The iQ200 Microscopic Analyzer is valuable tool for evaluation of urinary sediment at transplanted patients

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Abstract: The value of urinary cytology in the diagnosis of different pathological conditions in renal transplantation is particularly important. Manual microscopic urinalysis is a high-volume procedure that currently requires significant labour. *Objective:* To automate the sediment evaluation and to make this more accurate using the Iris Diagnostics Automated Urine Microscopy Analyzer (iQ200). Our goal was to compare the manual and automated microscopic data to apply iQ200 in renal function monitoring. *Method:* The iQ200 uses digital imaging and Auto Analyte Recognition software to classify urine constituents into 12 analyte categories and quantitatively report. *Results:* We determined cut-off values of urine particles in every category, which correlated well with manual microscopic results. The iQ200 was more sensitive for pathological casts than manual microscopic analysis. iQ200 helped the operator to differentiate between isomorphic and dismorphic erythrocytes and between lymphocytes and granulocytes, too. Every pathological constituent could be recognized, which is very important for early recognition of renal impairment, graft rejection and urinary tract infection. *Conclusions:* The iQ200 system automatically classifies 12 particles, significantly reducing the need for additional sample preparation, manual microscopic review achieving a high degree of standardization in urinalysis.

Keywords: urinary sediment, urine microscopy analyzer, renal function monitoring, rejection, infection

Introduction

Urine contains microscopically observable particles that can indicate certain types of urinary tract system disease [1]. In several clinical situations the examination of urine sediment was the key test that revealed a particular condition [2]. The value of exfoliative urinary cytology in the diagnosis of different pathological conditions in renal transplantation is widely recognized and especially important. The method, however, has not yet gained full acceptance, mainly because identification of different cells is not always possible by means of standard staining techniques. Image-based analysis system has been developed several years ago, to improve accuracy and increase the speed of throughput and later an automated urine microscopy analyzer (iQ200) has been introduced [3] Fogazzi et al. demonstrated that examination of urine sediments can still provide useful information in a wide range of clinical situations [2]. Urine cytology reliable, noninvasive, fast and simple method is appropriate as a first diagnostic line of renal allograft dysfunction, as well as for monitoring of the graft function. Immunocytology of urinary sediments, which is a noninvasive technique, has enormous clinical potential for the differential diagnosis of acute rejection, acute tubular necrosis, and Cyclosporin toxicity [4]. Acute rejection of allograft is one of the most serious complications of renal transplantation that requires fast and precise diagnostic approach. Automated instruments that can examine urine for cells and particles have reduced the need for labor-intensive manual microscopy [5].

Our goal was to automate sediment evaluation by using the Iris Diagnostics iQ™200 Automated Urine Microscopy Analyzer (iQ200; Iris Diagnostics, USA) with high-throughput system, and to evaluate the clinical performance and applications of this analyzer, to increase standardization and accuracy of the results, to compare the manual and automated microscopic data to evaluate the acceptability of applying the iQ200 in graft function monitoring. Our laboratory was the first, who applied and tested this system in Europe. The initial difficulties were solved by the collaboration of our laboraty and the staff of Iris Diagnostics.

Material, Method and Patients

The microscopic analysis of urine sediment provides essential information to clinicians about disease state in patients. Manual microscopic urine sediment analysis traditionally has been the reference method of urine sediment analysis. Unfortunately, it lacks precision and has wide interobserver variability. Automation of urinalysis has been recently introduced since manual microscopic methods require well-trained staff. The new Iris Diagnostics iQ200 analyzer processes specimens in 10-position racks by mixing, sampling and analyzing automatically.

Flowcell digital imaging was used as the method of measurement. Particle constituents from a 2 uL sample were imaged in a planar flowcell that hydrodynamically oriented and constrained particles within the focal plane of a microscope objective. A CCD digital camera captured five hundred 884 × 680 micrometer fields with 0.68 micrometer resolution. Individual particle images were isolated within each frame. From 3 ml of unspun urine is aspirated only 1 ml (2 uL × 500 images) into flow microscope. The sample passed through a flow cell, in front of a microscope, while a CCD digital camera captured 500 frames per sample.

Size, shape and texture features were used to classify each images, using the system's Auto Particle Recognition (APR^{TM}) software, into one of twelve categories: Red Blood Cells, White Blood Cells, WBC Clumps, Hyaline Casts, Pathological Casts, Squamous Epithelial Cells, Non-squamous Epithelial Cells, Bacteria, Yeast, Crystals, Mucus, Sperm and the non-analyte category "artifact". Particle concentration was calculated using the number of images and volume scanned providing a true walkaway system. Based on user-defined criteria certain classes of particles may be sub-classified and the results can be verified and reported very rapidly by the laboratory expert. iQ200 provides visual results that can be reviewed or edited by the operator.

The 840 urine specimens of kidney transplant patients were examined by both methods simultaneously and were compared for the most frequent clinically relevant urine particles.

We studied freshly collected, unspun urine samples submitted for diagnostic urinalysis to our laboratory according to the European Urinalysis Guidelines. After routine diagnostic microscopic urinalysis was performed by the iQ200, each sample was analyzed by manual microscopic method the same day. To reduce interobserver variability, the same technologist performed all the microscopic urinanalyses by using the same microscope (Hund Wetzlar). The areas of a high-power field (HPF) were determined with a measured scale to be able to correlate the cells or particles seen in a microscopic field (usually measured as cells or particles/HPF). Carryover analysis was performed by analyzing the specimen in triplicate, followed by three blank specimens.

Results

We could recognize each pathological constituent [cast (Fig. 1), renal cells (Fig. 2), yeast (Fig. 3) etc.] in the microscope and on the monitor screen as well. We determined the cut-off values of urine particles per high power field in each category (Table I) The cut-off values correlated well with those used in the manual method. The iQ200 was more sensitive for pathological casts than the manual method.

The size of the cylinders varies between wide ranges; and various granulates and cells are imbedded into the matrix, this way it should not be taken a surprise that their determination is partly the job of the validator. All cylinder types are more easily distinguishable on the screen (alwasys in the artificial/ART/ or amorph/AMOR/ cathegory; Fig. 1), than under the light microscope. Discrimination of cellular cylinders is also possible on the screen of iQ200. The artifact category must always be checked – even in case of small amounts – to get real quantitative and qualitative results. The granular and cellular cylinders have to be taken seriously in all cases, since their presence is often an indicative of active illness.

The iQ200 helps the operator in discriminating isomorphic and dysmorphic erythrocytes (Fig. 4). We could recognize the acanthocytes (Fig. 5) more easily and pre-

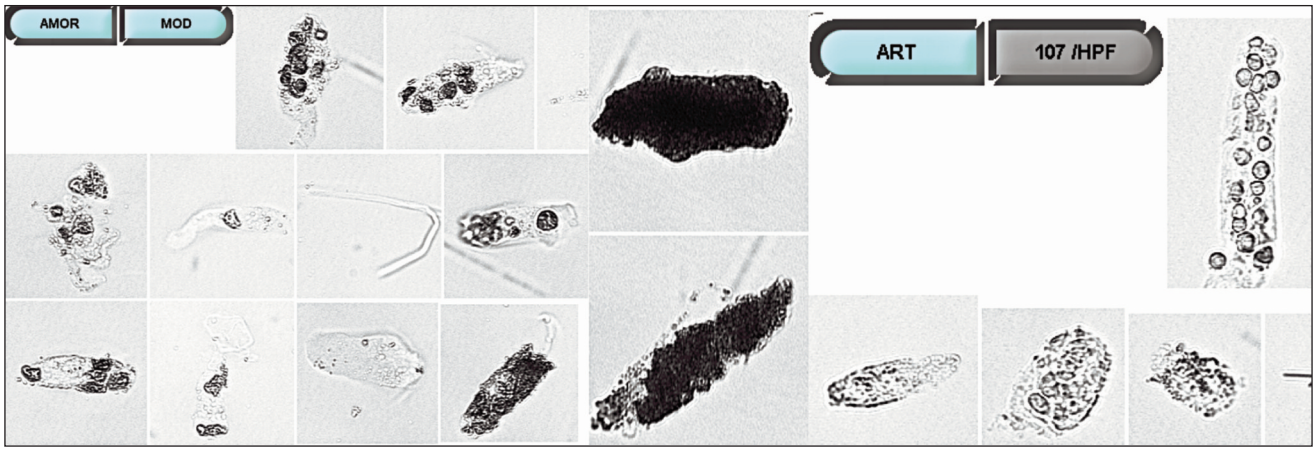

Fig. 1. Granular and cellular casts in the urine sediment. The presence these casts is always abnormal

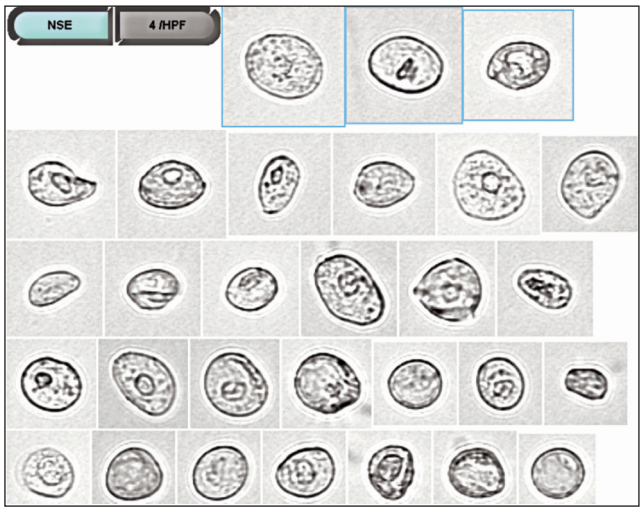

Fig. 2. Renal epithelial cells. Increased number of proximal and distal convoluted renal epithelial cells is seen in case of acute tubular necrosis and certain drug or heavy metal intoxication

Fig. 3. Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals. Most often they are Candida, which may colonize bladder, urethra, or vagina

cisely on the monitor of the iQ200 than in the microscopy. We could predict the rate of dysmorphic red blood cells of the investigated urine sediments for the clinicians.

With adequate experience we could discriminate lymphocytes from granulocytes in most cases (Fig. 6). The ability to detect these constituents is of utmost importance for early recognition of kidney impairment, graft rejection and urinary tract infection. In case of rejection the urine is likely to be full with lymphocytes in contrast to an infection, when granulocytes can be seen in the urine. This discrimination cannot be applied by manual method. Rarely, budding yeast and erythrocytes can be confused by the iQ200. In this case the specimen result can be edited at the monitor. The accuracy of the automatic microscopic analysis on the iQ200 was 76% at the beginning and 99% at the end of our study (when the operator became an expert in the review procedure of images at the on-screen visual display of the iQ200) 64% and 62% of the total investigated urine sediment samples were reported negative by the manual microscopic method and the iQ200, respectively. The iQ200 was more sensitive for pathological casts and renal tubular cells than the manual method. Several times it was the iQ200 that drew our attention to the presence of casts and renal tubular cells.

Conclusion

Urine cytology as a reliable, non-invasive, fast and simple method is appropriate as a first diagnostic line of renal allograft dysfunction, as well as for monitoring of the graft function [6]. Manual microscopic examination of centrifuged urine has several variables associated with sample preparation, centrifugation and counting variation [3]. The automated iQ200 analyzer provided more accurate results at very low and high concentration of cell analyzed. The possible reason of this the process of centrifugation, recantation, resuspension may cause a loss of cell as a lysis or adhesion to the surface of the test tube and is limited by the number of particles that be counted per HPF [3].

Morphological evaluation showed a small number of cells in patients with stable renal function; a larger num-

Table I The cut-off values correlated well to those used in the manual method. The iQ200 was more sensitive for pathological casts than the manual method

Upper limit of formed elements in urine sediment (formed element/high powerfield)	
Formed elements	Cut-off
Red blood cell	8/HPF
White blood cell	15/HPF
White blood cell clump	1/HPF
BYST	8/HPF
Hyphae yeast	2
Squamous epithelial	20
Transitional epithelial	1
Renal epithelial	1
Fat	1
Casts (waxy, fatty, cellular, granular, red blood)	No cut-off value, drop into the artifact category

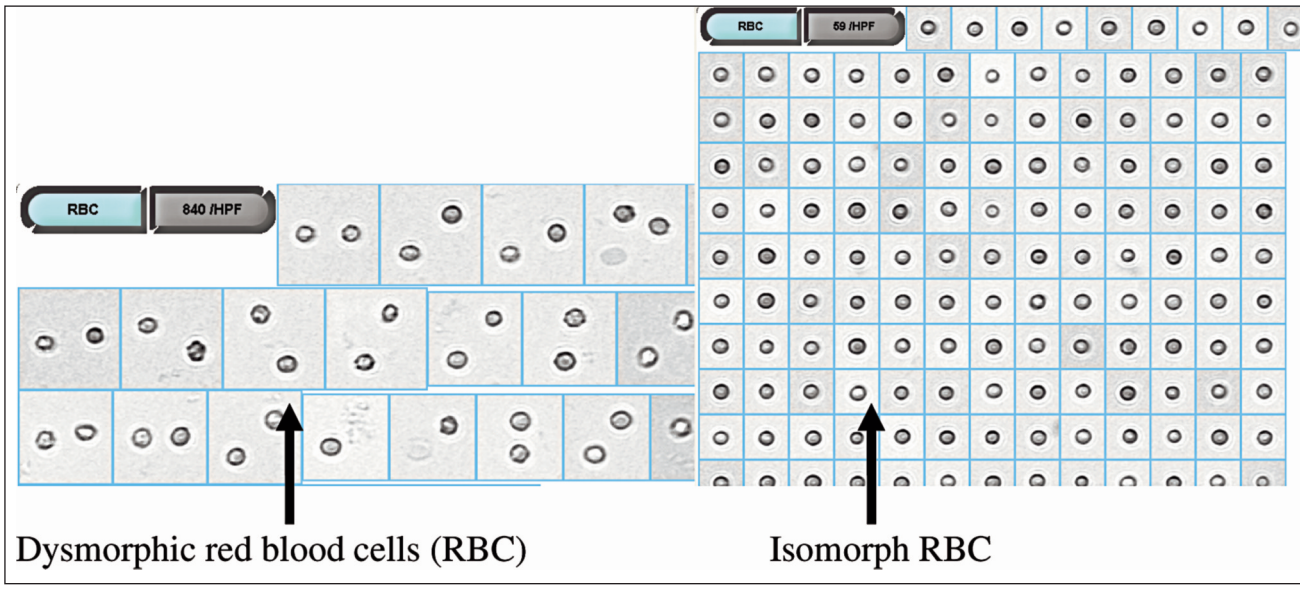

Fig. 4. This figure shows abnormal (dysmorphic) and normal (isomorph) RBC's. The presence of dysmorphic RBC's in urine suggests glomerular disease such as glomerulonephritis

ber of cells, with predominance of lymphocytes [7], during acute rejection episodes; an absolute predominance of neutrophils during bacterial infection. The discrimination of white blood cells is only possible by the iQ200. Large-sized cellular debris in cases of post-transplant tubular necrosis and small cell debris in cases of cyclosporine cytotoxicity were easily detected only by iQ200. Every pathological particle was recognized by iQ200, unlike by manual method. A typical cytologic profile of acute tubular lesion consists of tubular cells. The sediment has a high scatter pattern, i.e. high density and large particles [8], isomorphic erythrocytes, casts, cellular and/or amorphic debris. Excretion of urothelial cells was also significantly increased during most episodes of acute rejection suggesting that rejection of ureters occurs concomitantly with rejection of the kidneys [9]. Recently numerous fungal casts were identified in the urine of a patient who had undergone renal allograft transplantation. The recognition of fungal casts permitted an unequivocal diagnosis of systemic fungal infection [10]. Casts represent an important, diagnostic component of urinary sediment, and may signal renal parenchymal disease in asymptomatic individuals [11]. The iQ200 analyzer was more sensitive for budding yeast cells, than the manual method. These particles have various shapes that could create false positive results. Therefore, the operator should review the storage images or microscopic evaluation before submitting the final report. The morphological differences between the dysmorphic erythrocytes from a glomerular and the isomorphic erythrocytes from a non-glomerular source of bleeding, have been described, but the causes of red-cell dysmorphism are not fully known [12]. It has been suggested that the altered shape and variable size of erythrocytes from pathological nephrons are the result of a dual injury: haemolytic injury when entering the nephron, and exposure within the tubular lumen to osmotic forces. The microscopic search for dysmorphic erythrocytes (acanthocytes), casts or leukocytes in the sediment is a helpful technique for deciding the how to proceed with further diagnostic measures. Morphological changes in more than 17% of the erythrocytes are highly suggestive of a glomerular cause. Also, the presence of various casts points to kidney disease [13]. Determining these various types of sediments by manual operation is a cumbersome and time-consuming task. Manual microscopic urinalysis is a high-volume procedure that currently requires a high degree of training and expertise, as well as significant effort to examine microscopic sediment [14]. The iQ200 system automatically classifies 12 particles, significantly reducing the need for additional sample preparation and manual microscopic review and achieves a high degree of standardiza-

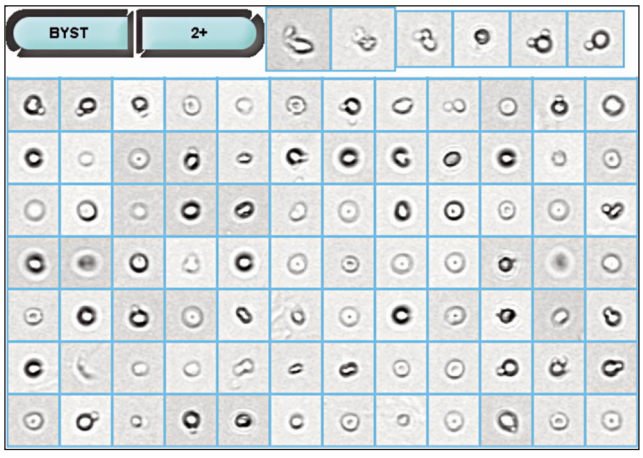

Fig. 5. Acanthocytes. A type of dysmorph RBC's that are relatively rare, but their presence in the urine is an indicative of abnormal conditon. The classification was failed (not into "BYST" category) by iQ200. The operator had to review the storage images and validate the results

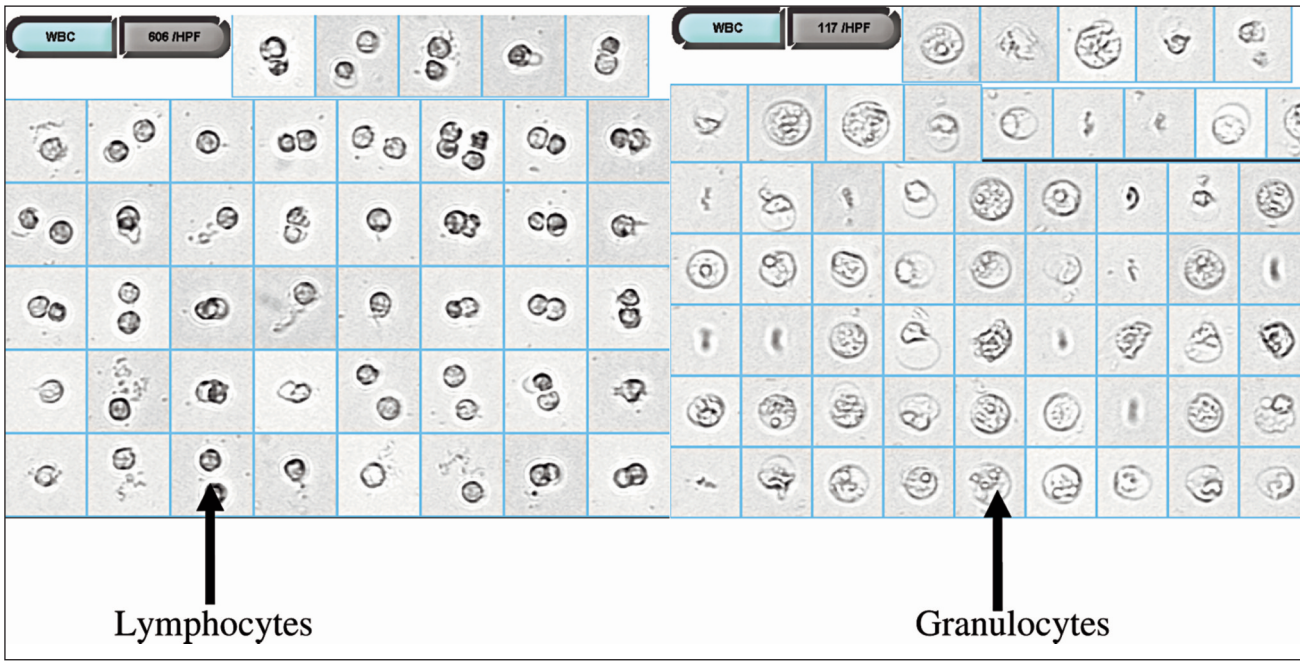

Fig. 6. The ability to differentiate lymphocytes from granulocytes is very important for early recognition of graft impairment, rejection and urinary tract infection

tion in urinalysis. Manual analysis of urine sediment, although clinically useful, is fraught with methodological problems [14]. At least 11 different factors may contribute to imprecision in manual urinalysis [15], ranging from centrifugation to the different interpretation of a cell or cast in urine sediment by different technologists [16]. To this end, there have been attempts to automate the process, thereby improving accuracy, precision, and throughput. With this iQ200 system the pathologic constituents can be detect very accurately, which is of utmost importance for early recognition of kidney impairment, graft rejection and tract infection.

In summary, based on our results from 840 samples, we concluded that the automated iQ200 displayed a good correlation for analyzing major cellular elements. For the pathological particulars the operator should visually inspect and edit images on the screen before releasing the results towards the clinicians. This tool is useful for evaluation of allograft dysfunction, or monitoring graft function after kidney transplantation.

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