THE EFFECT OF METFORMIN ADD-ON THERAPY IN PREVENTING SYSTEMIC LUPUS ERYTHEMATOSUS FLARES. A RANDOMIZED PLACEBO CONTROLLED TRIAL

Ghada Mohamed Hasan EL-kannishy[1], Khaled Farouk Abel Alaziz EL-Dahshan[1], Amani Mostafa Ismail[2], Eman Mahmoud El Sayed El Refaie[*2]

Article History: Received: 02.04.2023 **Revised:** 05.05.2023 **Accepted:** 10.05.2023

Abstract

Systemic lupus erythematosus (SLE) is a chronic inflammatory systemic autoimmune disease. Recurrent relapses of disease and development of long-term organ damage are two key unsolved clinical problems. The pathogenesis of SLE is complex, and it is increasingly recognized that the overactive immune system driving the disease presents metabolic abnormalities, offering novel therapeutic opportunities.

Although both the short- and long-term survival rates of SLE patients have increased tremendously over the past 50 years, the mortality rate of SLE patients remains 2–4 times higher than that of healthy individuals.

Recently, a number of mechanistic studies have identified potential benefits of metformin in the treatment of SLE. Metformin has been repurposed for multiple autoimmune conditions because it reverses aberrant metabolism in an array of immune cell lineages, such as T-helper 1 cells, T follicular helper cells, T-helper 17 regulatory cells, plasmablasts, neutrophils, and plasmacytoid dendritic cells.

The mode of action of metformin is multifaceted. It can exert a regulatory effect through the suppression of oxidative phosphorylation by inhibiting mitochondrial electron transport chain complex 1, and through the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway, or via AMPK-independent pathways.

Keywords: Systemic lupus erythematosus, Metformin.

1. Department of Internal Medicine, Faculty of Medicine, Mansoura University.
2. Department of Consultant of clinical pathology Urology and Nephrology Center, Faculty of Medicine, Mansoura University
[*]Corresponding Email: dr.e.refaie@gmail.com

1. LUPUS NEPHRITIS

SLE is a multi-systemic autoimmune disease with high heterogeneity. The hallmark of SLE pathogenesis is the production of autoantibodies (1), which results from a combination of genetic, epigenetic, environmental, hormonal, and immune-regulatory factors (2). The heterogeneity is expressed with different clinical phenotypes that range from which organs are inflicted to the way that disease is caused at a specific organ and can be attributed to different autoantibody profiles, genetic variants, and interferon levels (3).

Kidney involvement in SLE (LN) is a common and potentially life-threatening form of the disease. There are diverse ways with which SLE can cause kidney disease, such as lupus podocytopathy (4), tubulointerstitial disease (5), and syndromes like thrombotic thrombocytopenic purpura (6), but the usual form of kidney involvement is lupus nephritis (LN). LN is a form of glomerulonephritis in SLE patients, which is characterized by the presence of stains for immunoglobulin G (IgG), immunoglobulin M (IgM), C3, and C1q in the immunofluorescence (IF) (7). Patients with LN have been shown to have higher rates of morbidity and mortality compared with patients without renal involvement. There are different classes of LN that present different clinical signs which have different prognosis.

Lupus nephritis

Table (1): mechanism and pathogenesis of LN (8)

Mechanism	Way of activation	Effect
Surplus apototic material	Reduced renal DNAse1	Production of autoantibodies
Autoantibodies	Surplus apoptotic material	Activation of macrophage dendritic cells
B cells	Cytokines by macrophages	Production of autoantibodies Aggregation of production of tertiary lymphatic cells

NETs	Autoantibodies	Production of autoantigen Complement activation Tissue damage
Type1 interferon	Plasmacytoid dendritic cells	Overexpression of toll- like receptor 7
Complement	Surplus apoptotic material Reduced ability of complement to remove apoptotic material	Tissue damage

Histopathology and clinical phenotypes of LN

Lupus nephritis is an immune complex disorder of the kidney that may present with many faces. Periods of remission and exacerbation are typically found during the course of the disease. The pathological features can also be varied, including glomerular lesions, but also tubulointerstitial and vascular lesions. The major pathological findings are described in the LN Classification of 2003 by a consensus meeting of renal pathologists, nephrologists, and rheumatologists of the American Society of Nephrologists (ISN) and Renal Pathology Society (RPS), while previous classification schemes had been proposed by pathologists and nephrologists under the auspices of the World Health Organization (WHO) (9).

Class I: is characterized by mesangial immune deposits in IF, but no morphological changes in light microscopy, according to the classification of ISN/RPS 2004. Urinary abnormalities are minimal and include microscopic hematuria with mild proteinuria, while renal function is normal. This is the mildest glomerular lesion in LN and is relatively rare, since these patients generally have no essential clinical renal abnormalities and are not referred to nephrologists for biopsy.

Class II: is defined by purely mesangial hypercellularity of any degree, or mesangial matrix expansion by LM, with mesangial immune deposits. No subendothelial deposits visible by light microscopy are allowed for this class. Only few subendothelial or subepithelial deposits visible by IF or EM are allowed. Urinary abnormalities are mild and include microscopic hematuria with mild proteinuria, while renal function is usually normal. If nephrotic syndrome is observed, in an otherwise typical case of class II nephritis, with no subepithelial deposits, the possibility of lupus podocytopathy should be examined.

Class III: includes active or inactive focal and segmental endocapillary and/ or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits with or without mesangial alterations. Microscopic or macroscopic hematuria and severe proteinuria are usually seen. Lupus serologies are usually active.

Class IV includes active or inactive diffuse segmental and/or global endocapillary and/or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits with or without mesangial alterations. These patients have the most severe and active clinical renal presentation. Proteinuria can reach nephrotic level, and many patients (up to 50%) can present with nephrotic syndrome. Urine sediment is active, while red blood cell (RBC) casts are common. Renal insufficiency can be demonstrated by glomerular filtration rate (GFR), although serum creatinine can be normal, especially in young women with little muscle mass. Hypertension can be observed, while lupus serologies are active.

Class V: includes membranous LN with global or segmental subepithelial immune deposits by LM and IF or EM, with or without mesangial alterations. Severe proteinuria or nephrotic syndrome is usually seen in many cases accompanied by microscopic hematuria. Renal insufficiency is uncommon.

Class VI: includes advanced sclerosing LN. Urinary abnormalities consisted of proteinuria of varying degree with inactive sediment, while renal function is impaired. Hypertension is common, while lupus serologies may be inactive (i.e., "burnt-out" lupus). There are also mixed classes in LN that include classes III and V and **Classes IV and V**. In addition, an activity and chronicity index has been proposed to determine the severity of disease, providing prognostic as well as therapeutic indications for patients' management. Commonest classes in biopsies samples, according to various studies are classes III, IV, and V. (10). Among the first five classes, **Classes III and IV** have the worst prognosis. Classes III and IV are characterized by high activity. "Wire" loops (thickened eosinophilic glomerular membranes occupied by deposits), eosinophilic "hyaline" thrombi, and numerous inflammatory cells into capillary lumens including neutrophils, nuclear "debris," membranoproliferative pattern, glomerular crescents, and/or necrosis can be seen **Figures (1).**

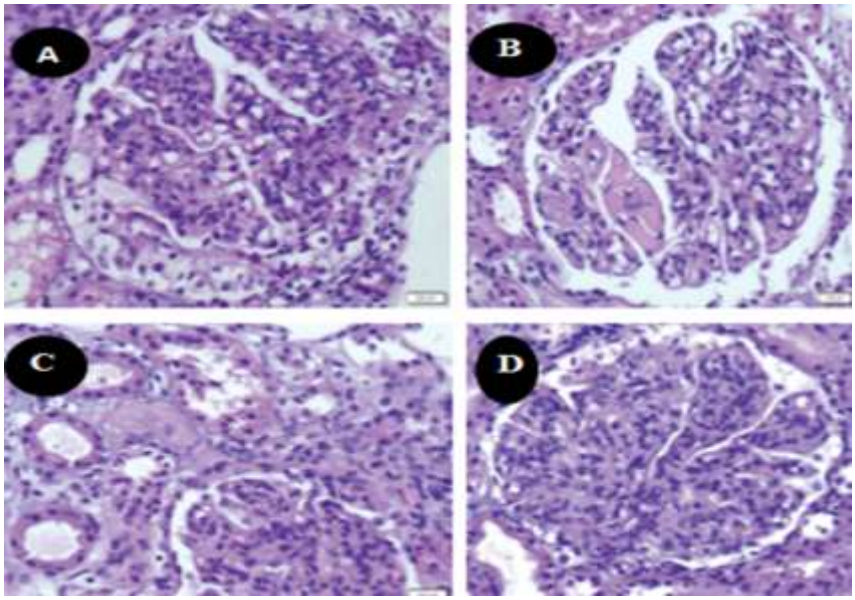

Figure (1A): Severe endocapillary cellularity/proliferation in association with crescent formation in the left corner [H&E X400]. **Figure (1B):** Mesangial and endocapillary cellularity/proliferation in association with "hyaline" thrombi into glomerular lumens [H&E X400]. **Figure (1C):** Immune complex deposits in an arteriole, the so-called lupus "vasculopathy" [H&E X400]. **Figure (1D).** Membranoproliferative pattern in LN [H&E X400] (11).

In repeat biopsies, a "transformation" phenomenon has been described, from one class to another, usually after treatment, or spontaneously. Class III to class IV is a common transformation in repeat biopsies, but many authors prefer to interpret it as a transition along a disease continuum, rather than a true transformation (12).

Mesangial proliferation is often seen after the treatment of class III or class IV LN, although ultrastructurally residual irregularities of the glomerular basement membrane consisted of resorbed and organized subendothelial deposits can be seen. Virtually, all directions of transformation have been described.

Some investigators have proposed that class IV-S is pathogenetically distinct from other LN. (13) described a category of "severe segmental glomerulonephritis," in which the glomerular inflammation was predominantly segmental.

2. Management of Lupus Nephritis

1. Class I (minimal mesangial) and class II (mesangial proliferative):

These patients have an excellent renal prognosis, and there is no reason to treat with immunosuppressive therapy (14) in the absence of extrarenal manifestations. An exception is warranted for patients with nephrotic syndrome or nephrotic range proteinuria, who have class I or II in histology. These patients probably have lupus podocytopathy. In this regard, electron microscopy is helpful to establish the diagnosis by demonstrating podocyte effacement. The usual treatment consists of oral prednisolone 1 mg/kg once daily (maximum dose of 80 mg) for one to four months followed by gradual tapering after achieving remission (15).

2. Class III (focal) and class IV (diffuse) lupus nephritis:

Class III and IV LN are aggressive diseases that require a quick and effective implementation of the therapeutic strategy. The therapeutic goal of patients with the above histological classes is the achievement of complete response, which translates to the recession of immunologic and inflammatory activity.

The clinical criteria of defining a response to therapy are somewhat controversial and not universal, because a series of clinical studies and/or associations have defined different goals for a complete response. Nevertheless, all response criteria agree on the reduction of proteinuria and the improvement of the kidney function. We most commonly use the criteria published by the Improving Global Outcomes (KDIGO) Consensus Conference guidelines for glomerulonephritis, namely the reduction of proteinuria to <0.5 g/day measured by 24-hour urine collection or by the protein-to-creatinine ratio, the stabilization or improvement of the kidney function (±10% of the baseline) in a period of 6–12 months of therapy, as well the normalization of the urine sediment to red blood cells (RBCs) to ≤10 high-power field and absence of RBC cast.(16) Therapy must be initiated promptly after the acquisition of the diagnosis because a delay is related to irreversible kidney damage (17).

We most commonly initiate the therapy with the administration of 0.5–1 mg/kg/day prednisolone (maximum dose 80 mg/day) followed by a gradual tapering at three to six months. When the clinical or histological findings are more severe (worsening of kidney function and crescents formation), then a therapeutic opening with intravenous daily pulses of 0.5–1 g methylprednisolone for three days is preferred (18).

3. Class V (lupus membranous nephropathy):

The majority of patients with this histological class are presented with nephrotic syndrome or nephrotic-range proteinuria. Lupus patients with nephrotic syndrome due to membranous nephropathy should receive immunosuppression. Patients with nephrotic-range proteinuria despite the use of renin-angiotensin system blockers and/ or patients with worsening of their kidney function should also receive immunosuppressive therapy (16).

Calcineurin inhibitors that is, cyclosporin or tacrolimus, should be given cautiously in patients with impaired kidney function considering its potential for nephrotoxicity. According to the KDIGO and the EULAR guidelines, MMF is a reasonable first line of choice in these patients. However, if MMF is proven ineffective, cyclophosphamide may be used for six months in an effort to induce long-term remission (19). Long term calcineurin inhibitor or rituximab may also be tried if the patient had prior significant exposure to cyclophosphamide or if there are other contraindications.

The dose of MMF and CYC is the same as for the treatment of class III and IV LN. Cyclosporine, when used, is started at 3–5 mg/kg/day in two divides doses and tacrolimus at 0.05–0.1 mg/kg/day in two divided doses. Consequently, we measure whole blood trough cyclosporine or tacrolimus levels, and 2 hours after receiving dose [C2] levels for cyclosporine to navigate through therapy. The desired trough levels range from 100 to 200 ng/ml for cyclosporine and 4–6 ng/ml for tacrolimus, whereas it is 600–800 ng/ml for C2 cyclosporine levels.

Patients who have concurrent lupus membranous nephropathy and focal or diffuse LN are treated with the same approach as used for those with focal or diffuse LN alone **Table (2).**

Table (2): induction therapy of LN class III, IV, III+V or IV+V (16)

Treatment	Dose	Line of treatment
Cyclophosphamide (NIH)	0.5-1g/ m^2 Monthly for six months	First line
Cyclophosphamide (Eurolupus)	0.5 g every two weeks for three weeks	First line
Mycophenolate	3 g/day	First line
Glucocorticoids	0.5-1 mg/kg/day Tapering for three to six months	First line
Tacrolimus	4 mg/day	Part of multi target therapy
Belimumab	10mg/kg per 28 weeks	Add on regular therapy

Class VI (advanced sclerosing lupus nephritis):

Class VI disease is characterized by global sclerosis of more than 90% of glomeruli. The immunosuppressive therapy is highly unlikely to benefit them, and it will only produce adverse effects. Hence, these patients need to be treated as chronic kidney disease to control the blood pressure, to reduce the proteinuria by using renin-angiotensin system blockers, and to prepare for the next step, when it is needed, the kidney replacement therapy.

3. GENERAL MANAGEMENT:

General supportive measures in all patients with LN, as with other patients with glomerulonephritis, include the restriction of dietary sodium intake to <2 g/day, the restriction of protein intake to 0.8 g/kg/day for patients with chronic kidney disease with a GRF < 60 ml/min/1.73 m2, blood pressure control with a goal of <120– 130/80 mmHg, the use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker to maximally tolerated or allowed daily dose for the minimization of proteinuria and for the concomitant control of the blood pressure, the treatment of hyperlipidemia with lifestyle modifications (exercise, weight reduction, and smoking cessation), and the use of statins when needed, thrombosis prophylaxis for patients with nephrotic syndrome, and prophylaxis for Pneumocystis jirovecii pneumonia with trimethoprim/sulfamethoxazole, and the minimization of bone loss and osteoporosis prophylaxis due to the long-term glucocorticoid treatment (16).

Management of complications (ESKD)

Patients who develop ESKD can be managed with kidney transplantation, hemodialysis, or peritoneal dialysis. As with the other causes of ESKD, kidney transplantation is the best modality, with the best overall prognosis and survival, and so is preferred over hemodialysis or peritoneal dialysis (20). A preemptive transplantation may be carried out when the extrarenal manifestations do not bear any contraindication for surgery (21).

The recurrence rate of LN at the kidney allograft was examined using the United Network for Organ Sharing files, and it was found in 2.4% (167 of 6780 patients) (22). Among the patients who are on hemodialysis or peritoneal dialysis, there is no difference regarding the survival rates and mortality (23). It must be noted, interestingly, that the development of ESKD and the initiation of a kidney replacement therapy are in the majority of patients associated with a complete or partial remission of the extrarenal manifestations of SLE (24).

Prognosis of LN and risk factors for progression

The percentage of patients that achieve a complete remission within six months of therapy is 30% (25). Although the rates over the last decades have been becoming better, up to 20% of patients with LN will ultimately develop ESKD (26). Thus, the ability to predict the long-term renal outcome is of vital importance. A better long-term prognosis is associated with attaining the complete response of active LN. What favors the long-term renal outcome is the early decrease of proteinuria levels over six months of treatment compared with patients with persistently high-grade proteinuria(27) .

Regarding the demographic risk factors, caucasians have the best prognosis and Africans the worst, whereas Asians have an intermediate prognosis. Black patients present worse outcomes with increased rates of ESKD and mortality (27).

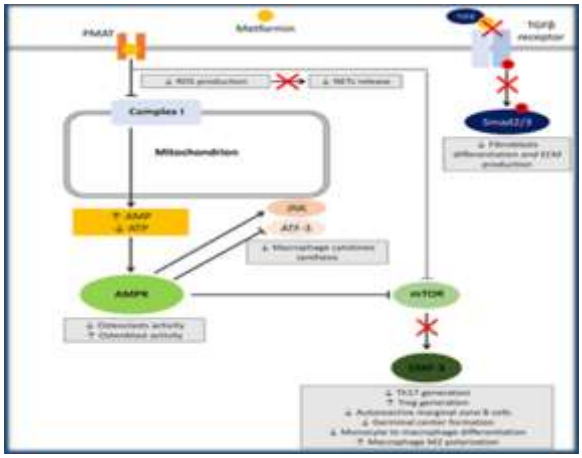

Figure (2): Mechanism of action of metformin. (28)

Metformin exerts anti-inflammatory effects by regulating autophagy and apoptosis. It was suggested in an earlier study that metformin could exert anti-inflammatory effects by targeting lysosomal proteolysis, similar to chloroquine (29) . The difference was that chloroquine swells lysosomal vesicles and raises the vacuolar pH, whereas metformin binds to metal ions and synergizes their inhibitory action. In another study, metformin improved impaired autophagy to exert anti-inflammatory effects in autoimmune arthritis (30). (31) (Kim et al) found that metformin normalized metabolic dysfunction in obese CIA mice by inducing fibroblast growth factor 21 (FGF21) expression and brown adipose tissue differentiation. Splenocytes co-cultured with brown adipose tissue reduced Th17 cell differentiation, supporting the therapeutic role of metformin in autoimmune arthritis and SLE.

Actions of metformin in the liver:

Metformin exerts direct effects on hepatic glucose and lipid metabolism. Metformin suppresses gluconeogenesis mainly through AMPK-dependent activation of key enzymes, whereas it enhances glucose uptake and glycolysis through the activation of hexokinase and pyruvate kinase.

Actions of metformin on skeletal muscle:

Metformin may moderately increase basal and insulin-stimulated glucose uptake. The former effect appears to be mediated by AMPK activation and subsequent aPKC and GLUT-4 activation, while the latter effect may involve the enhancement of insulin signaling either directly or indirectly through the attenuation of glucotoxicity, lipotoxicity, and inflammation.

Actions of metformin on the adipose tissue:

Metformin may inhibit agonist-induced lipolysis in adipocytes via inhibition of ERK1/2 phosphorylation, but it may counteract adipose tissue expansion through AMPK-dependent stimulation of FA oxidation and inhibition of lipogenesis in subcutaneous fat depot.

4. PHARMACOKINETICS OF METFORMIN

Metformin has an oral bioavailability of 50–60% under fasting conditions and is absorbed slowly. Peak plasma concentrations (Cmax) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations. The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution. Steady state is usually reached in one or two days (32).

Metformin has low lipophilicity and, consequently, rapid passive diffusion of metformin through cell membranes is unlikely. Metformin is not metabolized. It is cleared from the body by tubular secretion and excreted unchanged in the urine. Metformin is undetectable in blood plasma within 24 hours of a single oral dose. The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to (and appears to accumulate in) red blood cells, with a much longer elimination half-life: 17.6 hours (reported as ranging from 18.5 to 31.5 hours in a single-dose study of non-diabetic people) (33).

5. MEDICAL USES OF METFORMIN:

a. **Type 2 diabetes:**
b. **Polycystic ovary syndrome (PCOS):**
c. **Prevention of weight gain.**
d. **Cancer prevention.**

Adverse effects of metformin

1. **Gastrointestinal:**
2. **Lactic acidosis:**
3. **Hormonal effects:**
4. **Vitamin B12 deficiency:**

Contraindications of metformin:

Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including:

- Kidney disorders:
- Furthermore, metformin should be avoided or used with caution in patients with acute or chronic kidney disease, as it can increase the risk of lactic acidosis, a rare but serious side effect of metformin that can occur in patients with impaired renal function.
- Lung disease and liver disease.
- Unstable or acute congestive heart failure
- Metformin is recommended to be discontinued before radiographic study involving iodinated contrast agents, (such as a contrast- enhanced CT scan or angiogram).

6. METFORMIN IN SLE

Metformin controlled adaptive immune responses in SLE animal models. In experiments with murine SLE, metformin decreased autoantibody titers and target organ inflammation, including lupus nephritis (34).

Metformin inhibited germinal center reaction, controlled B cell differentiation into plasma cells, and regulated T cell differentiation by reducing Tfollicular helper (Tfh) and Th17 cells and increasing regulatory T cells (Treg cells). The regulation of B and T cells by metformin was associated with AMPK activation, and mTOR and STAT3 inhibition. In addition, metformin enhanced the immunomodulatory effects of other treatment (35).

Metformin-treated mice had increased Treg cells, and decreased Th17 cells, Tfh cells, germinal center B cells, and pro inflammatory cytokine production, along with mTOR and STAT3 suppression in skin fibroblasts. As gout is an inflammatory arthritis induced by monosodium urate (MSU) crystals deposited in tissues, including joints, and where the inflammation is mediated by mTOR signaling in monocytes, inhibition of mTOR might have therapeutic benefits in gout (36). mTOR inhibition by metformin resulted in reduced MSU crystal-induced monocyte death (pyroptosis) and inflammation in vitro; gout patients exhibited a decreased frequency of gout attacks (36).

Metformin inhibits the proliferation and migration of (FLS) Fibroblast-Like Synoviocytes, regulates Th17 and Treg cell differentiation, suppresses osteoclast differentiation and activity, and reduces inflammatory cytokine production. Metformin can also enhance autophagy and improve mitochondrial functions. Metformin decreases chondrocyte apoptosis and cartilage catabolism. These effects are mediated by the modulation of multiple interacting intracellular pathways, such as PI3K-AKT-mTOR, mTOR, STAT3, HIF1α, NF-κB, and SIRT signaling. By controlling synovitis and joint destruction in RA, and attenuating cartilage degradation in OA in vitro and in vivo, metformin has demonstrated therapeutic benefits in both diseases. Metformin enhances the immunomodulatory, anti-inflammatory, and chondroprotective effects of conventional treatments, suggesting its role as an adjunctive therapy (37).

In clinical studies, metformin treatment was associated with a reduced risk and severity of RA and OA. Metformin reduced the rates of admission and joint replacement surgery, and attenuated cartilage damage in obese and diabetic patients with RA or OA. These results suggest that metformin may have clinical efficacy in RA and OA (38).

Metformin acts primarily on T lymphocytes' mitochondrial dysfunction and oxidative stress in SLE. Treatment of TC lupus-prone mice with metformin inhibits mitochondrial metabolism due to the inhibition of complex I of the mitochondrial electron transport chain, fixed aberrant T cell metabolism, decreased IFNc production, and reestablished the IL-2 production (39).

Metformin also normalized in vitro IFNα production in CD4+T cells isolated from patients with SLE (39) . The normalization of T cell metabolism through the dual inhibition of glycolysis and mitochondrial metabolism has been proven using a combination of metformin plus glycolytic inhibitor 2-DG (39).

7. REFERENCES

1. Catalina MD, Owen KA, Labonte AC, Grammer AC, Lipsky PE. The pathogenesis of systemic lupus erythematosus: harnessing big data to understand the molecular basis of lupus. Journal of autoimmunity. 2020; 110:102359.
2. Rasa S, Nora-Krukle Z, Henning N, Eliassen E, Shikova E, Harrer T, et al. Chronic viral infections in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Journal of translational medicine. 2018; 16:1-25.
3. Goulielmos GN, Zervou MI, Vazgiourakis VM, Ghodke-Puranik Y, Garyfallos A, Niewold TB. The genetics and molecular pathogenesis of systemic lupus erythematosus (SLE) in populations of different ancestry. Gene. 2018; 668:59-72.
4. Karagiannis M, Drouzas K, Liapis G, Lionaki S. Lupus Nephritis: Clinical Picture, Histopathological Diagnosis, and Management. 2023.
5. Wang H, Xu J, Zhang X, Ren Y, Cheng M, Guo Z, et al. Tubular basement membrane immune complex deposition is associated with activity and progression of lupus nephritis: a large multicenter Chinese study. Lupus. 2018;27(4):545-55.
6. Abu-Hishmeh M, Sattar A, Zarlasht F, Ramadan M, Abdel-Rahman A, Hinson S, et al. Systemic lupus erythematosus presenting as refractory thrombotic thrombocytopenic purpura: a diagnostic and management challenge. A case report and concise review of the literature. The American journal of case reports. 2016; 17:782.
7. Wang M, Liang Y, Hu Z, Chen S, Shi B, Heidari AA, et al. Lupus nephritis diagnosis using enhanced moth flame algorithm with support vector machines. Computers in Biology and Medicine. 2022; 145:105435.
8. Obrișcă B, Sorohan B, Tuță L, Ismail G. Advances in lupus nephritis pathogenesis: from bench to bedside. International Journal of Molecular Sciences. 2021;22(7):3766.
9. Truong L, Seshan SV. Lupus nephritis: The significant contribution of electron microscopy. Glomerular Diseases. 2021;1(4):180-204.
10. Weening JJ, D'agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney international. 2004;65(2):521-30.
11. Tsokos G. Systemic lupus erythematosus: basic, applied and clinical aspects: Academic Press; 2020.
12. Moroni G, Quaglini S, Maccario M, Banfi G, Ponticelli C. "Nephritic flares" are predictors of bad long-term renal outcome in lupus nephritis. Kidney international. 1996;50(6):2047-53.
13. Haas M, Rastaldi MP, Fervenza FC. Histologic classification of glomerular diseases: clinicopathologic correlations, limitations exposed by validation studies, and suggestions for modification. Kidney international. 2014;85(4):779-93.
14. Chavarot N, Verhelst D, Pardon A, Caudwell V, Mercadal L, Sacchi A, et al. Rituximab alone as induction therapy for membranous lupus nephritis: a multicenter retrospective study. Medicine. 2017;96(27).
15. Hu W, Chen Y, Wang S, Chen H, Liu Z, Zeng C, et al. Clinical–morphological features and outcomes of lupus podocytopathy. Clinical Journal of the American Society of Nephrology. 2016;11(4):585-92.
16. Li X, Zhang X. Comments on the 2021 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for anticoagulant therapy in patients with membranous nephropathy. Kidney International. 2022;101(1):186-7.
17. Gatto M, Zen M, Iaccarino L, Doria A. New therapeutic strategies in systemic lupus erythematosus management. Nature Reviews Rheumatology. 2019;15(1):30-48.
18. Cattran DC, Feehally J, Cook HT, Liu ZH, Fervenza FC, Mezzano SA, et al. Kidney disease: improving global outcomes (KDIGO) glomerulonephritis work group. KDIGO clinical practice guideline for glomerulonephritis. Kidney International Supplements. 2012;2(2):139-274.
19. Li P, Jiang M, Li K, Li H, Zhou Y, Xiao X, et al. Glutathione peroxidase 4–regulated neutrophil ferroptosis induces systemic autoimmunity. Nature immunology. 2021;22(9):1107-17.

20. Rovin BH. Kidney Failure in Systemic Lupus Erythematosus: A Fraught Complication in a Fraught Disease. American Journal of Kidney Diseases. 2022;79(5):617-9.
21. Portalatin GM, Gebreselassie SK, Bobart SA. Lupus nephritis–An update on disparities affecting african americans. Journal of the National Medical Association. 2022;114(3):S34-S42.
22. Morales E, Galindo M, Trujillo H, Praga M. Update on lupus nephritis: looking for a new vision. Nephron. 2021;145(1):1-13.
23. Derner O, Kramer A, Hruskova Z, Arici M, Collart F, Finne P, et al. Incidence of kidney replacement therapy and subsequent outcomes among patients with systemic lupus erythematosus: findings from the ERA Registry. American Journal of Kidney Diseases. 2022;79(5):635-45.
24. Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, et al. KDIGO clinical practice guideline on the evaluation and management of candidates for kidney transplantation. Transplantation. 2020;104(4S1):S11-S103.
25. Parodis I, Tamirou F, Houssiau FA. Prediction of prognosis and renal outcome in lupus nephritis. Lupus Science & Medicine. 2020;7(1):e000389.
26. Zhang L, Lee G, Liu X, Pascoe EM, Badve SV, Boudville NC, et al. Long-term outcomes of end-stage kidney disease for patients with lupus nephritis. Kidney international. 2016;89(6):1337-45.
27. Rovin BH, Teng YO, Ginzler EM, Arriens C, Caster DJ, Romero-Diaz J, et al. Efficacy and safety of voclosporin versus placebo for lupus nephritis (AURORA 1): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial. The Lancet. 2021;397(10289):2070-80.
28. Ursini F, Russo E, Pellino G, D'Angelo S, Chiaravalloti A, De Sarro G, et al. Metformin and autoimmunity: a "new deal" of an old drug. Frontiers in immunology. 2018;9:1236.
29. Ge X, He X, Liu J, Zeng F, Chen L, Xu W, et al. Amelioration of type 2 diabetes by the novel 6, 8-guanidyl luteolin quinone-chromium coordination via biochemical mechanisms and gut microbiota interaction. Journal of Advanced Research. 2022.
30. Lu G, Wu Z, Shang J, Xie Z, Chen C. The effects of metformin on autophagy. Biomedicine & Pharmacotherapy. 2021; 137:111286.
31. Kim E-K, Lee SH, Lee S-Y, Kim J-k, Jhun JY, Na HS, et al. Metformin ameliorates experimental-obesity-associated autoimmune arthritis by inducing FGF21 expression and brown adipocyte differentiation. Experimental & molecular medicine. 2018;50(1):e432-e.
32. Heller M, Duchêne A. Treating language as an economic resource: Discourse, data and debate. Sociolinguistics: theoretical debates. 2016;139:156.
33. Beard DK, Bristol S, Cosby K, Davis A, Manning C, Perry L, et al. Crystal structure of a hypothetical protein from Giardia lamblia. Acta Crystallographica Section F: Structural Biology Communications. 2022;78(2).
34. Park Y-H, Jang Y-J, Choi Y, Lee K, Kim HJ, Cho O, et al. Combination of LMT-28 and metformin improves beneficial anti-inflammatory effect in collagen-induced arthritis. Pharmacology. 2021;106(1-2):53-9.
35. Franciosi M, Lucisano G, Lapice E, Strippoli GF, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. PloS one. 2013;8(8):e71583.
36. Vazirpanah N, Ottria A, Van Der Linden M, Wichers CG, Schuiveling M, Van Lochem E, et al. mTOR inhibition by metformin impacts monosodium urate crystal–induced inflammation and cell death in gout: a prelude to a new add-on therapy? Annals of the rheumatic diseases. 2019;78(5):663-71.
37. Feng X, Pan J, Li J, Zeng C, Qi W, Shao Y, et al. Metformin attenuates cartilage degeneration in an experimental osteoarthritis model by regulating AMPK/mTOR. Aging (albany NY). 2020;12(2):1087.
38. Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, et al. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circulation research. 2007;100(11):1589-96.
39. Muñoz-Urbano M, Quintero-González DC, Vasquez G. T cell metabolism and possible therapeutic targets in systemic lupus erythematosus: a narrative review. Immunopharmacology and Immunotoxicology. 2022:1-14.