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ORIGINAL ARTICLE

A novel surface plasmon resonance approach to assess anti-dsDNA antibody kinetics and disease severity in systemic lupus erythematosus

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Abstract

Objectives. Anti-dsDNA antibodies are established biomarkers in systemic lupus erythematosus (SLE), yet the clinical utility of conventional assays remains limited by variable analytical performance. We evaluated a novel Fibre-Optic Surface Plasmon Resonance (FO-SPR) biosensor for real-time profiling of anti-dsDNA antibody–antigen interactions and investigated whether kinetic parameters are associated with disease severity, particularly lupus nephritis (LN). **Methods.** Sera from 26 SLE patients (including 12 with LN), 14 disease controls and 16 healthy donors were analysed using FO-SPR biosensors coated with biotinylated dsDNA. The sensors generated real-time kinetic profiles from which maximum binding response (B_{max}), association rate and dissociation constant were computed. FO-SPR kinetic results were compared with a conventional chemiluminescent anti-dsDNA assay (CLIA) and correlated with disease activity and renal involvement. **Results.** The FO-SPR platform enabled reliable detection of anti-dsDNA antibodies and clearly distinguished SLE patients with renal involvement: Eight of 12 LN patients showed B_{max} values above the 90th percentile of the non-SLE control group. In these patients, kinetic parameters revealed high-affinity anti-dsDNA profiles characterised by significantly higher association rates and maximal shifts and lower dissociation constants. The AUC-ROC for SPR-derived affinity parameters was 0.82 ($P = 0.006$), outperforming the CLIA assay to discriminate lupus patients with versus without nephritis. Furthermore, FO-SPR binding responses correlated with disease activity score in LN. **Conclusion.** This study highlights the value of an innovative FO-SPR biosensor for real-time characterisation of anti-dsDNA antibody affinity. The kinetic profiles captured by this platform provide clinically relevant insights, particularly for distinguishing LN patients from other SLE phenotypes.

Keywords: anti-dsDNA, lupus nephritis, SLE, surface plasmon resonance

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterised by a wide spectrum of clinical manifestations, ranging from isolated cutaneous involvement to severe, life-threatening damage to multiple organ systems. This clinical heterogeneity contributes to variability in disease phenotype and therapeutic response. The course of SLE is also largely unpredictable and characterised by periods of remission and disease exacerbation. Identifying reliable biomarkers to stratify patient subgroups and guide personalised treatment strategies remains a significant challenge in SLE research.[1,2]

Anti-double-stranded DNA (anti-dsDNA) antibodies are pivotal biomarkers for the diagnosis, classification and monitoring of SLE. These antibodies can often be detected years before clinical manifestations emerge, highlighting their potential in early disease identification.[3] Since 1982, the detection of 'antibodies to native DNA in abnormal titre' has been included in the American College of Rheumatology's (ACR) classification criteria for SLE.[4]

In patients with recent-onset rheumatic symptoms, the detection of anti-dsDNA antibodies using different assay techniques yields substantial discrepancies in both test outcomes and their correlations with clinical and biochemical manifestations. These methodological variations, characterised by differences in performance, complicate the interpretation of anti-dsDNA results and may impact their utility as diagnostic or prognostic biomarkers.[5,6]

The Farr radioimmunoassay (RIA) has long been considered the gold standard for anti-dsDNA detection because of its high specificity for high-affinity antibodies. However, its application is limited by the use of radioactive materials and relatively low sensitivity.[7] To address these limitations, solid-phase immunoassays (SPA), such as enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CLIA), have been developed, offering higher sensitivity. Nonetheless, these assays detect antibodies irrespective of their avidity to dsDNA. Those antibodies may not all be clinically relevant as biomarkers for SLE.[8] High-affinity/avidity anti-dsDNA antibodies are considered more clinically relevant and specific markers for SLE.[9–11] The 2019 EULAR/ACR classification criteria for SLE established new criteria for anti-dsDNA testing, requiring the use of an immunoassay with a demonstrated specificity of at least 90% for SLE compared to relevant disease controls.[12] This highlights the need for alternative approaches to profile high-affinity anti-dsDNA antibodies in SLE patients.

Surface Plasmon Resonance (SPR) has emerged as a powerful technology, particularly because of its ability to measure interactions in real time with high sensitivity and without the need for labels.[13] It enables direct visualisation of antigen–antibody interactions, allowing for the monitoring of antibody affinity through kinetic binding measurements. SPR has been proposed for the characterisation of monoclonal anti-dsDNA antibodies to establish new laboratory testing standards.[14] Despite its utility in characterising autoantibody parameters and its advantage over RIA in terms of occupational and environmental safety, SPR is not currently suitable for routine marker screening. Conventional biosensor technologies remain limited by high cost and complexity of use.[13]

Vixen Bio™ has developed a Fibre-Optic SPR (FO-SPR) biosensor system that allows label-free quantification and kinetic affinity measurements on a flexible benchtop instrument, combining the performance and speed of SPR. This upgraded technique has the advantage of working with a dip-in biosensor, avoiding microfluidics and sample clogging. The system is versatile, enabling the analysis of multiple targets in one device (proteins, antibodies, nanobodies, complex particles, microvesicles, etc.) with a rapid time-to-result even in crude samples such as serum, plasma or blood.[15–17] Moreover, it is user-friendly and cost-effective compared with competing biosensor technologies.

This project aimed to assess the applicability of the FO-SPR system in the field of autoimmunity, with a specific focus on characterising the complex interactions between anti-dsDNA antibodies and their targets in patients with SLE. Characterising the affinity and kinetic profiles of anti-dsDNA antibodies in SLE patients may provide valuable insights into cases with non-specific clinical presentations or limited manifestations. Moreover, higher-affinity antibodies are often correlated with increased disease activity and

organ involvement, including renal damage.[10] Therefore, assessing antibody kinetic profiles may help stratify patients according to disease severity and guide therapeutic decisions.[18]

RESULTS

Patients

Twenty-six SLE patients were included in this study, of whom 12 had LN. No significant differences in age or gender distribution were observed between the two SLE subgroups (median age non-nephritis SLE: 39 years [28.3–48.6], median age LN: 36.5 years [25.6–47.2]). The female-to-male ratio was 12:2 in the non-nephritis SLE group and 12:0 in the LN group.

In the LN group, two patients were classified as class II, four as class III, and six as class IV according to the revised 2018 ISN/RPS classification.[19] Nine LN patients were receiving treatment at the time of sampling (seven with hydroxychloroquine, three with azathioprine, four with methylprednisolone and three with methotrexate).

The control group consisted of 14 participants aged between 40 and 79 years. These individuals were diagnosed with autoimmune diseases other than SLE, including systemic sclerosis (n = 5), Sjögren's disease (n = 3), mixed connective tissue disease (n = 1), rheumatoid arthritis (n = 1), myositis (n = 1) and overlap syndromes (n = 3). They exhibited elevated levels of autoantibodies, but none specific to SLE.

Specific detection of anti-dsDNA antibodies using an innovative FO-SPR biosensor

The first step of the study involved validating the innovative analytical benchtop FO-SPR biosensor using the anti-dsDNA reference serum from the CDC (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Two sources of synthetic biotinylated antigenic dsDNA were evaluated in this study (data not shown): a long fragment (~48.5 kb) from Lumicks™ (The Netherlands) and a shorter fragment (~700 bp) from IDT™ (Belgium). The extensive length of the Lumicks dsDNA required lower shaking speeds during immobilisation, which in turn reduced diffusion rates and extended binding times. By contrast, the shorter IDT fragment allowed higher shaking speeds and functioned effectively at lower concentrations. Consequently, we selected the IDT biotinylated dsDNA for our subsequent experiments.

A significant and distinguishable signal on the sensorgram was observed when using the CDC reference serum (maximum binding response = 5.02), compared to the median signal obtained from healthy donor (HD) samples (median maximum binding response = 1.095 (0.825–1.268) nm, n = 16) (Figure 1a). This shift confirms the specific binding of anti-dsDNA antibodies to the immobilised biotinylated dsDNA on the sensor probes.

Employing a 1:10 dilution of the CDC reference serum provided the most distinct separation between

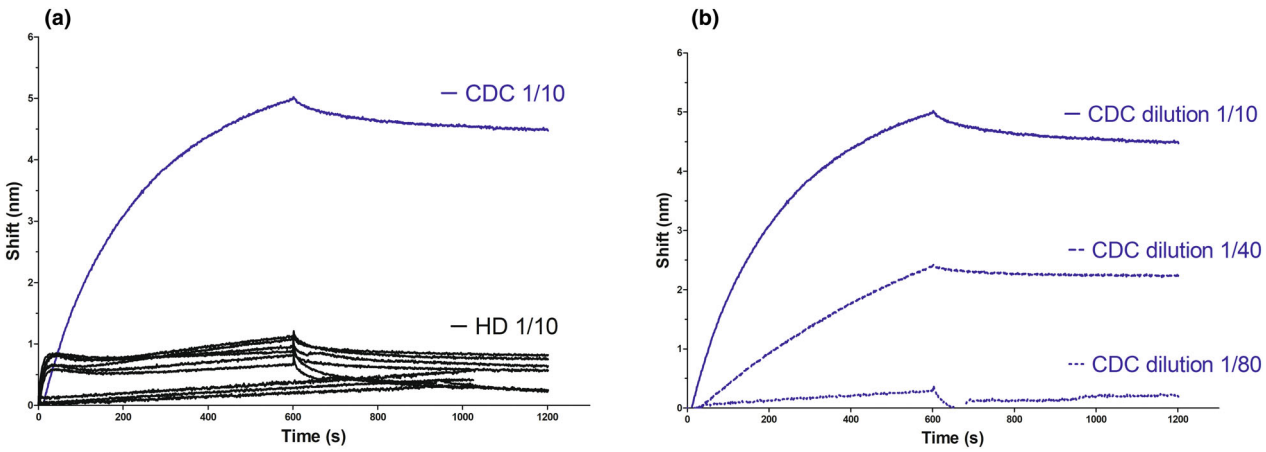

Figure 1. Kinetic curves using the CDC reference serum. **(a)** FO-SPR sensorgram showing a strong and specific binding response when using the CDC anti-dsDNA reference serum compared to healthy donor (HD) samples. **(b)** Comparison of FO-SPR sensorgrams obtained with diverse dilutions of CDC reference serum.

the HD group (lacking anti-dsDNA antibodies) and the CDC-positive control signal (Figure 1b). Consequently, all subsequent experiments were conducted using a 1:10 dilution of the samples.

FO-SPR enables discrimination of SLE patients based on real-time antibody binding

Real-time binding of all samples to the sensor probes was recorded. Neither the HD group nor the control disease (CD) group exhibited significant binding signals [B_{max} HD 1.095 nm (0.825–1.268), $n = 16$; B_{max} CD 1.895 nm (1.403–2.190), $n = 14$] (Figure 2a and b).

Representative binding curves from patients with SLE are shown in Figure 2c and d. One-third of the lupus patients (11/26) displayed a significant and clearly distinguishable anti-dsDNA binding signal compared with both control groups (42% of SLE patients had a B_{max} exceeding the 90th percentile of the non-SLE B_{max}). Interestingly, the majority of these patients (73%, 8/11) had lupus nephritis. Within the LN subgroup, eight out of 12 patients showed a B_{max} value above the non-SLE 90th percentile [B_{max} in LN: 2.955 nm (1.210–5.453), $n = 12$].

FO-SPR biosensor delivers reliable results across runs

An intra-run precision assay was conducted using samples from two patients with LN, presenting different anti-dsDNA concentrations as measured

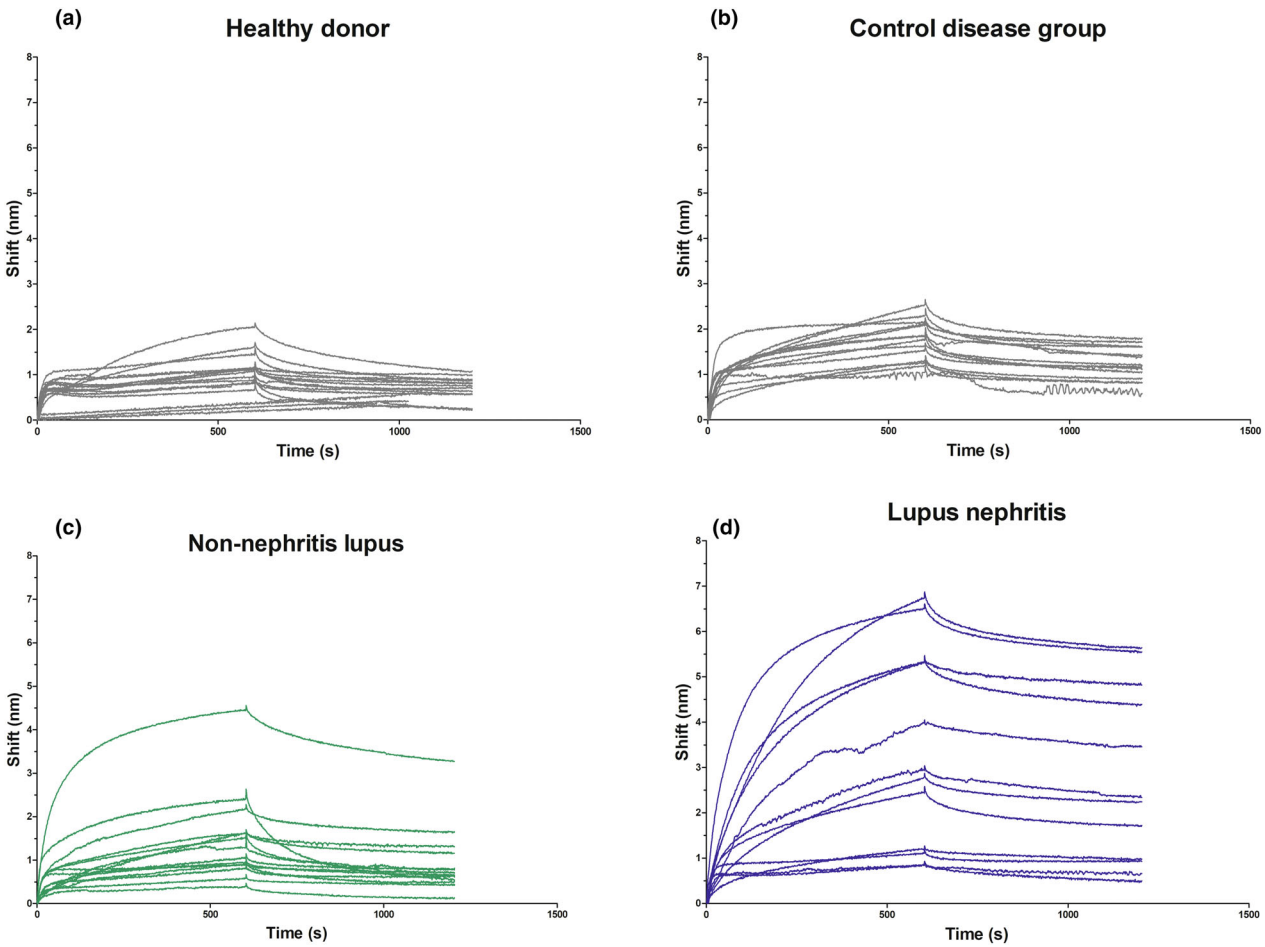

Figure 2. Kinetic curves from disease and control groups. Representative sensorgrams illustrating kinetic curves from four sample groups: **(a)** healthy donors, **(b)** control disease group, **(c)** lupus patients without nephritis and **(d)** lupus nephritis patients. Each panel displays the real-time binding response of the respective group, highlighting differences in kinetic profiles associated with disease status.

by the BIO-FLASH platform (Werfen), along with a healthy donor sample. Each sample was measured four times within the same experimental run. The coefficient of variation (CV) for the maximum binding response ranged from 1.2% to 12.0%, with all values remaining below 20%, indicating acceptable intra-run variability.

Reproducibility across runs (inter-run assay) was assessed using samples from four SLE patients with anti-dsDNA concentrations ranging from 248 to 1846 U mL^{-1}. Each sample was measured five times in separate runs performed on different days. The resulting kinetic curves were consistent across runs, with CVs ranging from 6.2% to 15.7%, all below the 20% threshold.

These results demonstrate that the biosensor provides reliable and reproducible measurements across both intra- and inter-run conditions, meeting standard requirements for repeated sample analysis.

Kinetic characteristics of anti-dsDNA antibodies in SLE

Kinetic analyses were subsequently performed only in the lupus cohort, as these samples exhibited binding signals high enough to allow reliable kinetic fitting. Indeed, kinetic characterisation is feasible only in lupus patients with sufficiently elevated anti-dsDNA antibody levels. No statistically significant differences in anti-dsDNA antibody concentrations, as measured by CLIA (Figure 3d), were observed between the two lupus groups. Interestingly, however, distinct kinetic profiles were detected between the different lupus phenotypes. A significantly higher maximum binding response was observed in the LN group compared with the non-nephritis lupus group (B_{max} non-nephritis 1.350 nm [0.970–1.845], B_{max} LN 2.955 nm [1.210–5.453], $P = 0.03$) (Figure 3a). Furthermore, the initial slope of the association phase, reflecting the association rate, was also significantly increased in the LN group (non-nephritis 0.002 [0.000–0.004], LN 0.006 [0.002–0.015], $P = 0.029$) (Figure 3b). A distinct difference in dissociation rates (k_{off}) was also noted between the two groups: the LN group exhibited a median k_{off} almost a half lower than the non-nephritis group ($P = 0.018$), indicating higher affinity binding interactions in the LN patients (Figure 3c).

Taken together, the three kinetic parameters—association rate, maximum binding response and dissociation rate—consistently demonstrated that patients with the more severe clinical phenotype (LN) exhibited significantly higher kinetic parameters than those without renal involvement, indicating a more matured affinity of the autoantibodies towards their antigen.

Data representation on a rate map enables visualisation of the relationship between the association rate and the dissociation rate constant across the samples, where a higher association rate and lower k_{off} value indicate higher affinity interactions (lower right quadrant below the diagonal line). This analysis reveals two distinct subgroups among lupus patients: those without

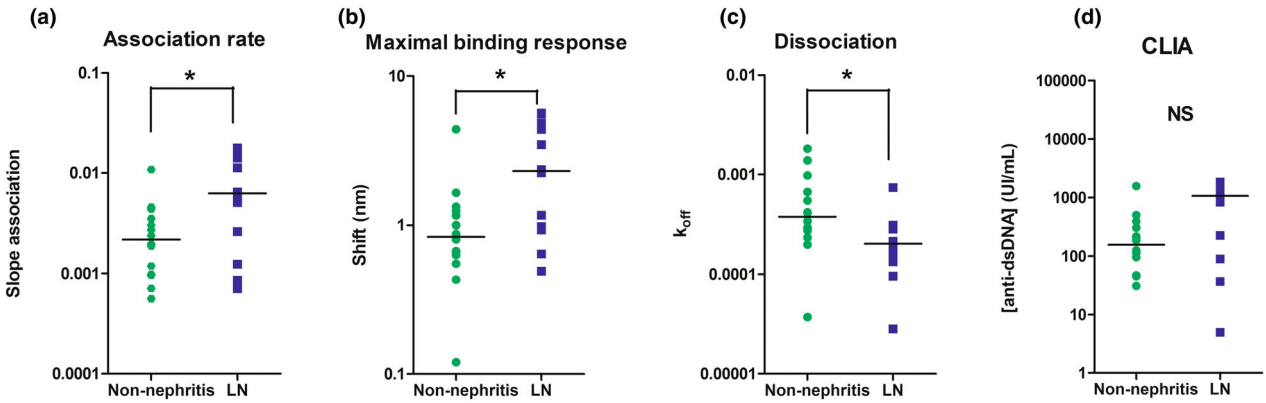

Figure 3. Comparison of anti-dsDNA autoantibodies levels and kinetic parameters between LN and non-nephritis lupus patients. **(a)** Maximum binding response during the association phase, showing a significantly higher response in the LN group (*$P < 0.05$). **(b)** Initial slope of the association curve, reflecting the rate of antibody binding to the sensor surface; the LN group exhibited a significantly steeper slope (*$P < 0.05$). **(c)** Dissociation phase analysis showing distinct k_{off} values between the two groups, indicating differences in antibody–antigen complex stability (*$P < 0.05$). **(d)** Anti-dsDNA levels measured by CLIA.

nephritis exhibit anti-dsDNA antibodies with lower affinity, whereas patients with lupus nephritis display antibodies of higher affinity (Figure 4).

The receiver operating characteristic (ROC) analysis demonstrated that kinetic parameters (association rate, B_{max} and dissociation constant) provided superior discrimination (AUC $\geq$ 0.75, $P < 0.05$) between non-nephritis lupus patients and those with LN compared to anti-dsDNA antibody concentrations measured by a routine CLIA technology (AUC: 0.71, $P = 0.064$) (Table 1). The use of a ratio of kinetics (association rate/dissociation constant) even allowed achieving an AUC of 0.82 ($p < 0.01$) for discriminating between the two lupus groups (Supplementary Figure 1).

However, when comparing all lupus patients to the combined control group (including HD and individuals with other autoimmune diseases), the FO-SPR device demonstrated lower discriminative power than conventional routine laboratory assays (Table 1). This suggests that the SPR assay may be more appropriately utilised as a second-line diagnostic tool to achieve more precise discrimination of lupus patients.

Routine renal markers in SLE

Routine clinical parameters including haemoglobin, proteinuria, haematuria, plasma albumin, plasma creatinine and GFR were recorded and analysed in both lupus patient groups (Table 2). No significant differences were observed between the non-nephritis and nephritis groups, except for haematuria, which was significantly more prevalent in patients with LN.

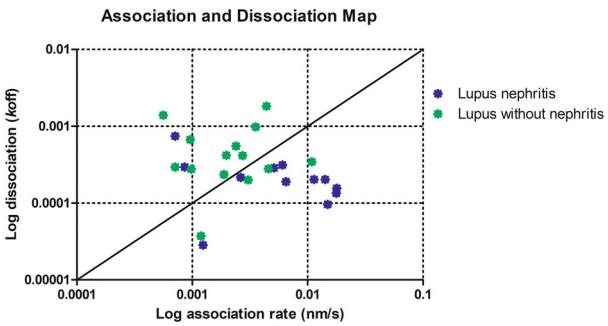

Figure 4. Association and dissociation rate map.

Correlation of SPR kinetics with activity score in lupus nephritis

At the time of renal involvement diagnosis and prior to initiating treatment, five patients with LN underwent SPR kinetic assessments. Interestingly, an association was identified between the maximal binding response observed in SPR measurements and the NIH activity score (sr = 1, $P < 0.05$) (Figure 5). In contrast, no correlations were found between standard biological parameters (UPCR, haematuria or GFR) and the NIH activity score (data not shown). Also, anti-dsDNA IgG levels measured by standard CLIA did not show correlation with the disease activity score (sr = −0.1, P = ns) (Figure 5).

Table 2. Comparison of routine clinical parameters between non-nephritis and lupus nephritis patients. This table illustrates the comparative analysis of routine renal function parameters [median (IQR)] between non-nephritis lupus patients and lupus nephritis patients

	Lupus without nephritis (n = 14)	Lupus nephritis (n = 12)
UPCR (g g^{-1} creatinine)	0.21 (0.06–0.35)	0.37 (0.14–1.61)
Hematuria (cells μL^{-1})	0 (0–2.75)	**8 (0–146)***
GFR (mL min^{-1} $1.73m^{-2}$)	90 (90–90)	90 (81–90)
Plasma creatinine (mg dL^{-1})	0.78 (0.69–0.80)	0.70 (0.55–0.93)
Plasma albumin (g L^{-1})	39.5 (37.0–42.3)	37.0 (33.9–40.3)
Haemoglobin (g dL^{-1})	12.2 (10.8–13.7)	11.5 (10.4–12.6)
Anti-dsDNA IgG (UI mL^{-1}) CLIA	157.0 (46.8–328.0)	1061 (124.4–1215)

Parameters shown in bold are statistically significant.
*P<0.05.

Table 1. AUC of each parameter to discriminate non-nephritis lupus and nephritis lupus

ROC curves Area under curve (AUC) (P-value)	Association (slope)/ dissociation (k_{off})	Dissociation (k_{off})	Association (slope)	Endpoint (B_{max})	CLIA (concentration)
Lupus without nephritis vs lupus nephritis	**0.82 (P = 0.006)**	**0.78 (P = 0.017)**	**0.75 (P = 0.033)**	**0.75 (P = 0.029)**	0.71 (P = 0.064)
All lupus patient vs collective control group	—	—	—	0.59 (P = 0.240)	0.95 ($P < 0.0001$)
Lupus nephritis vs collective control group	—	—	—	0.76 (P = 0.010)	0.91 ($P < 0.0001$)

Parameters shown in bold are statistically significant.

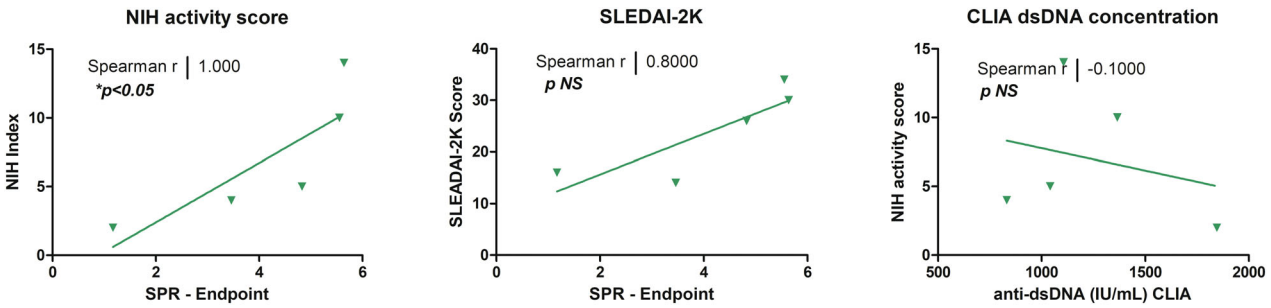

Figure 5. Correlation between SPR maximal binding response and disease activity in lupus nephritis patients. Data points represent individual patients; the solid line denotes the linear regression fit.

DISCUSSION

In this study, we evaluated the kinetic parameters of anti-dsDNA antibodies in patients with SLE using a novel FO-SPR biosensor. Our findings demonstrate that affinity-related characteristics of these autoantibodies, namely the association rate, maximum binding response and dissociation rate, discriminate SLE patients with LN from those without renal involvement.

The FO-SPR biosensor evaluated in this work uses an automated immersive format for real-time and label-free affinity characterisation of multiple targets (proteins, nanobodies, microvesicles, etc.) including antibodies.[20,21] Here, we applied for the first time this technology to the analysis of polyclonal anti-dsDNA antibodies in a complex serum matrix from SLE patients. Compared with conventional SPR technology, it offers the advantage of operating without microfluidics, thereby eliminating the risk of clogging and the need for cleaning between samples. This underscores the user-friendly nature and practical value of this innovative device.

A critical step in the development of solid-state biosensor surfaces is the immobilisation of the ligand (dsDNA) onto the gold substrate. To ensure stability, a covalent immobilisation strategy is preferred.[22] In our approach, we employed a biotin/streptavidin coupling chemistry for rapid and efficient capture of the ligand on streptavidin-coated chips, ensuring rigid surface presentation of the dsDNA. As reported by Buhl *et al.*,[14] antibody avidities vary depending on the antigenic DNA used, particularly with respect to its length. We selected a short, synthetic, biotinylated DNA with lower required concentrations, making it more cost-effective and compatible with higher shaking speeds, which accelerates binding kinetics. We postulate that longer DNA molecules may wrap around the sensor surface, thereby hindering the full binding capacity of anti-dsDNA antibodies. In contrast, immobilising a shorter dsDNA antigen enhances assay performance by improving accessibility and binding efficiency of the antibodies.

The technology showed a discrimination power between reference CDC serum not only for anti-dsDNA antibodies and HD but also for sera from SLE patients and disease controls. Technically, the FO-SPR platform demonstrated high reproducibility and robustness, with intra- and inter-assay variation below 20%.

FO-SPR measurements generate a sensorgram, a real-time plot of the signal (reflecting refractive index changes) over time, which visualises the binding events between antibodies and surface-immobilised antigens. This approach provides information on binding kinetics (association/dissociation), affinity and binding specificity.[23] By capturing antibody–antigen interactions in real time, the FO-SPR technology provides functional resolution not accessible through conventional assays.[24] In contrast to RIA, it avoids the use of radioactive materials, and unlike solid-phase immunoassays, it allows discrimination with low-affinity antibodies with uncertain clinical significance.[25]

LN is a type of major organ involvement in SLE patients that leads to higher rates of morbidity and mortality.[26,27] In this study, SPR sensorgrams revealed distinct anti-dsDNA binding kinetics across SLE subgroups. A subset of SLE patients showed a clearly distinguishable binding signal compared with controls, and this subgroup was predominantly composed of patients with lupus nephritis. Three kinetic parameters were derived

from the sensorgram (association rate, maximal binding response and dissociation rate), all of which significantly discriminated patients according to disease severity. Anti-dsDNA antibodies from the LN group exhibited a significantly higher association rate and a lower dissociation constant compared with the non-nephritis lupus group, suggesting differential molecular interactions. The AUC values for distinguishing LN from non-nephritis SLE exceeded 0.75 for all three kinetic parameters. Interestingly, we found that combining kinetic parameters (association rate/dissociation constant) provided an even better discrimination among lupus groups, with an AUC of 0.82. In contrast, anti-dsDNA levels measured using a conventional CLIA could not significantly discriminate among groups' severity. Also, standard biological markers (creatinine, eGFR, UPCR and pCr) were less discriminative, with the exception of haematuria. The FO-SPR appears to be a promising second-line diagnostic tool, specifically useful for evaluating lupus phenotype.

The course of proliferative LN is marked by alternating flares of activity and periods of quiescence. An accurate assessment of disease activity is essential for guiding therapy [2]. Interestingly, in the LN group, we observed a significant correlation between FO-SPR maximal binding response and the NIH activity score at the time of LN diagnosis, further highlighting the clinical relevance of this approach. High shifts in the sensorgrams of LN patients correlated with a higher disease activity. Currently, there is a substantial discordance between treatment response based on routine clinical parameters and histopathological findings in post-treatment kidney biopsies, and persistent activity in repeat kidney biopsies is associated with treatment failure and LN flare. Kidney biopsy being an invasive procedure, there is a significant unmet need for the identification of new biomarkers of kidney histology in peripheral blood or urine, in order to noninvasively evaluate the treatment response and to guide the intensity of immunosuppression.[2,28] The results of our study suggest that FO-SPR kinetic parameters could be a reasonable noninvasive biomarker candidate of persistent activity in kidney biopsy. A larger prospective study is needed in order to confirm the worth of FO-SPR parameters as a non-invasive biomarker candidate for persistent activity in renal biopsy.

Our data support the hypothesis that high-affinity autoantibodies are associated with more active and potentially more pathogenic disease, especially in the context of renal involvement. This observation is consistent with previous studies suggesting that the affinity of anti-dsDNA antibodies, rather than their concentration, may be the key determinant of their pathogenic potential.[7,18,29]

Nevertheless, our study has limitations. The relatively small sample size, particularly in the LN subgroup, limits the generalisability of our findings. We used a physician-diagnosed SLE as an entry criterion that potentially makes the study population more heterogeneous and reduces the power to detect clinical changes. However, even by restricting the analysis to patients who fulfilled the 2019 EULAR/ACR classification criteria for SLE,[12] a significant response in binding curves remained between the two lupus groups and kinetic parameters significantly discriminated lupus with or without renal involvement. Furthermore, although the observed correlation between kinetic parameters and disease activity is promising, prospective longitudinal studies are needed to evaluate the predictive value of these measurements in treatment monitoring. The lack of international standardisation of kinetic measurement techniques also represents a current barrier to clinical implementation.

In conclusion, this study demonstrates the applicability of a new user-friendly device for measuring the kinetic profiling of anti-dsDNA antibodies in SLE patients. Parameters describing the kinetics of antibody binding by FO-SPR allow to distinguish patients with severe lupus phenotypes, especially those with nephritis, compared with standard immunoassays. These findings open the door to the use of kinetic measurements as a novel stratification tool in SLE, with the potential to guide personalised treatment strategies. Larger prospective studies are warranted to validate these findings and to assess their long-term clinical utility.

METHODS

Patients

Twenty-six patients diagnosed with SLE and admitted to Brugmann University Hospital (CHU Brugmann, Brussels, Belgium) between 2016 and 2023 were retrospectively included in the study. Diagnoses have been provided by expert clinicians based on a holistic assessment including clinical, urinary and laboratory blood parameters, and regular follow-up of the progression of the disease. Twelve

of the 26 SLE patients had renal biopsy-proven lupus nephritis according to International Society of Nephrology (ISN)/Renal Pathology Society (RPS) scheme.[19] The control groups consisted of 14 patients with other autoimmune diseases (disease control group) recruited from CHU Brugmann and 16 healthy individuals (healthy donor group).

All serum samples were leftover specimens from routine diagnostics and were stored at −20°C until analysis. The study was approved by the Ethics Committee of Brugmann Hospital (reference number CE2024/40).

Instrumentation

Biosensor measurements were accomplished using the analytical benchtop WHITE FOx.

FO-SPR biosensor from Vixen Bio (Gent, Belgium) at a working temperature of 26°C. All measurements were performed on sensor chips provided by Vixen Bio, consisting of a gold surface with pre-immobilised streptavidin.

Method

Gold-coated fibre-optic probes with a streptavidin-functionalised surface were first rehydrated in phosphate-buffered saline (PBS) buffer, followed by a baseline measurement. This was performed by incubating the streptavidin-coated probes in a capture buffer (Tris pH 7.4; 1 mM EDTA; 1 M NaCl; 0.01% Tween-20) at 112 *g* for 180 s.

Subsequently, biotinylated double-stranded DNA (dsDNA) with a final concentration of 0.1 ng μL^{-1} (700 bp; 5′ biotin-labelled; obtained from IDT) was immobilised onto the streptavidin-coated surface. Measurements were taken at 1000 rpm for 900 s. After a washing step to remove unbound dsDNA, a target binding buffer with a reduced sodium chloride concentration (Tris-EDTA pH 7.4; 0.1 M NaCl; 0.01% Tween-20) was introduced to stabilise the signal prior to the antibody binding phase. This stabilisation step was carried out at 1000 rpm for 120 s.

Following signal stabilisation, the antibody samples, diluted to a final 1:10 ratio, were introduced, and kinetic measurements were recorded for 600 s at 1000 rpm. Finally, the dissociation phase was measured by immersing the probes in a dissociation buffer (Tris-EDTA pH 7.4; 0.1 M NaCl; 0.01% Tween-20) at 1000 rpm for an additional 600 s.

Biosensor measurements

Signal variations, induced by the antigen–antibody interaction, across the different steps were monitored over time as SPR sensorgrams, expressed as wavelength shifts (nm). The resulting data were processed using the WHITE FOx Biosystems Data Processor, which generated the corresponding sensorgrams (Vixen Bio, Gent, Belgium). These sensorgrams were subsequently analysed with the TraceDrawer software 1.10.1 (Ridgeview Instruments AB), which enables kinetic analysis of real-time binding interactions.

Kinetic parameters of anti-dsDNA antibodies

Three parameters were analysed to assess the kinetic profiles of anti-dsDNA antibodies from sensorgrams.

The association phase of the kinetic curve displays two components: an initial phase corresponding to the genuine physical interaction between autoantibodies and their antigens, followed by a secondary phase probably representing non-specific background interactions of serum proteins or, because of the patient's polyclonal autoantibody response, the engagement of a second, lower affinity binding site of the antigen. To compare the association rate reflecting antibody affinity, the slope of the kinetic curve was evaluated over the 0- to 250-s interval.

The endpoint of the association phase corresponds to the signal shift at maximal binding during the association phase (B_{max}) and depends not only on the concentration of anti-dsDNA antibodies but also on their affinity for the ligand. It was compared across the different sample groups.

The dissociation rate constant (k_{off}) is independent of analyte concentration and can therefore be used to rank interaction stabilities across different samples. It was calculated using the DissociationRate model within TraceDrawer, which is based on a one-to-one binding interaction model. The initial 100 s of the dissociation curve (from 600 to 700 s) likely reflect non-specific interactions, characterised by a steep slope, possibly because of secondary bulk effects from serum proteins binding to the sensor surface. Therefore, the k_{off} was calculated for each sample over the 700- to 1200-s interval.

Routine renal markers in SLE

For all lupus patients, different biological parameters were recorded. Plasma creatinine (pCr) and plasma albumin were measured using the Roche® Cobas analyzer, and glomerular filtration rate (GFR) (mL min^{-1} $1.73m^{-2}$) was assessed according to the pCr-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Urinary protein to creatinine ratio (UPCR) (g g^{-1} creatinine) was evaluated by turbidimetry on Roche® Cobas (Roche Diagnostics, Mannheim, Germany). Haemoglobin was measured on the Sysmex analyzer XN (Sysmex, Kobe, Japan). Haematuria was assessed on the sediMAX (77 Elektronika, Budapest, Hungary). Levels of serum IgG anti-dsDNA were assessed using a CLIA conducted on the BIO-FLASH analyzer (Werfen, Barcelona, Spain).

Kidney biopsies had been read by an experienced kidney pathologist and classified according to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) scheme. Also, description of both National Institutes of Health (NIH) activity index and NIH chronicity index had been reported in lupus nephritis kidney biopsy reports, using a semiquantitative scoring system between 0 to 24 and 0 to 12 respectively.[19] SLE activity was evaluated by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2 K),[30] a widely used binary scoring system. SLEDAI-2 K assigns scores based on the presence or absence of specific manifestations (24 descriptors across nine organ systems), producing a cumulative score ranging up to 105.[31]

Statistical analysis

Nonparametric Mann–Whitney U test was used for the comparison of two groups and nonparametric Kruskal–Wallis for the analysis of variance. A Dunn's multiple comparison test was performed for the comparison of more than two groups. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the ability to discriminate between lupus groups, as measured by the area under the curve (AUC). The nonparametric Spearman rank correlation coefficient (r_s) was used for correlation analysis. Statistical analysis of data was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA) (*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ns, nonsignificant).

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AUTHOR CONTRIBUTIONS

C.N. and F.C. conceived and designed the study. C.N., H.B.K.Z. and S.P. performed the experiments. C.N., M.T. and F.C. analysed the data. M.T. and V.B. recruited the patients and analysed clinical data. C.N., M.T., N.G. and F.C. wrote the manuscript. All authors read, revised, and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no competing financial interests. There was no financial support or financial link with Vixen Bio.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.