Background: Lupus nephritis is a severe and life-threatening manifestation of systemic lupus erythematosus. Tubulointerstitial hypoxia is a key factor in the progression to end-stage renal disease. Numerous aquaporins are expressed by renal tubules and are essential for their proper functioning. Several mouse models have shown their involvement in the regulation of renal inflammation during acute stress episodes. However, the expression of aquaporins (AQPs) in human lupus nephritis has been poorly studied.

Objectives: The aim of this study is to characterise the tubular expression of AQP1, AQP2 and AQP3 which could provide a better understanding of tubulointerstitial stress during lupus nephritis.

Methods: This retrospective monocentric study was conducted at Erasme-HUB Hospital, Brussels with the approval of the ULB-Erasme Ethical Committee (P2020/710). A total of 37 lupus nephritis samples and 9 healthy samples collected between 2000 and 2020 were obtained from the biobank of the pathology department. Kidney biopsy sections were reviewed according to the ISN/RPS 2018 classification. Immunohistochemistry was performed to detect AQP1, AQP2 and AQP3 and followed by digital image analysis. Digital image quantification analysis was performed using ImageJ software.

Results: We observed weak expression of AQP1 in glomeruli and strong expression on the apical side of proximal convoluted tubules in the cortex. In the medulla, it was confined to the descending loop of Henle and the vasa recta. AQP2 was exclusively expressed on the apical side of collecting tubules in both the cortex and the medulla. AQP3 was not detected in glomeruli, weakly expressed on the basolateral side of proximal convoluted tubules, and strongly expressed on the basolateral side of distal convoluted tubules and collecting tubules. No difference in staining location was found between healthy and lupus nephritis kidney biopsies. By digital quantitative analysis, we observed a significant decrease in AQP1 expression in the renal cortex (p<0.0001), a non-significant trend towards a decrease in AQP2 expression and a significant cortical and medullary decrease in AQP3 expression (p<0.001). This decrease was more pronounced in the subgroup of membranoproliferative glomerulonephritis (class III/IV), particularly for AQP3 (p<0.05). Within the subgroup of membranoproliferative glomerulonephritis, there was a strong negative correlation between both cortical and medullary AQP2 expression and interstitial inflammation (r=-0.5238; p<0.01). Decreased cortical AQP3 expression was strongly negatively correlated with interstitial fibrosis (r=-0.6651; p<0.001) and tubular atrophy (r=-0.6651; p<0.01).

Conclusion: We found a significant decrease in the expression of several AQPs in the parenchyma of lupus nephritis. We believe that this decrease is a feature of tubulointerstitial damage. Immunohistochemical analysis of AQPs on lupus nephritis samples could help to assess the tubulointerstitial hypoxia and cell damage and therefore renal prognosis.

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Fig. 1 Aβ2GPI antibodies bind to NETs

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Antibodies: Anti-phospholipid syndrome, Cell biology, Autoantibodies

Keywords: Anti-phospholipid syndrome, Diet and nutrition, -Oncics

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Background: NETosis has been described to play a role in the pathogenesis of APS. IgG and sera of APS patients are known to induce NETosis [1], however the direct role of a(2)GPI on NETosis is not fully known.

Objectives: The aim of this study is to evaluate the interplay between NETosis and a(2)GPI antibodies.

Methods: HD neutrophils were stimulated either with polyclonal a(2)GPI isolated from a serum pool of primary APS, with immunoglobulin isolated from HD (IgHD) or PMA. NETs were stained with anti-neutrophil elastase, SYBR green and DAPI. NET quantification was performed with NEOTQUANT, an automated approach. To evaluate the ability of a(2)GPI to bind to NET, HD neutrophils were stimulated with PMA and stained with anti-neutrophil elastase, SYBR green and with a(2)GPI and, as a control, with IgHD instead of a(2)GPI. Colocalization of a(2)GPI and NET signal was performed with Just Another Co-localization Plugin (JACoP). In order to evaluate the ability of a(2)GPI to bind to NETs and prevent DNA degradation, incubation with DNase I was also performed.

Results: Stimulation of HD neutrophils with a(2)GPI was able to induce a significantly higher number of NETs compared to stimulation with IgHD (77.6% vs 20%, p<0.0001). The prevalence of NETs in stimulated IgHD and unstimulated neutrophils was similar (20% vs 16%, p=ns). Two different shapes of NET were identified in neutrophils stimulated with a(2)GPI: “cloudy-like” and “spiky-like”. Unlike IgHD, a(2)GPI binds to NETs (Figure 1), and the a(2)GPI signal colocalized with the anti-neutrophil elastase signal at 93.6%. In addition, we showed that a(2)GPI binding to NETs did not prevent NETs degradation by DNase.

Conclusion: a(2)GPI antibodies are able to induce NETosis and to bind to NETs.


Antibodies: Aβ2GPI, Aβ2GPI iso-

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Background: In a systemic lupus erythematosus (SLE) murine model, the translocation of a gut pathobiotic induced an autoimmune response and death, which were prevented by antibiotics and a vaccine [1]. These findings suggest that the gut microbiota modulates SLE phenotype. Previously, we found increased circulating lipopolysaccharide (LPS) in SLE patients, which could be associated with decreased gut barrier integrity and translocation from the gut to the bloodstream [2]. We hypothesize that dysbiosis, impaired intestinal barrier integrity, and endotoxemia are crucial to the chronic activation of the immune system seen in SLE.

Objectives: To study diet, physical activity, body composition, gut microbiota, and gut permeability in SLE patients in comparison with healthy controls (HC).

Methods: Evaluation of HC and SLE patients (children and adults) who fulfill the 2019 EULAR/ACR SLE classification criteria. Individuals with inflammatory bowel disease, celiac disease, irritable bowel syndrome, diabetes, malignancy, or other immune-mediated diseases were excluded. The SLEDAI-2K score was used to evaluate disease activity. Diet and physical activity were assessed by three-day diet recall, PREDIMED, KIDMED, and the International Physical Activity questionnaires. Body composition was analysed by whole-body air-displacement plethysmography. Gut microbiota was studied by Next Generation Sequencing, with amplicon sequencing-based 16S rRNA analysis. The lactulose/mannitol test, which directly assesses intestinal permeability, was quantified by mass spectrometry (LC-MS/MS). Serum markers of gut permeability and inflammation (zonulin, sCD14, IFABP) were measured by ELISA. The biological activity of LPS was assessed through serum-induced toll-like receptor 4 (TLR4) stimulation in a reporter cell line.

Results: We studied 16 HC (median age 35.5Y [14-50Y]; 88% females) and 45 SLE patients (11 children and 34 adults; median age 32Y [11-67Y]; 87% females; median age at diagnosis 19Y [8-43Y]; median disease duration 7Y [3M-29Y]; 64% had lupus nephritis; median SLEDAI-2K at sample collection 4).

SLE patients had lower physical activity and higher sitting time, lower adherence to the Mediterranean diet, and higher fat mass than HC (p<0.05). In addition, SLE patients had a lower intake of α-linolenic acid and manganese (p<0.05). A decreased α-diversity of gut microbiota (p<0.05) was identified in SLE patients, reflecting dysbiosis. Lower adherence to the Mediterranean diet, higher zonulin levels, and longer SLE disease duration were significantly associated with decreased gut diversity in this cohort (p<0.05). The lactulose/mannitol ratio was significantly higher in SLE patients compared to HC (p<0.05), reflecting greater gut permeability. Patients with lupus nephritis had a higher lactulose/mannitol ratio than SLE patients without renal involvement (p=0.05). Interestingly, we found that zonulin was significantly increased in SLE patients (p<0.05).

We also found significantly increased levels of sCD14 in SLE patients (p<0.05) and increased levels of IFABP, but only in adult patients (p<0.05). No significant correlation was observed between any evaluated biomarker and SLEDAI-2K.

The serum of SLE patients induced a significantly higher TLR4 response compared to HC (p<0.05), which may reflect endotoxemia.

Conclusion: Our data support the hypothesis that gut dysbiosis and higher intestinal permeability contribute to SLE pathogenesis, being two promising therapeutic targets in this disease.

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[1] PMID: 29590047
[2] PMID: 24796678

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POS1427

GENETICALLY PROXIED THERAPEUTIC INHIBITION OF ANTIHYPERTENSIVE DRUG TARGETS AND RISK OF SYSTEMIC LUPUS ERYTHEMATOSUS: A MENDELIAN RANDOMIZATION ANALYSIS

Keywords: Systemic lupus erythematosus, Genetics/epigenetics

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by production of autoantibodies directed against nuclear and cytoplasmic antigens, affecting several organs. SLE is associated with a high burden of cardiovascular disease. Hypertension is frequent among patients with SLE and studies show that SLE patients are at a higher risk of experiencing hypertension events than the general population [1]. Alpha-adrenoceptor blockers, Angiotensin converting enzyme inhibitors (ACEI), Beta-adrenoceptor blockers (BB) and/or Calcium channel blockers (CCB) are generally recommended as the first-line antihypertensive therapy. Evidence from large randomized controlled trials comprehensively comparing risk of SLE of different classes of antihypertensive drugs is lacking.

Objectives: We used Mendelian randomization to investigate the potential effects of different antihypertensive drug classes on SLE.

Methods: We identified SNPs to proxy the protein targets of antihypertensive drugs on the basis that they mimicked the action of that drug on the target. Systemic blood pressure data were retrieved from the International Consortium of Blood Pressure Genome-Wide Association Studies, based on 757,601 individuals of European ancestry [2]. For each drug target, single-nucleotide polymorphisms (SNPs) in the drug target gene region (± 100kb) that were associated with SBP (p< 5 × 10–8) were selected as proxies for drug target perturbation after clumping to a pairwise linkage disequilibrium threshold of r2 < 0.3 using the 1000 Genomes Europe reference panel. Summary level statistics of SLE were obtained from the latest and most extensive GWAS database, including 14,267 individuals of European ancestry (5,201 cases and 9,066 controls) [3]. Mendelian randomization was then performed using the inverse variance weighted (IVW), MR-Egger and Weight Median (WM). If there was only a single variant available, we used the Wald ratio to estimate the effect.

Results: We found that a 1-SD decrease in blood ADRA1A expression (target for Alpha-adrenoceptor blocker) was associated with a lower risk of schizophrenia (IVW method, OR, 0.686, 95% CI, 0.641-0.934, P = 6.845×10–6). Genetically predicted effects of CACNB2 (target for CCB) decreased SLE risk (MR-Egger method, OR, 0.774, 95% CI, 0.660-0.908, P = 0.004; WM method, OR 0.959, 95% CI, 0.873-0.988, P = 0.019) (Figure 1). And other classes of antihypertensives drug targets had no effect on SLE risk.

Conclusion: Mendelian randomization suggests that CCB and Alpha-adrenoceptor blocker is likely to increase the risk of SLE. These findings warrant greater pharmacovigilance and further investigation into the effect of CCB and Alpha-adrenoceptor blockers on SLE susceptible population, as well as SLE patients with cardiovascular complications.

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Figure 1: forest plot of the association between a 1-SD change in expression of 10 listed pressure-lowering drug target genes in blood with risk for systemic lupus erythematosus. Data are represented as odds ratios (ORs) with 95% CIs (inner bars).