

Markers for Diagnosis and Progression in Bladder Cancer*

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ABSTRACT

Bladder cancer is a common disease that is often detected late and has a high rate of recurrence and progression. The current standard of care for the primary detection and follow-up of NMIBC consists of urethro-cystoscopy associated with cytology. However, several clinical risk factors have been claimed to predict recurrence and progression, these factors have a predictive value on a population basis, but no parameter has been found that reliably predicts how an individual patient's tumor will behave. In the last years many markers have been described in order to decrease the number of cystoscopies and try to provide individualized risk-stratified decision-making. We have focused our review in tumor markers for primary diagnosis, surveillance of non-muscle-invasive bladder cancer, and predicting progression to muscle-invasive disease. After our review, we can conclude that to the date no non-invasive biomarker has proven to be sensitive and specific enough to replace cystoscopy, neither in the diagnosis nor in the follow-up. On the other hand, promising results have been reported of potential biomarkers for predicting recurrence, early progression and poor response to BCG, new studies should be promoted to validate these results and make possible to incorporate markers as a new tool in clinical guidelines.

Keywords: Bladder Cancer; Non-Muscle Invasive; Recurrence; Progression; Marker; Urinary Marker; Biomarker

1. Introduction

Approximately 380,000 cases of bladder cancer (BC) occur around the world each year [1]. In the United States and Europe, BC is the fourth most common cancer in men [2]. This tumor affects three times as many men as women. Women, however, often have more advanced tumors than men at the time of diagnosis. While, majority of the deaths from BC occur in patients with muscle-invasive disease (MID), most BC (75% - 85%) are non-muscle invasive (NMI) at first diagnosis [3]. Generally, the prognosis for patients with NMI bladder cancer is good, although (30% - 80%) will recur, and (1% - 45%) will progress to MID within 5 years depending on grade, stage, and associated clinical risk factors [4]. The conservative management with transurethral resection followed by intravesical treatments with chemotherapy or/and immunotherapy has demonstrated a high efficacy in patients with NMI disease decreasing the risk of recurrence and progression. It is mandatory, in patients treated conservatively, a strict, frequent, and cost follow-up [5]. Unfortunately, a significant proportion of patients of this group will not respond and will progress

to invasive tumors ($\geq T2$) despite appropriate therapy. Several studies have reported radical cystectomy is only curative in 50% of patients who progress from NMID to MID compared to 90% when this is performed in patients with superficial tumors [6]. On the other hand, this later group of patients will suffer up to 50% of overtreatment [7].

The current standard of care for the primary detection and follow-up of NMIBC consists of urethro-cystoscopy (UCS) as the gold standard and cytology as an adjunct [3,8]. Several clinical risk factors as: size, multifocality, localization, associated carcinoma in situ, and recently prostatic urethra involvement and female gender [9,10] have been claimed to predict recurrence and progression. But these factors have a predictive value on a population basis, but no parameter has been found that reliably predicts how an individual patient's tumor will behave.

In the last years, many markers have been described in order to improve patients' comfort and help clinicians in the primary diagnosis of this common cancer, follow-up of NMIBC, and try to provide individualized risk-stratified decision-making.

The aim of this review is to provide a summary of the current evidence of the evaluation of biomarkers for diagnosis, follow-up of NMIBC, and predicting progression to MID. The MEDLINE/PubMed databases were

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used to search for the most relevant articles both original and reviews, using different combinations of the following keywords and search terms: bladder cancer, non-muscle-invasive bladder cancer, diagnosis, follow-up, progression, markers, urinary markers, and biomarkers.

2. Urinary Biomarkers for Primary Detection and Surveillance of Non-Muscle-Invasive Bladder Cancer

Urinary biomarkers may help decrease the number of cystoscopies in primary detection and surveillance, improving patients' quality-of-life and reducing the cost of follow-up. Most of the patients diagnosed of NMIBC because the risk of recurrence and progression will need a life-long follow-up by cystoscopy. Urine cytology is widely and frequently utilized but suffers from a poor sensitivity (30% - 35%). All new urinary biomarkers have shown a better sensitivity but lower specificity compared to cytology. The main question, still not answered, is whether sensitivity and specificity showed by these markers is enough to use them routinely and in all type of tumors, in order to avoid invasive explorations, such as cystoscopy.

Many studies do not differentiate between primary and recurrent tumors, nor is the performance of the studied marker assessed separately in NMIBC and MIBC or high versus low grade tumors. Apart from that, the vast majority of these studies are case-control trials comparing artificially composed study cohorts in which the prevalence of the disease frequently exceeds 50% [11]. Therefore, some of the findings are not reproducible when we want to translate them into routine clinical use because the efficacy of a marker is well known that depends on the studied population [12].

The most commonly studied urine markers and approved by the US Food and Drug Administration (FDA) and Health Canada (BTA, NMP-22, FISH UroVysion, uCyt+/ImmunoCyt, Microsatellite analysis) have demonstrated a high sensitivity (58% - 75%) and low specificity (73% - 86%) compared to 94% of urine cytology [13]. However, as occurs with the sensitivity of the cytology, Van Rhijn *et al.* [14] demonstrated in the subanalysis performed per pathological grade, that this high sensitivity decrease in the detection of low stage and grade tumors, which are the main group at primary diagnosis and recurrence. The BTA test and NMP-22 test have a very limited role because of their high false-positive rate [15,16]. But with careful selection of patients, the specificity of NMP-22 can be improved, and can be used during follow-up to delay cystoscopy control [17,18]. UroVysion can replace cytology for high-grade tumors when experience with urinary cytology is lacking or when its result is inconclusive, but adds little to the surveillance of low-grade tumors and might be useful to

predict response to intravesical therapy [19,20]. ImmunoCyt has showed the highest sensitivity for low-grade (LG) tumors and is more rarely affected by other urological disorders. However, with a 55% - 60% detection rate for LG tumors remains inadequate to replace cystoscopy [21,22]. Microsatellite analysis can predict recurrence of LG tumors up to 80%, but it still lacks sensitivity [23,24].

Nowadays, many of the tests are performed routinely in addition to urinary cytology and no single test is used widely by urologist in the follow-up of patients with BC. The trend is to associate one or more urine markers with urine cytology in order to improve sensitivity without decreasing specificity. Li, H. X. *et al.* [25] recently demonstrated that using ImmunoCyt and Cytokeratin 20 immunochemistry as adjunct marker for cytology were able to improve the sensitivity of cytology in detecting UC, specially for low grade, without a significant decrease in specificity. Other non-FDA approved, urine-based test are being investigated as potential urothelial carcinoma biomarkers include: citokeratins-8-19-20, microsatellite mutations, telomerase, DNA methylation and histone acetylation [26-29]. But to the date no non-invasive biomarker has proven to be sensitive and specific enough to replace cystoscopy, neither in the diagnosis nor in the follow-up. Additional studies are needed to determinate the clinical scenarios where urinary markers can be useful. The future markers need to be better, easier to perform, cheaper, and faster for the interpretation of the results. The test should have a high positive predictive value to avoid unnecessary invasive explorations, and high negative predictive value to avoid the risk of failing to detect tumors. The main problem is that these parameters vary between populations with different incidences. Therefore, cannot be used for comparing methods and tests.

3. Biomarkers for Predicting Recurrence and Progression to Muscle-Invasive Disease in NMIBC

Bladder cancer is a genetic disorder driven by progressive accumulation of multiple genetic changes. The specific alterations in gene expression that occur as a result of cross-talk between various cellular pathways determine the biologic behavior of the tumor. Thus different molecular profiles have been associated with recurrence, early progression and the response to a specific therapy. Biological measures may help to elicit the individual patient's risk of future outcome. Hence, they may be useful to identify those patients with NMIBC who could benefit from immediate radical cystectomy and do not waste a critical time with conservative management or ones who should have a more strict follow-up.

Because of all of this, in the last years the search for biomarkers has been called into question and has ob-

tained notoriety in basic and transitional BC research. Recently several studies, with significant number of cases, have reported that different biomarkers, in diverse cellular function, should be taken in consideration as predictors for recurrence and progression to MID in patients with NMIBC. These studies include: Karyopherin- $\alpha 2$, a nuclear and cytoplasmatic protein which is thought to play an important role in the nucleocytoplasmatic transport and has also been suggested to be a transporter of tumor suppressors [30]; HMOX 1, one of the three isoforms of HMOX that catalyzes the degradation of heme to biliverdin, carbon monoxide and free iron [31]. GSTM1 and GSTT1 both polymorphisms in glutathione S-transferase enzyme [32,33]; C16orf74 a gene involved in inflammatory processes and strongly associated with an anti-TNF response and hypoxia [34]; and the well known HRAS, KRAS, p63, Her-2 [35,36]. Her-2 protein expression has been recently published as Ezrin protein expression and the methylation status of TSGs were able to distinguish patients responding to BCG from those who may require more aggressive therapeutic intervention [37-39]. The p53 mutation and p53 protein status were one of the first markers in showing promising results for predicting outcomes of patients with NMIBC, but finally they have been abandoned for clinical practice, even though many studies continue to show significant association between recurrence, progression and survival [40,41].

Although several biomarkers obtained from these different studies have showed promising results, the majority of the experts in the field consider it is unlikely that any single marker will be able to improve prognostication for patients with NMIBC. Similarly is happening with clinical risk factors, many of the authors advocated for a combination of multiple independent markers to predict outcome. Bryan *et al.* [42] published in 2005 a panel using seven independent biomarkers according to increased expression of proteins and growth factors who play critical and different roles in regulation of the cell cycle, apoptosis, DNA synthesis and repair, and angiogenesis. The same group in 2009 updated their results and confirmed that the combination of (VEFG, pRb, FGFR3, CK-20, Ki-67, EGFR and p53) was effective to predict, recurrence, progression, and poor outcome in patients with BC.

Other authors have proposed and present their good results using a group of markers involved in one pathway (apoptosis, cell cycle regulation, etc.) [43,44].

Since a role for inflammation in carcinogenesis is now well-established) some authors [45] have worked in the analysis of COX-2 expression. But it seems that even COX-2 expression is higher in high grade and MID compared to NMIBC, but this is not useful to predict progression in NMIBC.

Although multiple markers described, any one has been included in guidelines to help clinicians into clinical decision-making, and clinical risk factors are still the only risk calculator tool to decide conservative management versus radical cystectomy in high risk NMIBC.

4. Conclusions—Take Home Message

In general, the best urinary markers have higher sensitivity than urinary cytology, but specificity is usually lower. But to the date no non-invasive biomarker has proven to be sensitive and specific enough to replace cystoscopy, neither in the diagnosis nor in the follow-up. For this reason, UCS continues to be the mainstay in BC detection and follow-up. As cytology is highly specific, it is still important as an adjunct for the evaluation of patients with hematuria, in the detection of primary BC and also in the surveillance of patients with high grade NMIBC. However, new markers combinations associated with urinary cytology may play a role in future screening of patients with low grade NMIBC.

Many markers for predicting recurrence, early progression and poor response to BCG have been identified in the last years. While some authors proposed using a combination of them, others advocate for individual ones. Due to the fact that classical clinical risk factors are not specific enough for predicting NMIBC outcomes and that promising results have been reported of potential biomarkers, new studies should be promoted to validate these results and make possible to incorporate markers as a new tool in clinical guidelines. But to carry out the translation of knowledge into practice with maximal efficiency and effectiveness, it is essential to conduct these studies with appropriate designs.

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