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# Immunocytochemical staining for p53 and Ki-67 helps to characterise urothelial cells in urine cytology

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**Objective:** The presence of atypical cells in urine cytology is unsatisfactory for both cytologists and clinicians. The objective of this study was to test whether p53 and Ki-67 immunostaining could improve urothelial carcinoma (UC) detection on urinary cytology.

**Methods:** A total of 196 urine samples were analysed, 142 from the bladder, 41 from the upper tract and 13 from ileal bladder replacement. Cytology results were expressed as normal (N) ($n$ = 81), atypia cannot exclude low-grade UC (ALG) ($n$ = 25), suspicious for high-grade UC (SHG) ($n$ = 39) and high-grade UC (HG) ($n$ = 51). Actual diagnoses were confirmed by histopathological analysis, cystoscopic examination or follow-up for at least 1 year. Immunocytochemistry performed on CytoSpin™ slides allowed the determination of the percentage of positive cells with p53 and Ki-67.

**Results:** The median percentage values [first to third quartile] of p53 and Ki-67 were 0 [0–5] and 0 [0–1] for N cytology, 5 [0–40] and 2 [1–10] for ALG, 10 [0–30] and 6 [3–25] for SHG, and 30 [10–80] and 20 [10–30] for HG, respectively. Statistically higher values were observed for both tests ($P < 0.001$) in positive cytologies (ALG, SHG and HG). The optimal cut-offs were 5% for p53 and 3% for Ki-67. The sensitivity and specificity for the detection of all UC were 86.4% and 76.7% for cytology alone, 81.3% and 93.2% for cytology and p53, 75.7% and 88% for cytology and Ki-67, and 68.9% and 97.5% for cytology, p53 and Ki-67, respectively.

**Conclusion:** Using p53 and/or Ki-67 in addition to cytology increases the specificity without penalising the sensitivity.

**Keywords:** urine cytology, urothelial carcinoma, p53, Ki-67, immunocytochemistry

## Introduction

Urine cytology in conjunction with cystoscopy represents the 'gold standard' in urothelial carcinoma (UC) screening and follow-up techniques. Considered as the best screening procedure, cytology testing is a non-invasive method that optimally combines specificity and sensitivity.[1] Although the method can be used to identify efficiently high-grade UC, urine cytology has a lower sensitivity for the diagnosis of low-grade UC or low malignant potential urothelial tumours.[2–5] Other urine markers have been proposed as alternative diagnostic tools. Yet, despite their comparatively higher sensitivity, none of these markers has attained the high level of specificity offered by urine cytology.[6,7] However, in some cases, such as recurrence in patients with ileal bladder replacement or tumours occurring in the upper

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tract, cytology results are particularly important for the detection of cancer cells because a biopsy may be difficult to perform. For such cases, cytology and histology are both limited in their ability to accurately predict the correct tumour grade,[8] and upper tract imaging surveillance through computed tomography (CT) scans is not effective in the diagnosis of upper tract recurrence.[9] Urinary cytology alone shows a poor ability to predict high-grade upper tract UC.[10] Currently—in the absence of a consensus with regard to the cytological responses in urinary cytology—clinicians disagree on how to interpret the presence of atypical urothelial cells in urine. These atypical urothelial cells seem to have a higher predictive value for progression to high-grade UC in upper tract specimens.[11] For these conditions, immunostaining of cells from the urine may provide rapid insights and thus facilitate diagnosis. Using biopsy specimens, previous studies have identified certain immunomarkers as potential predictive markers for recurrence or as prognostic indicators of the severity of disease. Among these markers, p53 and Ki-67 have been investigated in detail.[12,13] Ki-67 is a nuclear antigen present in all cycling human cells and is a marker of active cell proliferation. Immunocytological staining of Ki-67 provides an index that serves to estimate the growth fraction of a population of cells.[14] Piaton *et al.*,[15] who used this marker for dual labelling with p16 in urinary cytology, demonstrated the positive staining of Ki-67 in high-grade cytological cases.

The p53 tumour suppressor is a transcription factor; its activity produces various cellular outcomes, most notably cell cycle arrest and apoptosis, eliminating cancer-prone cells from the replicative pool.[16] The p53 gene is mutated in about half of all human tumours.[17] Detected through immunocytochemistry, nuclear p53 expression is generally indicative of p53 gene mutation responsible for its nuclear accumulation.[18] The mutation of p53 is thought to be an early event in the development of urothelial dysplasia and in *in situ* carcinoma.[19,20] It has also been identified as a predictive marker for bladder tumours with a high risk of progression.[21] Gene mutations frequently associated with UC include loss-of-function *TP53* mutations.[22] The rate of *TP53* mutation is two-fold higher in high-grade UC than in low-grade tumours. Aberrant expression patterns of Ki-67 and p53 were seen in 5% and 2% of grade 1 tumours, and in 85% and 60% of grade 3 tumours, respectively.[23] These common pathological markers are therefore suitable as a cytological diagnostic aid.

The present study used cells collected from urine samples to investigate the role of immunocytochemistry in the diagnosis of UC. The Ki-67 proliferation index and p53 nuclear accumulation were used to improve UC detection through urine cytology with a positivity cut-off for both markers.

## Materials and methods

### *Cytology and histology*

This retrospective study was based on a sample of 196 patients who underwent urinary cytology testing for the detection of ($n$ = 84) or as a follow-up for ($n$ = 112) UC between January 2009 and October 2013. All investigations were carried out in accordance with the principles laid down in the Declaration of Helsinki. Informed consent was obtained from all patients during clinical examination at the time of urine collection. The study itself was not brought before the local ethics committee because cystoscopy and urine cytology were clinical requirements for diagnostic purposes.

Urine samples of 50 ml were obtained for each patient before or after cystoscopy. The samples were fixed in Carbowax (50% alcohol and 1% polyethylene glycol; VWR International, Pessac, France) solution (vol/vol) and concentrated through centrifugation to retain 3 ml of cells in Carbowax. Cytocentrifuge slides (CytoSpin™; Thermo Scientific, Villebon-sur-Yvette, France) were prepared with 500 µl of concentrated cells. Papanicolaou staining was used for two of the slides; the remaining slides were retained for immunocytochemical analysis, which was performed within 4 days. To permit a comparison of the cytological and pathological results, the study selected patients who had undergone a biopsy or resection procedure following urinary cytology diagnosis. However, biopsies were not usually performed for patients with normal cystoscopy results; instead, these patients received a 1-year follow-up and were classified as normal if they remained lesion free (cytology and cystoscopy negative). Urine samples were collected from the bladder ($n$ = 142), the upper tract ($n$ = 41) and from an ileal bladder replacement ($n$ = 13). Two trained pathologists (MC-S and JA, more than 15 years of experience) analysed the slides. The cytologists were aware of the clinical data and corresponding

cystoscopic findings (indicated on a data sheet). In accordance with urinary cytology standards, results were classified as normal or reactive (N), mild atypia cannot exclude low-grade UC (ALG), severe atypia suspicious for high-grade UC (SHG) and positive for high-grade UC (HG). The criteria used for the SHG category are similar to the 'atypical urothelial cells cannot exclude HG (AUC-H)' category proposed by Piaton *et al.*[24] In accordance with the 2004 World Health Organization classification system,[25] pathological results were classified as normal, low-grade (pLG) [including papillary urothelial neoplasm of low malignant potential] or high-grade UC (pHG).

*Quantitative immunocytochemistry*

The immunocytochemical analysis was performed using CytoSpin slides fixed in cold acetone for 10 minutes and stored at −20 °C until use. Slides were rehydrated and exposed to a heat-induced pretreatment in a water bath at 94 °C (1) for 40 minutes in a pH 6 citrate buffer for Ki-67 antibody and (2) for 10 minutes in a high-pH solution (target retrieval solution high pH; DAKO, Glostrup, Denmark) for p53 antibody. DAKO also provided the primary mouse monoclonal antibodies directed against human p53 (clone DO-7, 1 : 30 dilution) and Ki-67 (clone MIB-1, 1 : 50 dilution). These steps were performed in a DAKO autostainer equipped with the EnVision FLEX system using a peroxidase label that was visualised with diaminobenzidine.

Positive staining was defined as nuclei that were strongly stained with either the p53 or Ki-67 antibody. The percentage of positive nuclei in the urothelial cells was evaluated. At least 200 cells were counted on each slide.

*Statistical analysis*

Spearman's rank correlation coefficient was used to assess the relationship between p53 and Ki-67 and the urinary cytology classifications (N, ALG, SHG, HG).

For urinary cytologies classified as ALG, SHG or HG, receiver operating characteristic (ROC) curves were used to determine the optimal cut-off level for p53 and Ki-67. For all analyses, diagnostic results of the pathological classification or of the 1-year follow-up served as the reference standard for the test. The diagnosis was considered to be positive if the pathological classification was pLG or pHG. The sensitivity, specificity and positive and negative predictive values were assessed for cytology alone and for cytology in conjunction with p53 and/or Ki-67. These values were calculated comprehensively for all classifications and separately for cytologies classified as ALG, SHG and HG. The McNemar chi-squared test was used to compare the sensitivity, specificity and positive and negative likelihood ratios between the cytological classifications and p53 and/or Ki-67.

## Results

*Patient data*

Cytological, histological and/or follow-up results are indicated in Table 1.

For the 196 urine samples evaluated (34 females, 162 males; median age, 70 years; range, 16–93 years), cystoscopy and biopsy results revealed a bladder tumour in 108 patients, classified as pLG for 28 patients (14.3%) and as pHG for 82 patients (41.8%). Of the 13 samples collected from ileal bladder replacements, three samples were histologically confirmed as pHG. Of the 41 samples collected from the upper tract, six samples (14.6%) were classified as pLG and 18 samples (43.9%) were classified as pHG. Of the remaining 142 urine samples collected from the bladder, 22 samples (15.5%) were classified as pLG and 61 samples (42.9%) were classified as pHG.

Table 1. Comparison of cytological findings with histology and/or cystoscopy/follow-up

| | | Cytological results | | | |
|---|---|---|---|---|---|
| | Histology | N | ALG | SHG | HG |
| Bladder (urine) | Normal | 45 | 9 | 3 | 2 |
| ($n$ = 142) | Low-grade | 8 | 11 | 3 | – |
| | High-grade | 3 | 3 | 19 | 36 |
| Upper tract | Normal | 14 | – | 3 | – |
| ($n$ = 41) | Low-grade | 3 | 2 | 1 | – |
| | High-grade | 1 | – | 6 | 11 |
| Ileal replacement | Normal | 7 | 0 | 3 | – |
| ($n$ = 13) | Low-grade | – | – | – | – |
| | High-grade | – | – | 1 | 2 |
| All patients | Normal | 66 | 9 | 9 | 2 |
| ($n$ = 196) | Low-grade | 11 | 13 | 4 | – |
| | High-grade | 4 | 3 | 26 | 49 |

ALG, atypia cannot exclude low-grade urothelial carcinoma; HG, high-grade urothelial carcinoma; N, normal; SHG, suspicious for high-grade urothelial carcinoma.

Regardless of localisation, urinary cytology results were negative in 81 cases: 48 cases did not undergo biopsy and remained tumour free after a 1-year follow-up, 18 cases showed a lesion following biopsy, 11 cases were histologically confirmed as pLG and four cases were histologically confirmed as pHG. Of the 25 cases classified as ALG, 13 cases were confirmed as pLG, nine cases were negative and three cases were confirmed as pHG based on histology results. Of the 39 cases classified as SHG, 26 cases were confirmed as pHG, four cases were confirmed as pLG, five cases had normal histology results and four cases did not undergo biopsy. Of the 51 cases classified as HG, 49 cases were histologically confirmed as pHG, one case was inflammatory without the presence of a tumour and one patient died (Table 1).

*Cut-offs for p53 and Ki-67*

Figure 1 illustrates the immunocytochemical results of cells collected from urine classified as low grade and high grade and demonstrating positive staining for p53 and Ki-67.

Based on the histological determination of UC, ROC analysis indicated optimal cut-off values of 5% for p53 and 3% for Ki-67 to permit a diagnosis of UC with the highest sensitivity and specificity: 79.4% and 84.2% for p53, and 75.2% and 85.9% for Ki-67, respectively (results not shown).

Spearman's rank correlation coefficient between quantitative immunocytochemistry and the urinary cytology classification (N, ALG, SHG and HG) was 0.40 ($P < 0.001$) for p53 and 0.68 ($P < 0.001$) for Ki-67. Figure 2 shows the box plots of p53 and Ki-67 according to the urinary cytology classification

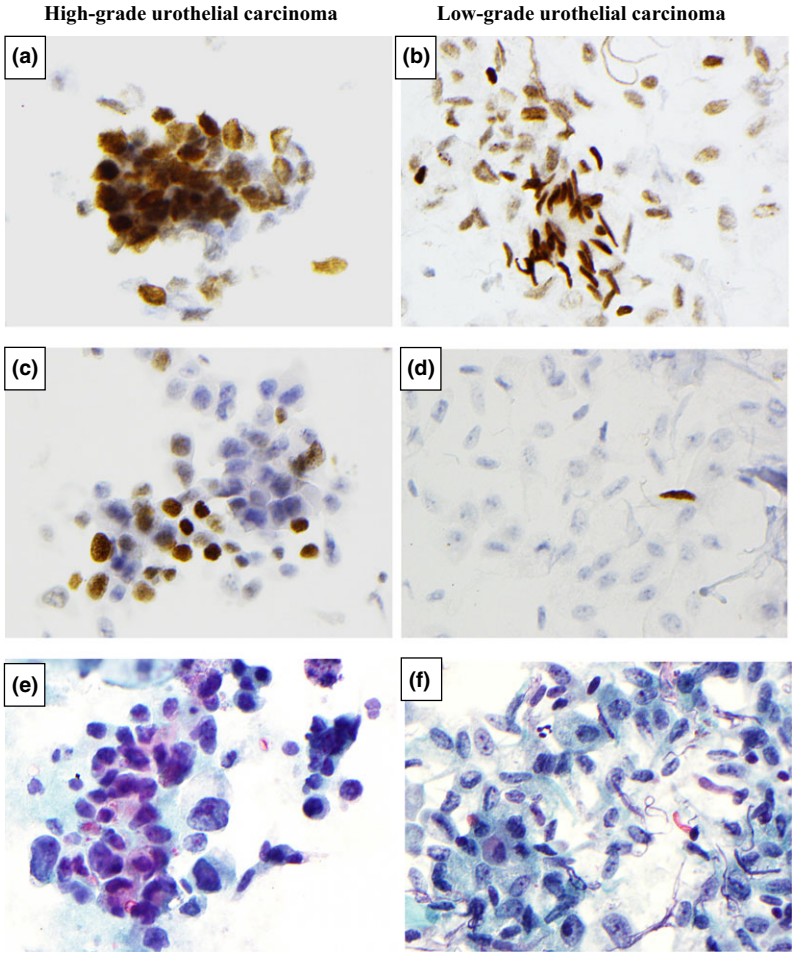


**Figure 1.** Immunostaining with p53 (a, b) and Ki-67 (c, d) of urine cells from high-grade and low-grade urothelial carcinoma, respectively. (e, f) Papanicolaou staining of the same cases. Positivity was evaluated at 90% (a) and 50% (b) of cells for p53 and at 40% (c) and 1% (d) for Ki-67.

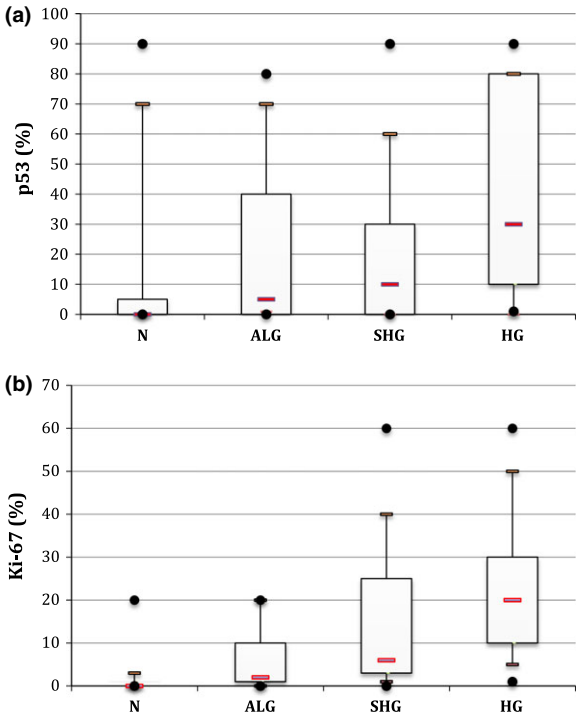


**Figure 2.** Box plots of p53 (a) and Ki-67 (b) according to urinary cytology classification. ALG, mild atypia, cannot exclude low-grade urothelial carcinoma; HG, high-grade urothelial carcinoma; N, normal cytology; SHG, suspicious for high-grade urothelial carcinoma.

(N, ALG, SHG and HG). Median values [first to third quartile] of p53 and Ki-67 were 0 [0–5] and 0 [0–1] for N cytology, 5 [0–40] and 2 [1–10] for ALG, 10 [0–30] and 6 [3–25] for SHG, and 30 [10–80] and 20 [10–30] for HG, respectively. Compared with normal cytologies, p53 and Ki-67 levels were statistically higher for ALG ($P < 0.001$), SHG ($P < 0.001$) and HG ($P < 0.001$).

*Diagnostic value*

To assess the diagnostic value of combining cytology (for all classifications) with p53 immunostaining, tests were considered to be positive if the cytology results were classified as HG, or if ALG or SHG was detected with at least 5% of p53-positive cells. Tests were considered to be negative if the cytology results were classified as N, or if ALG or SHG was detected with less than 5% of p53-positive cells.

To assess the diagnostic value of combining cytology (for all classifications) with Ki-67 immunostaining, tests were considered to be positive if the cytology results were classified as HG, or if ALG or SHG was detected with at least 3% of Ki-67-positive cells. Tests were considered to be negative if the cytology results were classified as N, or if ALG or SHG was detected with less than 3% of Ki-67-positive cells.

To assess the diagnostic value of combining cytology (for all classifications) with both p53 and Ki-67 immunostaining, tests were considered to be positive if the cytology results were classified as HG, or if ALG or SHG was detected with at least 5% of p53- and at least 3% of Ki-67-positive cells. Tests were considered to be negative if the cytology results were classified as N, or if ALG or SHG was detected with less than 5% of p53- or less than 3% of Ki-67-positive cells.

Sensitivity, specificity, positive and negative likelihood ratios and the number (and percentage) of false positives and negatives were calculated for cytology alone and for cytology in conjunction with immunostaining of p53 and/or Ki-67. The same parameters were also calculated for cytologies classified as ALG (detection of pLG), SHG and HG (detection of pHG) to permit a comparison of the test results according to the cytological classification. The results are shown in Table 2.

Cytology testing has a comparatively higher sensitivity for the detection of pHG than for pLG. Combining immunostaining with cytology (all cytologies) improves the specificity (76.7% for cytology alone, 93.2% for cytology with p53, 88.0% for cytology with Ki-67, and 97.5% for cytology with p53 and Ki-67) without penalising the sensitivity (86.4% for cytology alone, 81.3% for cytology with p53, 75.7% for cytology with Ki-67, and 68.9% for cytology with p53 and Ki-67). For ALG cytologies, combining cytology with p53 achieved optimal pLG detection. For SHG cytologies, combining cytology with Ki-67 achieved optimal pHG detection. For HG cytologies, combining cytology with both p53 and Ki-67 achieved optimal pHG detection. No statistical difference was observed with respect to localisation (bladder, upper tract, ileal replacement), screening or follow-up (data not shown).

## Discussion

Urine cytology efficiently identifies high-grade UC, but has a lower sensitivity for the diagnosis of low-grade tumours.[5] Atypical urothelial cells (ALG and SHG) should be considered as neither positive nor negative cytology results. The cytologist must use

Table 2. Performance of urine cytology alone and of cytology combined with p53 and/or Ki-67

| Performance | Sensitivity, % [95% CI] | Specificity, % [95% CI] | PPV, % [95% CI] | NPV, % [95% CI] |
|---|---|---|---|---|
| All cytologies (detection of UC) | | | | |
| Cytology alone | 86.4 | 76.7 | 82.6 | 81.5 |
| ($n$ = 196) | [81.4–91.3] | [75.8–77.7] | [82–83.3] | [80.5–82.4] |
| Cytology and p53 ≥ 5% | 81.3 | 93.2** | 94** | 79.1 |
| ($n$ = 169) | [76–86.5] | [92.5–93.8] | [93.4–94.5] | [78.1–80] |
| Cytology and Ki-67 ≥ 3% | 75.7 | 88** | 89** | 73.7 |
| ($n$ = 190) | [70.7–80.7] | [87.2–88.7] | [88.3–89.7] | [72.9–74.6] |
| Cytology and p53 ≥ 5% and Ki-67 ≥ 3% ($n$ = 184) | 68.9 | 97.5** | 97.3** | 71.2 |
| | [63.8–74] | [97.2–97.9] | [96.8–97.7] | [70.4–72] |
| ALG (detection of low-grade UC) | | | | |
| Cytology alone | 54.2 | 88.0 | 59.1 | 85.7 |
| ($n$ = 99) | [43.6–64.7] | [87.2–88.8] | [54.7–63.5] | [84.8–86.6] |
| Cytology and p53 ≥ 5% | 68.8 | 96.4** | 84.6** | 91.5* |
| ($n$ = 72) | [55.8–81.7] | [95.8–97.1] | [79.2–90.1] | [90.6–92.5] |
| Cytology and Ki-67 ≥ 3% | 46.2 | 96.8** | 75* | 89.7* |
| ($n$ = 76) | [31.8–60.5] | [96.3–97.4] | [64.4–85.6] | [88.8–90.6] |
| Cytology and p53 ≥ 5% and | 46.2 | 100** | 100** | 90* |
| Ki-67 ≥ 3% ($n$ = 76) | [31.8–60.5] | [100–100] | [100–100] | [89.2–90.8] |
| SHG (detection of high-grade UC) | | | | |
| Cytology alone | 86.7 | 83.5 | 66.7 | 94.3 |
| ($n$ = 109) | [77.2–96.1] | [82.6–84.5] | [64.3–69] | [93.6–94.9] |
| Cytology and p53 ≥ 5% | 73.1 | 96.4** | 90.5** | 88.5 |
| ($n$ = 82) | [63–83.2] | [95.8–97.1] | [87.7–93.2] | [87.5–89.5] |
| Cytology and Ki-67 ≥ 3% | 88.5 | 93.8** | 85.2** | 95.3 |
| ($n$ = 91) | [78.3–98.6] | [93.1–94.6] | [82.6–87.8] | [94.7–96] |
| Cytology and p53 ≥ 5% and Ki-67 ≥ 3% ($n$ = 89) | 65.4 | 100** | 100** | 87.5 |
| | [55.3–75.5] | [100–100] | [100–100] | [86.6–88.4] |
| HG (detection of high-grade UC) | | | | |
| Cytology alone | 92.5 | 97.1 | 96.1 | 94.3 |
| ($n$ = 121) | [85.4–99.5] | [96.6–97.5] | [95.3–96.8] | [93.6–94.9] |
| Cytology and p53 ≥ 5% | 89.8 | 98.2* | 97.8* | 91.5 |
| ($n$ = 104) | [82.4–97.2] | [97.7–98.7] | [97.1–98.4] | [90.6–98.8] |
| Cytology and Ki-67 ≥ 3% | 98.0 | 98.4* | 98.0* | 98.4** |
| ($n$ = 111) | [90.6–100] | [97.4–98.8] | [97.4–98.5] | [98–98.8] |
| Cytology and p53 ≥ 5% and Ki-67 ≥ 3% ($n$ = 113) | 89.8 | 98.4* | 97.8* | 92.6 |
| | [82.4–97.2] | [98.1–98.8] | [97.1–98.4] | [91.9–93.4] |

All cytologies, for bladder cancer detection; ALG, for low-grade bladder cancer detection; SHG and HG, for high-grade bladder cancer detection. *$P < 0.05$, **$P < 0.001$ when compared with cytology alone (in each group). ALG, atypia cannot exclude low-grade urothelial carcinoma; CI, confidence interval; HG, high-grade urothelial carcinoma; NPV, negative predictive value; PPV, positive predictive value; SHG, suspicious for high-grade urothelial carcinoma; UC, urothelial carcinoma.

additional tests to help determine an accurate diagnosis. Although many tests can be used to improve urinary cytological diagnosis, most of the available tests are time-consuming and costly.[22] In everyday practice, immunocytochemistry can be performed quickly and easily. The present study aimed to identify morphological markers capable of indicating urothelial tumour grades. For histological specimens, Ki-67 overexpression and p53 mutations have already been used as predictive markers for the recurrence or severity of UCs.[26,27] The same predictive potential has been observed with p53 for cytological specimens.[21] Recently, dual labelling with p16/Ki-67 has been proposed as a marker for the presence of high-grade cancer cells and to predict disease progression in urinary cytology.[15] Although

cytokeratin-20 expression in conjunction with urine cytology has been shown to increase sensitivity, urine cytology alone may miss high-grade tumours.[28] Testing this marker in the laboratory, we nevertheless obtained inadequate results because of the positivity of normal superficial cells. The study thus focused on p53 and Ki-67.

With respect to the overall sensitivity and specificity of cytology used alone, our results differ somewhat from those reported in the literature. In a meta-analysis, Mowatt *et al.* [29] reported a sensitivity of 44% and a specificity of 96% for urine cytology alone. This discrepancy may be the result of a selection bias favouring samples containing a sufficient number of cells to permit the immunocytochemical analyses. In fact, most false-negative results stem from an insufficient amount of cells in the urine sample. Moreover, cytologists at the participating institution were aware of the cystoscopic findings provided on a data sheet. Sensitivity, which is at best 30% for pLG and about 80% for pHG, is also dependent on the UC grade.[29] Our study confirmed this tendency. Indeed, the specificity of cytology alone (76.7%) may be lower when compared with other studies because we used the ALG category for the diagnosis of low-grade UC. However, specificity is higher (97.1%) for the detection of pHG.

We observed that combining p53 with cytology increased significantly both the sensitivity and specificity for ALG-classified cytologies. For SHG and HG, both sensitivity (88.5% and 98%) and specificity (93.8% and 98.4%) were increased significantly when Ki-67 was combined with cytology, and these values were higher when combining both tests (Table 2). Using p16/Ki-67 dual labelling for the detection of high-grade UC, no significant difference from urine cytology was noted according to sensitivity (82.5% and 80.8%, respectively) and specificity (94.9% and 94.9%, respectively).[15] Using fluorescence *in situ* hybridization (FISH) for the detection of high-grade UC, a slight increase in sensitivity when compared with urine cytology (75.5% and 71.2%) and in specificity (84.8% and 78.3%) was observed.[30] Therefore, our tests show a better performance when compared with p16/Ki-67 dual labelling or UroVysion FISH (Abbott Molecular, Abbott Park, Illinois, USA) for the detection of high-grade UC.

As expected, taking into account all cytologies for the detection of UC, combining cytology with at least 5% p53-positive urothelial cells and at least 3% Ki-67-positive urothelial cells allowed the highest specificity (97.5%) to be achieved, but the sensitivity decreased to 68.9%. However, these tests performed better than UroVysion for all UC (61.9% sensitivity and 89.7% specificity)[31] and better than p16/Ki-67 dual labelling (71% sensitivity and 92.4% specificity).[15]

To help determine indecisive urine cytology results, our laboratory now uses the established cut-offs, classifying positive results above these cut-offs as SHG when there is doubt between ALG and SHG. This is of particular interest for cytology samples collected from the upper tract. In such cases, cytology and histology provide less efficient methods of grading tumours than for bladder samples, partly because it is difficult to obtain sufficient material through biopsies.[8] Even UroVysion FISH analysis using voided urine samples does not reliably provide a high sensitivity (56%) and can produce false-positive results.[30,31] Because the treatment of upper tract UC depends on the grade and stage of the tumour,[32] positive cytology results have been recognised as a useful adjunct to histological interpretation.[33] Consequently, there is an important cytological distinction between ALG and SHG and between SHG and HG. Combining immunocytochemistry with cytology may therefore improve diagnostics.

A polyomavirus infection constitutes a limitation for the use of these markers because p53 and Ki-67 are systematically overexpressed as a result of viral replication.[34] It is particularly important to identify this cytopathogenic effect because a reactivated polyomavirus infection may be present concurrently with UC. However, the characteristic cytopathogenic effect of the polyomavirus is easily recognisable, and infection can be confirmed via polymerase chain reaction (PCR) assay.[35]

## Conclusion

In conclusion, the combined method—using the investigated markers in conjunction with cytology—increased specificity without penalising sensitivity. Combining cytology with at least 5% p53-positive urothelial cells and at least 3% Ki-67-positive urothelial cells allowed the highest specificity (97.6%) to be achieved, but the sensitivity decreased to 68.9%. However, these tests performed better than UroVysion [30] or p16/Ki-67 dual labelling [24] for the detection of all UC. The markers can easily be integrated into regular cytological practice.

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## Disclosure of Interest

The authors made no disclosures.

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