Automated Intelligent Microscopy for the Recognition of Decoy Cells in Urine Samples of Kidney Transplant Patients

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ABSTRACT

Background. BK virus (BKV)–associated nephropathy is definitely involved in allograft failure after kidney transplant. Thus, the need for an early control of viral reactivation in immunocompromised patients is well established. Determination of urinary release of decoy cells (DC) and BK viral load in plasma and urine by polymerase chain reaction (PCR) usually precedes renal biopsy. The aim of the study is to assess viral reactivation by BKV-DNA PCR and DC detection in urinary sediment using automated intelligent microscopy.

Methods. Seventy-eight kidney transplant patients were analyzed for the presence of plasma BKV-DNA by quantitative TaqMan real-time PCR. Additionally, automated intelligent microscopy was used for urine sediment analysis, allowing to count cells with decoy feature, confirmed by phase contrast microscopic review.

Results. Plasma BKV-DNA PCR was detected in 14 (17.9%) patients. DC were identified in 19 (24.3%) urine sediments by automated analyzers and confirmed by microscopic observation. Two patients were BKV-DNA–positive/DC-negative; conversely, 7 subjects were DC-positive/BKV-DNA–negative.

Conclusions. Plasma quantification of BK viral load is currently the best noninvasive method for the detection of viral reactivation. Nevertheless, automated methods to screen for the presence of DC in urine could facilitate early BK virus replication diagnosis and patient follow-up by quantitative and visual results.

BK virus (BKV) infection represents an important cause of allograft dysfunction after kidney transplant (KT), with a prevalence of about 20% to 80%. BKV infection can potentially evolve in 1% to 8% of cases in BKV-associated nephropathy (BKVAN), a severe condition connected with an overall risk of 10% to 80% of allograft failure. BKV infection severity is triggered by the use of immunosuppressive drugs: in a context of impaired immune response, viral replication is first observed in urine, and eventually viremia occurs in about 15% of infected patients [1].

Paramount aspects for disease control are its early diagnosis and the consequent treatment, usually based on immunosuppressant remodulation. Diagnostic process typically includes urine cytology for detection of BKV-infected cells (also called decoy cells [DC]); urine and serum BKV-DNA detection by polymerase chain reactions

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(PCRs); and allograft biopsy, which provides a definitive diagnosis of BKVAN [2].

Noninvasive methods for the early detection of patients with reactivation of BKV infection may be useful for the clinical surveillance of KT patients. The urinary cytology detection of DC, although a low positive predictive value (20%), has a high negative predictive value (99%) that makes their detection useful in early screening of KT patients [3,4]. DC can also be detected by phase contrast microscopy [5]. Microscopic examination remains the reference method for urinary sediment analysis; however, the use of automated analyzers has progressively increased in medical laboratories in recent years. The aim of this study was to verify whether the use of automated intelligent microscopy, routinely used in our laboratory, could improve diagnostics in BKV infection.

METHODS

Seventy-eight patients who received kidney transplants at our Transplant Unit from January 2015 to July 2017 were retrospectively reviewed, with the intent to analyze BKV-DNA plasma determinations and concomitant urinary sediment analyses performed in these cases. All the patients presented at least 6 months of follow-up at the moment of analysis.

Plasma BKV-DNA measurement is currently the initial screening method used in our institution because of its good correlation with BKVAN [6]. Blood samples were collected in ethylenediaminetetraacetic acid tubes and plasma was separated by centrifugation. DNA extraction was carried out from plasma samples by NucliSENS EasyMag instrument (bioMérieux S.p.A., Grassina, Italy) according to the manufacturer instructions. Extracted samples were then analyzed for the presence of BKV-DNA by quantitative TaqMan real-time PCR using commercially available kit (ELITechGroup S.p.A., Torino, Italy) using ABI Prism 7300 real-time PCR System (Applied Biosystems, Monza, Italy). The analytical sensitivity of the assay allows detection of about 10 gEq/reaction; the linear measuring range allows the quantification from 10 to 106 gEq/reaction. Computer software quantified the viral DNA load in starting samples, reported as genomic copies per milliliter, taking into consideration several parameters used in extraction and amplification steps. Samples with specific fluorescence corresponding to genomic copies lower than the lower linear measuring range value were reported as detected but low-positive (<250 genomic copies/mL) while those >250 copies/mL were reported as an integer value.

Urinalysis was performed according to European Guidelines [7] and IRIS iQ200 analyzer (Iris Diagnostics, Chatsworth, Calif, United States) was used for the automated urine sediment analysis; this system allows for a quantitative reporting of the elements present in urine providing visual results. A digital camera captured 500 frames per sample and each image was classified by size, shape, contrast, and element texture. After auto-classification, result images are reviewed and edited by the laboratory expert. The instrument can analyze 60 urine samples per hour and allow long-term storage of images. In our laboratory we perform urinalysis of KT patients during hospitalization and subsequent follow-up with a standardized procedure to highlight the presence of pathologic elements. The use of the automated system is supported by microscopic (phase contrast) confirmation.

Statistical Analysis

Qualitative variables were evaluated using the Fisher exact test. Comparison between DC positivity and plasma viral load was tested using the Pearson test. Receiver operating curve analysis was performed, investigating the diagnostic ability of DC when compared with the standard measurement of BK-DNA detection. Area under the curve (AUC), 95% CIs, sensitivity, specificity, positive predicting values (PPV), and negative predicting value were

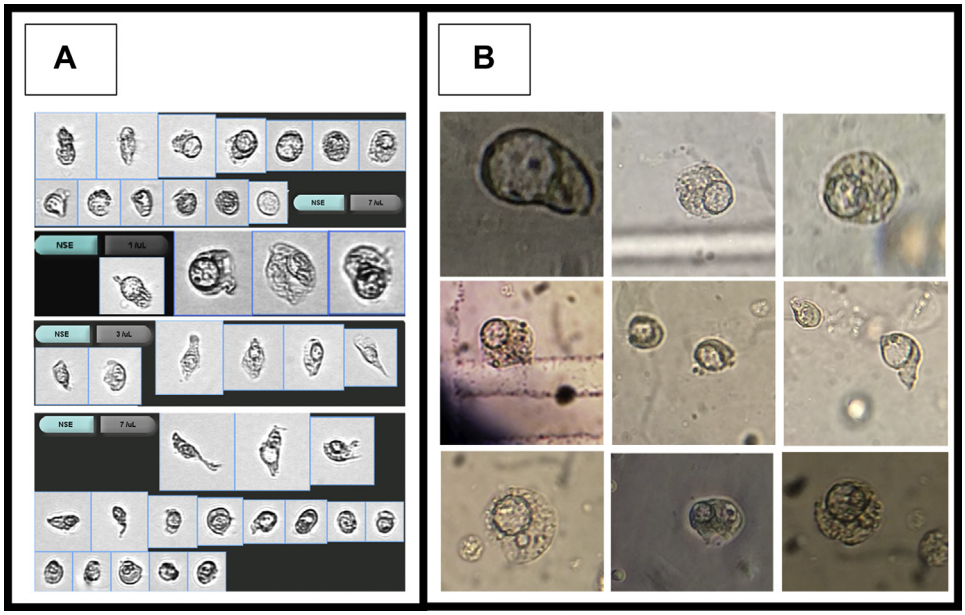

Fig 1. **(A)** Decoy cells observed by automated intelligent microscopy. **(B)** Decoy cells observed by phase contrast microcopy. Original magnification 400 ×.

reported. All statistical analyses were performed with SPSS 23.0 software (SPSS Inc, Chicago, Ill, United States).

RESULTS

The investigated population was composed of 78 individuals, with a slight prevalence of male sex (46/32; 59.0%/41.0%), and a median age of 53 years (interquartile range, 40–61 years) at the moment of KT. All patients had at least 6 months of follow-up after KT.

Plasma BKV-DNA PCR was detected in 14 (17.9%) patients, with viral load ranging from < 250 to 593,093 genomic copies/mL. DC were identified in 19 (24.3%) urine sediments by automated analyzers and then confirmed by phase contrast microscopy observation, with an excellent concordance mainly in negative cases ($P < .001$). Two patients were BKV-DNA–positive/DC-negative, and 7 individuals were BKV-DNA–negative/DC-positive. In all the samples in which DC were identified, the images of IRIS iQ200 agreed with the typical aspect of DC observed using a phase contrast microscope (ie, nuclear enlargement, ground-glass appearance, eccentric nucleus, and chromatin margination) (Fig 1). On receiver operating curve analysis, DC searching presented a high diagnostic power when compared with the standard-of-care research of BKV-DNA, with AUC = 0.844 (95% CI, 0.718–0.971; $P < .001$), sensitivity = 80.0%, specificity = 88.9%, PPV = 63.2%, and negative predicting value = 96.6%.

DISCUSSION

A growing use of automated urine analyzers has been observed in medical laboratories in recent years, with the main intent to save time and work for high-volume laboratories. Various studies have shown a good grade of agreement between automated instruments and manual microscopic analysis [8], while others showed their limits [9]. The value of urinary sediment in various renal diseases is well documented [10]. In KT patients, urinary sediment allows evaluation of allograft functionality in a repeatable and noninvasive way, detecting the presence of pathologic elements such as DC. The correlation between the presence of DC and urinary and/or plasma BKV-DNA has been described in various studies [11]. The diagnostic sensitivity and specificity of DC detection in urine sediment is lower than plasma viral DNA quantification [12], with a possible discordance between these different measurements. In the present study, 7 patients were only positive for DC but negative for the detection of BKV-DNA, thus showing PPV = 63.2%. A good agreement between methods came from the concordance of negative cases, confirming the excellent negative predictive value (96.6%). Overall, DC searching showed a high diagnostic power, with AUC = .844. Obviously, the reduced PPV value is caused by the fact that cells shedding in the urine represent a variable with high occurrence [13]. However, DC detection is a noninvasive and cost-efficient alert signal to better define the next diagnostic steps. As for the usefulness of detecting DC using automated intelligent microscopy, it looked to be a practical method to screen patients at risk during the usual medical control. In our experience, the IRIS iQ200 images supported highlighting the presence of DC, especially in a context with very few cellular elements. Moreover, the possible long-term storage of images is a helpful tool for patient follow-up.

CONCLUSIONS

Plasma quantification of BK viral load is currently the best noninvasive method for the detection of viral reactivation. Nevertheless, automated methods to screen for the presence of DC in urine could facilitate early BKV replication diagnosis and patient follow-up by quantitative and visual results.

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