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Urinary sediment analysis☆

Martha E. Baños-Laredo, Carlos A. Núñez-Álvarez*, and JavierCabiedes◆

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Laboratorio de Inmunología, Departamento de Inmunología y Reumatología, Mexico City, Mexico

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ABSTRACT

Urinary analysis is one of the most requested tests in the clinical laboratory. This test includes the physical, chemical and microscopic analysis of urine. This last one allows for the observation of urinary sediment (US) in search of formed elements (cellular cast, leukocytes, etc.), with different diagnostic uses. Urinary analysis can be assessed by manual or automated methods. In the laboratory diagnosis of autoimmune diseases, US analysis is mainly oriented towards the assessment of renal function in patients with lupus nephritis (LN) as this is a common clinical manifestation associated to systemic lupus erythematosus (SLE). Additionally, its value lies mainly for diagnostic criteria and evaluation of kidney injury, as well as for several damage indexes directed to patients with SLE. In the last years, several groups have sought to establish new urinary biomarkers of kidney damage in patients with SLE; however, this requires a greater number of studies to determine their true diagnostic value in this patients group.

Análisis de sedimento urinario

Palabras clave:
Sedimento urinario (SU)
Lupus eritematoso generalizado (LEG)
Nefritis lúpica (NL)

RESUMEN

El examen general de orina es una de las pruebas más solicitadas dentro del laboratorio de análisis clínicos e incluye el análisis físico, químico y análisis microscópico. En este último, se analiza el sedimento urinario en búsqueda de distintos elementos formes (leucocitos, cilindros, etc.) con diferente utilidad diagnóstica. El análisis de sedimento urinario se puede valorar mediante métodos manuales y automatizados. En el diagnóstico por el laboratorio de las enfermedades autoinmunes el análisis de sedimento urinario está principalmente orientado hacia el apoyo y valoración renal en pacientes con nefritis lúpica, una de las manifestaciones clínicas más frecuentes en pacientes con lupus eritematoso generalizado. Adicionalmente, su utilidad radica fundamentalmente en su valoración en la mayoría de los criterios diagnósticos y de afección renal, así como en los diferentes índices de daño en pacientes con lupus eritematoso generalizado. En los últimos años, diversos grupos de investigación han buscado nuevos biomarcadores urinarios de afección renal en pacientes con lupus eritematoso generalizado, sin embargo se requiere un mayor número de estudios para determinar su verdadero valor diagnóstico en este grupo de pacientes.

Introduction

From the clinical laboratory point of view, one of the most requested routine tests is the general urine examination (GUE), which covers a chemical analysis (pH, glucose, urobilinogen, etc.), a physical analysis (colour, aspect) and also the microscopic analysis of urinary sediment (US) in search of formed elements (erythrocytes, leukocytes, bacteria, casts, etc.).[1] Although it is considered a "routine" test, its correct interpretation is of great importance for it offers very important data. The goal of the present review is to focus on US analysis as an auxiliary tool for the diagnosis and monitoring of patients with systemic lupus erythematosus (SLE), based on a useful description for laboratory and clinical staff. This can be valuable due to the high incidence of renal damage in this group of patients. A brief description of the clinical importance of US analysis is presented, together with evidence that supports research for new biomarkers, useful for the diagnosis and follow-up of renal affectation in patients with SLE.

Urinary sediment analysis is one the most commonly requested laboratory tests for the study and/or evaluation of patients with

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* Corresponding author.
E-mail address: nuac80df@yahoo.com.mx (C.A. Núñez-Álvarez).

◆*In memory of our teacher and friend.*

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renal disorders. In general, renal and urinary tract diseases represent a significant public health problem; their late diagnosis affects the quality of life of the patient and, in the most severe cases, can lead to patient disability or even death.[2] In relation to rheumatic diseases, SLE has a multifactor origin (e.g. genetic, infections) in which there are high titres of antibodies directed against double-stranded DNA.[3] This can lead to the formation of antigen-antibody immune complexes and their subsequent deposit at a renal level, causing the activation and consumption of complementary proteins.[4] This scenario leads to lupus nephritis as a final outcome, and is one the most frequent complications in this group of patients.

On the other hand, it is worth mentioning that the kidney has a great functional reserve that allows it to withstand damage in up to 75% of its nephrons.[2] Nevertheless, due to its high complexity and delicate structure, affectation of more than 75% of its entirety leads to the presence of sudden clinical manifestations and loss of renal function.

Urinary sediment analysis methods

There are currently various methods to analyse urinary sediments,[5,6] which can be classified into: 1) traditional, or manual, and 2) automated methods. The first is relatively simple to carry out, semi-quantitative or quantitative, economical and can be performed by virtually any laboratory. However, ample experience is necessary for its interpretation and analysis. Manual methods are also so simple that they are poorly valued at present, when more advanced biochemical techniques based on sophisticated technology prevail. With regards to automated methods, these have been developed to reduce interobserver variability and are carried out with special equipment through differential cytometric analysis.[6] They make it possible to measure quantitative parameters (e.g., number of leukocytes/µl) and are relatively expensive compared with the traditional methods. From our point of view, their main disadvantage lies in their low differentiating power between some formed elements present in the US (casts, yeasts, parasites, etc.); they should not replace the traditional microscopic analysis that makes it possible to identify practically all formed elements of diagnostic usefulness (epithelial cells, erythrocytes, leukocytes, casts, crystals, etc.). A viable option is the combination of both methods to obtain the best results[7] and to avoid a greater number of false positives.[8]

To obtain and prepare a urine sample correctly, it is necessary to take into account some important aspects to securing a representative, reliable analysis of the sample.[9,10] With this in mind, the urine should always be collected in a perfectly clean container, and should be analysed within the first 2 h after urination, making it essential to record the date and time when the sample was collected. The urine can be collected by spontaneous urination, clean-catch technique and/or sterile catheterisation.

Briefly, the technique for US analysis is the following: approximately 10 ml of urine are placed in a urinalysis tube or, in its absence, in a clean test tube. The sample is immediately centrifuged at 3,500 rpm for 3 min. The supernatant is then decanted and the US is resuspended by manual mechanical agitation. A drop is placed on a clean microscope slide, extending it evenly. Lastly, a clean cover slip is placed above it and it is observed through a conventional microscope.

Microscopic analysis[1,5,9,10]

For microscopic analysis, the preparation should initially be observed with a final magnification of x100 (using x10 eyepiece and x10 lens) to obtain a general view of the US. All elements identified should be confirmed with x400 magnification (using x10 eyepiece and x40 lens) so as to avoid reading and/or reporting multiple artefacts. With this magnification, the various formed elements observed should be reported semi-quantitatively and quantitatively. The reference values for the different formed elements observed in US are shown in Table. The different parameters observed in US analysis are described briefly.

Erythrocytes. Their morphology is of great importance and provides valuable data (Figure 1). The quantity existing offers information about how chronic the pathological process is. Isomorphic erythrocytes (post-glomerular) and dysmorphic erythrocytes (glomerular) can be detected. Under non-pathological circumstances, these can be observed in small quantities. Dysmorphic erythrocytes are observed with certain regularity in patients with active lupus nephritis.

Leukocytes. Their importance lies in the quantity or number in which they are present and they can be an indicator of damage or chronicity of the pathological process involved (Figure 2). Pus cells, also known as wandering cells, can also be detected. These are leukocytes that present abundant granules with movement within their cytoplasm and their presence is an indicator of a probable

Table
Different parameters observed in US analysis

Parameter*	Reference value	Clinical usefulness
Bacteria	Absent	Indicator of infectious process
Leukocytes	0-5 per field	Indicator of inflammatory process
"Wandering" leukocytes	Absent	Indicate an acute process (pyelonephritis)
Erythrocytes	0-2 per field	Isomorphic (post-glomerular): intense exercise, trauma Dysmorphic: Inflammation, nephrolithiasis, glomerulonephritis, lupus nephritis
Cellularity	0-2 per field	Evaluate the integrity of the epithelium covering the renal tract
Plane epithelium	Male: scarce Female: variable in relation to menstrual cycle	Normal
Renal epithelium	Absent	Inflammatory process, glomerulonephritis, nephrolithiasis
Casts	Absent	Evidence of renal damage
Hyaline	0-1 per field	Hypersecretion of Tamm-Horsfall protein in renal tubules by probable renal involvement. Present in some healthy individuals (e.g. athletes)
Leukocytary	Absent	Leukocyte infiltration in renal tubules, pyelonephritis
Epithelial	Absent	Tubular damage, rejection of transplant
Erythrocytary	Absent	Glomerulonephritis
Granulous	Absent	Degeneration of cellular cast by stasis in the renal tubule caused by decrease in glomerular filtration
Waxy	Absent	Probable renal failure. Lack of glomerular filtrate flow

*The number of formed elements per field must be viewed and reported with a 400x increase. At least 10 visual fields must be counted, but the entire slide should be analysed.

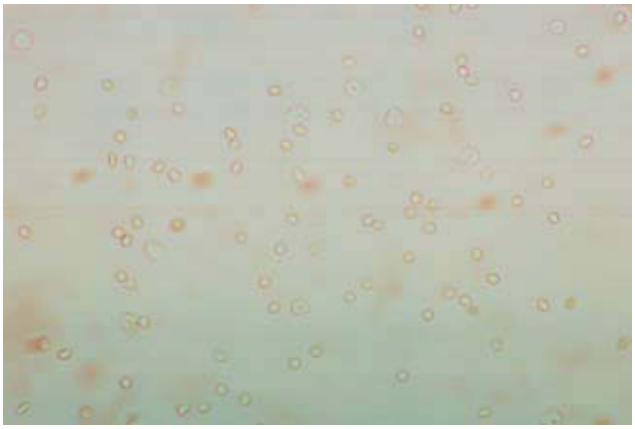

Figure 1. Erythrocytes in US (400×).

Figure 2. Leukocytes in US (400×).

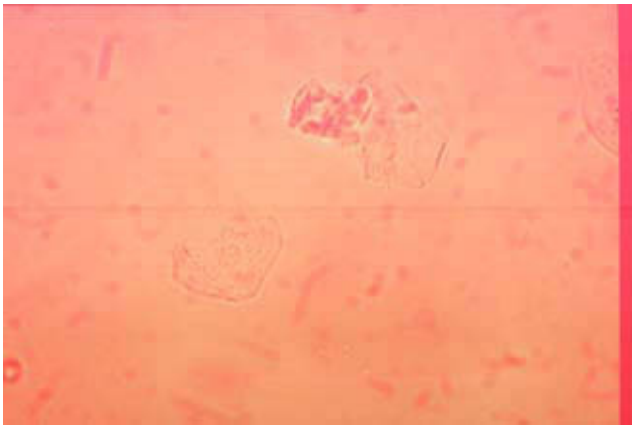

Figure 3. Epithelial cells in US (400×).

pyelonephritis. Under normal conditions, it is possible to observe up to 5 leukocytes per field.

Epithelial cells. Under normal conditions, they can be present in urinary sediment in greater or smaller number depending on physiological conditions and patient gender (Figure 3). Epithelial cells are of irregular size, elongated, and have a nucleus and granulation in their cytoplasm. While normally scarce in males, the quantity of epithelial cells in females varies depending on the menstrual cycle. Another kind of epithelial cells that can be present are renal or tubular cells, which are round, slightly larger than a leukocyte, with a large, round nucleus. Under normal circumstances, this type of cell should not be found and its presence is an indicator of renal damage.

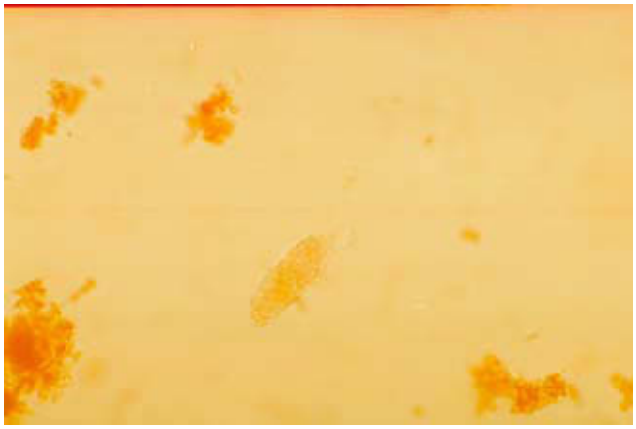

Figure 4. Granulous cast in US (400×).

Casts. Casts are a product of inflammatory processes with epithelial destruction. Their morphology is a result of their passage through renal tubules (distal, proximal and collector). The fundamental cast matrix consists of a high molecular weight glycoprotein called the Tamm-Horsfall protein, excreted exclusively by renal epithelium cells in the post-bend ascending portion of Henle's loop, in the distal tubule.[11-13] The physiological function of this protein has still not been fully established. It should be mentioned that under non-pathological conditions, there should be no casts in the US apart from the hyaline casts, which can be present under certain circumstances.

US analysis can also reveal different types of casts. They are briefly described as follows:

Hyaline casts. Of tubular morphology with rounded ends, they are elongated, transparent and weakly birefringent. They result from an increase in glomerulus permeability, which allows the passage of certain microproteins. These attach themselves to the Tamm-Horsfall protein, acquiring the previously-mentioned morphology.

A whole series of formed elements (erythrocytes, leukocytes, etc.) can probably be added to or included in this protein matrix, changing its aspect and name. These casts can be found in some healthy individuals after having performed intense exercise.

Granular cast. This is a hyaline cast with different degrees of saturation by granular material of proteinaceous origin and uniform size distributed along the cast (Figure 4). It can be observed in pyelonephritis, viral infection, chronic lead intoxication, etc.

Erythrocyte cast. With abundant erythrocytes in the interior, this looks like a hyaline cast and is an indicator of glomerulonephritis.

Leukocyte cast. A hyaline cast with the presence of abundant leukocytes. Its presence is an indicator of pyelonephritis.

Epithelial cast. A hyaline cast can be observed with an internal content consisting of epithelial cells from renal tubules. These casts are present in nephrosis, eclampsia, amyloidosis, acute tubular necrosis and rejection of renal transplants. When degeneration of the cellular material within the cast is detected, it is known as granular or coarse granular casts.

Wax cast. This is formed as a result of the lack of cast excretion, which causes continuous cellular degeneration (Figure 5). Its appearance resembles that of a hyaline cast with internal invaginations or notches. Its presence indicates chronic renal failure.

Crystals. Crystals can adopt multiple shapes depending on the chemical compound and the urine pH. Different types of crystals

Figure 5. Waxy cast in US (400×).

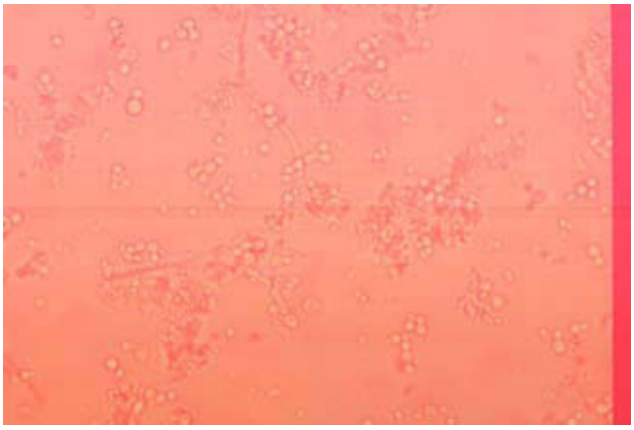

Figure 6. Yeasts in US (400×).

(e.g. uric acid, oxalate) can be observed in US. In comparison with the formed elements of urine, crystals have diagnostic significance only in some cases such as metabolic disorders and renal calculi.

Yeast. These are ovid-shaped colourless cells, with birefringent walls, and frequently present budding (Figure 6). They should not be present under normal conditions. The most commonly observed yeast is *Candida* sp.

Parasites. In urine, we can identify *Trichomonas vaginalis*, which is a flagellated protozoan parasite whose presence should be reported only when its characteristic movement due to its flagellum has been detected. Its presence indicates urogenital trichomoniasis.

Bacteria. These are frequently present in US due to urethral or vaginal contamination. Their presence in large quantities suggests urinary tract infection.

Mucus filaments. These are irregular structures with a long, thin, filamentary shape. They lack pathological significance.

Usefulness of urinary sediment analysis in SLE

The usefulness of US analysis lies fundamentally in its evaluation in the majority of criteria for diagnosis and renal affectation.[3,14] It is also useful in the different damage indices[15-17] in lupus patients. Among the laboratory criteria or aspects evaluated, those of renal assessment include US analysis with a special emphasis on erythrocyte presence and cast detection.

Urinary biomarkers related to lupus nephropathy

Lupus nephritis is the most severe form of renal failure in patients with SLE[18-20] and it is associated directly with morbidity and mortality in this group of patients. For this reason, there are currently various projects focused on the search for new renal damage biomarkers in patients with SLE.[21,22] These projects have not been successful to date, as they have failed to convincingly report any biomarkers as a predictor of renal failure or relapse. It is worth mentioning that finding a new biomarker with an adequate prognostic value is essential to reduce one of the most frequent clinical entities in patients with SLE. One advantage lies in the fact that it is a non-invasive technique and obtaining samples is a simple and accessible process. In addition, it would serve as an alternative to renal biopsy (considered the golden standard). Although there are various studies that analyse clinical importance of different urinary biomarkers associated to renal damage in patients with SLE, the present review will only mention them briefly and generally.

For the past few years, different urinary biomarkers or candidates associated to lupus nephropathy have been studied, including proteinuria and microalbuminuria, inflammation mediators such as interleukin-6, interleukin-8, interleukin-10, VCAM-1, P-selectin, chemokines such as CXCL-16, monocyte chemotactic protein-1 (MCP-I), IP10 and others such as NGAL (lipocaline-2), TNF-like weak inducer of apoptosis (TWEAK), among others.[22-24] In this context, interleukin-6 and interleukin-10 initially showed association with disease activity in lupus nephritis, but some studies did not find this association. MCP-1 has been found in urine from patients with active lupus nephritis. Other authors have reported lipocaline-2 as a urinary biomarker, because it shows correlation with renal damage activity in SLE patients.

Finally, there have been studies on different urinary biomarkers that show encouraging results in some cases. Nevertheless, none of them has been completely validated and further studies (longitudinal and/or controlled) are required to evaluate the true role played by the different urinary biomarkers associated to renal damage in this group of patients.[24]

Conclusions

The analysis of US is one of the most commonly requested tests in clinical laboratories. Although it is a relatively simple technique, it provides the physician with very important data to support the diagnosis of several pathologies. At present, there are different methods for US analysis (traditional, or manual, and automated). Although automated methods exist, they should not replace microscopic US examination. Microscopic US analysis of US makes it possible to identify different formed elements (casts, leukocytes, etc.) of varying diagnostic relevance. From the point of view of laboratory support for the diagnosis of autoimmune diseases, the analysis and adequate interpretation of US is of great usefulness as a support for the diagnosis and treatment of SLE patients, especially those with lupus nephropathy. Finally, during the past few years, different urinary biomarkers have been studied as an alternative to renal biopsy. More studies contributing an adequate diagnostic and prognostic value in this group of patients are required.

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