Clinical Controversies

Management of Patients with Normal Cystoscopy but Positive Cytology or Urine Markers

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Abstract
This presentation considers follow-up after successful transurethral resection of a high-grade non-muscle-invasive tumour, with normal cystoscopy, followed by bacillus-Calmette-Guérin (BCG) therapy. Focusing on two possible outcomes, a positive cytology but a negative urinary biomarker result, versus positive biomarkers but a negative cytology, we discuss what the evidence and guidelines recommend and which test is more robust.

Patient summary: Bladder cancer is usually assessed by examination of tissue taken from the bladder, either by surgery or by biopsy; however, trace elements in the urine, known as biomarkers, can also provide an assessment. The challenge arises when the two methods do not agree: the tissue sample is positive for cancer, but the biomarker is negative, or the reverse. For now, these authors conclude that the tissue examination is more reliable than the biomarker result.

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1. Case presentation
A 72-yr-old man, heavy smoker, presented with gross haematuria. Transurethral resection of the bladder tumour revealed a 1.5-cm Ta high-grade tumour. Detrusor muscle was present but not involved. Otherwise, there were no suspicious areas in the bladder.

The patient tolerated an induction course of bacillus Calmette-Guérin (BCG). Cystoscopy at 3, 6, 9, and 12 mo was clear of recurrence, and so he continued on maintenance BCG therapy for 1 yr. At this time, maintenance was stopped, as per the protocol in our centre.

His next 6-mo (18 mo after starting BCG) cystoscopy was normal, with no abnormalities in the bladder, and his upper tract was normal on the computed tomography (CT) scan. At this point, let us consider two possible real-life scenarios:

1. His cytology suggested urothelial cancer (high-grade cells seen), so he underwent another cystoscopy. This looked normal, but because of the positive cytology, one possible urine biomarker was tested and the result was negative.
2. The other possible follow-up outcome was that instead of a positive cytology but a negative marker, the result of the follow-up cystoscopy was a negative cytology but a positive biomarker result.

Considering each of these situations, what should the physician do now?
2. **Option A: my patient has normal cystoscopy and negative urine biomarkers, but positive cytology**

2.1. **Evidence**

This case presents a 72-yr-old male, a heavy smoker with gross haematuria, in whom transurethral resection of the bladder (TURB) revealed a Ta high-grade 1.5-cm tumour with muscle present but not involved.

A first consideration is the real meaning of the cytology result in a case such as the present one. Urinary cytology is the “gold standard” for non-muscle-invasive bladder cancer (NMIBC) detection and surveillance. It is usually called for when a high-grade malignancy or cancer in situ (CIS) is present; with an evidence level of 3, it has high sensitivity in a high-grade tumour, but low sensitivity in a low-grade tumour [1]. Barbotage cytology can improve the detection of bladder cancer [2], since it ensures more cells to test with, but it remains the case that the success of cytology is user dependent (Fig. 1).

The European Association of Urology (EAU) guideline [3] recommends the use of cytology as an adjunct to cystoscopy in high-risk disease situations, whereas the American Urological Association [4] recommends its use in both intermediate- and high-risk situations, and also for the assessment of the BCG response.

Comparing cytology with fluorescence in situ hybridisation (FISH), the sensitivity of FISH is higher (72% vs 42%, FISH vs cytology), but the specificity of cytology is better (96% vs 42%, cytology vs FISH) [1].

Therefore, using the standard marker for diagnostic follow-up of NMIBC, with a positive predictive value of 95% and a negative predictive value of 96%, and recalling that the accuracy of these values will be user dependent, for a patient similar to the one discussed here, with a positive cytology result but a normal cystoscopy (ie, negative for cancer) and negative urine biomarkers, there is a 95% probability that this is a case of urothelial transitional cell carcinoma UCC of the urinary bladder or urinary tract.

2.1.1. **First steps**

What are the first steps in this case? As cystoscopy is operator dependent, the first step is to ask whether the cystoscopy result was really normal. An investigation within the European Organisation for Research and Treatment of Cancer (EORTC) found that a “double-check” second cystoscopy may reduce the risk of 3-mo recurrence by 30–35%. A bladder diagram will help because it will usually result in a better TURB from a surgeon in a tertiary centre who will be operating on the next patient. Finally, a flexible cystoscopy is recommended because it can enhance the detection rate of tumours located in difficult places.

2.1.2. **Diagnosing the tumour**

About 12–48% of NMIBC and muscle-invasive bladder cancers occur in the prostate, which is a site of relapse in patients with NMIBC after BCG instillation [1]. Therefore, for patients with a positive cytology and a normal cystoscopy during follow-up, it is mandatory to evaluate the bladder and the prostatic urethra with multiple biopsies.

According to the EORTC [5,6], for accurate diagnosis of a case such as the present one, with a positive cytology, a normal cystoscopy, and bladder mapping, defining the location of the tumour requires 7-French (7F) biopsies, with

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**Urinary cytology**

- High sensitivity in high-grade tumors but low sensitivity in low grade tumors (level of evidence 3)
- It is useful when a high-grade malignancy or CIS is present
- Cytological interpretation is user dependent
- The evaluation can be hampered by UTI, stones or intravesical instillation

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<th>Table 8-10. Percentage of malignant cytology specimens in 74 consultations with a tissue-confirmed TCC tumor</th>
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Fig. 1 – Use of cytology to define tumour location. CIS = cancer in situ; PUNLMP = papillary urothelial neoplasm of low malignant potential; TCC = transitional cell carcinoma; UTI = urinary tract infection; WHO = World Health Organization. Adapted from Kamat et al. [1].
cold cup and TURB. (In my practice, the preference is for cold cup, plus transurethral resection [TUR] of the prostatic urethra, two retromedial biopsies, two lateral wall biopsies [one posterior and one superior], and the prostatic urethra mapped with a TUR.)

As per EORTC guidelines photodynamic diagnosis (PDD) should be used in cases similar to the present one, because it enhances the detection rate of TCC and CIS by 20% and 30%, respectively.

2.1.3. Defining tumour location

The location of tumour has a direct impact on detection accuracy. In renal pelvis tumours, the sensitivity is 78–94%, whereas for ureteral tumours it is 19–54%; further, in cases similar to this patient, it is important to exclude the upper urinary tract as the location of the tumour. The incidence of upper urinary tract cancer in a patient with NMIBC is 0.2–0.4%, but when the tumour is located on the trigone area, the incidence rises to 6–7% [5].

CT imaging is used primarily to visualise the upper urinary tract. It is a “gold standard” for this, with sensitivity of 88–100% and specificity of 93–100% [1], used in conjunction with bilateral washing of the upper urinary tract and biopsy with 7F urethral catheters.

According to the guidelines, in cases with doubtful CT, a ureteroscopy, and selective cytology, a biopsy of the suspicious area should be performed, using either a semirigido or a flexible instrument. Our centre likes a small semirigido instrument, which is better for the evaluation of calices. A uro-CT-negative bilateral selective cytology has a predictive value of >85%, but a ureteroscopy using PDD with a flexible instrument improved the detection rate of CIS.

Further improvement of tumour location can be obtained with confocal laser endomicroscopy (Cellvizio, Cell-viZio; Mauna Kai Technologies) [7]. This instrumentation was introduced over 15 yr ago as an optical biopsy system, providing real-time visualisation of tissue in the bladder and upper urinary tract. Used in conjunction with biopsy, the physician can physically examine the bladder while observing the screen. In this way, the cells can be visualised, but this requires interpretation of what appears on the screen. Interpretation of CIS cells and dysplasia is very difficult because it requires a differential diagnosis with inflammation, which is very difficult to detect and judge.

Confocal laser endomicroscopy was used for the first time for upper urinary tract by Bonnal et al. [7] in a phase II study, which demonstrated that the imaging is feasible and had no side effects. Breda et al. [8] have conducted another study to detect a suspicious area in the upper urinary tract and perform the biopsy there; the concordance between the screen and the final path was 75%. We performed a phase II study [9] where the objective was detection of dysplasia in CIS; the results showed 73% concordance between the screen diagnosis and the final path diagnosis.

2.2. Conclusion and management recommendation

In patients with a normal cystoscopy and negative urine biomarkers, but a positive cytology, a meticulous investigation of the bladder and the prostatic urethra with multiple biopsies [7] is mandatory. The upper urinary tract should also be evaluated by CT (specificity of 93–100%). In case of a doubtful CT result, ureteroscopy and selective cytology with biopsies of the suspicious areas should be performed. Finally, new technologies such as Cellvizio may be considered, and eventually used for real-time visualisation of the bladder mucosa and upper urinary tract tissue.

3. Option B: my patient with normal cystoscopy has negative cytology but positive urine biomarkers

3.1. Evidence

Urine biomarkers for the detection and surveillance of bladder cancer are not used in the UK. This reflects their poor overall utility (poor discrimination of bladder status) and lack of cost effectiveness. Therefore, in the following, I base my opinion using reviews in the literature [10].

3.1.1. Positive biomarker results, but a missed cancer?

Nuclear matrix protein 22 (NMP22) was approved by the Food and Drug Administration (as an adjunct to cystoscopy) based on a study of 668 patients with NMIBC (similar to this patient) [11]. The authors identified that the combined cystoscopy and NMP22 test improved recurrence detection from 91% to 99%. Shariat et al. [10] used these findings to examine 2222 patients, and create a risk-prediction model controlling for age and gender that predicted which urinary NMP22 levels correlated with a risk of disease progression or recurrence, which would require another cystoscopy to find the disease. My reading of this study was that for clinicians who would perform a cystoscopy at an NMP22-level-predicted risk of 5% for recurrence or 1% for progression, the biomarker did not aid clinical decision making. For less risk-averse clinicians, however, who would perform a cystoscopy only at a higher risk threshold of >8% for recurrence or >3% for progression, urinary NMP22 levels helped indicate which patients required cystoscopy and who could be spared this procedure. An increase of the NMP22-level risk prediction to 15% reduced the 581 cystoscopies by 229, while missing only 25 cancer recurrences per 1000 men with NMIBC and negative cytology.

In other words, the biomarker did not replace the necessity for correct physician decision making. The question here is, in the situation of a positive biomarker but a negative cytology, what does this mean? Is there a problem with the biomarker or is there a problem with our cancer detection?

3.1.2. The biomarker test is nonspecific

Several years ago, we looked at urinary DNA methylation as a biomarker [12]. We found significant methylation in the urinary DNA from patients with bladder cancer. This convinced us that we had found a good assay for disease detection. We then compared our findings with those in an age-matched control population (mostly symptomatic men) and found high levels of DNA methylation in the same cohort. To differentiate whether this might be a
phenomenon of ageing, we collected urine samples from young males with sexual infections. Once again, we found abnormal methylation of tumour suppressor genes in a smaller percentage of these men.

This highlights the paradox for urinary tests, because many of these features that are seen in cancer are normal pathological and cellular processes. Most mammalian cells evolve with time, and it is probably a combination of ageing plus the timing of the sampling that creates the ambiguous diagnosis.

Therefore, the question was, did the DNA methylation biomarker produce a false-positive test? Or was it that we do not understand the biology well enough? Probably, it is the latter in this case.

3.1.3. Another biomarker, the same ambiguity

The use of another biomarker is likely to raise the same problem of false positives. For example, an analysis of microRNA expression in urinary samples showed that there were many people with abnormal RNA expression but no cancer [13]. In other words, they had positive tests, but from knowing their natural history, it was certain that they never had cancer—again, a demonstration of a lack of specificity in a biomarker.

3.1.4. A cohort of commercial biomarkers and low specificity

In 2010, Shariat et al. [14] reported the sensitivity/specificity results of a selection of commercial biomarkers relating to cases of positive biomarker and negative cytology. Looking at the results, the specificities for cytology are good, at 94–99%, but the biomarkers are not as specific (68–84%). Again, this suggests a lack of understanding of the biology.

3.1.5. False-positive biomarkers

Finally, a prospective Dutch study [15] examined the sensitivity/specificity of various biomarkers in a population-based study of 1747 men. The authors found haematuria in 409 men, of whom 75 were positive for one of the biomarkers. Of these, only five of 71 had a cancer when fully tested. Therefore, in the real world, a lot of people test positive for a biomarker but do not have cancer. However, this case is different from a failure to find the cancer in the face of a positive cytology, when we assume that the cytology is correct but we simply have not detected the cancer. Rather, in this case I think that there is a real chance that the biomarkers are reporting false positive results.

3.1.5.1. The biomarker is correct, but the NMIBC has been missed. As discussed at the start, approval for NMP22 depended on its ability to enhance cancer detection. Better imaging, for example, with PDD, may improve cancer detection. Several years ago, Mowatt et al. [16] conducted a NICE/UK-funded health technology appraisal that showed that PDD found more diseases, with higher sensitivity, than white-light cystoscopy. It now appears that many of these initial studies were conducted in an era of poor-quality white-light cystoscopy, and it may be that the PDD and narrow band imaging have renewed interest in the quality of white-light cystoscopy. Currently, there is a national UK randomised trial, PHOTO [17], comparing white-light and enhanced cystoscopies.

Nevertheless, PDD appears to be superior for imaging disease: two more recent studies [5,18] have reported that 20% of significant lesions are missed by white-light cystoscopy. A 20% miss rate with white light means that we are missing disease, but fluorescence cystoscopy is highly promising for improving cancer detection.

3.1.5.2. Are we missing disease because we are not looking in the right place? A final important consideration is whether we are missing the presence of real cancer because it is not where we are looking. This comes back to the biology. While performing some microRNA work at Sheffield a few years ago, we compared tissue taken from some 20 patients having prostate cancer of their urothelium with 20 or 30 areas of macroscopically normal urothelium in people having bladder cancer elsewhere.

The result was a range of changes in the microRNA showing that the normal urothelium in people with bladder cancer is completely different genetically and epigenetically from normal urothelium in people without bladder cancer. However, when we simply examine the tissue both macroscopically and histologically, we see a significant amount of overlap between what macroscopic and histological tumours are, and what histologically normal urothelium is in people who have cancer.

In other words, their biomarkers would be positive, but even looking under a microscope, these patients do not have bladder cancer at that point in time—because the tumour tissue is outside the bladder.

One of the best papers on the presence of extra-bladder cancer is from the Studer’s group (refer to the work of Giannarini et al. [19]), which was looking at a cohort of patients whom it had been following up for a long time, through multiple courses of BCG. Cytology in this context was positive, and the group was looking for the cancer. What it found was that roughly half of the patients had upper tract disease and roughly half had prostatic disease that was being missed.

The Studer’s group [19] came up with an algorithm (Fig. 2), which is what I would follow in case of the other possibility in this patient, where the cytology is positive but the tumour cannot be located. The physician has to hunt carefully, and trying to find it in the upper urinary tracts will require washing of the upper tracts and a biopsy of the prostate.

3.2. Conclusion and management recommendation

What would I do? I would check the entire urothelium, beginning with CT of the upper urinary tracts. I would perform washing because I do not know whether ureteroscopy is going to be helpful in the absence of positive washing. I would biopsy the prostate and then I would watch very carefully, because the patient is obviously at risk for developing disease.
4. Discussion and management options

Early detection of bladder cancer recurrence in patients with a previous history of NMIBC remains a challenge in the urological community. The goal is to have molecular markers that are able to assess whether a patient should undergo cystoscopy and other invasive procedures.

Urine cytology is useful and remains the current standard for the detection of high-grade tumours because of its high specificity and sensitivity [14]. In the existing literature, urine cytology has high specificity ranging between 94% and 99%, and sensitivity of >80% in high-grade tumours [20]. This means that in patients with a positive cytology, the presence of a high-grade tumour in the bladder should be pursued. Despite its high specificity, however, Giannarini et al. [19] demonstrated that for a considerable percentage of patients with a positive cytology, the clinician failed to find any tumour in the bladder. This lack of concordance between cytology and bladder status suggests the need to evaluate the entire urothelium, making it clear that in any case of positive cytology, the urologist has to look for tumours in the bladder, the upper urinary tract, and also the prostatic and penile urethra [19].

The highest diagnostic power of cytology is in the detection of CIS, where sensitivity reaches 100% according to some authors [21]. While the most common finding in cases of positive cytology is CIS in the bladder, we should not forget that during follow-up of high-risk NMIBC, a "non-CIS" recurrence could occur. In this framework, the presence of a biomarker that is able to reasonably ensure the absence of a recurrent tumour could avoid unnecessary cystoscopies.

Unfortunately, most of the available markers are characterised by low positive predictive values that limit their application in routine clinical practice [22]. This limitation is mainly due to a lack of biological correlation between urothelial tumour cells and the expression of the biomarkers. Moreover, the relation between different

Fig. 2 – Algorithm for cytologically positive but apparently T0 cancer. CT = computed tomography; MRI = magnetic resonance imaging. Adapted from Giannarini et al. [19].
available biomarkers remains poorly understood. NMP22, a marker released in the urine as a consequence of apoptosis, has been found to be elevated in urothelial cancer cells. Recent studies have shown low sensitivity for detecting recurrence, for both qualitative and quantitative tests [23,24]. Similarly, urinary DNA methylation has been studied as a biomarker in bladder cancer, with initially promising results [12]. Despite the initial enthusiasm, however, when the DNA methylation was analysed in symptomatic men with sexual infections it showed high levels in this cohort of patients [12]. This low specificity and the high false-positive rates are one of the critical aspects that limit the application of most of the urine biomarkers in clinical practice.

Moreover, we should take into account that the “false positive” may be indicative of cell alterations, not macroscopically visible, which may develop as tumours in the follow-up, even though the real clinical utility of the biomarkers remains unclear [22]. Owing to these limitations, none of the available markers has been proved to have a greater clinical utility than cytology, mainly due to the low specificity [1].

Could we reach different results, depending on the marker used? First and foremost, we should not forget that the predictive power of biomarkers strictly depends on the question to be answered. If the main target is to avoid unproductive cystoscopies, then probably instead of looking for good sensitivity and specificity, which is not the case owing to the large number of false positives, we have to find a marker with a very high negative predictive value. A test that is a good predictor of the absence of a tumour will have great utility in daily clinical practice when there is no cystoscopy or cytology. It remains to be seen what will happen in the near future with the biomarkers currently being assessed in on-going studies.

5. Summary and management recommendation

Urine cytology still represents an essential complement of the follow-up of NMIBC. Patients with a positive cytology during follow-up of NMIBC have to be evaluated carefully with cystoscopy and multiple random biopsies of the bladder. In case of positive cytology and negative cystoscopy, cystoscopy with PDD is recommended in order to identify suspicious areas and avoid uninformative biopsies. The upper urinary tract as well as the urethra (prostatic and penile) should be evaluated in cases of negative bladder pathology and persistent positive cytology. The high number of urine markers under evaluation for NMIBC diagnosis and follow-up is representative of the increasing interest in this research area.

Unfortunately, none of the currently available biomarkers presents the diagnostic accuracy of urine cytology, mainly due to the risk of false-negative or false-positive results with the biomarkers currently available for clinical use, although in the future a high negative predictive value will probably be accepted for detecting those patients who do not need a follow-up cystoscopy. Accordingly, urinary biomarkers need further evolution in order to be accepted for use other than in combination with standard diagnostic procedures, and any recommendation for regarding to do when a biomarker is positive but the cytology is negative should be individualised to the particular patient situation.

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Analysis and interpretation of data: Palou.
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