Biomarkers in patients treated with BCG: an update

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Introduction: *Bacillus Calmette-Guerin (BCG) instillations are the recommended treatment for non-muscle invasive bladder cancer but high recurrence and progression rates remain after treatment. Despite patients risk stratification, BCG effectiveness remains unpredictable. A close, invasive and expensive follow up is mandatory. To improve or even replace this heavy surveillance in this high risk population, validated biomarkers were developed.*
Materials and methods: *To identify the useful tools for the urologist in monitoring bladder cancer patients, we reviewed the literature focusing on plasma and urinary biomarkers of BCG-therapy outcome. Articles dated from 1988 to 2013 including specific keywords (urinary bladder neoplasm, biological markers, intravesical administration, recurrence) were examined and relevant papers were selected.*
Results: *Before treatment initiation, genetic polymorphisms of multiple agents (cytokines, matrix-metalloproteinases) were found to become very useful to tailor therapy and monitoring. Those biomarkers belong to personalized medicine which is a topic of great interest today, but still need to be validated in cohorts from different ethnicities.*
During instillations, cytokines (IL-2, IL-8, IL-6/IL-10) were reported to be reliable to determine treatment response and efficacy. Further studies are needed to confirm results and standardize thresholds.
After treatment, UroVysion, the FDA-approved fluorescence in situ hybridization (FISH), appeared to be the most robust marker of all the clinical parameters reviewed; but is not yet validated for BCG-treated patients.
Conclusions: *No recommendations for everyday practice can be established today, but a combination of several markers and clinicopathological characteristics may be the future. As bladder cancer diagnosis and management are evolving, practicing urologists should be aware of and utilize bladder cancer markers in clinical practice.*

Key Words: non-muscle invasive bladder cancer, BCG, biomarkers

Introduction

The International Bladder Cancer Group recommend intravesical bacillus Calmette-Guerin (BCG) instillations as adjuvant treatment for patients with intermediate and high risk non-muscle invasive bladder cancer (NMIBC).[1] Despite treatment, 30%-50% of patients fail to respond, and 15%-30% experience progression to muscle-invasive disease.[2]

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Current guidelines recommend risk stratification for NMIBC management based on actual available prognosis factors (number of tumors, tumor size, T category, presence of concurrent CIS, tumor grade). Patients are stratified into three risk groups that facilitate treatment choices: low risk tumors, intermediate risk tumors and high risk tumors.[1]

Despite those nomograms, BCG effectiveness remains unpredictable. Thus cystoscopy and cytology are recommended for monitoring patients treated with BCG, but with a low level of evidence (Grade C).[1] Moreover, there is no consensus regarding an upper limit to the number of repeat transurethral resection of the bladder and the maximum interval between the initial diagnosis and surgery.[3] These limits

TABLE 1. **Biomarkers in bladder cancer treated by BCG**

Biomarkers	Type of marker		Results				Comments
	Urinary	Serum	Response	Recurrence	Time to recurrence	Progression	
Predictive of response: genetic polymorphism							
Cytokines							
- TGF-β 25 GG		x				OR = 7.17 p = 0.004[10]	Turkish population
- IL-10 1082 GG		x				OR = 5.47 p = 0.05[10]	Turkish population
- IL-10 1082 GCC/GCC		x				OR = 8.4 p = 0.025[10]	Turkish population
- TNF-α T-1031C CC		x		HR = 0.38 p= 0.024[11]			North Indian population
- IL-8 251T AA		x		HR = 0.12 p < 0.001[12]			
- IL-8 NFκB ATTG Del/Del		x		HR = 2.53 p = 0.049[12]			
-IL-6 GC + CC		x		HR = 4.60CI$_{95\%}$ (1.2-17)[09]			
MMP-2 1306 TT		x		HR = 4.32 p = 0.006[14]	34 vs 45 mo. p = 0.039[14]		North Indian population
During BCG							
IL-2	x	x	5.9 ± 9.9 ng/mL vs. 0 p < 0.01[24]	p = 0.003[26]	p = 0.0001 # IL-2[29]		IL-2[29] = mRNA in blood samples
IL-8	x		p < 0.0002[31]	p = 0.47[30] p = 0.0209[32] p = 0.001[33]	34.9 vs. 18.8 mo. p = .006[30]		
IL-18	x		p = 0.0464[32]				
IL-6/IL-10	x			HR = 3.62 p < 0.001[35] p = 0.002[36]	p = 0.003[36]		
After BCG							
Survivin				100% sensitivity 78% specificity[38]			
ImmunoCyt	x		Reliable[43]/ Not reliable[44]				Opposite results
UroVysion	x			HR = 2.7 p = 0.017[48] HR = 4.6 p < 0.001[49] HR = 6.7 p < 0.001[47]	17.1 mo. vs. 19.4[48]	HR = 8-13 p < 0.01[46] HR = 9.4 p = 0.01[49]	A negative FISH result in case of a negative or equivocal cytology does not exclude low grade urothelial neoplasia[50]

underline three major issues. First, as clinicopathologic parameters are not able to sufficiently identify patients at risk of progression, the decision to perform radical cystectomy in patients with high grade NMIBC recurrence is challenging and one of the most difficult management issues in urological oncology. Second, treatment costs for non-muscle invasive bladder cancer in the United States are estimated at $157.5 M over 5 years. Bladder cancer has the highest lifetime treatment costs per patient of all cancers because of the high recurrence rates and long survival of NMIBC requiring lifelong surveillance and treatment of recurrence by expensive modalities.[4] Finally, in addition to economic considerations, surveillance carries some morbidities and limitations. Cystoscopy, currently the gold standard, is an invasive procedure which potentially generates complications and can give inconclusive results. Although urinary cytology is a non-invasive test with historically high specificity for bladder cancer (more than 95%), poor sensitivity has been recently reported[5] (51% for high grade tumors) together with specificity ranged from 83% to 88%.[5] Furthermore, its accuracy is highly dependent on the operator's skills and expertise[6] and is affected by BCG induced inflammation.[7]

The tremendous human, psychological and economic burden of bladder cancer underscores the importance of optimizing diagnosis and treatment protocols. Consequently, a plethora of promising new marker candidates have been generated to diagnose and monitor bladder cancer. The application of biomarkers to patients undergoing BCG therapy is particularly compelling because this population has high grade disease with a significant risk of progression. Moreover, in the inflammatory environment created by the BCG treatment itself, biomarkers already FDA-approved or validated for bladder cancer diagnosis, may no longer be relevant. To validate a molecular marker, rigorous epidemiological design and appropriate statistical methods are required as in pharmacological clinical trials.[8] Markers are expected to be non-invasive, simple, efficient, highly sensitive and specific.

Although biomarkers are not at the stage of clinical application yet, we wanted to update physicians about useful tools soon to be everyday practice in monitoring of bladder cancer patients. Thus, we focused on plasma and urinary predictive biomarkers of BCG treatment outcome. We divided this review to analyze the utility of markers into three circumstances. First, predictive use: who will respond to BCG therapy? Second, monitoring during therapy and third, monitoring after completion of therapy.

Materials and methods

A literature search was conducted using the PubMed database to identify the published English language articles related to biomarkers in treated patients with BCG for a bladder cancer. The free-text search was extended by adding these keywords: urinary bladder neoplasms, biological markers, intravesical administration, recurrence, prognosis, progression, cytokine, polymorphism genetics, and gene signature. Articles reporting biomarkers based on biological elements other than blood, plasma or urine were excluded. All articles dated from 1988 to 2013 were examined by the authors and relevant papers were selected.

Results

Before initiating treatment by BCG: who will respond to instillation?

Biomarkers assessed at diagnosis, before any treatment decision, would be particularly useful for patient presenting with pT1G3 tumors. Indeed, these tumors are considered as high risk tumors by guidelines.[1] But, data are not available to recommend either intravesical full-dose BCG or cystectomy to manage these patients;[1] while this choice is fundamental for the patient prognosis as delaying cystectomy is life-threatening.[3]

Today, genetic polymorphism is becoming a very serious option to characterize potential BCG-responsive population. Currently, the study of single nucleotide polymorphism (SNPs) is the subject of increasing interest. It is defined as a genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered. Promising prognostic candidates of responsiveness to BCG are presented below, Table 1; some of them will perhaps be part of the basic pretreatment analysis in the future.

Cytokine family members help mediate many of the effector phases of inflammatory responses,[9] Figure 1. Cytokines are a diverse group of non-antibody proteins produced by immune and non-immune cells, which act as mediators and regulators between cells of immune processes. Genetic polymorphisms in several cytokine genes have been described to regulate the production of certain cytokines. Studies revealed correlations between a number of cytokine gene polymorphisms and response to BCG therapy.[9] IL-10 (Interleukin-10) and TGF-β (Transforming growth factor) have been described as anti-inflammatory cytokines, Figure 1. Particular polymorphisms were reported to have higher rates in the progression group than in the remission

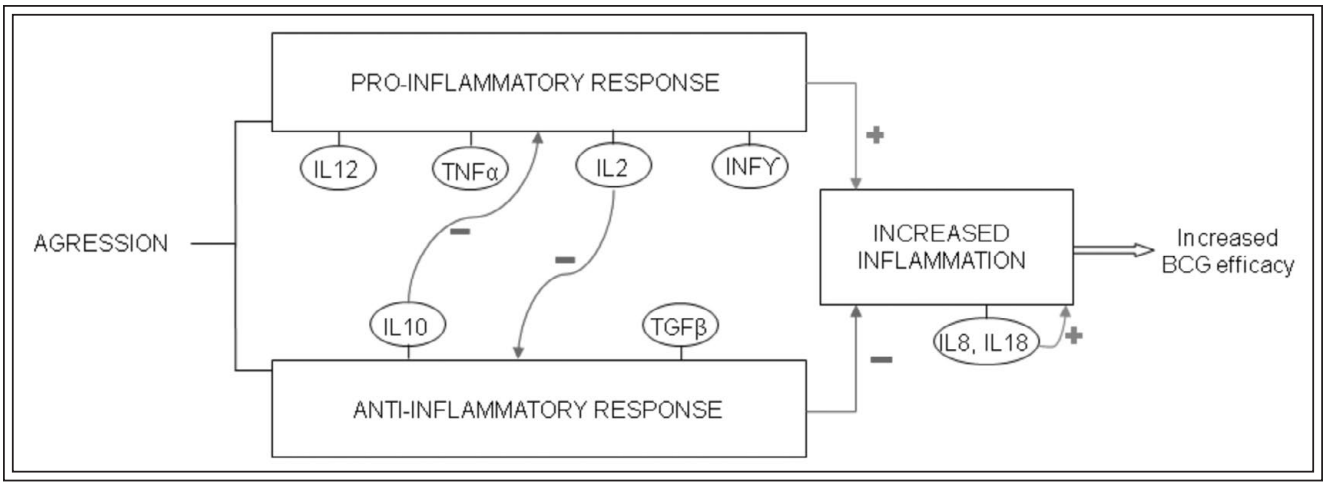

Figure 1. Functions of cytokines in BCG inflammatory response.

group in a Turkish population (TGF-β-25GG: 92.85% versus 64.44% (p = 0.04); IL-10-1082GG: 28.5% versus 6.8% (p = 0.05); and IL-10-1082GCC/GCC: 28.57% versus 4.5% (p = 0.025)).[10] TNF-α (tumor necrosis factor alpha) has various functions and is pivotal in recurrent inflammatory reactions encountered in bladder cancer, Figure 1. In a population of North India, it was reported that a specific polymorphism was associated with susceptibility to bladder cancer and may significantly reduce the risk of recurrence compared with the wild type genotype in patients with high grade NMIBC after BCG immunotherapy (HR = 0.38 $CI_{95\%}$ (0.14-0.98), p = 0.024).[11] IL-8 (Interleukin 8) is also a member of the cytokine superfamily. It attracts neutrophils and macrophages to urinary tract and manifests a wide range of pro-inflammatory effects (angiogenesis, tumor growth, invasion, and potential metastasis). In the same North Indian population, depending on the polymorphism, after BCG-treatment, they reported an association with reduced risk of recurrence or with an increased risk and with susceptibility to bladder cancer.[12] IL-6 (Interleukin 6) is an interleukin that activates mainly a proinflammatory reaction. Furthermore, high levels of IL-6 may favor a T-helper–2 (Th2) pattern of humoral immune response, which does not contribute to combating cancer. In white subjects, an association was found between variant IL-6 genotypes with increased recurrence risk in patients treated with maintenance BCG treatment (HR = 4.60 $CI_{95\%}$ (1.24-17.09)).[9] It appears that knowing a patient's genetic make-up related to cytokine production may allow the clinician to predict response to BCG therapy, and therefore guide treatment before progression occurs.[10]

Matrix metalloproteinases (MMPs) are key molecules for tumor growth, invasion and metastasis in many cancers. Measurements of MMPs in urine before any treatment, especially MMP-2 and MMP-9, demonstrated a superior diagnostic performance compared with cytology and have been identified as prognostic markers of bladder cancer.[13] Genetic polymorphisms of several MMPs were studied and showed promising results which need to be confirmed in a heterogeneous population. Indeed, in a population of North India, MMP-2 specific polymorphisms (1306TT genotype) was found to be associated with a high risk of recurrence in BCG treated patients (HR = 4.32 (p = 0.006)) and with reduced recurrence free survival (p = 0.039) compared with wild type genotype (CC).[14]

Susceptibility to some infectious diseases such as tuberculosis seems to be dependent on the natural resistance-associated macrophage protein 1 (NRAMP1) gene. This gene regulates intracellular pathogen proliferation and macrophage inflammatory response. Data show that the alteration in the NRAMP1 protein appears to have a major impact on the response to BCG therapy. In a cohort of Caucasian patients at high risk of recurrence, following BCG treatment, on a multivariate analysis, specific polymorphisms showed an association with increased recurrence risk and recurrence-free survival.[15] In contrast, in a predominantly Chinese population, the same genotype was associated with better responses to intravesical BCG.[16] A possible explanation of the conflicting results are the differences between the studied populations and the small sample sizes. These results suggest that ethnicity may be an important factor in determining BCG response.

Human glutathione peroxidase 1 (hGPX1) is a selenium-dependent enzyme that participates in the

detoxification of hydrogen peroxide and has been suggested to be involved in detoxifying cigarette smoke-derived oxidative radicals. In the previously quoted Chinese population, a specific genotype was associated with decreased recurrence interval after BCG therapy.[16] In contrast, Zhao et al had reported that the same variant of hGPX1 genotype had a borderline protective role in recurrence, with the association being stronger in Caucasians, but only 57% of their patients received intravesical BCG.[17]

Nucleotide excision repair (NER) is one of the major cellular DNA repair pathways protecting against damage from carcinogens in tobacco smoke. Associations between specific polymorphisms (XPA and ERCC6) and recurrence-free survival were found in Caucasians patients treated by BCG.[18] Whereas, in the well studied population of Northern India, other polymorphisms (XPC,[19] ERCC2[20] and XRCC1[21]) were found to be associated with increased recurrence risk. However, only a few studies have been validated in specific ethnic cohorts with reduced median follow up (21[18] and 14 months[19-21]) and complementary results are needed.

Based on the published literature, it would appear that further investigation of a combination of high risk alleles to predict BCG response would be useful.

During BCG therapy: who is responding?

BCG instillations are recommended to be administrated according to the empirical 6-weekly induction schedule followed by a maintenance schedule (1 year minimum and up to 3 years).[1] Thus, being able to evaluate response during the treatment seems essential in order to be able to switch to the surgical option as soon as possible. Indeed, as we mentioned above, losing weeks or years before radical treatment is not desirable, as earlier cystectomy improves the long term survival of patients with high risk superficial bladder tumors in whom BCG therapy fails.[3]

Current thoughts on BCG antitumor activity are that it relies on the BCG-induced inflammatory response. Different steps crucial to BCG activity have been identified and are related to inflammatory markers. Initially, BCG organisms bind to both tumor and normal urothelium via fibronectin.[22] The activated urothelial cells release pro-inflammatory cytokines. The activated neutrophils stimulate macrophages, which in turn initiate phagocytosis of infected urothelium, and release both pro-inflammatory and anti-inflammatory cytokines.[23]

Therefore, cytokine urinary levels are increased after each subsequent BCG instillation in patients responding to treatment in proportion to response to treatment. Depending on when cytokines are elaborated in the process, they peak at different times after the instillation (2 hours for Interleukin-12, 4 hours for IL-2 (Interleukin-2) and TNF-α, 6 hours for IL-6 and IL-8, and IL-10 (Interleukin-10) after the eighth instillation[24]). Moreover, they are more or less specific to inflammation subsequent to BCG therapy or those occurring during urinary tract infections. Alone or in combination, cytokines were reported to predict response to BCG, bladder cancer recurrence or disease-free survival. Currently, only few well-studied cytokines seem relevant, Table 1.

IL-2

IL-2 is a pro-inflammatory cytokine secreted by T cells upon their activation, Figure 1. A pivotal role for IL-2 in the mechanism of action of BCG in the treatment of bladder cancer is strongly suggested by all the studies. IL-2 can be measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits in urine or blood samples.

Urinary levels of IL-2 after BCG therapy were associated with a better clinical outcome. Indeed, urinary IL-2 levels after instillations of BCG may serve as a valuable prognostic factor of treatment response.[24,25] During maintenance BCG therapy, high urinary levels of IL-2 were associated with protection from recurrence,[26] pointing to the relevance of routine IL-2 monitoring during maintenance therapy.[27] Furthermore, during the extended BCG induction cycle, the favorable urinary IL-2 overproduction, gradually switched to a less favorable IL-10 profile, Figure 1. This suggested some patients may not benefit from extended BCG courses and that decision of maintenance BCG instillation may be adapted to individual urinary cytokine levels.[28] Moreover, by monitoring IL-2 urinary levels, it was demonstrated that the pro-inflammatory responder profile was observed earlier with higher IL-2 during reinstillations of BCG than during the first course, confirming the mini-maintenance reinduction concept.[25]

IL-2 levels in blood samples were also relevant. The induced expression of the IL-2 gene was also studied. It was reported that the inducibility of IL-2 mRNA, determined at the time of treatment with BCG, served as a powerful predictive indicator of the subsequent response and an independent predictive parameter of the patient remission (sensitivity = 95.6% and specificity = 70%) and disease-free interval ($p = 0.001$)[29], Table 1.

IL-8 and IL-18

IL-8 is a proinflammatory cytokine, chemoattractant of neutrophils and macrophages. IL-8 levels measured

after intravesical instillation may have value in detecting a response to intravesical therapy[30-32] and the possibility to predict a future recurrence.[30,32,33] However, its use is limited by the narrow timing of urine collection,[30] non standardized cutoff values[30] and a specificity potentially altered by urinary tract infections.[34]

IL-18 is a recently identified cytokine, produced by macrophages, which participates in innate and acquired immunity and activates natural killers cells and cytotoxic T lymphocytes, a sub-group of T cells that induce the death of cells that are infected (with BCG for example). Elevated levels of urinary IL-18, measured within the first 12 hours after BCG administration, were significantly associated with increased response rates.[32]

Thus, IL-8 and IL-18 secretion in the urine after the first intravesical BCG instillation may reflect individual patient presensitivation status to mycobacteria such as after vaccination, potency of the BCG strain used or the potential of the individual immune system to respond to mycobacterial pathogenic exposition. This observation seems promising, but needs further investigation.

TNF-α

TNF-α is a multifunctional, pro-inflammatory cytokine, which regulates immune cells. It activates macrophages, a major event associated with the activity of BCG. It was found that higher TNF-α levels had a strong tendency towards the absence of recurrence ($p = 0.07$), with a mean follow up of 54.1 months[35] and in a simple regression Cox's hazard regression model ($p = 0.012$).[24]

IL-6/IL-10

IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is a multifunctional cytokine produced by cells in response to several inflammatory conditions such as BCG instillation. Significantly higher production of IL-6 was observed in responder patients in a simple regression Cox's hazard regression model ($p = 0.023$)[24] but, urinary tract infections were reported to be associated with non-specific elevations of IL-6.[34]

IL-10 is an important modulator of immune-mediated events and inhibits BCG-induced macrophages cytotoxicity, Figure 1.

IL-6 and IL-10 ratio (IL-6/IL-10) have been showed to be prognostic marker of recurrence in patients affected by NMIBC (HR = 3.62 $CI_{95\%}$(2.80-4.92) $p < 0.001$,[35] $p = 0.00236$[35,36]) and an independent prognostic factor of time-free recurrence[36] by the same authors.

After BCG therapy: monitoring patients

Monitoring high risk of recurrence or progression patients with relevant biomarkers would help to decrease invasiveness, missed diagnosis and cost, and increase accuracy. It would select patients who require more intensive follow up, and allow patients to undergo invasive procedure only if a high risk was detected or for histological diagnosis.

Survivin

Survivin, a novel inhibitor of apoptosis, is a gene that is up-regulated in bladder cancer. While survivin is generally undetectable in terminally differentiated adult tissues, survivin is expressed in bladder cancer and its expression has been established as a prognostic factor in several tumor types.[37] Urinary survivin levels were reported to be higher in patients who had recurrence compared with those who achieved remission prior to, during and after intravesical treatment, Table 1. Indeed, the presence of urinary survivin 1 month after the completion of treatment was reported to predict bladder cancer recurrence with 100% sensitivity and 78% specificity (92% after 1 year).[38] Furthermore, no difference was found in survivin expression between patients who received intravesical therapy and those who did not.[39] Those results should be interpreted with caution, because of small sample size and lack of standardized technology and the analyzing process for urinary survivin.

FDA-approved tests

The bladder tumor antigen (BTA) test was the first approved by the FDA in 1995 for surveillance and initial diagnosis of bladder cancer. The test is an ELISA which detects bladder tumor associated antigen in human urine. Specificity of the test was decreased by former (28%-40% versus 82.8%)[40] and current BCG instillations (65.3% versus 80.7%, $p = 0.023$).[41] Therefore it has been suggested that the BTA tests should not be recommended for patients having received BCG because of the high rate of false-positive results.[40,41]

Nuclear mitotic apparatus protein 22 (NMP22) is member of the family of Nuclear Matrix Proteins (NMPs) and provides support for the nuclear shape. NMP22 is much more prevalent in malignant urothelial cells. The quantitative and qualitative NMP22 tests are FDA-approved as adjunctive tests for use in the initial diagnosis and surveillance of patients with bladder cancer. Few articles studied the prognostic value of these tests after intravesical instillations of BCG, but false positive rates were found to be considerably higher (43.7% versus 34%).[42] Thus,

NMP22 has significant limitations for monitoring patients following BCG therapy.[34,42]

ImmunoCyt/uCytt (Sci-medxInc, Denville,NJ, USA) is an immunocytochemical test that detects specific cellular markers of bladder cancer in exfoliated urothelial cells in voided urine. It was approved by the FDA for the surveillance of patients with bladder cancer. This marker has been poorly studied in patients treated by BCG. It was reported that sensitivity and specificity were not affected significantly by a single instillation therapy after transurethral resection or by intravesical long term treatment with epirubicin and BCG.[43] In addition, it was found to have a greater sensitivity than cytology. Presence of cells expressing the antigens after a weekly course of instillation with epirubicin and BCG was reported to be able to identify non-responders or a group of tumors that really benefit from longer cycles of instillation.[43] However, ImmunoCyt was also reported to be less sensitive in the follow up of patients undergoing BCG therapy. But, combination with cytology led to a sensitivity of 100% for recurrences for CIS.[44] Complementary tests seem necessary before validating the use of this FDA approved biomarkers for the monitoring of patients after BCG instillations.

UroVysion (Vysis,DownersGrove, IL, USA) utilizes fluorescence in situ hybridization (FISH) to detect common chromosomal anomalies in exfoliated bladder cancer cells associated with malignant development before it is clinically evident by cystoscopy. UroVysion is FDA-approved for initial diagnosis and surveillance. It has significantly higher sensitivity than cytology for detecting bladder cancer, while maintaining the high specificity of cytology.[45] Furthermore, chromosomal integrity is not affected by BCG therapy, urinary tract infections, hematuria, or any instrumentation process, thus allowing for the interpretation of FISH results.[44] In multiple studies (including one prospective[46] trial and one multicentric[47]), patients with a positive FISH result after BCG therapy were more likely to relapse (38.3% versus 17.8% (p = 0.020),[46] HR = 2.7 $CI_{95\%}$ [1.18-6.15] (p = 0.017),[48] HR = 4.6 $CI_{95\%}$ [1.9 to 11.1](p = < 0.001),[49] HR = 6.7 $CI_{95\%}$ [2.1-22.1] (p < 0.01)),[47] progress (HR = 8-13 (p <0.01),[46] HR = 9.4 $CI_{95\%}$ [1.9-45.3] (p = 0.001)[49]) and tended to recur earlier (17.1 months versus 19.4),[48] Table 1. Moreover, patients with positive pre and post-BCG FISH results had a greater risk of recurrence than patients whose FISH status changed from positive to negative after BCG treatment.[48] Additionally, the earlier the conversion from a negative to positive FISH result, the higher the risk of disease recurrence.[46,49] Finally, it was reported that a positive FISH result at baseline was not predictive of early tumor recurrence (during first surveillance at 3 months); it was predictive of overall recurrence.[46] However, a negative FISH result in case of a negative or equivocal cytology does not exclude low grade urothelial neoplasia.[50]

Discussion

In the management of patients being considered for or being treated with BCG, biomarkers are needed because this cohort has high grade disease with a significant risk of progression and without identification of relevant prognostic factors. The development of predictive biomarkers to assess patients who are most likely to respond to BCG therapy is important in this population and is one of the most challenging missions of genetic research.

Predictive markers could avoid missing a "window of opportunity" for cure. Thus, biomarkers could lead to personalized medicine which is a topic of great interest today. A customized healthcare strategy would be tailored for each individual considering his susceptibility to a particular disease or their response to a specific treatment. It would allow therapeutic interventions to be concentrated on those who will benefit, sparing expense and side effects for those who will not. Before treatment initiation, genetic polymorphisms of cytokines or MMPs were found to become very useful to tailor adapted therapy and monitoring. But although this solution is very attractive because of its non-invasiveness (blood samples), it is still limited by the ethnic specificity of the studied populations. Indeed, some genotype frequencies vary dramatically from one ethnicity to another, limiting generalization of the results. Those biomarkers still need to be validated in larger samples from different ethnicities.

During instillations, monitoring cytokines levels in blood and urine have been reported to be promising markers to predict BCG responder status, recurrence and recurrence free survival. Moreover, it seems to be a useful tool to tailor individual BCG instillation schedules (necessity of extended courses or not). It could lead to personalized instillation schedules depending on cytokine status. An important goal is to identify why non-responders have lower or higher specific cytokines levels. Immune response is categorized as either type 1 (Lymphocytes T Helpers 1: Th1) or type 2 (Lymphocytes T Helpers 2: Th2), based on the profile of cytokines produced by T cells. A Th1 cytokine profile (IL-2) is more often associated with cell-mediated immunity and is the most suitable for eradication of malignant cells. In contrast to Th1 lymphocyte, Th2 lymphocytes release the functionally opposite cytokine (IL-10), which suppresses delayed-

type hypersensitivity and is downregulated by the Th1 reaction. Thus, two antagonistic responses coexist as in a chronic infection. BCG involves the generation of an enhanced Th1 cytokine immune environment in the bladder wall. Individuals unable to generate a Thelper 1 lymphocyte response are considered immunologically unresponsive to the treatment.

Thus, pro-inflammatory and anti-inflammatory cytokine levels reflect the balance between an efficient response and its antagonist. Future studies should combine "opposite" cytokine in order to improve accuracy of markers.

For patients undergoing surveillance, the Food and Drug Administration (FDA) has approved four urinary biomarkers for clinical use, but, active inflammatory condition at the time of the testing is specified as a limitation. Since inflammation is part of the mechanism of action of BCG, it is important to recognize the limitations of these markers in BCG treated patients. In this specific population, UroVysion appeared to be the most valuable FDA-approved test to predict relapse, time to recurrence and progression. Moreover, it was reported to be a useful predictive marker before initiating any treatment. Notably, a high number of false-negative tests were reported.[48] It is hypothesized that as FISH depends on the presence of a sufficient number of malignant cells in the sample; the aggressive exfoliation of the vesical mucosa during BCG-therapy, tumor cells that do not exfoliate, or low tumor burden could all decrease the number of cells. Furthermore, UroVysion is limited to the detection of four chromosomes (3, 7, 9, 17), which are only some of those most frequently altered in bladder cancer.

Work on biomarkers adapted for BCG-treated patients is still in its research stages but preliminary results show much promise, especially the potential use of a combination of markers or "personalized" markers to more precisely predict response and progression in bladder cancer patients treated with intravesical BCG.

Conclusion

To conclude, even though urinary interleukins seem promising markers, future studies with larger samples, combining promising biomarkers and focusing on patients treated by BCG are needed. It's important to emphasize that while these non-invasive means of monitoring BCG-treated patients are still at a research level, we've never been closer to clinical applications, and consequently, everyday prescription. That's why it is critical for the physician to be updated on bladder cancer biomarkers with particular attention to their application to this high risk population of patients. □

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