Mertens, Laura S.; Neuzillet, Yann; Horenblas, Simon; van Rhijn, Bas W.G.
BIOMARKERS FOR ASSESSING THERAPEUTIC RESPONSE IN BLADDER CANCER
Archivos Españoles de Urología, vol. 66, núm. 5, junio, 2013, pp. 495-504
Editorial Iniestares S.A.
Madrid, España

Available in: http://www.redalyc.org/articulo.oa?id=181031087011
BIOMARKERS FOR ASSESSING THERAPEUTIC RESPONSE IN BLADDER CANCER

Laura S. Mertens¹, Yann Neuzillet¹, ², Simon Horenblas¹ and Bas W.G. van Rhijn¹.

¹Department of Urology. The Netherlands Cancer Institute. Antoni van Leeuwenhoek Hospital. Amsterdam. The Netherlands.

Summary.- Reliable markers for assessing therapeutic response are needed to select the most effective treatment strategy for bladder cancer patients. We analyzed the role of biomarkers predicting response of non-muscle invasive bladder cancer (NMIBC) on BCG induction, and of non-organ confined muscle invasive bladder cancer (MIBC) on neoadjuvant chemotherapy. A critical, non-structured review of the literature was conducted.

For assessing BCG therapy outcome, measurement of urinary IL-2 levels seems to be the most potent marker of all the clinical parameters reviewed. Measurements of urinary interleukins IL-8, IL-18, and tumour necrosis factor apoptosis-inducing ligand levels seem promising as well. Immunohistochemical markers (ie, TP53, Ki-67, and Rb) display contradictory results and seem unsuitable. Gene polymorphisms need to be studied more thoroughly before their clinical relevance can be determined.

Regarding assessing and predicting response of MIBC to neoadjuvant chemotherapy, a set of potent markers has been studied. However, no conclusive evidence is yet available on their additional value over the established clinicopathological variables.

Prospective trials are needed to validate the clinical benefit of molecular markers to predict response to BCG (NMIBC) and neoadjuvant chemotherapy (MIBC) before predictive biomarkers can become part of clinical practice.


Resumen.- Son necesarios marcadores fiables para evaluar la respuesta terapéutica en pacientes con cáncer vesical, para seleccionar la estrategia terapéutica más efectiva. Analizamos el papel de los biomarcadores prediciendo la respuesta del cáncer vesical no muscular invasivo a la inducción con BCG, y del cáncer vesical muscular invasivo no órgano confinado a la quimioterapia neoadyuvante. Se llevó a cabo una revisión crítica no estructurada de la literatura.

Para evaluar el resultado de la terapia con BCG, la medición de los niveles de IL-2 parece ser el marcador
INTRODUCTION

Bladder cancer is one of the most common malignancies of the urinary tract. It is the fourth most common malignancy in men, accounting for 7% of all newly-diagnosed cancers in men, and 3% of all cancer-related deaths in the United States (1). Bladder cancer includes several histological variants. In the Western World, urothelial carcinoma is the most commonly diagnosed type of bladder cancer. Of those, approximately 70% will be non-muscle invasive bladder cancer (NMIBC).

NMIBC is treated by transurethral resection (TUR) of the bladder tumour. The risks of recurrence and progression of NMIBC may be estimated for individual patients using a scoring system based on 6 histopathological and clinical variables (2,3). The stratification of patients into low-, intermediate-, and high-risk groups for recurrence and progression is crucial for the recommendation of adjuvant treatment and follow-up schemes (3). Patients with an intermediate or high risk of recurrence and/or progression should receive one immediate instillation of chemotherapy followed by a minimum of 1 year of bacillus Calmette-Guérin (BCG) intravesical immunotherapy. Restaging TUR after 4-6 weeks allows for a better selection of patients for BCG therapy (4). Despite treatment, 30–50% of the NMIBC patients fail to respond to BCG, and 15–45% have progression to muscle-invasive disease depending on their initial risk category (5-9). A radical cystectomy (RC) may be offered to the highest risk patients, and is recommended in BCG-failure patients (3). Predicting response to BCG is important to identify which patients fail to achieve response and to enhance their clinical management.

For muscle invasive bladder cancer (MIBC), in particular non-organ confined bladder cancer (Stage T3, T4, or node-positive disease), treatment of choice is an RC, including a bilateral pelvic lymph node dissection (PLND). Prospective studies have shown that neoadjuvant chemotherapy followed by RC and PLND significantly improves survival (10-14). Complete clinical and in particular complete pathological response to neoadjuvant chemotherapy is associated with improved survival whereas patients with clinical progression under neoadjuvant chemotherapy will not benefit from extensive surgery (15,16). Consequently, predictive markers may indicate who will and who will not benefit from neoadjuvant chemotherapy.

Biomarkers for predicting response to BCG and neoadjuvant chemotherapy are necessary to individualize the treatment regimen. In this review, we analyzed the role of biomarkers predicting response of NMIBC on BCG induction, and of MIBC on neoadjuvant chemotherapy.

Response of NMIBC to intravesical BCG

A theory on the working mechanism of intravesical BCG instillation, is that a local immune response is mounted against the tumour in the bladder. Zuiverloon et al. recently reviewed the current knowledge on markers predicting response to BCG immunotherapy in NMIBC patients (17). In analogy to their report, 4 groups of markers predicting response of NMIBC to BCG are reviewed: clinicopathological, inflammatory, cell cycle, and gene polymorphisms.


1. Clinicopathological markers

Non-responding patients can be clinically identified by refractory disease after BCG induction (4). Higher risks of recurrence and progression are seen in patients in whom a tumour was visible at first cystoscopy (18-20), in patients with a history of bladder cancer, older age, concomitant CIS, multiplicity, and high-grade tumours (20-23). The role of tumour size and BCG toxicity as prognostic factors is ambiguous (20,21,24). In a review by Saint et al. no other independent clinicopathological prognostic...
factors for bladder tumour response to BCG have been identified (25). Clinicopathological variables alone are not able to predict response to BCG and therefore other types of markers are needed.

2. Inflammatory markers

BCG’s mechanism of action is not completely understood. However, it is assumed that exposure to BCG results in a local immune response and massive inflammation, in which neutrophils, antigen-presenting cells and inflammatory cytokines are involved.

A high number of urinary dendritic cells after BCG induction, is suggested to have a positive effect on the outcome of BCG treatment. Patients without recurrence showed to have greater increased urothelium expression of antigen-presenting molecules and chemokines after BCG treatment (26,27). Cytokines that have been hypothesized to play a role in BCG response in NMIBC are IL-2, IL-8, IL-18, polymorphonuclear neutrophil granulocytes (PMN) and tumour necrosis factor apoptosis-inducing ligand (TRAIL).

It has been demonstrated that expression of the IL-2 gene in peripheral blood mononuclear cells is essential in BCG-induced antitumour response; 95% of the BCG-non responders lacked inducibility of IL-2 mRNA (28). High levels of urinary IL-2 after BCG administration are associated with a favourable outcome (28-30). Measurement of urinary IL-8 and IL-18 concentrations also seems promising in predicting BCG response (31,32); however, cut off values need to be validated and the optimal time point for the measurement needs to be established. In a group of BCG-treated mice, depletion of PMN cells impaired the effect of BCG and resulted in a decreased survival compared with non depleted mice. High levels of urinary tumour necrosis factor apoptosis-inducing ligand (TRAIL) were present in BCG-responders.

Taken together, urinary IL-2 levels currently seem to be the most potent marker of all the parameters reviewed.

3. Molecular biomarkers

• Cell cycle markers

Retinoblastoma (Rb) is a tumour suppressor protein which is dysfunctional in several major cancers (33). Rb underexpression may be predictive of non response and cancer recurrence after intravesical BCG therapy (34,35). Tumour protein 53 (p53) is another tumour suppressor protein. It responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence and DNA repair. The predictive value of p53 for BCG response in NMIBC is unclear, since several studies show opposite results (34-41). Ki-67 is a nuclear protein associated with cellular proliferation (42). It is not considered suitable to predict response to BCG therapy either, due to the contradictory results reported in the literature (36,38,39). More evidence is needed before recommendations can be made, regarding the use of cell cycle markers for this purpose.

• Gene polymorphisms and signatures

Nucleotide excision repair (NER) and inflammation-related gene polymorphisms are currently the most investigated gene polymorphisms with regard to BCG treatment response in NMIBC patients. NER is a DNA repair mechanism, of particular importance by removing the vast majority of DNA damaging and consequently preventing unwanted mutations. Several variant alleles of NER genes have been observed in BCG-treated patients with a decreased RFS (43-46).

Polymorphisms of TNF and IL-8 and IL-6 were suggested to influence outcome after BCG immunotherapy and to be associated with decreased recurrence risk and increased survival (47,48). Polymorphisms of peroxisome proliferator-activated receptor _ (PPARG) and tumour growth factor ß (TGF-ß) were also found to be associated with a favourable outcome (49,50). Accordingly, two polymorphism variants in NRAMP1 showed an association with RFS following BCG treatment (51).

Microarray technology has not yet been fully used in the search for BCG response biomarkers. Until now, only one paper reported a gene signature that could possibly predict BCG response and progression in primary T1 bladder tumours (52). Efforts are currently being made to enhance this technology.

Response of MIBC to neoadjuvant chemotherapy

The use of neoadjuvant or induction chemotherapy for non-organ confined MIBC has been advocated. Its rationale is twofold: Reducing the size of tumour, allowing a complete surgical resection of the cancer and eradicating occult metastasis prior to surgery. Treatment of choice is cisplatin-based combination chemotherapy. Cisplatin-based neoadjuvant chemotherapy has demonstrated a survival benefit and a down staging effect with a complete pathological (pCR) of approximately 25% (53-56). Responders, especially those with a complete pathological response have a more favourable prognosis (16,57). By contrast, a large subset of patients does not respond, and non
responders fare poorly. Furthermore, chemotherapy has considerable toxicity. Biomarkers predicting response to neoadjuvant chemotherapy could allow for identification of responders as early as possible.

1. Clinicopathological markers

The American Joint Committee on Cancer TNM staging system is the most commonly used tool to predict outcomes of patients with MIBC (58). Nomograms have been developed to improve prediction of outcomes, such as tumour recurrence after cystectomy. Recently, an algorithm based on preoperative serum levels of CEA, CA 125 and CA 19.9 was developed to predict non-organ confined disease in ≤cT2 patients (59). This algorithm may possibly aid in selecting patients who would benefit from neoadjuvant chemotherapy before cystectomy. Regarding therapeutic response assessment and/or prediction, no such algorithms are currently used.

Clinical tumour response to neoadjuvant chemotherapy is usually evaluated using contrast-enhanced-CT (CE-CT) scanning, according to RECIST 1.1 (60). Such estimation of tumour response is subjective and may be confounded by chemotherapy-related tissue changes and residual non malignant masses. Post-treatment assessment of metabolic tumour response according to EORTC recommendations (61) is increasingly employed in treatment protocols for other malignancies and offers better and earlier therapy monitoring than CE-CT (62-70). A recent meta-analysis shows good accuracy of FDG-PET/CT in staging of MIBC (71). Unfortunately, there are currently no data available on FDG-PET/CT for response evaluation of neoadjuvant chemotherapy in MIBC yet.

Besides optimizing the assessment of therapeutic response in bladder cancer, it would be interesting to identify a set of biomarkers which allows for prediction which patients are most likely to respond to neoadjuvant chemotherapy. Ideally, these biomarkers may allow identification of responders and non-responders before administration of neoadjuvant chemotherapy.

2. Inflammatory markers

In a series of 30 MIBC patients receiving neoadjuvant chemotherapy, it has been investigated that patients who responded, the pre-treatment values of T lymphocytes, CD4+/CD8+ ratio, and NK cell levels were significantly higher than in non responders (72). No conclusions may be drawn from this small, single study, and further prospective studies are needed to confirm these findings.

3. Molecular biomarkers

• Cell cycle markers

Several studies have been carried out to investigate the role of apoptosis-related proteins in prediction of response to neoadjuvant chemotherapy or chemoradiation for MIBC. Sarkis et al. reported a threefold increase in mortality risk in case of overexpression of p53 in tissue sections after cisplatin-based neoadjuvant therapy. However, the authors did not study the expression level of p53 before chemotherapy (73). Results from other studies regarding the predictive value of p53 immunoreactivity in patients treated with neoadjuvant chemotherapy are contradictory (74-77).

The relation between p21 expression and response and survival following neoadjuvant chemotherapy is ambiguous (74,75), as well as the relation between Ki-67 expression and survival (76,78). In the study by Garcia del Muro, the decrease in expression of RB1 was studied but was not correlated with survival (75). In a single study, the Bax/Bcl-2 ratio did show a significant association with the complete response rate (78). Moreover, a high S-phase fraction (SPF) has shown to be significantly associated with increased overall and disease free survival after treatment with neoadjuvant chemotherapy (79).

One of the problems regarding the studies on the abovementioned markers is the suboptimal study designs. Quite some observations are made in retrospective studies or in studies limited by the small number of patients. It has been suggested that expression levels of p53 and p21, when simultaneously assessed, exhibit independent predictive value for long-term bladder preservation and survival in patients with MIBC treated with neoadjuvant chemotherapy (75). However, this was investigated in a single study among patients who underwent bladder preserving therapy. Other combinations of expression levels have been proposed, but also suffer the same limitations (80).

A different category of intracellular proteins include the heat shock proteins. Heat shock proteins (HSP) have a cytoprotective function (81), but also contribute to the inflammatory reaction: following stress, HSP can be presented on the cell surface or released to the surroundings, thereby activating the immune system (82) and mediating the production of proinflammatory cytokines (83). Pre-chemotherapy expression of HSP-60 was found to be marginally associated with good pathological response after neoadjuvant chemoradiation for MIBC or high-risk NMIBC (84). It is unclear whether HSP60 expression may also be useful for predicting response to neoadjuvant chemotherapy only.
Of other proteins, for instance, multidrug resistance protein 1 (MRP1) and lung resistance related protein/major vault protein (LRP/MVP), it has been suggested that high expression levels correlate with respectively high and low response rates (85). Grossman et al. have shown that microvascular markers (CD34 and HPCA-1) were correlated with survival after treatment with neoadjuvant chemotherapy and cystectomy (76). The decrease in the microvasculature may explain a decrease in access of chemotherapy to cancer cells.

Cell cycles markers were correlated to response and outcome of bladder cancer patients treated with neoadjuvant chemotherapy, however, they were mostly evaluated in single studies, in a wide variety of settings. Consequently, conclusive clinical evidence regarding the predictive value of these markers is lacking at this time.

**Gene expression**

Cisplatin-based chemotherapy acts by damaging DNA. Levels of DNA repair genes could predict response. One of the repair genes is excision repair cross complementing 1 (ERCC1). Measuring ERCC1 activity may have utility in clinical cancer medicine because one mechanism of resistance to platinum chemotherapy drugs correlates with high ERCC1 activity. Bellmunt et al. evaluated messenger RNA expression levels of ERCC1 in tumour DNA from MIBC patients treated with cisplatin-based chemotherapy. They found a significantly higher survival in patients with low ERCC1 levels (86).

Takata et al. analyzed gene expression profiles of biopsy materials from 27 MIBC patients using a cDNA microarray consisting of 27,648 genes, after populations of cancer cells had been purified by laser microbeam microdissection. They selected and validated a set of 14 genes that showed the most significant prediction of response (87,88). In another study by Als et al., 55 genes that correlated with survival time after chemotherapy could be identified: Emmprin and survivin gene expression were found to independent prognostic markers for poor outcome (89). Favourable outcomes have been reported for MIBC patients with expression of the apoptosis-associated XAF1 gene or with overexpression of cyclo-oxygenase (COX)-2, treated with neoadjuvant chemotherapy (90,91). Decreased BRCA1 gene expression was correlated with a better response to neoadjuvant chemotherapy and overall survival in bladder cancer patients (92). In this study, mRNA analysis was employed to quantify BRCA1 expression, instead of immunohistochemical studies.

Gene expression markers may provide opportunities for achieving more personalized treatment. In particular, information obtained from DNA microarrays might allow for identification of candidate genes whose expression profiles present predictive value for response to chemotherapy in MIBC. Further analyses are necessary to substantiate the value of these potential markers.

**The COXEN model**

The US National Cancer Institute has screened more than 100,000 anticancer compounds using a panel of 60 diverse human cancer cell lines (NCI-60). Lee et al. utilized these data to develop the co-expression extrapolation (COXEN) algorithm (93). COXEN compares microarray gene expression profiles between cell lines and patient tumours, to generate signatures predictive of sensitivity or resistance (93). Its value for predicting the outcome of approximately 500 patients with 3 types of cancers (including bladder cancer) has been investigated and validated (94). It has been shown that COXEN can accurately predict drug sensitivity of bladder cancer cell lines treated with commonly used chemotherapeutic drugs (93). The Takata et al. neoadjuvant chemotherapy patients (as discussed in the previous paragraph) were included in the analysis.

COXEN, together with other advanced bioinformatic predictive strategies, seem to hold promise for the ability to select patients who would benefit the most from neoadjuvant chemotherapy.

**CONCLUSIONS**

Bladder cancer is a heterogeneous disease, with a highly variable prognosis, depending on the stage and the grade of the tumour. Biomarkers may help elucidate biologic features to identify patients at high risk for recurrence and progression after treatment.

It is essential to predict which patients will or will not respond to BCG as early as possible. Currently, this is based on routine clinicopathological parameters. Of all the biomarkers studied, urinary IL-2 levels appear to be the most potent predictive markers of BCG response: high IL-2 levels after BCG treatment correlate with a more favourable outcome.

The use of neoadjuvant chemotherapy has been advocated for over a decade, however its use in common practice is relatively sparse. Markers may improve selection of patients for chemotherapy possibly leading to higher utilization. Whereas the potential for predicting response and survival of NMIBC
after BCG therapy has been studied extensively, the assessment and prediction of response to neoadjuvant chemotherapy has lagged behind. However, it has been acknowledged that those biomarkers can be of great utility. Therefore, recently, several studies have made great progress towards this end. Although there seems to be a potential for molecular biomarkers to predict the response to neoadjuvant therapies, prospective trials need to be initiated to validate the reliability and the clinical benefit of these markers. To date, molecular markers have not conclusively proven to have additional value over the established clinicopathological variables and have not yet become part of (routine) clinical practice. Microarray technology might have potential in establishing molecular differences between responders and non-responders allowing patient-tailored treatment in MIBC.

BIBLIOGRAFÍA y LECTURAS RECOMENDADAS (*lectura de interés y ** lectura fundamental)

18. Andius P, Holmang S. Bacillus Calmette-Guerin therapy in stage Ta/T1 bladder cancer: progno-
tic factors for time to recurrence and progression. BJU Int 2004; 93(7):980-984.


