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Liver injury correlates with biomarkers of autoimmunity and disease activity and represents an organ system involvement in patients with systemic lupus erythematosus

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Abstract

Liver disease (LD), defined as 2-fold elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT), was examined in a longitudinal study of systemic lupus erythematosus (SLE) patients. Among 435 patients, 90 (20.7%) had LD with a greater prevalence in males (15/39; 38.5%) than females (75/396; 18.9%; $p = 0.01$). SLE disease activity index (SLEDAI) was greater in LD patients (7.8 ± 0.7) relative to those without (5.8 ± 0.3 ; $p = 0.0025$). Anti-smooth muscle antibodies, anti-DNA antibodies, hypocomplementemia, proteinuria, leucopenia, thrombocytopenia, and anti-phospholipid syndrome were increased in LD. An absence of LD was noted in patients receiving rapamycin relative to azathioprine, cyclosporine A, or cyclophosphamide. An absence of LD was also noted in patients treated with N-acetylcysteine. LFTs were normalized and SLEDAI was diminished with increased prednisone use in 76/90 LD patients over 12.1 ± 2.6 months. Thus, LD is attributed to autoimmunity and disease activity, it responds to prednisone, and it is potentially preventable by rapamycin or N-acetylcysteine treatment.

Keywords

Liver disease; Autoimmunity; Lupus; Disease activity; Treatment

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Contributorship statement

All authors contributed meaningfully to this study.

Competing interests

The authors have no competing interests.

Ethical approval information

This study has been approved for protection of human subjects by the SUNY IRB.

Data sharing statement

All data have been submitted either in the main body of the manuscript or in the supplemental materials section.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that causes inflammation in multiple organ systems with diverse clinical manifestations [1]. It has been reported that patients with SLE have a 9.3% to 59.7% chance of developing abnormal liver function tests (LFT) during follow-up periods of multiple years [2–5]. Two commonly measured LFTs are aspartate transaminase (AST) and alanine transaminase (ALT). These transaminases participate in amino acid metabolism and are normally found at low levels in plasma serum. However, upon hepatocyte damage, these liver enzymes are released, and abnormal levels can be detected in the circulation [6]. In addition to indicating liver damage, the ratio of AST to ALT can help differentiate the etiology [7].

Management of SLE patients with persistent AST and ALT elevations is challenging. Physicians may be confronted with long-lasting abnormal liver enzymes, which cannot be explained by any obvious causes after excluding viral hepatitis, alcohol toxicity, and potentially harmful drugs. Discerning the cause of liver dysfunction and the safety of immunosuppressant treatments are difficult in these patients. Although the association between SLE and liver disease has been observed on multiple occasions, the relationship of liver disease to co-morbidities and drugs has not been well established. Our study has been initiated to determine the causes of liver disease with a focus on the contributions of SLE disease activity and medication use. This initiative was prompted by the common dilemma that the clinician face in daily practice with respect to handling of liver enzyme elevations. Recent studies set the threshold for drug-induced liver injury at a 2-fold elevation of ALT or AST, depending on the patient population involved [8,9]. In immunocompromised patients, such as those infected by human immunodeficiency virus (HIV) or hepatitis C virus (HCV), the threshold of liver injury was set at a 2-fold elevation of ALT or AST [8,9]. Therefore, we have undertaken a longitudinal study of ALT and AST elevations in SLE patients by excluding subjects with alcohol abuse, hepatitis and human immunodeficiency virus infection, or thyroid disease, all of which can cause liver disease independent of SLE [10–12]. None of these confounding factors have been previously excluded in previous studies of LFT elevation with respect to disease activity and medication use in patients with SLE. The results of this conservatively defined longitudinal study of 435 SLE patients indicate that LD, which is delineated as a 2-fold elevation of ALT or AST, may represent a manifestation of lupus disease activity and respond to continued immunosuppression and introduction of prednisone rather than caused by drug toxicity.

2. Methods

2.1. Human subjects

Patients who satisfied the American College of Rheumatology criteria for a definitive diagnosis of SLE [13,14] among those seen and treated at SUNY Upstate Medical University Hospital from October of 1999 to December of 2011 were included in this study. The clinical protocol was approved by the Institutional Review Board. All patients of our lupus cohort are screened for antibodies to hepatitis A, B, or C virus. Patients with evidence of hepatitis A, B, or C virus infection, human immunodeficiency virus (HIV) infection, IgM-positive recent parvovirus B19 infection, and those with a history of alcohol abuse have

been excluded. Alcohol abuse diagnosis was made using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (*DSM-5*) and definition of a problematic pattern of alcohol consumption [15]. Allowable alcohol consumption was considered as an average of one drink per day. One drink was defined as 12 oz of beer, 4 oz of wine, or 1 oz of liquor which do not elicit AST or ALT elevation [16]. To rule out non-hepatic disease as a cause of abnormal liver function tests, SLE patients who possessed elevated creatine phosphokinase (CPK) and thyroid-stimulating hormone (TSH) laboratory values were also excluded from analysis. We defined liver disease (LD) as a 2-fold or greater elevation of serum AST or ALT above the upper limit of the normal range. Patients with a greater than normal, but less than 2-fold elevation in AST or ALT were defined to have intermediate liver disease (iLD). The remaining patients who never had an elevated LFT value were classified as normal. As markers of autoimmune hepatitis (AIH) and primary biliary cirrhosis, anti-smooth muscle antibody (SMA) and anti-mitochondrial antibody (AMA) were assessed. Patient demographics, such as gender, age at the time of LD, ethnicity, and body mass index (BMI) were noted. We analyzed the prevalence of liver disease with respect to diabetes. 27 patients had diabetes, 5 of them type 1. 18 patients were treated with insulin, while 9 patients were only treated with oral anti-diabetic medications, 6 with metformin, 2 with glipizide, 2 with glyburide, and 2 with sitagliptin. The medications taken by SLE patients with LD were recorded for the day when the patient exhibited a 2-fold elevation of liver enzymes and for the next follow-up date when the patient's LFT have normalized. In addition, we reviewed liver imaging studies and liver biopsies when available.

2.2. Routine laboratory tests

AST (normal range: males, <37 U/L; females, <31 U/L), ALT (normal range: males < 41 U/L; females < 31 U/L), CPK (normal range: males, 20–200 U/L; females, 20–180 U/L), TSH (normal range: 0.270–4.200 μ IU/mL), C3 (normal range: 90–180 mg/dL), and C4 (normal range: 10–40 mg/dL) were measured on a Roche/Hitachi Modular Analyzer (*Roche Diagnostics*, Indianapolis, IN). Platelets (normal range: 150–400 K/ μ L) and WBC (normal range: 4.0–10.0 K/ μ L) were counted on a Beckman–Coulter LH 750 Hematology Analyzer (Brea, CA). Lupus anticoagulants were assessed by Staclot LA (delta < 10 s), Staclot® dRVV (<1.2 normalized ratio) manufactured by Stago Diagnostics (Parsippany, NJ, USA). Platelet neutralization assay has been developed in house (delta < 1 s) using a STA-R Evolution instrument by Diagnostica Stago Diagnostics. Cardiolipin and β 2-glycoprotein 1 (β 2-GP1) antibodies were measured by Quest Diagnostics (Madison, NJ). Antinuclear antibody (ANA; normal range > 1:50 dilution) was detected in the HEP-2 Test System by Zeus Scientific (Raritan, NJ). The immunofluorescent ANA test was used for diagnosis of all patients [17]. Anti-mitochondrial antibody (AMA; normal range > 1:50 dilution) and anti-smooth muscle antibody (SMA; normal range > 1:50 dilution) were measured using NOVA Lite® ANA Plus Mouse Kidney & Stomach assay manufactured by Inova Diagnostics (San Diego, CA). Hepatitis viral antibody tests were performed on an ARCHITECT *i*1000SR Immunoassay Analyzer manufactured by Abbott Diagnostics (Abbott Park, IL).

2.3. Biomarkers of lupus disease activity

As markers of active SLE, the presence of proteinuria, glomerulonephritis, anti-double stranded DNA antibodies (anti-DNA), hypocomplementemia, leukopenia, thrombocytopenia, and antiphospholipid antibodies (APLA), lupus anticoagulant, and antiphospholipid syndrome (APS) was recorded. APS was confirmed by a clinical thrombotic event and positive tests for cardiolipin or β 2-GP1 antibodies or lupus anticoagulant that was persistent for 12 weeks [18]. SLE disease activity was assessed by using systemic lupus erythematosus disease activity index (SLEDAI) [19]. Patients that were positive for proteinuria had urine protein levels greater than 0.5 g/day [19]. Hypocomplementemia was classified as low C3 or low C4. Patients with leukopenia or thrombocytopenia had white blood cell (WBC) counts less than 4000/L and platelet counts less than 100,000/L, respectively.

2.4. Statistical analyses

Statistical assays were performed with two-tailed χ^2 -test, Fisher's exact test, and correlation analysis using the GraphPad Prism Version 5 software (San Diego, CA). A two-tailed p value <0.05 was considered significant.

3. Results

3.1. Prevalence of LD is increased in male SLE patients

20.7% (90/435) of SLE patients met our criteria of LD, 29.0% (126/435) had iLD, and 50.3% (219/435) had normal AST and ALT (Table 1). Assessment of demographic differences included ethnicity, age, and gender. Among these parameters, ethnicity did not influence the prevalence of LD or iLD in SLE (Table 1). Out of 435 SLE patients, 39 were male and 396 were female. Thus, 38.5% of male patients had LD, while only 18.9% of female patients had LD relative to subjects with normal LFTs (χ^2 p = 0.01; Table 1). The prevalence of LD was also increased in males relative to subjects with iLD (χ^2 p = 0.02; Table 1). Age was not significantly different between the three cohorts of LD, iLD, and normal LFTs. However, when comparing patients based on gender and age it revealed that subjects with iLD (45.2 ± 1.1 years) were slightly older than those with LD among females (41.1 ± 1.8 years; p = 0.045; Table 2). The average AST/ALT ratio was 1.21 ± 0.04 in all patients, and it was not affected by age, ethnicity, or gender (Table 2). Body mass index (BMI) was not significantly different between patients with LD, iLD, and normal LFTs (Table 2). No significant differences in BMI were noted either when male and female patients were separately analyzed (Table 2). As shown in Table S1, concurrent diabetes did not influence the prevalence of liver disease.

3.2. LD is associated with lupus disease activity characterized by increased SLEDAI, anti-DNA and hypocomplementemia

The greatest differences in biomarkers of lupus disease activity were observed when the LD cohort was compared to lupus patients with normal LFTs. SLEDAI was greater in LD patients (7.8 ± 0.7) relative to those with normal LFT (5.8 ± 0.3 ; p = 0.0025; Fig. 1A). SLEDAI of iLD patients (6.452 ± 0.4827) was not significantly different from those with normal LFT (p = 0.251) or LD (p = 0.0938). Even after excluding anti-DNA and

complement values, the SLEDAI scores remained elevated in LD patients (7.1 ± 0.7) relative to those with normal LFT (5.5 ± 0.3 ; $p = 0.0210$; Fig. 1B). SLEDAI of iLD patients (6.1 ± 0.5) was not significantly different from those with normal LFT ($p = 0.2688$) or LD ($p = 0.2571$). When clinical and laboratory components of SLEDAI were separately analyzed, proteinuria ($p = 0.007$), leukopenia ($p = 0.0200$), and thrombocytopenia ($p = 0.0003$) as well as anti-DNA antibodies ($p = 0.0050$) and hypocomplementemia ($p = 0.0200$) were all increased in SLE patients with LD (Table 3). The prevalence of SMA was also increased in LD patients ($p = 0.03$). When compared to iLD, LD patients still demonstrated a few significant differences, showing higher prevalence of leukopenia ($p = 0.0100$) and thrombocytopenia ($p = 0.0470$). When iLD and LD patients were grouped together as a single cohort, SMA ($p = 0.0200$), proteinuria ($p = 0.03$), anti-DNA antibodies ($p = 0.0480$), and thrombocytopenia were all increased in patients with abnormal LFT ($p = 0.0070$; Table 3). Traditional markers of lupus disease activity, anti-DNA and hypocomplementemia, were not associated significantly with iLD alone (Table 3).

Not only was the prevalence of SLE disease activity markers increased in LD patients, but AST and ALT were also significantly correlated with the lupus biomarkers (Fig. 2). AST levels were positively correlated with anti-DNA titers ($r = 0.34$, $p < 0.0001$, Fig. 2A). Along this line, LD patients also had higher anti-DNA antibody titers (LD: $114.8 \text{ U/ml} \pm 32.8$; normal: $55.7 \text{ U/ml} \pm 11.8$; $p = 0.03$; Fig. 2B). C3 values negatively correlated with both AST and ALT (Fig. 2C). Additionally, C3 was significantly lower in LD than iLD subjects (LD: $106.8 \text{ mg/dL} \pm 5.3$; iLD: $119.4 \text{ mg/dL} \pm 3.7$; $p = 0.044$; Fig. 2D). When the different SLE biomarkers were compared to one another, C3 and C4 were negatively correlated with anti-DNA antibody titers (Fig. S1: C3: $r = -0.17$, $p = 0.004$; C4: $r = -0.13$, $p = 0.03$). As expected, the prevalence of proteinuria ($\text{N}0.5 \text{ g/day}$) was significantly higher in anti-DNA-positive patients ($p = 0.0030$; Table S2). APS was more common in patients with LD relative to those with iLD ($p = 0.0390$) or normal LFT ($p = 0.0025$; Table 3).

3.3. LD was correlated with use of azathioprine, cyclophosphamide, cyclosporin A and prednisone treatment

Use of azathioprine (AZA; $p = 0.0460$), prednisone (PRED; $p = 0.0002$), cyclophosphamide (CTX; $p = 0.0300$), and cyclosporine A (CsA; $p = 0.0261$) was all increased in patients with LD (Table 4). In comparison to subjects with normal LFT, those with iLD or LD showed a greater use of azathioprine (AZA; $p = 0.0200$) and prednisone ($p = 0.0020$). Use of mycophenolic mofetil (MMF; $p = 0.0300$), prednisone ($p = 0.03$), and CsA ($p = 0.0020$) was also increased in LD subjects relative to those with iLD. Use of rapamycin (Rapa) was not different between patients with LD, iLD, or normal LFT. However, when these traditional immunosuppressant medications were compared between patients with normal LFT and LD, an absence of LD was noted in patients receiving Rapa relative to AZA (two-tailed Fisher's exact test $p = 0.0237$), CsA (two-tailed $p = 0.0060$), and CTX (two-tailed $p = 0.0047$; Table S3). An absence of LD was also noted in patients treated with NAC relative to CsA (two-tailed $p = 0.0164$) and CTX (two-tailed $p = 0.0143$; Table S3). These findings suggest that Rapa and NAC may prevent LD in SLE. However, due to the small numbers of patients treated with Rapa and NAC, these results should be viewed with caution and considered

tentative only, which require confirmatory follow-up studies. NSAID use was reduced in patients with LD in comparison to those without ($p = 0.02$; Table 4).

3.4. Resolution of LD is associated with increased prednisone use and diminished SLEDAI

To evaluate the potential mechanisms of LD, patients were followed longitudinally until LFTs normalized. Upon follow-up, LFTs normalized in 76 of 90 (84.4%) LD patients (two tailed Fisher's exact test $p < 0.0001$). The normalization required 12.1 ± 2.6 months. At the peak of LD, SLEDAI correlated with AST (Pearson's $r = 0.4796$; $p < 0.0001$) and, to a lesser extent, with ALT (Pearson's $r = 0.2680$; $p = 0.0283$; Fig. 3A). Upon normalization of LFTs, SLEDAI scores were reduced from 8.8 ± 0.9 to 5.2 ± 0.9 ($p = 0.001$; Fig. 3B). SLEDAI scores without anti-DNA or hypocomplementemia were also reduced from 7.6 ± 1.0 to 4.4 ± 0.9 ($p = 0.032$; Fig. 3C). Although hypocomplementemia was not significantly affected (data not shown), anti-DNA titers were also reduced with normalization of LFT (two-tailed $p = 0.079$; Fig. 3D).

Given that LD was associated with increased disease activity and a greater need for immunosuppressants, we examined medication use at study visits preceding and following 2-fold elevation of AST or ALT. Normalization of LFTs was not associated with introduction or discontinuation of immunosuppressive medications. Among LD patients, 7/7 remained on AZA, 1 additional patient received CTX, and 2 additional patients were given CsA when LFTs normalized. The only statistically significant difference between LD patients and their normalized states was an increase in prednisone use (Table 5). Thus, a greater proportion of LD patients was taking prednisone in their normalized state ($p = 0.043$; Table 5). Given that this study was not an interventional clinical trial, no formal pill counts of medications were conducted. Therefore, cumulative prednisone doses could not be reliably determined. Reversal of liver disease was associated with introduction of rapamycin in two patients and NAC in one patient, however, these differences was not significant (Table 5). 7/14 patients whose 2-fold elevated LFTs failed to normalize had imaging studies. 2 patients had abdominal computer to-mogram (CT) study that revealed liver metastasis secondary to lung cancer, 1 patient had sonogram indicating steatosis of the liver, while the remaining subjects had normal imaging studies.

3.5. Histological evidence of inflammation in the liver of patients with SLE

Liver biopsy was performed in four SLE patients with LD. In two of the patients, only mild lymphocytic infiltrations were noted (data not shown). In one of these two patients, LFTs normalized in 6 months with administration of HCQ alone. In a second patient with patchy lymphoid infiltrates, LFTs normalized in 13 months with administration of HCQ and MMF. In a third patient with a >2-fold elevation of AST at 85, an ALT at 65, an increased SMA titer at 1:1250, and an elevated anti-DNA at 157 (normal: 99 U/mL), liver biopsy revealed chronic portal inflammation with infiltrating eosinophils, lymphocytes and plasma cells as well as macro and microvesicular steatosis (Fig. S2). LFTs normalized with administration of HCQ and MMF. Liver histology in a fourth patient demonstrated extensive inflammation with lymphocytic, plasma cell and eosinophilic infiltration both in hematoxylin and eosin and trichrome stained sections (Figs. S3A–C). Reticulin staining showed that tissue architecture was disrupted in areas of lymphocyte invasion (Fig. S3D). Mitotic figures and

apoptotic hepatocytes were detected (Figs. S3E and F). Lastly, bile duct proliferation was observed in areas of lymphocytic infiltration (Figs. S3G and H). These findings were consistent with aggressive autoimmune hepatitis [20,21]. The liver pathology occurred with elevation of AST at 1438 U/L (normal: 35 U/L) and active SLE, as evidenced by malar rash, arthritis, elevated anti-DNA of 1308 U/ml (normal: 99 U/ml) and SMA at 1:1250. High-dose prednisone of 60 mg/day, azathioprine (100 mg/day), mycophenolate mofetil (1.5 g/day), and hydroxychloroquine (400 mg/day) were all used in this patient over more than 2 years without any benefit. However, after 6 months of rapamycin treatment (2 mg/day), the LFTs began to normalize, as demonstrated by an AST value of 32 U/L and ALT value of 47 U/L, and prednisone could be tapered to 10 mg/day.

4. Discussion

The liver is often overlooked as a target organ in SLE, and its pathologies are usually considered rare [22] and secondary to toxicity of therapies [5,23] or, less commonly, to disease activity of SLE [3,4]. Significant liver disease is generally regarded as unusual in SLE and not a major cause of mortality [24] despite the fact that elevated liver enzymes are frequently encountered [3]. After exclusion of infectious and metabolic causes, such as thyroid disease, 49.7% of our SLE cohort had iLD with one elevated transaminase and 20.7% of SLE patients had LD with a 2-fold elevation of AST or ALT. The AST/ALT ratio of LD patients was not significantly different from that in patients with normal LFTs (LD: 1.26 ± 0.11 ; normal: 1.18 ± 0.03 ; $p = 0.32$), but it did confirm that our study successfully excluded patients with alcoholic hepatitis, which would have exhibited AST/ALT ratio >2.0 [6,7]. Our results suggest that LD is a consequence of SLE, as LFTs strongly correlated with biomarkers of disease activity, and the normalization of LFTs in 76/91 patients occurred with maintenance of immunosuppressant regimens and additional treatment with prednisone. Therefore, abnormal LFTs alone should not be a justification to withhold immunosuppressive treatment, especially when SLE is active. Our findings concur with a smaller retrospective study from Italy [25], which also suggested that LD respond to prednisone in patients with SLE. Nevertheless, it's important to state that these associations of improved LFT with diminished disease activity upon prednisone use do not necessarily prove causality.

AST but not ALT remarkably correlated with elevated anti-DNA in this study. Anti-DNA has been previously associated with chronic liver diseases [26]. Elevated AST relative to ALT has been associated with alcoholic hepatitis as well as progression of chronic viral hepatitis to cirrhosis [8,27]. Recently, we noted an elevation of AST but not ALT in liver disease caused by predisposition to oxidative stress-induced necrotic cell death in transaldolase deficiency [28]. Necrotic cell death is overall increased in SLE patients due to oxidative stress-induced mTOR-dependent expansion of CD3⁺CD4⁻CD8⁻ double-negative (DN) T cells [29,30]. These T cells also play critical roles in pathogenesis of SLE patients [31]. Importantly, DN T cells are expanded in the liver of lupus-prone mice [32,33]. In turn, mTOR blockade with rapamycin [29, 34] or NAC reduces the expansion of DN T cells and production of anti-DNA [35]. Given that reactive oxygen intermediates propagate via high diffusible aldehydes through the bloodstream in SLE [36], they may also underlie the disease-activity related elevation of AST rather than ALT in SLE. Oxidative stress in the

liver causes the elevation of AST over ALT [28]. Thus, increased AST may be related to oxidative stress-driven autoantigenesis in SLE [36]. This includes the oxidative modification of DNA that is detectable in circulating immune complexes of patients with SLE [37]. Thus, oxidized DNA released from the injured liver may serve as a danger signal and trigger of anti-DNA in SLE [38]. In turn, the increased production of leptin, which may originate from stellate cells in the liver [39], contributes to T-cell hyper-reactivity in SLE [40].

The decrease in the number of significant laboratory values after combining iLD and LD into one group shows that the iLD cohort dilutes the differences between normal and LD patients. Our regression analysis demonstrates a significant positive correlation between LFTs and anti-DNA antibodies and a negative correlation between LFTs and C3. These results are consistent with our parametric (t-test) and binomial (χ^2) analysis showing that LD patients are more likely to be positive for anti-DNA antibodies and hypocomplementemia. The lack of correlation between LFTs and C4 levels can be attributed to genetic causes that commonly underlie the deficiency of this complement factor in SLE [41].

A retrospective study of 206 SLE patients found a similar, 20.9%, rate of liver disease in 1980 [52]. While this study utilized stricter criteria, requiring 4 separate determinations of 2-fold increase in either total bilirubin, AST, ALT, lactate dehydrogenase, or alkaline phosphatase, it did not exclude alcoholic and viral hepatitis [52]. The incidence of mucosal ulcers, cytopenia, and thyroid disease was significantly higher, while arthralgia was significantly less common in the group with liver disease [52]. It is conceivable that infection by hepatitis virus or thyroid disease contributed to the disease manifestations in the latter study [52]. In contrast, our study excluded alcoholic and viral hepatitis as well as thyroid disease, because increased serum TSH is often seen in liver cirrhosis [60, 61]. We also excluded hepatitis viruses A, B, and C as well as HIV and parvovirus B19 in our cohort, because the pathologies of these infections can mimic the presentation of SLE [59]. Thus, by excluding infectious and metabolic causes, we found the prevalence of anti-DNA antibodies, hypocomplementemia, SMA, leucopenia, thrombocytopenia, and proteinuria as well as SLEDAI to be markedly increased in SLE patients with LD in comparison to SLE patients with no liver involvement. In contrast to several earlier retrospective studies summarized in Table S4, these findings strongly support the notion that LD is primarily a consequence of disease activity in SLE.

Another retrospective study of liver enzyme patterns in 81 patients with SLE from 1981 documented a 55% prevalence of abnormal LFTs [42]. Excluding salicylate toxicity, alcohol abuse, and viral hepatitis cut the prevalence to 23% of patients who had no explanation for abnormal LFTs other than SLE [42]. After filtering out patients with less than a 2-fold elevation in LFTs, only 8.6% of these SLE patients met our criteria for LD [42], which is significantly lower than the 20.7% LD prevalence documented in this study. A prospective study from 1984 found elevated LFTs in 61 of 260 SLE patients, however, 40 of the 61 patients with elevated LFTs had a documented non-SLE liver pathology or toxicity [53]. Based on our study's criteria, only 10% of these SLE patients had elevated LFTs [53], which is significantly lower than the 49.8% prevalence rate of elevated AST or ALT found in our study.

Our study has been initiated to determine the causes of liver disease with a focus on the contributions of SLE disease activity and medication use. This initiative was prompted by the common dilemma that the clinician face in daily practice with respect to handling of liver enzyme elevations. Recent studies set the threshold for drug-induced liver injury at a 2-fold elevation of ALT or AST, depending on the patient population involved [57,58]. In immunocompromised patients, such as those infected by HIV or HCV, the threshold was set at a 2-fold elevation of ALT or AST [57,58]. Our results clearly suggest that such enzyme elevation may represent a manifestation of lupus disease activity and may respond to continued immunosuppression and introduction of prednisone rather than caused by drug toxicity.

Alternatively, a recent comprehensive study assessed the causes of chronic liver disease in Canadian patients with SLE. Here, subjects with 1.5 times elevated AST and/or ALT on 2 consecutive visits within a 2-year span were included [43]. Among 1533 patients reviewed, 134 (8.7%) met the inclusion criteria. Compared to controls, cases were higher in BMI, anti-DNA antibody, prevalence of hypertension, antiphospholipid syndrome, and use of immunosuppressive medication, especially azathioprine and methotrexate; they were lower in IgM. Although this study had different goals, the association of LFT elevation with anti-DNA and APLA is in agreement with our findings. It has not been documented whether anti-DNA was higher in patients with AST or ALT elevation [43]. Although our study has been initiated with the goal that was to help with decision making of clinicians facing 2-fold elevation of ALT or AST in patients with SLE, which is commonly considered as a sign of drug-induced liver injury [57,58], we also examined other potential causes of chronic liver disease unrelated to SLE in our cohort. The available records of 7/14 patients whose 2-fold elevated LFTs failed to normalize revealed 2 patients with liver metastasis secondary to lung cancer and 1 patient with steatosis of the liver, while the remaining subjects had normal imaging studies. These analyses suggest that a single 2-fold elevation of ALT or AST in patients with SLE commonly represents a treatable manifestation of lupus activity rather than chronic liver disease.

A retrospective study from 2014, which excluded patients with alcohol abuse and viral hepatitis, reported any elevation in one LFT test in 9.3% (47/504) of SLE patients in Guangzhou, China [51]. This is significantly lower than the prevalence of liver disease in our cohort, which corresponds to 49.7% in our lupus population with iLD and LD that included all patients with any elevation of AST or ALT (216/435; two-tailed χ^2 $p < 0.0001$). In this Chinese study, SLEDAI values were similar in patients with or without LFT elevation, nevertheless, liver disease was more common in patients with SLEDAI >4 , and those with elevated anti-DNA or hypocomplementemia [51]. Therefore, despite the markedly lower prevalence, LFT elevation also appears to be a consequence of lupus disease activity in China [51]. In contrast, another study from 2013 showed LD in 59.7% of SLE patients in Japan [54]. In this study, LD was defined as an elevation of any two of the following enzymes: AST, ALT, γ -glutamyl transpeptidase (GGT), or alkaline phosphatase [54]. The slightly greater prevalence of liver disease may be attributed to the inclusion of elevated gamma-glutamyl transferase or alkaline phosphatase because these markers have been implicated in extrahepatic biliary pathologies [44,45]. Notably, similar to our study, LD was also more common in Japanese males than females with SLE [54]. In a recent study of

MRL/lpr mice prior to lupus disease onset, mitochondrial dysfunction was found in the liver to be more severe in males than females [46]. Thus, more investigation is warranted to determine why males with SLE are more at risk for developing LD and whether this results from mitochondrial dysfunction.

Despite the fact that the liver possesses critical roles in the immune system, including autoimmunity [47], the relationship between SLE and this organ is not well characterized. Given the fundamental role of the liver in metabolism, which has been also recognized as a regulator of immune responses [48,49], the injury of this organ may contribute to pathogenesis in SLE. In comparison to 42 matched healthy subjects, metabolome changes in peripheral blood lymphocytes (PBL) of 36 lupus patients have recently unveiled a prominent impact on the pentose phosphate pathway, which reflects a greater demand for nucleotides and oxidative stress [50]. Among 42 metabolites, which exhibited altered concentration in lupus PBL, adenosine was reduced by 54% in 13 of these patients, who also had liver disease, with respect to patients with normal LFTs (two-tailed t-test raw $p = 0.000093$; $p = 0.031$ after Bonferroni-correction for 34 significantly changed metabolites in SLE). Notably, adenosine has significant anti-inflammatory properties [51], including an inhibitory effect on the development of SLE [52–55]. Given that adenosine is produced and released into the bloodstream by hepatocytes [56], it may represent a potential metabolic link between liver disease and SLE.

In the current study, anti-DNA, hypocomplementemia, proteinuria, thrombocytopenia, and leukopenia as well as SLEDAI disease activity scores were significantly associated with LD, which therefore appears to be a component of pathogenesis at least in a subset of patients. This notion is further supported by a resolution of LD upon continued treatment with immunosuppressants and increased use of prednisone. In our cohort, no patient was found to have primary biliary cirrhosis, which involves the autoimmune destruction of intra-hepatic biliary ducts and thus leads to cholestasis and cirrhosis [57]. AMA is rarely detected in healthy individuals, but it is found in over 90% of primary biliary cirrhosis patients [57]. In our study, LD was not associated with the presence of AMA. On the other hand, SMA was associated with LD in our lupus cohort, which implies that autoimmune hepatitis is a manifestation of SLE. Indeed, AIH and SLE have been documented to possess several overlapping serological markers and clinical presentations, including positive ANA, SMA, and poly-arthritis [58,59]. Although the specificity of SMA for autoimmune hepatitis is high, its sensitivity is only moderate [60]. Thus, the sole presence of a positive SMA is insufficient to confirm the presence of AIH as a confounding factor in SLE. Nevertheless, all of our four SLE patients with biopsy-proven AIH also had SMA. While one LD patient had mild AIH on biopsy and responded to the introduction of HCQ alone, two patients with AIH only responded to a combination treatment with HCQ and MMF. In contrast, a 4th patient with AIH revealed severe inflammation and 50-fold elevated AST, and she only improved upon treatment with rapamycin (sirolimus). Along these lines, a conspicuous absence of prior exposure to rapamycin was noted in LD patients relative to other immunosuppressants, such as AZA, CsA, and CTX. This is consistent with the documented effectiveness of rapamycin in SLE [29,34] and AIH [61–63]. There was also a conspicuous absence of LD in patients treated with NAC relative to those treated with CsA or CTX. However, the significance of these findings is limited due to the small numbers of patients

on any given medication and the retrospective nature of this analysis. LD, as defined in this study, did not develop in any of the patients enrolled in prospective clinical trials with rapamycin [29] or NAC [35]. Given that NAC has well-established hepato-protective effects [64–66], and it showed safety and clinical efficacy in a double-blind placebo-controlled pilot study of 3-month duration, follow-up studies of longer duration are clearly warranted in SLE patients [35]. Although our study suggest that LD may be more common than generally perceived, liver biopsy does not appear to be routinely justified given the overwhelming responsiveness to persistent immunosuppression and administration of prednisone. Nevertheless, the facts that only 4 of our patients have undergone liver biopsy, two of whom showed aggressive hepatitis, indicate that significant liver disease is rare in SLE.

NSAIDs were used more frequently in patients with normal LFTs. While NSAIDs are commonly employed to treat mild arthritis in SLE, they can elicit liver injury [67]. Therefore, we associate the inverse correlation between LD and NSAID use with a purposeful avoidance and lack of effectiveness of this therapy in patients with more severe lupus.

Interestingly, hepatocellular carcinoma (HCC) has increased prevalence in SLE with an elevated standardized incidence ratio of 1.7 [68] to 2.6 [69]. The pathogenesis of HCC is characterized by chronic inflammation driven by glutathione depletion and oxidative stress [28,70], which have also been documented in patients with SLE [36,71,72]. Interestingly, APS was significantly more common in our lupus cohort with LD (Table 3), which concurs with meta-analyses of liver involvement in patients with APS [73,74]. Mitochondrial mass and oxidative stress are increased in PBL of lupus patients with APS [30]. Along these lines, NAC, a precursor of glutathione and potent antioxidant, was found to be beneficial for liver dysfunction [28] as well as SLE [35]. Therefore, further studies aimed at delineating common mechanisms underlying oxidative stress in liver disease and SLE appear to be justified. It is conceivable that NAC may be particularly beneficial in SLE patients with liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clim.2015.07.001>.

Abbreviations

AIH	autoimmune hepatitis
AMA	anti-mitochondrial antibody
Anti-DNA	anti-double stranded DNA antibody
ANA	antinuclear antibody
Anti-TNF	tumor necrosis factor blocker
ALT	alanine aminotransferase
APLA	anti-phospholipid antibody
APS	anti-phospholipid syndrome
AST	aspartate aminotransferase
AZA	azathioprine
BMI	body mass index
C3	complement factor
C4	complement factor 4
CPK	creatine phosphokinase
CsA	cyclosporine A
CTX	cyclophosphamide
HCQ	hydroxychloroquine
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIV	human immunodeficiency virus
iLD	intermediate liver disease
LD	liver disease (LD)
LFT	liver function test
MMF	mycophenolate mofetil
MPA	mycophenolic acid
mTOR	mechanistic target of rapamycin
NAC	N-acetylcysteine
NSAID	non-steroidal anti-inflammatory drug
PBL	peripheral blood lymphocytes
PRED	prednisone

Rapa	rapamycin/sirolimus
SLE	systemic lupus erythematosus
SLEDAI	systemic lupus erythematosus disease activity index
SMA	anti-smooth muscle antibody
TSH	thyroid-stimulating hormone
WBC	white blood cell

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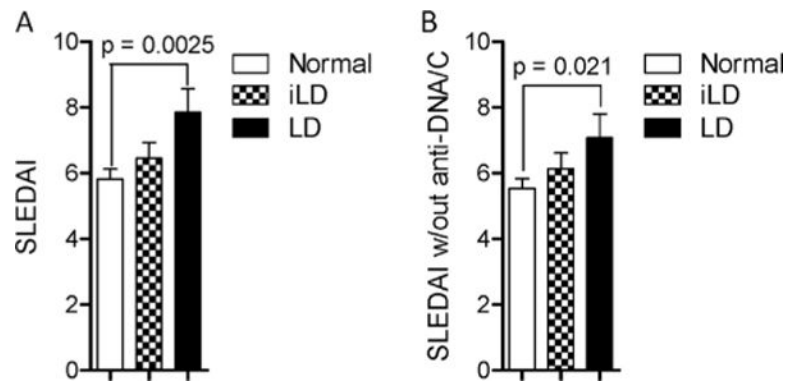


Fig. 1. LD is associated with increased disease activity in SLE. A) Assessment of disease activity by SLEDAI in 90 patients with LD defined as 2-fold elevation of AST or ALT, 126 patients with iLD defined as <2-fold elevation of AST or ALT, and 219 patients with normal LFTs. B) Modified SLEDAI scores in patients with LD, iLD, and normal LFTs after excluding anti-DNA and complement values. Data represent mean \pm SEM. p values indicate comparison with two-tailed unpaired t-test.

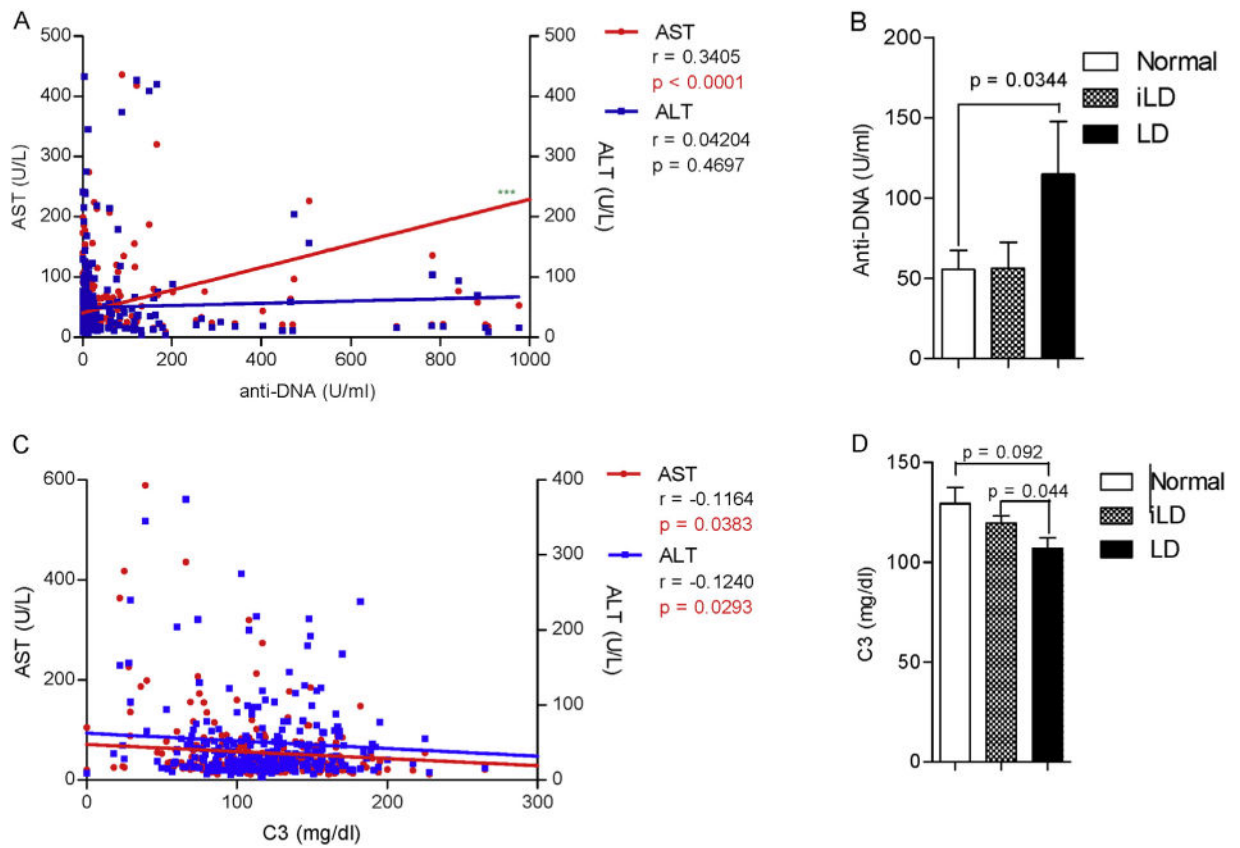
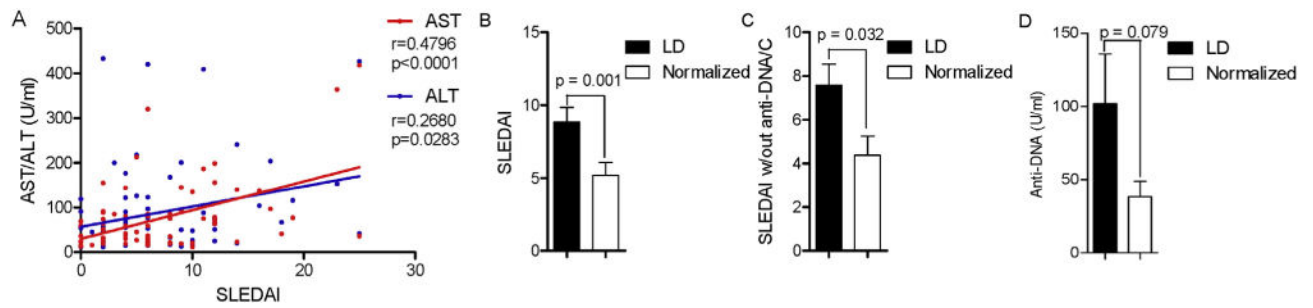


Fig. 2. Increased production of anti-DNA autoantibodies and hypocomplementemia in lupus patients with LD. A) Correlation of AST but not ALT with anti-DNA. B) Increased anti-DNA production in SLE patients with LD. C) Correlation of C3 with AST and ALT. D) C3 hypocomplementemia in SLE patients with LD. Pearson r values and corresponding two-tailed p values are indicated for correlation dot plots. Data represent mean \pm SEM in bar charts; p values indicate comparison with two-tailed unpaired t -test.

**Fig. 3.**

Reduction of SLEDAI with reversal of LD in patients with SLE. A) Correlation of SLEDAI with AST and ALT in lupus patients with LD. Pearson r values and corresponding two-tailed p values are indicated. B) Assessment of disease activity by SLEDAI in LD patients with normalized LFT. C) Modified SLEDAI scores with exclusion of anti-DNA and complement values in patients with LD upon normalization of LFTs. D) Diminished anti-DNA antibody production in LD patients upon normalization of LFTs. Data represent mean \pm SEM. p values indicate comparison with two-tailed paired t-test.

Prevalence of liver disease (LD), intermediate liver disease (iLD) and normal liver function (N) in 435 SLE patients. p values reflect two-tailed χ^2 test. p values <0.05 are in italics.

Table 1

Patients	Normal		iLD		LD		p		Total
	N	%	N	%	N	%	N vs. iLD + LD	N vs. LD	
All	219	50.3	126	29.0	90	20.7			435
Male	16	41.0	8	20.5	15	38.5	0.74	0.01	39
Female	203	51.3	118	29.8	75	18.9		0.22	396
Caucasian	166	52.5	84	26.6	66	20.8	0.13	0.87	316
African American	41	45.6	32	35.5	17	18.9		0.24	90
Native American	1	50.0	1	50	0	0			2
Asian	4	28.6	5	35.7	5	35.7			14
Hispanic	7	53.8	4	30.8	2	15.4			13

Analysis of body mass index (BMI), age, AST/ALT ratio relative to LD in SLE. p values reflect two-tailed χ^2 test. p values <0.05 are in italics.

Table 2

Mean	Normal	iLD	PN vs. iLD	LD	PN vs. LD	PiLD vs. LD	Overall
<i>BMI</i>							
All patients	29.9 ± 0.5	31.1 ± 0.7	0.20	30.4 ± 1.0	0.4	0.5	30.2 ± 0.5
Males	32.3 ± 2.0	27.9 ± 2.8	0.5	32.4 ± 2.2	1.0	0.5	31.3 ± 1.2
Females	29.4 ± 0.5	31.3 ± 0.7	0.10	30.2 ± 0.9	0.8	0.4	30.2 ± 0.4
PMales vs. Females	p = 0.4	p = 0.6		p = 0.5			p = 0.6
<i>Age</i>							
All patients	44.7 ± 1.0	45.1 ± 1.1	0.78	41.4 ± 1.7	0.09	0.052	45.4 ± 0.7
Males	41.4 ± 3.9	44.3 ± 4.8	0.66	42.9 ± 4.2	0.80	0.84	42.6 ± 2.4
Females	45.0 ± 1.1	45.2 ± 1.1	0.88	41.1 ± 1.8	0.07	0.045	44.3 ± 0.7
PMales vs. Females	p = 0.38	p = 0.84		p = 0.69			p = 0.49
<i>AST/ALT</i>							
All patients	1.18 ± 0.03	1.23 ± 0.12	0.58	1.26 ± 0.11	0.32	0.89	1.21 ± 0.04
Males	1.14 ± 0.08	1.04 ± 0.39	0.55	1.28 ± 0.20	0.50	0.44	1.17 ± 0.05
Females	1.18 ± 0.03	1.25 ± 0.13	0.54	1.26 ± 0.20	0.41	0.97	1.21 ± 0.05
PMales vs. Females	p = 0.69	p = 0.69		p = 0.93			p = 0.79

Associated of clinical and laboratory factors with LD in SLE. p values reflect two-tailed χ^2 test. p values < 0.05 are in italics.

Table 3

	Normal (N)	iLD	LD	PN vs. iLD	PN vs. iLD + LD	PiLD vs. LD	PN vs. LD
SMA (+)	10 (13.0%)	11 (26.8%)	13 (29.5%)	0.06	0.02	0.78	0.03
SMA (-)	67 (87.0%)	30 (73.2%)	31 (70.5%)				
AMA (+)	4 (5.1%)	3 (7.3%)	1 (2.3%)	0.62	0.92	0.28	0.47
AMA (-)	75 (94.9%)	38 (92.7%)	42 (97.7%)				
Proteinuria (+)	20 (9.2%)	16 (12.8%)	18 (20.5%)	0.30	0.03	0.13	0.007
Proteinuria (-)	197 (90.8%)	109 (87.2%)	70 (79.5%)				
Anti-DNA (+)	20 (11.9%)	13 (14.9%)	17 (27.4%)	0.49	0.048	0.06	0.005
Anti-DNA (-)	148 (88.1%)	74 (85.1%)	45 (72.6%)				
Hypocomplementemia	43 (25.9%)	27 (28.7%)	28 (41.8%)	0.62	0.10	0.08	0.02
Normal Complement	123 (74.1%)	67 (71.3%)	39 (58.2%)				
WBC <4000 K/ul	6 (2.8%)	2 (1.6%)	8 (8.9%)	0.49	0.30	0.01	0.02
WBC >4000/ul	211 (97.2%)	124 (99.2%)	82 (91.1%)				
Platelet <100,000/ul	6 (2.8%)	7 (5.6%)	12 (13.3%)	0.17	0.007	0.047	0.0003
Platelet >100,000/ul	211 (97.2%)	119 (94.4%)	78 (86.7%)				
APS (+)	28 (14.0%)	20 (17.0%)	25 (30.0%)	0.52	0.038	0.039	0.0025
APS (-)	171 (86.0%)	98 (83.0%)	58 (70.0%)				

Table 4

Relationship of medication use to LD in SLE. p values reflect two-tailed χ^2 test. p values <0.05 are in italics.

	Normal (N)	iLD	LD	PN vs. iLD	PN vs. LD + LD	PIED vs. LD	PN vs. LD
HCQ (+)	109 (50.0%)	56 (44.8%)	37 (41.1%)	0.35	0.16	0.59	0.16
HCQ (-)	109 (50.0%)	69 (55.2%)	53 (58.9%)				
MMF (+)	21 (9.6%)	8 (6.4%)	15 (16.65%)	0.42	0.75	<i>0.023</i>	0.08
MMF (-)	197 (90.4%)	117 (93.6%)	74 (83.3%)				
MPA (+)	9 (4.1%)	8 (6.4%)	3 (3.3%)	0.35	0.62	0.31	0.74
MPA (-)	209 (95.9%)	117 (93.6%)	87 (96.7%)				
AZA (+)	6 (2.8%)	9 (7.2%)	7 (7.8%)	0.053	<i>0.02</i>	0.87	<i>0.046</i>
AZA (-)	212 (97.2%)	116 (92.8%)	83 (92.2%)				
PRED (+)	52 (23.9%)	39 (31.2%)	41 (45.6%)	0.14	<i>0.002</i>	<i>0.03</i>	<i>0.0002</i>
PRED (-)	166 (76.1%)	86 (68.8%)	49 (54.4%)				
MTX (+)	12 (5.5%)	13 (10.4%)	5 (5.6%)	0.09	0.23	0.21	0.99
MTX (-)	206 (94.5%)	112 (89.6%)	85 (94.4%)				
CTX (+)	0 (0.0%)	1 (0.8%)	2 (2.2%)	0.19	0.08	0.38	<i>0.03</i>
CTX (-)	218 (100%)	124 (99.2%)	88 (97.8%)				
CsA (+)	1 (0.5%)	0 (0%)	4 (4.4%)	1.00	0.213	<i>0.0287</i>	<i>0.0269</i>
CsA (-)	217 (99.5%)	125 (100%)	86 (95.6%)				
Rapa (+)	6 (2.8%)	4 (3.3%)	0 (0%)	1.00	0.75	0.141	0.1858
Rapa (-)	212 (97.2%)	121 (96.7%)	90 (100%)				
NAC (+)	4 (1.8%)	2 (1.6%)	0 (0%)	0.87	0.64	0.76	0.65
NAC (-)	214 (98.2%)	123 (98.4%)	90 (100%)				
Rituximab (+)	1 (0.5%)	1 (0.8%)	0 (0%)	0.69	0.99	0.40	0.52
Rituximab (-)	217 (99.5%)	124 (99.2%)	90 (100%)				
Anti-TNF (+)	0 (0%)	1 (0.8%)	1 (1.1%)	0.19	0.15	0.81	0.12
Anti-TNF (-)	218 (100%)	124 (99.2%)	89 (98.9%)				
NSAIDs (+)	86 (39.4%)	44 (35.2%)	23 (25.6%)	0.435	0.07	0.13	<i>0.02</i>
NSAIDs (-)	132 (50.6%)	81 (64.8%)	67 (74.4%)				

Abbreviations: AZA, azathioprine; CsA, cyclosporine A; CTX, cyclophosphamide; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; MPA, mycophenolate acid; NAC, N-acetylcysteine; NSAID, non-steroidal anti-inflammatory drug; PRED, prednisone; Rapa, rapamycin/sirolimus; Anti-TNF, tumor necrosis factor blocker.

Table 5

Medication changes with normalization of LD in SLE. p values reflect two-tailed χ^2 test. p values <0.05 are in italics.

Patient count	LD	Normalized	p
Hydroxychloroquine (+)	37	32	0.90
Hydroxychloroquine (-)	53	44	
Mycophenolic mofetil (+)	14	12	0.97
Mycophenolic mofetil (-)	76	64	
Mycophenolic acid (+)	3	2	0.79
Mycophenolic acid (-)	87	74	
Azathioprine (+)	7	7	0.74
Azathioprine (-)	83	69	
Prednisone (+)	41	47	<i>0.043</i>
Prednisone (-)	49	29	
Methotrexate (+)	5	3	0.63
Methotrexate (-)	85	73	
Cyclophosphamide (+)	2	3	0.52
Cyclophosphamide (-)	88	73	
Cyclosporine (+)	4	6	0.75
Cyclosporine (-)	85	83	
Rapamycin (+)	0	2	0.155
Rapamycin (-)	90	88	
N-acetylcysteine (+)	0	1	1.00
N-acetylcysteine (-)	90	89	
Rituximab (+)	0	0	NA
Rituximab (-)	90	76	
Anti-TNF (+)	1	0	0.36
Anti-TNF (-)	89	76	
NSAIDs (+)	23	12	0.12
NSAIDs (-)	67	64	