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Biomarkers in biological fluids and their potential use as indicators of lupus nephritis in individuals with systemic lupus erythematous

Lisandro Pacheco-Lugo[1], Yirys Díaz-Olmos[2], Gustavo Aroca-Martínez[3]

[1] Researcher,Nephrology Group, Faculty of Medicine, Universidad Simón Bolívar, Barranquilla, Colombia.
[2] MA Student, Universidad Simón Bolívar, Barranquilla, Colombia.
[3] Scientific Director,Clínica de la Costa. Researcher, Faculty of Medicine, Universidad Simón Bolívar, Barranquilla, Colombia.

Abstrac

Lupus nephritis is one of the most severe manifestations of systemic lupus erythematous. Renal involvement in patients with systemic lupus erythematous is an important cause of morbidity and mortality. The pathogenesis of lupus nephritis involve multiple factors, wich include genetic predisposition, epigenetic regulation and environmental interaction. Conventional clinical parameters such as creatinine clearance, proteinuria, urinary sediments, antibodies anti-double-strand DNA and complement proteins they are not enough sensitive or specific to detect disease activity. In the last decades, "Omics" technologies (Proteomic, genomic, transcriptomic, metobolomic) have been used in an extensive way looking for biomarkers, which allowed to discovery variants associated with systemic lupus erythematous and lupus nephritis. Such findings have expanded our knowledge about molecular basis of disease and they have been very important to identification of potential therapeutic targets to prediction of disease and early treatment. In this review, we resume some of recent studies focused in identification of biomarkers associated with lupus nephritis in diverse biological fluids.

Key words: Lupus nephritis, systemic lupus erythematous, biomarkers, renal involvement.

Biomarcadores en fluídos biológicos y su potencial uso como indicadores de nefritis lúpica en individuos con lupus eritematoso sistémico

Resumen

La nefritis lúpica es una de las manifestaciones más severas del lupus eritematoso sistémico. El compromiso renal en pacientes con lupus eritematoso sistémico es un causal importante de morbilidad y mortalidad. La patogénesis de la nefritis lúpica involucra múltiples factores, entre los que se incluyen predisposición genética, regulación epigenética e interacción ambiental. Los parámetros clínicos convencionales tales como eliminación de creatinina, proteinuria, sedimentos urinarios, anticuerpos anti-ADN de doble cadena y niveles del complemento no son lo suficientemente sensibles o específicos para detectar actividad de la enfermedad. En las últimas décadas, las técnicas basadas en "Ómicas" (proteómica, genómica, transcriptómica, metabolómica) han sido utilizadas de manera extensa para la búsqueda de biomarcadores, las cuales han permitido descubrir una amplia variedad de variantes que son asociadas con lupus eritematoso sistémico y nefritis lúpica. Esos descubrimientos han expandido nuestro entendimiento de las bases moleculares de la enfermedad y han sido muy importantes para la identificación de potenciales blancos terapéuticos para predicción de la enfermedad y tratamiento temprano. En esta revisión, resumimos algunos de los estudios recientes enfocados en la identificación de biomarcadores asociados a nefritis lúpica en diversos fluidos biológicos.

Palabras clave: Nefritis lúpica, lupus eritematoso sistémico, biomarcadores, compromiso renal.

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Correspondencia: Lisandro Pacheco Lugo, lpacheco28@unisimonbolivar.edu.co

Introducción

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies against a wide range of autoantigens, including DNA, RNA, histones, and other nuclear components.In most patients, vital organs and tissues are involved, including kidney, brain, cardiovascular system, joints, and skin. Lupus nephritis (LN) is a serious and very common complication among individuals suffering from SLE and is associated with significant morbidity and mortality rates in patients with SLE. Approximately 74% of patients with lupus will develop LN at some point during the course of their disease[1,2].

The pathogenesis of LN is a complex process that involvesdeposition of autoantibodies in the glomerulus, activation of complements and macrophages, cell proliferation, and production of proinflammatory cytokines and chemokines, which are then linked through multiple mechanisms to cause tubular damage, tubulointerstitial inflammation, and fibrosis.For all this, LN continues to be one of the most severe manifestations of SLE[3]. The medical therapy for LN depends on the severity of the disease. Thus, identifying reliable biomarkers for LN will help to assess disease activity, identify patients at risk for renal damage, and facilitate early diagnosis and intervention that leads to favorable outcomes[1,4].

Current laboratory markers for LN such as proteinuria, urine protein-to-creatinine ratio, creatinine clearance, anti-double-stranded DNA antibodies, complement levels, anti-C1q antibodies are not reliable enough. All of them lack sensitivity and specificity for differentiating renal activity and damage in patients with LN (Table 1).Kidney damage can occur before renal function is affected and long before it can be detected by clinical laboratory parameters. Persistent proteinuria does not necessarily indicate inflammation in the kidneys and may be due to pre-existing chronic lesions or recent renal damage during the course of the disease.

Relapses in patients with LN may occur without any observable and recent increase in the degree of proteinuria. Renal biopsy is the gold standard for providing information on the histological classes of LN and the relative degree of activity and chronicity in the glomerulus.However, this procedure is invasive and serial biopsies are impractical for monitoring LN. Thus, novel biomarkers that are capable of discriminating lupus renal activity and its severity, predicting renal relapses, and monitoring treatment response and disease progression are urgently needed. In this review we presentthe recent advances and discoveries of new molecules that could potentially serve as biomarkers of the degree of renal involvement in patients with lupus nephritis.

Table 1

Performance of historically used biomarkers to predict lupus renal activity[5–7].

Test	Sensitivity	Specificity
Anti-double-stranded DNA antibodies	53-100%	50-69%
C3	56-79%	51-64%
C4	53-74%	64-65%
Anti-C1q	53-81%	64-71%

Source:Adaptedfrom Reyes-Thomas et al.[8]

The ideal biomarker

Biomarkers are defined as biological, biochemical or genetic events whose alterations are correlated with pathogenesis or disease manifestations, and which can be qualitatively or quantitatively evaluated in laboratories.The ideal biomarker should 1) be biologically active and pathophysiologically relevant, 2) be simple to use in routine practice, and 3) change sensitively and accurately with disease activity[9,10].In addition, and not less importantly, biomarkers must be cost-effective, including those used in follow-up tests.In LN, biomarkers should identify patients at risk for relapse so that therapy can be tailored to individual situations and the duration of treatment can be precisely determined[11].

Serum biomarkers for lupus nephritis

Some serum markers of SLE include: anti-double-stranded DNA antibodies (anti-dsDNA), anti-C1q antibodies, and circulating levels of complement factors such as C3 and C4. These, however, are useful for diagnosis but limited for monitoring renal activity[11]. Nucleosomes play an important role in the development of renal lesions by mediating the production and binding of autoantibodies to basal membranes. A meta-analysis revealed that anti-nucleosome antibodies are a highly accurate diagnostic markers for both SLE and LN[12]. In addition, serum anti-C1q antibodies are also valuable non-invasive markers forpredicting renal histopathology in patients with LN. In fact, the levels of serum anti-C1q antibody can be used as a marker for renal activity with higher sensitivity and specificity than traditional markers of renal activity such as C3/C4 consumption and anti-dsDNA[13].

Panda et al. reported that high and intermediate levels of mannose-binding lectin are significantly associated with LN in patients with SLE[14]. In addition, serum complement factor H levels have been shown to be associated with renal activity in patients with LN[15].

Large-scale disease analyses using high-throughput autoantigen microarrayhave facilitatedthe discovery of disease biomarkers on a global scale. By using a glomerular proteome array, Li et al. identified "clusters" of autoantibodies that more sensibly predict active SLE and LN[16]. Some of these autoantibody clusters, such as anti-chromatin, anti-DNA, anti-Ro, and anti-RNP, were associated with nephritic disease activity. These researchers also found that the presence of immunoglobulin M autoantibodies in patient's serum was associated with reduced LN severity[17]. Some studies have suggested that ribosomal anti-P antibodies in association with anti-dsDNA antibodies correlate with much higher nephritic activity in patients with SLE. However, our group has shown that ribosomal anti-P antibodies are not associated with lupus nephritis in patients suffering from SLE[19].

Circulating miRNAs in serum, plasma and urine

MicroRNAs (miRNAs) are short, non-coding RNA sequences of approximately 20 nucleotides long that regulate gene expression at a post-transcriptional levelthrough binding to 3' untranslated regions, coding sequences or 5' untranslated regions of target messenger RNAs, leading to the inhibition of translation or degradation of the messenger RNA.miRNAs have been predicted to control approximately 30% of the protein-coding human genome[20]. miRNAs control the expression of genes involved in various biological processes such as apoptosis, proliferation, differentiation, and metastasis.Variation in miRNA levels could cause the deregulation of a wide range of target genes, which are associated with disease. Circulating miRNAs in biological fluids have many advantages when evaluating their potential use as biomarkers: miRNAs are stable in different biological fluids, the sequences of most miRNAs are conserved among different species, the expression of some miRNAs is tissue-specific, and the level of miRNAs can be easily assessed by various methods, including polymerase chain reaction (PCR), which is a method that is routinely used in clinical laboratories and even in health institutions.Changes in miRNA levels in plasma, serum, urine, and saliva have been associated with different diseases[21–23], which potentiates their use as biomarkers of disease, and may even be useful for monitoring the progression of certain pathologies.The varied expression of miRNAs in kidney during pathological processes makes miRNAs a valuable new tool for understanding, diagnosing, and discovering therapeutic options for SLE and lupus nephritis.In the case of LN, several studies have shown a relationship between circulating miRNAs and lupus nephritis. Table 2 shows some miRNAs that have been associated as biomarkers of lupus nephritis.

Table 2

MicroRNAs associated as potential biomarkers in patients with lupus nephritis

Associated MicroRNA	Biological fluid in which it was evaluated	Author
hsa-miR-371-5P, hsa-miR-423-5P, hsa-miR-638, hsa-miR-1224-3P and hsa-miR-663	Blood	Te, et al.[24]
mir-146-a	Blood	Dai, et al.[25]; Tang, et al.[26]
miR-155	Blood	Wang, et al.[27]
miR-224	Blood	Lu, et al.[28]
miR-21	Blood	Stagakis, et al.[29]
miR-125-a	Blood	Zhao, et al[30]
miR-200[a], miR-429	Urine	Wang, et al. [31]

Cytokines and chemokines and their role in lupus nephritis

Cytokines

LN is an important clinical manifestation of SLE. Although numerous abnormalities of the immune system have been proposed, overexpression of certain cytokines plays an essential role in the pathogenesis of LN[32].In the early stage of the disease, immune deposits or autoantibodies induce the production of cytokines in resident renal cells, ultimately leading to the expression of inflammatory cytokines/chemokines and leukocyte infiltration and activation.Then, infiltrated leukocytes such as macrophages and dendritic cells secrete a variety of cytokines and activate virgin T cells, which shifts the profile of secreted cytokines towards a Th1, Th2 and/or Th17 profile. Recent studies have revealed these inflammatory processes in experimental animal models as well as in human LN models. Intervention directed against cytokines may have therapeutic potential to treat LN[33].

Serum levels of interleukin-17 (IL-17) and IL-23 are increased both in active and inactive SLE patients, while IL-22 levels decrease in patients with active lupus[34]. Elevations in IL-6 and IFN-alpha levels appear to be associated with active renal disease. Also, elevated levels of IL-2Ra are associated with severe lupus nephritis; thus, if these findings are confirmed, this biomarker can be very useful[35]. Serum levels of soluble IL-7 receptor are markedly increased in patients with LN, making this molecule a good biomarker for SLE, especially in LN[36].

Chemokines

Fu et al. measured interferon-inducible chemokines (RANTES, CXCL-11, MIG, IP-10, CCL-19, MCP-1) and IL-8 as biomarkers foractive disease.Levels of these chemokines were higher in patients with SLE compared to healthy controls and controls with rheumatoid arthritis. CXCL-13 appears to show a decrease pattern in patients with SLE, although patients with LN show different behavior, with increased CXCL-13[37].

Of the molecules mentioned above, the best studied is MCP-1. There is increasing evidence that MCP-1 plays an important role in the progression of renal failure based on different murine models and in various stages of proliferative LN[38]. MCP-1 is synthesized mainly by mesangial cells and excreted in the urine. Therefore, MCP-1 in the urine is a promising biomarker for LN activity[39].In additionto its chemoattractant and releasing properties on mononuclear cells, MCP-1 appears to play an important role in situ in inducing the production of proinflammatory cytokines such as IL-6 and ICAM-1 by renal and mesangial tubular cells[40].

Recently, Sánchez-Muñoz et al. suggested that vanin-1 could be a potential biomarker for active nephritis in individuals with SLE. Vanin-1 belongs to a new enzymatic pathway involved in inflammation, oxidative stress, and cell migration via cysteamine. This protein has broad tissue expression, but its peak expression is in the tissue epithelium and in peripheral leukocytes. Vanin-1 levels are useful to sense acute renal injury induced by organic solvents, drugs, and diabetes[41].Using real-time PCR and ELISA, these researchers found significantly elevated levels of blood and urinary vanin-1 in SLE patients with renal activity, compared torheumatoid arthritis-

patients without renal involvement.However, in this study only 20 patients with SLE were evaluated, 7 of whom had renal activity, which does not allow for an exhaustive analysis of the results. It is necessary to replicate this study with a much larger sample.

Urine biomarkers

Generally, urinary substances reflect kidney damage better than serum components. Urine is a source of biofluid which is easy to collect and urine biomarkers usually reflect the renal function directly in various types of nephritic diseases.Proteomic approaches, such as two-dimensional electrophoresis and mass spectrometry, have been widely used to analyze potential urine biomarkers that are associated with renal damage caused by LN. ET-1, for example, is a 21-amino acid peptide produced in vasculature and participates in cell proliferation, inflammation, vasoconstriction, and fibrosis.Fractional excretionlevels of ET-1 show a significant increase during the progression of chronic kidney disease and LN. Levels of this molecule decreased after therapy in patients with LN, thus this biomarker can be very useful[42].

Lipocalin-2

Lipocalin-2, secreted by leukocytes and epithelial cells, is important for iron transport. Urinary levels of this molecule were evaluated in 70 patients with SLE (with or without nephritis) and in healthy controls. Urinary levels of lipocalin-2 were predictive of active nephritis[43].

VCAM-1

Wu et al. compared urine samples from 38 patients with SLE vs. 15 normal controls and 6 control patients with rheumatoid arthritis, and found that SLE patients with active nephritis had higher urinary VCAM-1 levels than controls[44].Similar results were reported by Kiani et al., who found that urinary VCAM-1 levels strongly correlated with higher renal activity, hematuria, proteinuria, and pyuria in 81 patients with SLE[45].

IL-6

Urinary levels of IL-6 have also been evaluated as potential biomarkers for renal damage in patients with SLE. Levels of IL-6 in urine were evaluated in 29 patients with active LN, and patients with class IV LN were found to have higher levels of this interleukin than patients with other type of nephritis[46,47].

IP-10

Interferon-gamma inducible protein-10 (IP-10), also known as CXCL10, is a chemokine secreted by endothelial cells stimulated by interferon-gamma, fibroblasts, and monocytes. Along with its receptor CXCR3, IP-10 promotes the migration of T cells to inflammation sites and is also known to play a role in the down-regulation of angiogenesis. Given the known pathogenic relevance of IP-10 and the increased levels found in the serum of patients with SLE, Avihingsanon et al. studied 26 patients, 14 of whom had class IV LN, and 12 of them had class II, III, V or VI of lupus.They examined the urinary mRNA levels of IP-10, CXCR3, transforming growth factor beta (TGF-β) and vascular endothelial growth factor, and found that levels of all four mRNAs were increased in patients with class IV lupus nephritis when compared to the other classes of nephritis.Interestingly, patients who responded to therapy had significantly lower levels of IP-10, suggesting that IP-10 could be used as anindicator for treatment efficacy[48].

TWAEK

The TWEAK cytokine was discovered in 1997[49]. It was assigned to the tumor necrosis factor alpha (TNF-a) family based on sequence motifs. In kidney cells, TWEAK is involved in important biological events, including modulation of cell survival and up-regulation of proinflammatory mediators[50].

Schwartz et al. examined urinary TWEAK levels in a study group of patients with lupus. They found that TWEAK levels were significantly higher in patients with active nephritis, compared to patients with inactive nephritis[51].They also found a significant correlation between urinary TWEAK and SLEDAI scores, indicating a linear relationship between

TWEAK levels and the threshold for nephritic activity. The same group reported similar results in another cohort of SLE patients[52].

Suzuki et al., using mass spectrometry, identified a series of increased proteins in the urine of children with LN compared to individuals without renal activity. The proteins identified were: transferrin, alpha-1-acid glycoprotein, lipocalin prostaglandin-D synthase, albumin and albumin-related fragments[53]. These results were confirmed by Brunner et al. in a subsequent study[54]. Ceruloplasmin or ferroxidase, as it is officially known, is the main copper transport protein in blood. This protein was found in higher levels in individuals with active LN compared to SLE patients without nephritis[54].

Conclusions

The current treatment of severe LN is unsatisfactory in terms of disease resolution and toxicity. To improve the efficacy and decrease the adverse effects of immunosuppression, it would be ideal to be able to predict the course and pathology of LNin orderto adjust the therapy appropriately. This requires biomarkers that reflect disease activity. Recently, significant effort has been put into trying to identify circulating biomarkers that can anticipate renal relapse in patients with LN.

The sampling of molecules reviewed in this article exemplifies the growing interest in finding a true biomarker for LN. Although many of the studies are preliminary, the results are quite motivating.With recent advances in proteomics and increasingly robust ultrasound technologies, there is optimism that a biomarker will soon emerge with the potential to contribute to disease management and to decrease in high rates of morbidity and mortality.

Nevertheless, at present, no one has evaluated these potential biomarkers in large longitudinal cohorts of patients. In this regard, it might be much better to conduct clinical studies that determine the predictive power of theknown molecules in large multi-center studies with SLE patients, instead of continuing the search for new molecules.Only then will we discover whether these promising biomarkers are a hope for improving the quality of life of SLE patients[8].

In our view, it is probable that a combination of biomarker profiles, rather than individual biomarkers, will emerge to help predict the severity of inflammation, the level of fibrosis, the degree of drug response, and other variables. This approach has the potential to limit the use of renal biopsy. Additionally, this should improve therapeutic efficacy and limit toxicity.

IP-10 as biomarker is very promising in its diagnostic capacity, as it appears to be quite specific for patients with class IV LN.If this information is validated in other studies with much larger patient cohorts, IP-10 could potentially be used instead of renal biopsy to order treatment or change in disease management. Undoubtedly, more studies are needed to validate its potential use as a candidate biomarker[8].

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

SLE, systemic lupus erythematosus

LN, lupus nephritis

DNA, deoxyribonucleic acid

dsDNA,double-stranded deoxyribonucleic acid

RNA, ribonucleic acid

RNP, ribonucleoprotein

miRNAs, microRNAs

PCR, polymerase chain reaction

IL, interleukins

ELISA, enzyme-linked immunosorbent assay

VCAM, vascular cell adhesion molecule

ICAM, intercellular cell adhesion molecule

TWEAK,tumor necrosis factor-like weak inducer of apoptosis

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