Combining molecular and pathologic data to prognosticate non-muscle-invasive bladder cancer

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Abstract

Non-muscle-invasive (NMI) bladder cancer (BC) is a chronic disease with varying oncologic outcomes requiring frequent follow-up and repeated treatments. Recurrence (in up to 80%) is the main problem for pTa NMI-BC patients, whereas progression (in up to 45%) is the main threat in pT1 and carcinoma in situ (CIS) NMI-BC. In a recent European Organization for Research and Treatment of Cancer (EORTC) analysis, multiplicity, tumor-size and prior recurrence rate are the most important variables for recurrence. Tumor grade, stage, and CIS are the most important variables for progression to muscle-invasive (MI)-BC. However, reproducibility of pathologic stage and grade is modest, which is a major concern to clinicians.

Molecular markers are promising for predicting clinical outcome of NMI-BC, especially because clinicopathologic variables are not sufficient for individual prediction of prognosis. Several obstacles and opportunities have been linked to molecular markers. The role for molecular markers to predict recurrence seems limited because multifocal disease and incomplete treatment probably are more important for recurrence than the molecular features of a removed tumor. Prediction of progression with molecular markers holds considerable promise. Examples are the combination of immunohistochemical markers, gene expression signatures, and molecular grade (based on FGFR3 mutation status and Ki-67 expression). Nevertheless, the value of molecular markers over clinicopathologic indexes is still being questioned and their clinical use limited. © 2012 Elsevier Inc. All rights reserved.

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Introduction

The global incidence of urinary bladder cancer (BC) was approximately 357,000 cases in 2002 [1]. The highest incidence rates are observed in North-America and Western-Europe [1,2], while the lowest rates are found in the Asian countries (China, Japan, Korea) and central Africa [1,3]. Variation in registration of pTa (low-grade) tumors may partly be the cause of these differences [4]. In the USA, it is the fourth most common cancer in men and the tenth in women [2].

Most (75%) BCs are non-muscle-invasive (NMI) at first diagnosis [pTa, pT1, carcinoma in situ (CIS)] [5]. In NMI-BC, around 70% present as pTa, 20% as pT1, and 10% as CIS lesions [6]. Generally, the prognosis of NMI-BC is good, although 30%–80% will recur and 1%–45% will progress to muscle-invasion (MI) within 5 years [5–9]. Consequently, NMI-BC is a chronic disease with varying oncologic outcomes requiring frequent follow-up and repeated treatments, making the cost per patient from diagnosis to death the highest of all cancers [10,11]. Treatment ranges from transurethral resection (TUR) followed by a single chemotherapy instillation in low-risk NMI-BC, to sometimes re-TUR and adjuvant intravesical therapy in intermediate- and high-risk patients, and to (early) cystectomy for (treatment-refractory) high-risk NMI-BC [12].

MI-BC (pT2-pT4) is completely opposite from NMI-BC. If left untreated, it is a deadly disease, usually within a short time frame. Treatment often requires a multidisciplinary approach with extensive surgery, radiotherapy, and chemotherapy as cornerstones [13]. Despite these life altering treatments, the prognosis of MI-BC patients who do not have radiological signs of metastasis at time of diagnosis is still dismal. Roughly half of these patients will develop metastasis and eventually die of their disease [13].

Currently, decision making and prognosis of BC still relies heavily on tumor, node, metastasis (TNM) stage...
and pathologic grade [12,13]. However, the reproducibility of pathologic assessments is modest [12–15]. Therefore, intense research efforts are being made to identify and characterize molecular markers for various malignancies. Molecular markers hold considerable promise to predict clinical outcome of NMI-BC. Nevertheless, the value of molecular markers over clinicopathologic indexes is still being questioned and their clinical use limited.

This review summarizes the current status of clinicopathologic data and molecular markers for prediction of recurrence and progression of NMI-BC. In addition, reproducibility of pathology and molecular markers [immunohistochemistry, gene signatures, and molecular grade (mG)] are discussed.

**Prognostic factors recurrence**

*Clinical and pathologic prediction of recurrence*

Clinical and pathologic factors for NMI-BC recurrence have been studied extensively over the years [5,8,16–21]. Although studies vary in the number of included patients, duration of follow-up, the variables analyzed, and statistical analysis, the most important variables for prediction of NMI-BC recurrence are multiplicity, tumor-size, and prior recurrence rate [8,16–21]. Sylvester reviewed 19 articles, of which most compared various intravesical chemotherapy drugs and regimens [19]. In an attempt to individualize prediction of recurrence, Sylvester et al. [8] calculated the probability of recurrence using data of 2,596 patients who participated in 7 European Organization for Research and Treatment of Cancer (EORTC) trials. Different variables were important for recurrence and progression of NMI-BC. Each variable was assigned a weighted score per endpoint (recurrence or progression). The total score per endpoint was based on 6 variables (i.e., grade, stage, CIS, multiplicity, size, and prior recurrence rate). This risk calculator is available at: http://www.eortc.be/tools/bladdercalculator. The European Association of Urology (EAU) subsequently adopted this system in their guidelines and determined the scores for patients at low, intermediate, and high risk for recurrence [5,8]. Recently, Fernandez-Gomez et al. [21] reported the data on prognostic factors from 4 Club Urológico Español de Tratamiento Oncológico (CUETO) trials, in which 1,062 patients received bacille Calmette-Guerin (BCG). Multiplicity, prior tumor, female gender, and CIS were significant predictors for recurrence in multivariate analysis [21]. Taken together, multiplicity is of utmost importance to predict recurrence. The chances on definitive cure of a patient with primary NMI-BC rely on a high quality TUR, removing all (pre)malignant lesions. Multiple tumors probably have more lesions than we are able to visualize and resect at the TUR.

**Molecular prediction of recurrence**

Over the years, a wide variety of markers has been identified and linked to BC prognosis. However, most molecular markers show a relation to grade, stage, and progression and not to recurrence of BC [20,22–24]. Even the recently identified FGFR3 (fibroblast growth factor receptor 3) mutation, which allows selective identification of favorable NMI-BC, did not predict recurrence in 2 studies with more than 200 patients either alone or in combination with other molecular markers [25,26], as multiplicity was the only significant predictor for recurrence in multivariate analysis [25]. For P53, opposite results are found. Shariat et al. [27] found the mutant genotype to be a predictor for recurrence, whereas Moonen et al. [28] found more recurrences in patients with wild type tumors. Both studies had adequate follow-up and included around 100 patients. A combination of multiple biomarkers or analyses of multiple genes may be of interest to identify specific markers (survivin [29], Mcm3 [30]) or gene classifiers to discern recurrent from nonrecurrent NMI-BC [27,29–31]. However, validation with 404 patients from 5 European countries of the previously published 26-gene signature for recurrence was not successful [32]. The role for molecular markers to predict recurrence seems limited. Multifocal disease and incomplete treatment by TUR may be more important for recurrence of NMI-BC than the molecular features of a removed tumor.

**Prognostic factors progression**

*Clinical and pathologic prediction of progression*

As for recurrence, clinical, and pathologic factors for NMI-BC progression have been studied extensively over the years [8,16–21]. Fortunately, progression to MI-BC is not as frequent as recurrence. Nevertheless, 5-year probabilities range from <1% to 45%, depending on prognostic factors [5,8]. In larger series, usually the same patient populations were used to predict recurrence and progression [8,19]. The most important variables for prediction of progression in NMI-BC, however, are CIS, grade 3, and stage T1 and, therefore, very different from the most important ones for recurrence [8,17,19]. Sylvester et al. [8] calculated the probability of progression using the same 2,596 patients who participated in 7 EORTC trials. The weighted score for progression based on the same 6 variables as described in this review was used. The EAU also adopted this system in their guidelines [5]. Scores for patients at low, intermediate and high risk for progression to MI-BC were defined [5,8]. The main limitation of the EORTC risk tables is that most patients were treated by “old” intravesical chemotherapy regimens. Improvements of intravesical chemotherapy, use of a single chemotherapeutic instillation after TUR, and the increased use of (maintenance) BCG may reduce the pre-
dictability of these tables, especially for progression. Fernandez-Gomez et al. [21] reported the data on prognostic factors from 4 CUETO trials, in which 1,062 patients received BCG. A recurrence at first cystoscopy, stage, grade, and prior tumor were the prognostic variables in the multivariate analysis for progression. Concomitant CIS was significant in univariable analysis [21]. Additional aggressive factors, which are rare but may be found in NMI-BC, are micropapillary BC and lymphovascular invasion (LVI). Micropapillary NMI-BC is BCG-resistant, and two-thirds of patients NMI-BC progressed to MI [33]. If LVI is present in an NMI-BC specimen at TUR, it indicates understaging and possible lymph-node involvement [34]. Moreover, these authors reported LVI in 9% of their TURs as opposed to 36% of their matched cystectomy specimens [34].

The decision to perform a radical cystectomy for high-risk NMI-BC remains a major challenge for urologists because a randomized comparison between immediate cystectomy and first-line conservative management is not available [12]. Moreover, prognostic factors as outlined above are not conclusive for individual prediction in patients with (high-risk) NMI-BC and, consequently, frequent follow-up with cystoscopy is required [5,8,12,25,35].

Molecular prediction of progression

The important pathologic variables for prediction of progression in NMI-BC are CIS, grade 3, and stage T1. These factors reflect the biological aggressiveness of the disease and are associated with the high risk NMI-BC phenotype [5,8,19]. Molecular alterations are often linked to these high-risk pathologic features and not to multiplicity and size, which are clinical variables and more important for recurrence.

A wide variety of molecular markers (oncogenes, tumor suppressor genes, cell cycle regulators, epigenetic changes, proliferation antigens, cell adhesion molecules, chromosomal alterations, and signaling proteins, etc.) has been investigated for a better assessment of NMI-BC prognosis [20,22,24,25,35–37]. Some of these markers have shown to be associated with biological aggressive disease and hold promise to predict progression in NMI-BC [20,22,24,25,37]. In this review, some crucial developments will be highlighted.

p53 is the most frequently investigated marker to predict progression [25,27,28,38]. Most, but not all, studies found that a p53 mutation or p53 overexpression is associated with progression. The number of patients and methods of analysis cause the discrepancies between studies [38].

Two genetic events (loss of parts of chromosome 9 and FGFR3 mutations) in NMI-BC are both frequent and present in early NMI-BC. Loss of heterozygosity of chromosome 9 has been found in up to 60% of cases [24,36,37]. However, no association with clinical parameters or progression has been found [24,37]. Conversely, the FGFR3 mutation has been found to be a selective marker for favorable disease in several reports [25,26,35,39]. In pTaG1, 88% had a mutation as opposed to 19% of pT1G3 cases [25]. Moreover, in several independent studies, the FGFR3 mutation protected against progression [25,26,35,39]. This led to the proposal of the FGFR3 mutation as the genetic event responsible for the favorable pathway in BC (Fig. 1) [25]. Furthermore, based on FGFR3 mutation status and the expression of the proliferation marker Ki-67, molecular grade (mG1-3) was introduced as a highly reproducible and prognostic tool in BC progression. The first prospective data confirmed the prognostic value of molecular grade (mG) for progression [26]. Moreover, in a study with long-term (median; 8.6 years) follow-up, the addition of mG to the multivariable model for progression significantly increased its predictive accuracy and mG provided valuable prognostic information next to the EORTC risk-scores for progression [35].

The story of p53 has taught us that a combination of molecular markers is necessary to predict progression [38]. mG as discussed above is an example of this. Another example is the differential expression of multiple immuno-histochemical markers from 1 tissue sample using a tissue micro array (TMA). Shariat et al. [27] investigated p53, pRB, P21, and P27 and found that the number of altered markers was independently associated with an increased risk of progression. The technique is easy, its analysis has been automated, is possible in any pathology laboratory, and enhances prediction of progression [27,40].

Another example of combining genetic information is the construction of a gene classifier using microarrays [31]. This approach has the potential for individual prediction of prognosis. In an international validation study, which is currently the largest study on gene-expression microarrays, 2 classifiers, 1 based on 52 genes and another on 88 genes, were significant in multivariate analysis for progression [32]. Although these gene classifiers enhanced prediction of progression next to clinicopathologic indexes, sensitivity and specificity to predict progression were both only 66%. Hence, a gene classifier on its own is not enough to predict progression in individual cases.
Prospective data using well-defined patient cohorts and endpoints will give us valuable information on mG [25,35] and the (tissue/gene) microarray technologies [27,31,32,40] in the future. In addition, more work is needed on reproducibility, easy access to these technologies, and simplification and combination of methods. Until then, the value of molecular markers over traditional histopathology is not clear and, therefore, these markers are currently not used in clinical decision making.

Reproducibility

Observer variability and prognosis in pathology

Reproducibility of pathologic assessment has not been studied as extensively as clinicopathologic prediction of recurrence and progression [12]. Nevertheless, reproducibility is of utmost importance to compare clinical results between institutes and also if we want to individualize the prognostic value of NMI-BC grade and stage.

The largest study on stage review was published in 2000 [15]. Local pathology and review pathology were compared in 1,400 patients who participated in 5 EORTC trials. Although agreement between pathologists varied from 36% to 90%, differences in prognosis were slight. The authors recommended pathology review in high-risk NMI-BC. This conclusion was confirmed by a study with 164 T1 NMI-BC patients treated with BCG whose original slides were reviewed [41]. Close to 20% of T1 tumors were up-staged or down-staged, and the reviewed stage strongly predicted patient’s prognosis [41]. Hence, pathology review in the high-risk NMI-BC category identifies patients with different prognoses who may benefit from other treatment strategies than BCG.

Pathologic grade carries significant prognostic information, especially for prediction of progression [5–9,12–21]. However, its poor reproducibility is a recognized problem and a major concern. Grade assessment (WHO 1973) varied from 42% to 73% between local and review pathologists in the 5 EORTC studies mentioned above [15]. A new classification system for grade was adopted by the WHO in 2004 [12]. Next to high observer variability, the main reasons to propose a new system were the lack of clear definitions for the three WHO 1973 grades and the high percentage of NMI-BC classified as G2 (the default diagnosis) [12]. Although the new system contains detailed histologic criteria to decrease observer variability, direct comparison of both classification systems for grade did not result in a better prediction of progression and/or better reproducibility [12,14]. The main criticism of the new system is that it has been accepted despite lack of clinical evidence and proper studies with long-term follow-up to assess its reproducibility and prognostic value compared with the “old” WHO 1973 system [12]. Therefore, the AUA and EAU guidelines advocate the use of both systems until the 2004 system has been properly validated [12,16]. However, the advantage of the new system over the WHO 1973 system will probably not be large as interobserver variability remains high in both systems.

Recently, the mean grade of a pathologist was found to be constant for 1 pathologist but highly variable between pathologists (up to 0.7 grade), irrespective of the classification system used [14]. In this study with 173 NMI-BC cases, the mean-grades for the WHO 2004 system were 0.3–0.5 grades higher than those of WHO 1973. Mean grade distinguished low- and high graders among the pathologists, and this knowledge was strongly linked with the risk of progression for each grade category [14]. This new mean grade concept allows a better assessment of the prognostic value of grading. Mean grade has the potential of becoming a tool for quality assurance in pathology and clinical trials [14].

Stage and grade are the gold standards on which treatment decisions in NMI-BC are based. The poor reproducibility of pathologic stage and grade is a recognized problem and a major concern for clinicians. It is probably the main reason that individual decision-making based on grade and stage is troublesome in individual cases.

Reproducibility of molecular markers

Studies on reproducibility of molecular markers are rare. Hereunder, some aspects of reproducibility of immunohistochemistry, gene signatures, and molecular grade (mG) for NMI-BC are discussed.

The National Cancer Institute (NCI) conducted a very well-designed study on the reproducibility of p53 immunohistochemistry [42]. Fifty high-grade, primary BCs were subjected to several sources of variability (i.e., the scorer, the staining laboratory, and another tumor-block). The percentage agreement ranged from 83% (2 scorers, 2 slides, 2 staining laboratories) to 95% (1 scorer, 2 slides, 1 staining laboratory). In another study, the percentage agreement (2 scorers, 1 slides, 1 staining laboratory) for Ki-67, p53, and P27 were 91%, 88%, and 85%, respectively [25]. These values are higher than the percentages of agreement for pathologic grade but it should be noted that immunohistochemistry is either positive or negative (2-tier system) and pathologic grade usually is a 3-tier system. In the new WHO 2004 classification system, for grade the first 2 categories are sometimes lumped together, resulting in a 2-tier system. Using such an approach, the percentages of agreement for pathologic grade ranged from 68% to 87% (4 pathologists, 1 slide, 1 laboratory). These values come close to but do not completely reach the reproducibility of immunohistochemistry.

Several groups have constructed a gene classifier using microarrays to predict clinical outcome [31,32,43,44]. The classification performances show very similar accuracies to predict NMI-BC progression but the studies show only little overlap in the identified genes that are part of the classifier [31,32,43,44]. Some explanations for this phenomenon may
include heterogeneity between the analyzed tumors, poor reproducibility of this method, and/or a cutoff value issue when scaling down from expression profiles of ±20,000 genes to less than 100 genes to construct a gene classifier. The main issue that remains is which genes to choose in a classifier to predict a certain clinical endpoint.

Molecular grade (mG), based on FGFRI mutation status and Ki-67 expression, has been proposed as an alternative to predict progression [25,35]. A recent study was the first to compare the reproducibility of pathologic grading and mG on the same series of patients [35]. The reproducibility of mG was almost perfect (κ: 0.76), whereas reproducibility for pathologic grade was only fair to substantial (κ: 0.17–0.58) [35]. Taken together, mG (composed of 3 mg) proved more reproducible than pathologic grade assessment, making it a reliable and robust tool to assess progression in NMI-BC.

The reproducibility of molecular markers in NMI-BC is an understudied subject, which deserves more attention if molecular markers are to become important in clinical practice.

Conclusions

Prognosis of NMI-BC relies heavily on clinical variables (multiplicity, size, and prior recurrence rate) to predict recurrence, and pathologic variables (TNM-stage, grade, and CIS) to predict progression. The role for molecular markers to predict recurrence seems limited. Prediction of progression with molecular markers holds considerable promise.

Reproducibility is understudied compared with prognostic analyses for recurrence and progression of NMI-BC. Nevertheless, it is highly important. If the gold standard (stage, grade) is not reproducible, prognostic analyses may be biased. Moreover, the fair to substantial reproducibility of pathology in NMI-BC may be a critical obstacle for the introduction of molecular markers in clinical practice.

Several opportunities have recently been identified. First, knowledge of the pathologist’s mean grade may be a step forward for quality control in pathology and clinical trials. Second, prospective data using well-defined patient cohorts and endpoints will give us valuable information on mG and (tissue/gene) microarray technologies in the near future. The reproducibility of prognostic (clinical and molecular) markers in NMI-BC has been understudied and deserves more attention.

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