MCP-1 in urine as biomarker of renal lupus in absence of cytokines, interferon-γ and growth factors

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Objective: To characterize 17 immunological markers in the Urine of patients with SLE.  
Introduction: Lupus nephritis is an inflammatory disease affecting the renal parenchyma. Cytokines and chemokines are key immune mediators that have been related with the pathogenesis of the disease. Obtaining non invasive prognosis markers is a highly desirable objective in order to improve the clinical management of these patients.  
Patients and methods: In this study we profiled 17 immune mediators (Th1, Th2, Th17 cytokines, chemokines and growth factors) in the urine of 25 patients with systemic lupus erythematosus with active renal disease by using a Biorad® 17-plex kit on a Luminex® platform. A group of healthy volunteers of similar age and comparable sex distribution was recruited as control (n=10).  
Results: Results evidenced that the only detectable mediators in urine were IL-8, MCP-1 and MIP-1β. When levels of these mediators were compared between patients and controls, significantly higher levels of MCP-1 were observed in the urine of the patients. MCP-1 levels in urine correlated positively with the SLEDAI score and negatively with plasma levels of complement C4.  
Conclusions: Our results reinforce the role of MCP-1 in urine as biomarker of disease activity in renal lupus, excluding the detection of other soluble immune mediators such as Th1, Th2, Th17 cytokines and growth factors as suitable markers in this non invasive sample.

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MCP-1 en orina como biomarcador de lupus renal en ausencia de citocinas, interferón-γ y factores de crecimiento

Resumen  
Objetivo: Perfilar 17 mediadores inmunológicos en la orina de pacientes con LES.  
Introducción: La nefritis lúpica es una enfermedad inflamatoria que afecta al parénquima renal. Citoquininas y quimiocinas son los mediadores inmunes dominantes relacionados con la patogénesis de la enfermedad. La obtención de marcadores de pronóstico no invasivos es un objetivo sumamente deseable para mejorar el manejo clínico de estos pacientes.  
Pacientes y métodos: En este estudio nosotros perfilamos 17 mediadores inmunológicos (citoquinas Th1, Th2, Th17, quimiocinas y factores de crecimiento) en la orina de 25 pacientes con lupus y enfermedad renal activa usando un kit Biorad® 17-plex en plataforma Luminex®. Como grupo control se seleccionaron (n = 10) voluntarios sanos con similar edad y sexo que los casos.  
Resultados: Los resultados evidenciaron que los únicos mediadores perceptibles en orina eran IL-8, MCP-1 y MIP-1β. Cuando los niveles de estos mediadores fueron comparados entre los pacientes y los controles, MCP-1 en la orina de los casos fue el único que aumentó de forma significativa (p < 0,05) con respecto a los controles. Estos niveles de MCP-1 en orina se correlacionaron de forma positiva con la puntuación de SLEDAI y de forma negativa con los niveles en plasma de la proteína C4 del sistema de complemento.  
Conclusiones: Nuestros resultados refuerzan el papel de MCP-1 en orina como biomarcador no invasivo de actividad de enfermedad en el lupus renal, no evidenciando la detección de otros mediadores inmunológicos solubles, como son las citoquinas Th1, Th2, Th17 y factores de crecimiento.

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**Introduction**

Lupus nephritis is an inflammatory disease affecting the renal parenchyma. Cytokine and chemokines are the key immune mediators that have been related with the pathogenesis of the disease. Obtaining non-invasive prognostic markers is a key objective to improve the clinical management of these patients. The presence of chemokines in the urine of patients with lupus nephritis has been proved. The multiplex assays available today allow for the simultaneous detection of a great variety of mediators in a very small amount of urine. Monocyte chemoattractant protein-1 (MCP-1) is considered a renal inflammation marker, but it is not exclusive to lupus. It is present in high levels in the urine of patients with diabetes mellitus, IgA nephritis, and certain types of vasculitis, among other conditions.

**Material and methods**

In this study, we profiled 17 molecules present in urine, from the first morning urination, of 25 patients with systemic lupus erythematosus (SLE) with active renal disease by using a Biorad® 17-plex kit on a Luminex® platform. The selected mediators included Th1 cytokines (IL-2; IL-12p70); Th2 cytokines (IL-4; IL-5; IL-10; IL-13); Th17 cytokines (IL-6; IL-17); growth factors (GM-CSF; GCSF); chemokines (IL-8; MCP-1; MIP-1β); and also IL-7. Patients with SLE were selected from the Autoimmune Disease Unit at our hospital. A group of 10 healthy people who work at the University of Valladolid (Spain) of similar age and comparable gender distribution to that of the group of patients was selected as control group. The definition of patients with SLE was that of the American College of Rheumatology. All patients with SLE were receiving immunomodulator treatment at the time of sample collection, either with non-steroidal anti-inflammatory drugs, steroids, chloroquine, or immunosuppressive medication. Disease activity in each patient was assessed at the moment of sample collection using the SLE Disease Activity Index 2000 (results expressed as median [interquartile range]: patients (124.7 [138.6] pg/mL) and controls (56.8 [75.0] pg/mL) (P < 0.05). No difference was found between patients and controls for IL-8 and MIP-1β. The positive correlation between the MCP-1 levels in urine and the score of the SLE Disease Activity Index 2000 with statistical significance (expressed as Spearman's correlation coefficient; P = 0.569; 0.004) and the negative correlation with plasma levels of C4 protein of the complement system (r = −0.389; 0.05) were an interesting discovery. Just as interesting is the discovery of a positive association between the urine levels of MCP-1 and IL-8 (0.436; 0.029) and between MCP-1 and MIP-1β (0.478; 0.016), which somehow suggests coordinated secretion of these chemokines in this disease.

**Discussion**

Chemokines, such as MCP-1 and IL-8, are powerful chemotactic factors of monocytes in the glomerulus when there is renal damage. Furthermore, MCP-1 stimulates the increase of intracellular calcium, which induces the freeing of superoxide anions and lysosomal enzymes and stimulates the production of adherent molecules and cytokines. Many cytokines and pro-inflammatory mediators could cause the production of MCP-1 in the mesangial cells of the glomerulus, the endothelial cells of tubules, and the monocytes. It has been proven that increases of tubular MCP-1 expression were strongly associated to infiltration of monocytes and fibrosis in the interstice of patients with lupus nephritis. This suggests that MCP-1 takes part in the pathogenesis of tubular-interstitial damage, as it recruits monocytes and the fibrosis of the interstice takes place.

One method to detect the expression of MCP-1 in glomerular disease is to measure the levels of MCP-1 in urine, which is high in patients with glomerulopathies, and more acutely so in patients who present inflammatory glomerulopathies. Our results reinforce the role of MCP-1 in urine as a biomarker of disease activity in lupus nephritis; the production of MCP-1 is minimal or undetectable in the renal tissue of subjects without renal affection, excluding the detection of other soluble immune mediators such as Th1, Th2, and Th17 cytokines and growth factors as non-invasive markers.

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**Conflict of interest**

The authors declare no conflict of interests.

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References