($P = 4.37E-02$) levels showed significant variation with changes in disease activity. mS-2K: modified SLEDAI-2K; ANOVA: analysis of variance.
antibodies varied significantly ($P = 3.5 \times 10^{-3}$ and $P = 4.4 \times 10^{-2}$, respectively) in patients with a change in clinical state. These results suggest that temporal fluctuations in these parameters might mirror changes in disease status. To further explore this issue, a linear regression analysis was performed and comparisons between models were carried out utilizing AIC (Table 3). Disease activity was the defined outcome, while the immunological markers were treated as fixed effect variables. Analysis of the individual biomarkers demonstrated that anti-nucleosome antibodies had the best fit with disease activity ($\text{AIC} = 1702.12$, $P < 10^{-4}$). The combination of C3 and anti-nucleosome antibodies outperformed all other models ($\text{AIC} = 1661.42$, $P < 10^{-4}$), including the conventional pairing of C3 and anti-dsDNA antibodies ($\text{AIC} = 2957.00$, $P = 0.0004$). These findings indicate that, independently and in combination with C3, anti-nucleosome antibodies displayed the strongest association with disease activity and may serve to forecast the onset of flares or monitor response to treatment.

To determine the ability of the biomarkers to predict subsequent clinically meaningful changes in disease activity we used kNN with leave-one-out cross-validation [17]. The prediction accuracy of the three individual biomarkers was poor, with anti-nucleosome antibodies identifying only 23% of clinically meaningful fluctuations (e.g. $|\Delta mS-2K| > 4$). For C3 and anti-dsDNA the prediction accuracy for these clinically significant events was further reduced to 19% and 7%, respectively. Inclusion of all three biomarkers yielded a similar prediction frequency to anti-nucleosome antibodies alone (prediction accuracy of approximately 25% for clinically meaningful fluctuations). These findings suggest that fluctuations in these biomarkers are relatively poor predictors of subsequent changes in clinical disease activity.

**Discussion**

There is a paucity of validated SLE biomarkers that contemporaneously mirror disease activity and, more importantly, forecast impending flares. Currently anti-dsDNA antibodies and hypocomplementaemia are the only serological parameters that are routinely utilized as activity-specific biomarkers in SLE patient care. However, these biomarkers are insensitive in certain clinical situations, such as the reliable identification of active patients and longitudinal monitoring of disease activity. Various autoantibodies [7, 18, 19], including those targeting nucleosomes [7, 20], have been proposed as activity-specific biomarkers. Although cross-sectional studies have shown that anti-nucleosome antibodies are increased in SLE patients, particularly in active disease, limited data exist as to their suitability to monitor activity over time. In this study we examined the utility of anti-nucleosome antibodies as a biomarker of SLE activity with an emphasis on its performance as a longitudinal biomarker.

The analysis presented here supports the inclusion of anti-nucleosome antibodies for the diagnosis of SLE. Six (12%) SLE patients were found to be exclusively anti-nucleosome antibody positive, with five patients remaining so for the duration of the study. These patients were clinically distinct, with non-renal, milder SLE (e.g. dermatological, musculoskeletal). These results mirror those of a longitudinal study in which 13.9% of patients remained anti-nucleosome antibody positive [21] and suggest that anti-dsDNA and anti-nucleosome antibodies may not identify identical patient populations. Incorporation of anti-nucleosome antibodies into a screening panel may limit delays in diagnosis arising from serological discrepancies.

The identification of patients with active SLE is anchored on the presence of clinical symptoms, with compatible serological abnormalities bolstering the clinical impression. A requisite characteristic of activity-specific biomarkers is a high specificity to effectively exclude patients with inactive disease. By this measure, anti-nucleosome antibodies (specificity = 0.83) outperformed both anti-dsDNA antibodies (specificity = 0.67) and C3 (specificity = 0.5), demonstrating higher specificity than either traditional biomarker and supporting its potential role as an activity-specific biomarker. Our conclusions are supported by a meta-analysis that showed a significant
correlation between anti-nucleosome antibodies and disease activity [8].

We hypothesized that the poor performance of traditional biomarkers was a consequence of differences in the methodologies utilized to define their thresholds (reference thresholds) versus anti-nucleosome antibodies. Selected to discriminate between health and disease, reference thresholds favoured sensitivity over specificity, the converse of the operator characteristics desired for an activity-specific biomarker. In order to standardize comparisons between the three biomarkers, a Youden’s index was performed to determine activity-specific thresholds for anti-dsDNA antibodies and C3. Application of the calculated thresholds improved on the performance characteristics of anti-dsDNA (specificity = 0.83) and C3 (specificity = 1), rendering both comparable to anti-nucleosome antibodies. This exercise highlights the importance of establishing context-specific biomarker thresholds. For example, in the case of anti-dsDNA antibodies, application of the reference threshold optimizes its performance as a diagnostic tool, whereas use of the calculated cut-off improves its utility as an activity-specific biomarker. Determination of distinct thresholds will enhance the overall clinical utility of individual biomarkers.

Having refined the thresholds for traditional biomarkers, we examined whether their performance was improved by the inclusion of anti-nucleosome antibodies. Incorporation of anti-nucleosome antibodies, in lieu of or in addition to anti-dsDNA antibodies, did not enhance the discriminatory capacity of current biomarkers. This is likely a consequence of the high degree of concordance between the two autoantibodies, allowing them to function in an interchangeable manner in a cross-sectional analysis. Although these cross-sectional findings support the use of the traditional pairing of anti-dsDNA antibodies and C3 as disease-activity biomarkers, some caveats are warranted. The method of detection of anti-dsDNA antibodies has a significant impact on performance. In this study, a Farr assay rather than an ELISA was utilized. Although studies have demonstrated that the Farr assay has a better correlation with global disease activity than the ELISA [14], there is a growing trend favouring antibody-based detection techniques as a more cost-effective, radioactivity-free alternative. As these methods become standard, the discriminatory capacity of anti-dsDNA antibodies will require re-evaluation, as changing assay methodologies may favour the use of anti-nucleosome antibodies. Recent studies have suggested that simultaneous positivity for anti-dsDNA and anti-nucleosome antibodies may have prognostic utility, as this immunological phenotype is associated with proliferative renal lesions and a poorer overall clinical outcome [22]. These observations further support the routine measurement of anti-nucleosome antibodies.

Although a cross-sectional analysis showed that both autoantibodies demonstrate similar performance characteristics, fluctuations in anti-nucleosome antibody levels more accurately reflect clinically significant changes in disease activity. Changes in disease activity were not mirrored by changes in anti-dsDNA antibodies. While older studies suggested that an increase in anti-dsDNA antibodies heralds disease exacerbation [23], our findings parallel that of a recent study that found no such association [24]. In longitudinal modelling, the traditional pairing of anti-dsDNA and C3 performed more poorly than models that included anti-nucleosome antibodies and C3. In this latter example, anti-nucleosome antibodies were noted to bear the greatest responsibility for the fidelity model. Taken together, these results argue for the serial monitoring of anti-nucleosome antibodies, as fluctuations in this biomarker may be a more accurate reflection of changes in disease activity.

Several reports have addressed the utility of anti-nucleosome antibodies to monitor disease activity with varying findings. The largest of these examined 101 patients over a 2 year period with three serological assessments and reported that the mean anti-nucleosome antibody level for the patient population at each visit remained stable despite a modest decrease in overall disease activity [21]. As this study did not examine the relationship between changes in disease activity and autoantibody levels in individual patients, detection of variations in antibodies with clinically significant changes might have been masked by a preponderance of individuals with no change in disease activity or a balance between individuals with flaring and remitting disease. The strength of our study was the use of several modelling strategies that provide an unbiased global evaluation of the relationship between changes in disease activity and fluctuations in biomarker levels. Using these approaches, we show that while fluctuations in the levels of anti-nucleosome antibodies do not correlate perfectly with changes in disease activity in all patients, they outperform conventional biomarkers. The same findings were seen in another longitudinal study that examined a small number of patients (n = 20) using a similarly unbiased statistical approach [24].

Although changes in anti-nucleosome antibody levels correlated contemporaneously with disease activity, they were poor predictors of impending clinical changes. An inability to predict consequent clinical events was found for all three biomarkers and indicates that fluctuations in biomarker levels either closely precede (e.g. within <3 months) or occur synchronously with alterations in disease state. This suggests that longitudinal tracking of biomarkers may be more useful for informing clinical decisions at the time of assessment than forecasting flares.

As a final note, this cohort was weighted towards patients with renal involvement. Although previous studies have reported a preferential relationship between LN and anti-nucleosome antibodies [7, 9], a meta-analysis draws this association into question [8]. A reasonable interpretation of these conflicting reports is that anti-nucleosome antibodies may correlate with the severity of disease rather than reflecting a specific relationship with LN. In the future it will be necessary to replicate our results in a clinically more heterogeneous SLE patient population to address this possibility.
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Clinical vignette

Dual-energy computed tomography molecular imaging of pigmented villonodular synovitis

A 35-year-old woman presented with a 1 year history of non-traumatic gradual swelling of the first ray of the left foot, which had been painful for several weeks. Physical examination revealed a firm, slightly tender mass in the medial planar soft tissues. Laboratory investigations, including levels of uric acid and CRP, were normal. Radiographs showed a soft tissue mass without calcification or involvement of bones and joints. The patient underwent dual-energy CT (DECT) for further evaluation. Spectral analysis with two-material decomposition demonstrated an X-ray attenuation characteristic of iron within the distal synovial sheath of the flexor hallucis longus tendon (Fig. 1A and B), consistent with localized pigmented nodular tenosynovitis. The diagnosis was subsequently confirmed by percutaneous core needle biopsy (Fig. 1C).

Pigmented villonodular synovitis (PVNS) encompasses a family of rare, usually benign, neoplastic processes arising from the synovium of joints, bursae and tendon sheaths, with pigmented nodular tenosynovitis occurring most commonly in the digits. While DECT has been proved effective for the detection, characterization and quantification of uric acid deposits in patients with gout [1], additional musculoskeletal applications have been suggested, including the detection of iron deposition in PVNS [2]. To our knowledge, this is the first description of non-invasive molecular imaging of PVNS using DECT.

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References


Fig. 1 Dual-energy CT and histopathology images of localized pigmented nodular tenosynovitis

(A) Axial and (B) three-dimensional volume-rendered dual-energy CT images of the left foot illustrate the presence of iron material (colour coded in blue) within the distal synovial sheath of the flexor hallucis longus tendon, consistent with localized pigmented nodular tenosynovitis, also referred to as giant cell tumour of the tendon sheath. (C) Photomicrograph of the core needle biopsy specimen revealed intra- and extracellular haemosiderin, bland mononuclear cells and scattered multinucleated giant cells (Prussian blue stain; original magnification 100×).