Active NET formation in Libman-Sacks endocarditis without antiphospholipid antibodies: A dramatic onset of systemic lupus erythematosus

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Running head: NETosis in SLE with Libman-Sacks endocarditis

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ABSTRACT

Although neutrophil extracellular traps (NETs) have been highlighted in several systemic inflammatory diseases, their clinical correlates and potential pathological role remain obscure. Herein, we describe a dramatic onset of systemic lupus erythematosus (SLE) with clear-cut pathogenic implications for neutrophils and NET formation in a young woman with cardiac (Libman-Sacks endocarditis) and central nervous system (psychosis and seizures) involvement. Despite extensive search, circulating antiphospholipid autoantibodies, a hallmark of Libman-Sacks endocarditis, could not be detected. Instead, we observed active NET formation in the tissue of the mitral valve, as well as in the circulation. Levels of NET remnants were significantly higher in serially obtained sera from the patient compared to sex-matched blood donors ($p=0.0011$), and showed a non-significant but substantial correlation with blood neutrophil counts ($r=0.65$, $p=0.16$). The specific neutrophil elastase activity measured in serum seemed to be modulated by the provided immunosuppressive treatment. In addition, we found anti-Ro60/SSA antibodies in the cerebrospinal fluid of the patient but not NET remnants or increased elastase activity. This case illustrates that different disease mechanisms mediated via autoantibodies can occur simultaneously in SLE. NET formation with release of cytotoxic NET remnants is a candidate player in the pathogenesis of this non-canonical form of Libman-Sacks endocarditis occurring in the absence of traditional antiphospholipid autoantibodies. The case description includes longitudinal results with clinical follow-up data and a discussion of the potential roles of NETs in SLE.

Keywords: Anti-dsDNA antibodies; Elastase activity; Libman-Sacks endocarditis; Neuropsychiatric lupus; Neutrophil extracellular traps; NET remnants; Systemic lupus erythematosus
INTRODUCTION

Nowadays the role of neutrophils in the etiopathogenesis of systemic lupus erythematosus (SLE) attracts highest interest [1, 2]. However, with the LE cell test as a key exception, granulocytes have historically been rather neglected in the context of SLE [3, 4]. One reason of this paradigm shift is that low-density granulocytes (LDGs), a subset of neutrophils which is increased in SLE, show the capacity to produce type I interferons (IFN) and spontaneously form neutrophil extracellular traps (NETs) [5, 6]. The latter consist of decondensed chromatin decorated with neutrophil-derived proteases and antimicrobial peptides that may trap and kill pathogens [7]. Furthermore, ‘classical’ lupus autoantigens, such as histones and double-stranded (ds) DNA have been identified as constituents of NETs and their corresponding autoantibodies may potentially protect the NETs from degradation [8]. In MRL/lpr mice, inhibition of NET release containing mitochondrial DNA ameliorates lupus-like autoimmune disease by down-regulating the type I IFN response [9].

The established increased risk of cardiovascular disease in SLE has also been mechanistically linked to LDG-NET by a direct cytotoxic effect of these on endothelial cells [5]. In addition, the oxidation of high-density lipoproteins increases their pro-atherogenic activities [10]. Several pathways leading to NET formation require the activation of NADPH oxidase activation [11]. The Ncf1 gene, encoding the p47phox subunit of the phagocyte NADPH oxidase (NOX2) complex, is critical for the induction of reactive oxygen species (ROS). A single nucleotide polymorphism of the Ncf1 gene has recently been linked to SLE, and other autoimmune conditions, and was mechanistically shown to reduce the ROS production in neutrophils from patients with SLE [12, 13].

Non-bacterial thrombotic endocarditis (NBTE) constitutes a pathologic entity of sterile vegetative endocarditis, which is found in up to 4% of autopsies [14]. Formation of these vegetations has been reported in association with several systemic conditions, including malignancies, severe burns, and states of hypercoagulation [14, 15]. The term Libman-Sacks endocarditis refers to a specific subclass of NBTE which almost exclusively affects individuals with antiphospholipid autoantibodies (aPLs), i.e. primary or secondary antiphospholipid syndrome (APS) [16]. The vegetations found in Libman-Sacks endocarditis range in size from
microscopic to large and verrucous and consist of platelet-fibrin thrombi, immune complexes and mononuclear cells [17, 18].

The accumulated prevalence of Libman-Sacks endocarditis in SLE remains uncertain, but 10-15% of SLE patients are affected according to some well-characterized cohorts and its presence has been strongly associated with stroke and concomitant APS [19, 20]. Further, an association of Libman-Sacks endocarditis with neuropsychiatric SLE (NP-SLE) was found in a six-year longitudinal study of 76 patients with SLE [21]. However, patients with Libman-Sacks endocarditis are typically asymptomatic until embolization occurs, whereas the vegetations may be discovered by echocardiography (ECG).

Herein, we describe a rare and very dramatic onset of SLE with Libman-Sacks endocarditis accompanied by central nervous system (CNS) involvement. Clinical follow-up data, as well as results of blood and cerebrospinal fluid (CSF) biomarkers, mitral valve histopathology and brain imaging are provided. Potential pathological roles of NETs in relation to the clinical vignette are discussed.

Case description
This woman of Swedish origin, born in 1991, had no family history of rheumatic disease. She suffered from episodic migraine since her teenage years and developed a deep vein thrombosis during her first pregnancy 2009. At that time, the test for lupus anticoagulant test (LA) was negative. Over the following three years, she developed hypothyreosis, arthralgia (without swellings) and photosensitivity. In 2014, the migraine was deteriorated, but no abnormalities were found on brain magnetic resonance imaging (MRI).

During the spring of 2016, at the age of 25, she became very sensitive to stress and cognitively affected with disturbed personality. She was unable to perform her daily work as an assistant nurse. In September of the same year, she sought care at the emergency room, with a fever of 38.5° C. She was perceived to be neurologically affected and psychotic. A previously unrecognized systolic regurgitation was identified. ECG detected a mobile vegetation (12x5 mm) close to the median mitral commissural. A lumbar puncture was performed and CSF showed signs of intrathecal IgG production, without cells or increased
albumin levels. MRI of the brain was performed and was abnormal with multiple lesions (see below). Septic emboli were suspected and several intravenous antibiotics were given on the suspicion of bacterial endocarditis. Samples for analyses of antinuclear antibodies (ANA) and aPLs were taken, but the thoracic surgeon went on to mitral valve replacement before results of the autoantibody tests were at hands. The patient received a biologic mitral prosthesis and the rheumatology unit was first consulted when the autoantibody test results were reported.
MATERIALS AND METHODS

Imaging
In total, four MRI and one computed tomography (CT) brain examinations were performed before (2014), at disease onset (2016), and during follow-up (2017). All MR examinations were performed on a 1.5T MR scanner including the following sequences: T1-weighted spin-echo (SE) in axial and sagittal plane, axial T2-weighted turbo spin-echo (TSE) and T2/ Fluid attenuation inversion recovery (FLAIR), diffusion weighted images (DWI), and axial, coronal and sagittal T1-w post contrast administration. The two initial MR examinations in 2014 and in September 2016 included also MR angiography (MRA) of the intracranial vessels using a 3D time-of-flight (TOF) sequence. In addition, at the time of the acute disease onset in September 2016 a CT (pre- and post-contrast administration) was performed.

ANA, complement proteins and cell counts
ANA was analyzed as previously described by indirect immunofluorescence microscopy using multispot slides with fixed HEp-2 cells (ImmunoConcepts, Sacramento, CA, USA) as antigen substrate, and fluorescein-isothiocyanate (FITC) conjugated gamma-chain-specific anti-human IgG as detection antibody (DAKO, Glostrup, Denmark) [22]. ANA fine-specificities were detected in sera and CSF using the addressable laser bead immunoassay (ALBIA; FIDIS Connective Profile kit; Theradiag, France) whereas plasma complement proteins (C3 and C4) were detected by turbidimetry, and cell counts with Cellavision Diffmaster at the Clinical Immunology and Clinical Chemistry Units, Linköping [23].

aPLs
Anti-cardiolipin (aCL) and beta-2-glycoprotein-I (anti-β2GPI) antibodies were analyzed in sera at the Clinical Immunology Unit (Linköping), using fluoroenzyme-immunoassays (Phadia-250, Thermo-Fisher Scientific Phadia AB, Uppsala, Sweden) [24]. LA was determined by the dilute Russell’s viper venom time (dRVVT) method (Siemens Healthcare GmbH, Erlangen, Germany) in Linköping. In addition, anti-phosphatidylserine/prothrombin (PS/PT) antibodies were analyzed by ELISA with kind help from Inova Diagnostics, San Diego, USA.

Tissue preparation
Tissue samples of the mitral valves were routinely immersed in a solution of 4% formaldehyde in a phosphate buffer pH 7.3 (molarity 0.1) and left for 12 and up to 72h. The samples were then dehydrated through a series of graded ethanol solutions, immersed in xylene, followed by melted paraffin with 60° C overnight. Finally, the samples were embedded in paraffin blocks and allowed to cool down. The embedding AutoTec instrument (Sakura Finetek, CA, USA) was used during the dehydration process. Additional paraffin sections were cut at the laboratory in Erlangen, Germany, to be used in investigations of NET and immune complex staining.

**Routine histopathology**

Paraffin-embedded tissue specimens were stained with hematoxylin & eosin, periodic acid-Schiff-diastase (fungal staining) and Giemsa stain (bacterial staining) employing routine procedures.

**NET and immune complex staining**

Paraffin-embedded tissue sample slides were deparaffinized followed by an antigen retrieval step using 10 mM sodium citrate pH 6 for 20 min at 95° C. Slides were then blocked with 5% normal goat serum (ab7481, Abcam, Cambridge, UK) in PBS (14190250, Thermo Fisher Scientific, Waltham, MA, USA) for 1h at RT. Primary antibodies detecting C1q (F0254, Agilent Technologies (Dako), Santa Clara, CA, USA), C3c (F0201, Agilent Technologies (Dako)), IgG (209-545-088, Jackson Immuno Research Labs, West Grove, PA, USA), neutrophil elastase (ab68672, Abcam) or citrullinated histone H3 (ab5103, Abcam) were incubated overnight at +4° C. Secondary detection antibodies AffiniPure goat anti-rabbit IgG (H + L) Cy5-conjugated (111-175-144, Jackson Immuno Research Labs) or AffiniPure goat anti-mouse IgG (H + L) Cy5-conjugated (115-175-146, Jackson Immuno Research Labs) were incubated together with propidium iodide (P4864, Sigma-Aldrich, St. Louis, MO, USA) for 1h at room temperature. Slides were then embedded in DAKO fluorescent mounting medium (S3023, Agilent Technologies) and analyzed using a BZ-X700 microscope (Keyence Corporation, Osaka, Japan).

**CSF markers of tissue damage and immune activation**
One aliquot of the CSF sample was used for the determination of cell counts, CSF/serum albumin ratio, IgG index, IgG synthesis index and the isoelectric focusing profile, according to clinical routines performed at the Clinical Chemistry Unit, Linköping University Hospital. Within one hour, the remaining CSF was centrifuged (300g for 10 min), the supernatant was aliquoted in 0.5 mL polypropylene tubes, immediately frozen and stored at −70° C. IgG in CSF was measured by turbidimetry at the Linköping University Unit. Neurofilament light protein (NFL) (Uman Diagnostics, Umeå, Sweden) and Tau (Fujirebio, Ghent, Belgium) were analyzed using commercially available ELISAs at the Neurochemical Pathophysiology and Diagnostics, Sahlgrenska University Hospital, Mölndal, Sweden.

NET remnants in serum and CSF

NET remnants in serum and CSF were measured with a previously described method [25]. A mouse anti-nucleosome monoclonal antibody (B6.SLE-1) was used for coating. Standards (plasmapheresis sample from a vasculitis patient), sera and CSF were diluted in PBS containing 0.05% Tween 20. A rabbit anti-human MPO antibody (DAKO, Carpinteria, CA, USA) was used as detection antibody, followed by addition of an alkaline phosphatase-conjugated swine anti-rabbit antibody (DAKO), and a phosphatase substrate (Sigma-Aldrich, St Louis, MO, USA). The plate was read at 405 nm using a VersaMax ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Serum and CSF elastase activity

5 µl serum was added to 195 µl of PBS + 25 µl 1M fluorogenic substrate MeOSuc-AAPV-AMC (Santa Cruz Biotechnology, sc-201163) + 25 µl 3.3 mM sivelestat (S7198, Sigma-Aldrich) or PBS in black 96-well plates (ThermoFischer Scientific, 137101). For measuring neutrophil elastase activity in CSF, 100 µl CSF was added to 100 µl PBS + 25 µl 1M fluorogenic substrate MeOSuc-AAPV-AMC (Santa Cruz Biotechnology, sc-201163) + 25 µl 3.3 mM sivelestat (S7198, Sigma-Aldrich) or PBS in black 96-well plates (ThermoFischer Scientific, 137101). Fluorescent readings were collected on a TECAN Infinite 200 Pro using the filter set (excitation 360, emission 465) after 51h of incubation at 37 °C. Assays were performed with technical duplicates

Control subjects
For analyses of NET remnants and elastase activity in serum and CSF, 11 female blood donors (mean age 30.8 years, range 24–39) and 1 healthy male CSF donor (33.7 years) served as controls. The control mitral valve originated from an autopsy of a female individual (25.1 years).

**Statistics**
The GraphPad software (version 4.0; GraphPad Software Inc., San Diego, CA, USA) was used for preparing figures and for statistical evaluation. Mann-Whitney $U$-test was used to examine differences between groups. Correlation analysis was performed using Pearson’s correlation coefficient. Two-tailed $p<0.05$ was considered significant.

**Ethics approvals**
Informed consent was obtained from all subjects. The research protocol was approved by the Regional Ethics Review Board in Linköping, Sweden (Decision No. M75-08/2008) and Lviv National Medical University, Ukraine (Decision No. 2018.02.26/2).
RESULTS

Disease onset and SLE diagnosis
The ANA analysis was strongly positive with a combined homogenous/speckled staining pattern on HEp-2 cells and antibodies against dsDNA, Ro60/SSA and La/SSB were detected by ALBIA. Anti-C1q antibodies were negative, but levels of complement proteins C3 and C4 were initially reduced and direct Coomb’s test was positive without signs of hemolysis. Urinalysis was normal. Blood cultures were negative. Electroencephalography showed moderate general abnormality, including some occasions with suspected epileptic activity occipitally. Thus, based on the presence of the classification criteria (photosensitivity, psychosis/seizures, anti-dsDNA and ANA) accompanied by other relevant clinical symptoms like migraine, arthralgia, hypothyreosis and possible Libman-Sacks endocarditis, a diagnosis of SLE was set [26]. Surprisingly though, aCL and anti-β₂GPI antibodies were negative with regard to all tested isotypes (IgG, IgM and IgA); and just like in 2009, LA with dRVVT technique was negative. IgG anti-PS/PT antibodies were negative.

Brain imaging
No abnormalities were found on the MRI before SLE onset in 2014. The examination demonstrated a slightly irregular left vertebral artery just before the bifurcation to the basilar artery, but no abnormalities of the intracranial vessels. The CT scan in September 2016 at time of acute NP-SLE onset demonstrated no bleeding, and no focal mass or ischemic changes. The following MRA examination (same day) demonstrated small scattered cortical and subcortical hyper-intense lesions on T2 and FLAIR images as well as in the deep white matter in the centrum semiovale of both hemispheres. Several of these lesions demonstrated restricted diffusion, which most likely represented small ischemic lesions. Neither bleeding nor pathological contrast enhancement was noted (Figure 1A–E).

Autoantibodies and complement
As demonstrated in Figure 2A, the level of anti-dsDNA was elevated at SLE onset. A prompt reduction was observed after introduction of immunosuppression, but detectable anti-dsDNA levels were found during the first year. Anti-Ro60/SSA was positive, seemingly unaffected by immunosuppressive therapy, whereas anti-La/SSB decreased slightly over
time. The C3 and C4 levels were initially reduced, but returned to normal levels during the first weeks (Figure 2B).

**Routine tissue stainings**
Thick fibrotic layers coated with fibrin and inflammatory cells, e.g. lymphocytes and granulocytes, in the mitralis valve tissue was reported by the pathologist (Figure 3A). Stainings for fungi (periodic acid-Schiff-diastase) and bacteria (Giemsa) were negative. Hematoxylin bodies could not be identified. Thus, histopathology was not suggestive of infection but compatible with NBTE and Libman-Sacks endocarditis.

**NET and immune complex staining**
The immunostaining for the neutrophil elastase (NE) and citrullinated histone H3 (citH3) revealed a strong neutrophil infiltration in areas co-localizing with diffuse interstitial DNA staining (Figure 3B) suggestive of NETs. These intense infiltration areas were also positive for immune complex components IgG, C1q and C3c. The mitral valve from the control individual showed no neutrophil infiltration and conserved tissue architecture without diffuse interstitial DNA staining (Figure 3C).

**CSF markers of tissue damage and immune activation**
IgG, Tau and particularly NFL were significantly increased at disease onset (Figure 4A), but no oligoclonal bands, mononuclear cells or increased albumin were to be found. Significant reductions were observed for IgG, Tau and NFL at the 35-week follow-up. IgG then normalized (≤38 mg/L) whereas Tau (≤300 ng/L) and NFL (≤380 ng/L) were still slightly elevated. At disease onset, CSF-Ro60/SSA but no other ANA fine-specificity was detected at a level above the cut-off used for serum (≥60 units/mL) and it decreased during immunosuppressive therapy (Figure 4A). CSF from the healthy control was negative for all ANA fine-specificities (not shown).

**Serum and CSF NET remnants and elastase activity**
Circulating NET remnants were found at disease onset, but the levels were stable and did not correlate with the patient’s recovery during the first year (Figure 4B). The correlation between serum NET remnants and neutrophil count during the follow-up visits did not reach
statistical significance ($r=0.65$, $p=0.16$). However, levels of NET remnant were significantly higher compared to the sex-matched controls ($p=0.0011$). As shown in Figure 4B, NET remnants could not be detected in CSF of either the SLE patient or the healthy control, nor were neutrophils found in CSF.

The NE enzymatic activity was measured in serum and CSF samples of the patient. In sex-matched controls, the conversion of a fluoregenic substrate was shown to correlate with the percentage of inhibition by the specific inhibitor sivelastat ($p<0.003$). The baseline sample of the patient contained inhibitable amounts of NE which was markedly decreased in the successive samples (Figure 5). NE activity was not detectable in CSF samples of the patient (not shown).

Clinical follow-up

Post thoracic surgery, the patient received antipsychotics, antibiotics, glucocorticoids, hydroxychloroquine (HQ), and anticoagulation with warfarin. In addition, due to CNS involvement, cyclophosphamide was given according to the NIH protocol [27]. She exhibited a gradual recovery why cyclophosphamide was discontinued after four months and replaced by mycophenolate mofetil (MMF). During the two MRI follow-up examinations at weeks 11 and 46, a successive regression of the hyper-intense lesions both in size and number of lesions were noted. A regression of the diffusion abnormalities were observed already at week 11. No new lesions or pathological contrast enhancement were noted on the follow-up MRIs. At the 35-week follow-up, the neurologist reported her as completely recovered which was also reflected by the significant reduction of the CSF biomarkers (Figure 4A). According to the cardiologist, she is currently physiologically well and without clinical or ECG signs of mitral prosthesis dysfunction. Last visit at the rheumatology unit was in week 85. Her SLE was still clinically and serologically quiescent on a stable low-dose of prednisolone combined with HQ and MMF (Figure 2A–B).
DISCUSSION

In this case report, we describe a young female with some years of prodromal symptoms (including episodic migraine, thrombosis, hypothyreosis and arthralgia) before the prompt and dramatic onset of SLE with cardiac and CNS involvement. This case of Libman-Sacks endocarditis is very rare since it is aPL seronegative. We consistently received negative results for the classical APS tests: LA (dRVVT) as well as aCL and anti-β2GPI antibodies of IgG, IgM and IgA isotypes. In addition, IgG anti-PS/PT antibodies suggestive to be highly associated with APS were negative [28, 29]. The hypothesis of alternative, and possibly simultaneous, disease-driving mechanisms behind the SLE manifestations was the cause for our devoted interest in this case.

Although the physician ultimately sets the diagnosis of APS, laboratory tests are critical at many points of the diagnostic process [20]. According to the Sydney classification criteria, APS requires the co-existence of at least one specified clinical manifestation together with one positive laboratory test [30]. However, the term ‘seronegative APS’ has been suggested to describe patients who have a clinical profile suggestive of APS but persistently test negative for aPLs by the conventional routine assays [31]. Several research groups have investigated novel methodological approaches to detect non-traditional aPLs, i.e. different isotypes and/or other autoantigens, in patients with clinically suspected seronegative APS [32–34]. It cannot be excluded that the case we here describe, who in addition to Libman-Sacks endocarditis experienced migraine and a deep vein thrombosis during her first pregnancy, actually had aPLs not detected by the current routine methodology.

Another apparent and intriguing possibility is that the disease mechanism behind the cardiac manifestation is different from the one previously described in Libman-Sacks, i.e. highly associated with – and mediated via – aPLs [20]. Based on the histopathological findings with NET formation and abundant neutrophils in the mitral valve specimen, it is not far-fetched to suggest an important role of neutrophils in the disease process. In addition, compared to sex-matched controls, continuously raised levels of NET remnants and elastase activity were found. NET release was recently shown to be associated with the presence of ANA [35]. NETs have indeed been identified at the site of inflammation in nephritis and APL-associated
venous thrombosis of patients with SLE [6, 36]. However, whether this is a primary or secondary phenomenon, and what directs the neutrophils to the site of inflammation and facilitates the formation of NETs is still elusive. The in vitro release of NETs are reportedly triggered by e.g. purified antibodies or cytokines, including anti-β2GPI, anti-RNP, anti-LL37, anti-MMP9 antibodies, and by hyperacetylated histones microparticles and toll-like receptor-activated platelets [37–42]. It remains uncertain to which extent these findings can be translated into the in vivo situation. Yet, another possibility may be that the patient after all had a bacterial endocarditis despite the fact that no bacteria were found in blood or in tissues. However, the patient recovered with immunosuppression but was on the other hand simultaneously treated with antibiotics.

The finding of high levels of anti-dsDNA antibodies at disease-onset may also have important implications for active NET formation and reduced clearance since these autoantibodies reportedly interfere with the degradation process of NET-borne chromatin [8, 43]. Thus, it is plausible that the anti-dsDNA antibodies detected in the patient may have enhanced NET formation and/or hindered NET dismantling in the mitral valve. Interestingly, HQ constitutes a drug with inhibitory effect on NET in vitro [1]. Other drugs with similar potential have been reviewed recently [44].

In addition to Libman-Sacks endocarditis, the patient suffered from NP-SLE with psychosis and seizures. Despite decades of research, our current understanding of the underlying pathophysiology of NP-SLE remains limited [45]. Since we did not find neutrophils or NETs in the CSF, it is more likely that a different disease mechanism was responsible for the CNS involvement. Besides the induction of type I IFNs from plasmacytoid dendritic cells by immune complex-dependent signaling, autoantibodies exert a number of pathogenetic effects. One example is that neurotoxic autoantibodies, such as anti-dsDNA, can enter the CNS and bind to N-methyl-D-aspartate receptors (NMDARs), causing neurological manifestations [46]. Relevant to this, the anti-dsDNA levels as well as the CSF biomarkers of our patient followed the overall disease activity and declined over time in response to the immunosuppression. Possibly even more intriguing, as the only ANA fine-specificity, we detected anti-Ro60/SSA in CSF at the disease onset. Whereas the patient’s circulating levels of anti-Ro60/SSA remained unchanged over time, anti-Ro60/SSA in CSF normalized during
immunosuppressive treatment. Intrathecal synthesis of anti-SSA antibodies has previously been described in patients with both SLE and primary Sjögren’s syndrome with CNS involvement [47–49]. Circulating anti-SSA antibodies have also been seen in association with transverse myelitis and neuromyelitis optica [50, 51].

To conclude, separate disease mechanisms, possibly mediated via different circulating and intrathecal autoantibodies, may be of relevance for various SLE manifestations. Based on our findings, we propose that NET-based pathology can cause Libman-Sacks endocarditis without detectable aPLs.
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FIGURE LEGENDS

Figure 1 A-E: Axial FLAIR (A and B) demonstrate at time of the acute onset scattered hyperintensive lesions in the white matter of centrum semiovale and subcortically in both hemispheres. Some of these lesions demonstrated reduced diffusion on DWI (C) and ADC (D) maps suggestive of acute small ischemic events. The 3DTOF (Time-of-Flight) MR angiography (E) of the intracranial vessels demonstrated normal vessels without evidence of vasculitis or stenosis.

Figure 2 A-B: Laboratory data: (A) Autoantibodies detected by ALBIA; anti-dsDNA (ref. <80 IU/mL), anti-Ro60/SSA (ref. <50 IU/mL) and anti-La/SSB (ref. <40 IU/mL). (B) Complement proteins C3 (ref. 0.70–1.3 g/L) and C4 (ref. 0.13–0.32 g/L). Weeks indicate time since SLE onset.

Figure 3 A-C: Mitral valve samples show thick fibrin and fibrotic layers, lymphocytes and neutrophil granulocytes. No signs of fungi or bacteria and the findings were suggestive of non-bacterical trombotic endocarditis (A). Complement, IgG deposition and neutrophil markers (B and C). Fluorescence microscopic images of C1q, C3c, IgG, neutrophil elastase (NE) and citrullinated histone H3 (citH3) in the patient with Libman-Sacks endocarditis (B) compared to the individual with healthy mitral valve (C). Depicted are fluorescence images of tissue samples stained with PI (red), immunofluorescence images of C1q, C3c, IgG, NE and citH3 (Cy5, green) and an overlay of the respective images (Merge). The fluorescence images were taken at a magnification (20x, 300 µm x 300 µm) of the area indicated in the H&E staining (red box) of the complete sample in 4x magnification (B and C).

Figure 4 A-B: Laboratory of data: (A) CSF markers of damage and immune activation; IgG (ref. <38 mg/L), Tau (ref. <300 ng/L) and NFL (ref. <380 ng/L). (B) Left panel; serum NET remnants (filled circles) and neutrophil count (open circles) in serial blood samples from the patient. Weeks indicate time since SLE onset. Right panel; comparisons of NET remnants of the patient and healthy controls in sera and CSF, respectively. ** p=0.0011
**Figure 5:** The baseline sample (0) from the patient shows rather low neutrophil elastase (NE) activity but this activity was completely inhibitable by sivelestat. Follow-up samples (1-5) were not fully inhibitable by sivelestat. In healthy controls (HC) the amount of converted substrate correlates significantly \((p<0.003)\) with inhibition by sivelestat. These results suggest that the specific activity of NE in serum was strongly modulated during the disease course and provided therapies.