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DOES MANTOUX TEST RESULT PREDICTS BCG IMMUNOTHERAPY EFFICIENCY AND SEVERE TOXICITY IN NON-MUSCLE INVASIVE BLADDER CANCER.

Wojciech Krajewski,[1] Romuald Zdrojowy,[1] Jędrzej Grzegrzółka,[2] Piotr Krajewski,[1] Michał Wróbel,[3] Mateusz Łuczak,[1] Anna Kołodziej[1]

1. Department of Urology and Oncological Urology, Wrocław Medical University, Wrocław, Poland
2. Department of Histology and Embryology, Wroclaw Medical University, Wrocław, Poland.
3. Department of Urology and Oncologic Urology, Lowersilesian Specialistic Hospital, Wrocław, Poland

Abstract:

Purpose: To evaluate on a large group of patients whether Mantoux tuberculin skin test (TST) result is associated with BCG immunotherapy effectiveness and whether it can predict occurrence of moderate to severe toxicity.
Material and Methods: We analysed group of 823 patients with intermediate and high risk NMIBCs who were treated with BCG. The study included 412 patients with the history TST and 411 without TST. A standard dose of Statens Serum Institute tuberculin RT23 was used. The reaction was read 48-72 hours later by evaluating the diameter of palpable induration. The size of the induration was considered positive when the measurement was greater than or equal to 6 mm and excessively positive when bigger than 26 mm. Whole BCG immunotherapy schedule consisted of 27 instillations.
Results: The patients were followed for median 61 months. The 5 years recurrence and progression free survival (RFS, PFS) did not differ between the groups in both total study population and tumour subgroup analysis. TST result in both total study population and in subgroups was not statistically associated with RFS, PFS and cancer specific survival.
The moderate-to-severe toxicity was observed in 181(44%) TST patients, and in 196(47%) patients without TST. Incidence of toxicity was not statistically different and also not statistically associated with TST result in any of the tumour subgroups of TST group.
Conclusion: This study shows, that TST does not have value in prediction of bladder cancer recurrence, progression nor cancer specific survival. Also it doesn't have a value in predicting therapy toxicity.

Key words: Bacillus Calmette-Guerin; Mantoux test; non-muscle-invasive bladder cancer; progression; recurrence

INTRODUCTION

Standard of care in non-muscle invasive bladder cancer (NMIBC) includes transurethral resection of bladder tumour (TURB) and subsequent intravesical therapy, which, depending on the cancer risk level, may be a single cytostatic agent instillation with or without bacillus Calmette-Guerin (BCG) immunotherapy regimen [1,2]. BCG therapy has been proven to be effective for lowering the recurrences and progression rates of NMIBC including carcinoma in situ (CIS) treatment [3-5]. The BCG therapeutic effect is largely associated with cellular immunological mechanism, however, its precise way of action is still unidentified [6]. Despite the fact, that BCG immunotherapy is a widely accepted management of medium and high risk NMIBCs, there are many uncertainties with regard to treatment protocol, reduction of side effects and mechanism of action [6,7].

The Mantoux tuberculin skin test (TST), also known as test for purified protein derivative (PPD), is the standard method of tuberculosis diagnosis. The TST is performed by intradermal injection of tuberculin PPD into the inner surface of the forearm. Subsequently, the skin reaction (induration) is read between 48 and 72 hours after administration and the result is interpreted depending on induration size and one's risk of being infected with tuberculosis [8].

Prognostic value of TST reactivity in patients treated with BCG has been evaluated in some studies. Correlation between tumor free status and the presence of positive TST have been observed in these reports, however, the studies were based on small groups of patients receiving short BCG regimens [9-11].

The aim of the study was to analyse whether TST result was associated with BCG immunotherapy effectiveness and whether it could predict occurrence of moderate or severe side effects on a large group of BCG patients.

MATERIAL AND METHODS

Study population and inclusion criteria

We retrospectively analysed group of 823 patients with intermediate and high EORTC risk NMIBC who were treated in our outpatient BCG department between 1998 and 2016[12]. One team of three physicians qualified all patients for immunotherapy and one physician administered majority of instillations (AK). Patients observed for minimum 12 months with introduction and any maintenance courses (≥7 instillations) were included in the study analysis. Four hundred and twelve patients (80F/332M, age 64,2+/-9,2y) received TST before BCG immunotherapy introduction and 411 (81F/330M, age 66,4+/-9,4y) did not. During first three years of outpatient department functioning all patients were qualified for TST. Later, qualification for the test was random, however not purposely randomized, basing on test kits availability.

Procedures

TST was performed before BCG immunotherapy introduction. A standard dose of Statens Serum Institute (SSI) tuberculin RT23 was used. The reaction was read 48 to 72 hours later by evaluating the diameter (millimetres) of palpable, raised and hardened area in the forearm. In case of erythema without induration, the result was read as "0 mm". The size of the induration

was considered to be positive when the measurement was greater than or equal to 6 mm. Induration bigger than 26 mm was considered excessively positive [13].
Before first instillation every patient had chest X-ray, urine culture performed and complete blood count, creatinine, GOT and GPT levels measured.
BCG instillations were introduced at least 14 days following the last invasive bladder procedure.
Patients received immediate single instillation with a chemotherapeutic agent after primary TURB according to EAU guidelines (doxorubicine, mitomycin). A restaging TURB was performed according to guideline recommendations.
Whole BCG immunotherapy schedule consisted of 27 instillations divided into introducing course and seven maintenance courses. Introducing course was composed of 6 weekly given instillations. Maintenance courses were comprised of three weekly given instillation administered after 3, 6, 12, 18, 24, 30 and 36 months [14].
Before each course urine culture and GOT and GPT levels were measured. Chest X-ray and USG were performed every 6 months. Cystoscopy (CS) was performed every 3 months during first two years following TURB and then every 6 months. After five years CS was performed annually. Urine cytology was performed every 3 months if the primary tumour was poorly differentiated (HG, G3 and some G2) or in case of concomitant CIS or mucosal dysplasia. Bladder wall biopsy was performed I every case of recurrence suspicion and positive cytology. In case of primary CIS mapping biopsy was performed routinely after first maintenance course.

Evaluations

The patients were analysed in terms of toxicity occurrence, recurrence free (RFS), progression free (PFS), cancer specific (CSS) and overall survival (OS). Times to events were calculated taking the date of initiating BCG as time zero.
Toxicity was defined as clinical state requiring administration of antimycobacterial agents (fluoroquinolones, rifampicin, isoniazid etc.) and/or dose reduction and/or cessation of therapy.
A recurrence was defined as a reoccurrence of tumor of any stage and grade confirmed by TURBT and histologic or cytological assessment.
Progression was defined as a rise to T2 or higher tumour stage.

Statistical analysis

All statistical analyses were performed using Prism 5.0 software (GraphPad, CA, USA). For correlations of TST implementation and results with clinical endpoints chi-square test and Fisher exact test tests were used. For analysis of survival periods the Kaplan Meyer curves were performed and Mantel Cox test was used. Results were considered as statistically significant when $p < 0.05$ in all analyses.

RESULTS

The baseline patients' characteristics are included in **Table 1**. The study included 412 patients (80 Female/ 332 Male) with the history TST procedure and 411 (81F/330M) patients without TST who were age and gender matched. Average number of BCG instillations given was 18,98 in TST group and 19,22 in group without TST. The difference was not statistically significant. The groups did not differ statistically in terms of presence of muscle layer in

histopathological specimen, concomitants CIS, tumour focality and tumour size. The groups were not matched in terms of primary diagnosis ($P < 0.001$), therefore, subgroup analyses were performed.
The patients were followed for median 61 months [range 12-257, SD 55]. The groups were not matched in terms of observation time, so we performed a survival analysis, avoiding direct comparison of frequencies of observational end-points. The observation time difference is caused by the fact, that during earlier years of BCG outpatient department functioning, TST test was performed more frequently than in recent years.
The recurrence was observed in 139 pts. (33,8%) in group without TST and in 194 pts. with TST (47%). Thirty-nine pts. (9%) experienced the recurrence more than once during study follow-up in group without TST and 64 pts. in group with TST (15,5%). Progression of the cancer was observed in 70 pts. (17%) without and 113 pts. (27,4%) with TST. There were 31 (7,5%) and 72 (17,5%) cancer specific deaths in without TST and with TST groups respectively.
Results of Mantel Cox analysis of survival according to TST status are showed in **Table 2**, and analysis of survival according to TST result (negative/positive) in **Table 3**. None of the analysed parameters were statistically correlated.
The TST result (negative/positive/excessively positive) in both total study population and in subgroups was not statistically associated with RFS, PFS, OS and CSS (**Table 4**).
In the total study population analysis RFS for 12, 24 and 60 months concerned 81,1%, 73,4% and 62% of patients with TST and 80,9%, 73,9% and 62,1% of patients of without TST, respectively. PFS for 12, 24 and 60 months for all tumours concerned 91,5%, 87,3% and 79,7% patients in group with TST and 90,7%, 87% and 80,1% of patients without TST, respectively. Those results did not differ statistically for both overall and subgroup analysis.
The toxicity was observed in 181 (44%) of TST patients, and in 196 pts. (47%) in group without TST. In group with TST 115 pts. (28%) underwent dose reduction, 99 pts. (24%) needed fluoroquinolones administration, 68 pts. (16%) required more potent tuberculostatic agent, and in 83 pts. (20%) BCG immunotherapy was stopped because of toxicity. In group without TST 113 pts. (27%) experienced dose reduction, in 144 pts. (35%) fluoroquinolones were administered, 44 pts. (10%) needed potent tuberculostatic agent, and in 87 pts. (21%) BCG was detained because of toxicity. In the analysis of toxicity occurrence, when Chi-square test was performed, there was no statistical differences between group without TST and patients with positive and negative result of the TST ($P = 0.547$). When Fisher's exact test was performed in TST group for patients with positive and negative TST result, the subgroups where not statistically different ($P = 0.915$).

DISCUSSION

In this study we analysed the possible association between TST result with BCG efficiency and occurrence of moderate to severe toxicity in 823 patients with NMIBC.
The BCG immunotherapy is a widely accepted standard of care in intermediate and high risk NMIBCs. Its efficiency has been proved in numerous papers and its use is advocated by international clinical guidelines. However, despite the fact that BCG in NMIBC treatment was introduced more than 40 years ago, there are still many unknowns [(15)]. Precise way of action,

the best administration schedule, optimal dosage as well as makers of response and risk factors for complications still remain a conundrum.

It is well known that BCG action is based on immunological response. Components of the BCG instillation such as attenuated bacteria, dead bacilli and subcellular debris are characterized by powerful immunological properties. Increased immunological activity in the bladder after BCG instillation leads to the activation and migration of cytotoxic lymphocytes, macrophages and natural killer (NK) cells which finally results in tumor eradication [(6)].

Earlier papers indicated possible association between TST result and BCG efficiency and toxicity. Yet, these studies were performed on small group of patients, with various administration schedules and with short follow-up. Ultimately, there are no studies in populations with high risk of possible tuberculosis exposition.

Shortly after World War II, which was the time when the majority of current BC patients in Poland were born, Poland was a country with one of the highest incidence of tuberculosis in Europe. At present, despite the significant decrease in the number of cases, the differences between Poland and Western Europe are still visible. The regulations concerning the BCG compulsory vaccinations and the bacterial strain were different among various European countries. In Poland, until 1950 only the Dutch strain was used and after the introduction of the compulsory vaccination in 1955 the strain was changed for Brazilian Moreau. It is unclear what is the effect of the immunization during vaccination. Vast majority of our study population underwent compulsory vaccination with BCG vaccine during their life-time. All abovementioned factors make the Polish population very different in terms of tuberculosis immunization from the patients in other countries, especially, North America [(16,17)].

In the analysis of this study population, it was shown that preformation of TST was not associated with reduced risk of tumour recurrence. In the survival analysis, statistical significance was not reached in any tumour group (total study population, TaLG, TaHG, T1HG, T1LG, CIS) for neither 12, 24 nor 60 months RFS. Also, the median times to recurrence were not statistically different for subgroup analysis. TST result (negative/positive/excessively positive) in both total study population and in subgroups was not statistically associated with RFS.

Influence of TST on progression was also analysed. It was shown that that preformation of TST was not associated with reduced risk of cancer progression neither in total study population nor in subgroup analysis. PFS for 12, 24 and 60 months did not differ statistically. The median times to progression in subgroup analysis were also not statistically different. Similarly, as in recurrence analysis, TST result was not statistically associated with PFS neither for total study population, nor for subgroups.

Finally, OS and CSS were not proved to be dependent on TST preformation. Statistical significance was not reached for neither total study population nor tumour subgroups. What is more, TST result did not influence neither OS nor CSS. However, a number of reported deaths was not big and may by not statistically representative.

For the purposes of this analysis, the moderate and sever toxicity were defined as clinical situation requiring administration of antimycobacterial agents (fluoroquinolones, riphamazide, izoniazide etc.) and/or dose reduction and/or cessation of the therapy.

The toxicity was observed in 181 TST patients, and in 196 patients without TST. The difference was not statistically different. Incidence of toxicity was also not statistically associated with TST result in any of the tumour subgroups of TST group.
This study has some limitations that ought to be disclosed. Firstly, this study is limited by its retrospective nature. The long period of the observation raises doubts about whether a cohort effect may occur with present-day high-risk patients being dissimilar from those from early part of the study. However, during 20 years of the course, none of qualification, therapeutic and maintenance details did change. What is more, to avoid biases, only one team of physicians qualified all patients and one physician administered almost all of instillations (AK). Additionally, only two nurses were included in the treatment protocols.
Secondly, our Department is referral in urooncology, only one in the voivodeship which offers BCG immunotherapy. Therefore, population of our patients underwent resections in various centres. For that reason, TURBs and histopathological assessment could be performed with different quality. However, majority of the specimens were originally or were re-evaluated by our team of pathologists to avoid incorrect qualification.
Thirdly, despite random patient allocation, the study did not have precise randomization protocol for whole period.
Lastly, TaLG tumors represent big part of cases. However, all of those tumours were in intermediate risk group according to EORTC grading (e.g. because of size, multifocality and/or recurrent character). For that reason, subgroup analysis was performed.
Notwithstanding limitations our study has some clear strengths. Firstly, we present big Centro-European population which was not previously included in BCG trials. The study population is homogeneous in terms of immunization, vast majority of our patients underwent compulsory vaccination with BCG vaccine during their life-time. Secondly, our group have long and meticulous follow-up with minimal loss to observation. Thirdly, our patients received maintenance courses in 6+3 manner, which was not studied in previous reports. Finally, to our knowledge, this is the biggest study analysing aspect of TST in population of NMIBC receiving BCG immunotherapy.

CONCLUSION

This study shows that the Mantoux tuberculin skin test does not have value in prediction of bladder cancer recurrence, progression nor cancer specific survival. Also, it doesn't have a value in predicting therapy toxicity.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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Corresponding author**:**

Wojciech Krajewski, PhD, M.D.

Department of Urology and Oncological Urology, Wrocław Medical University, Borowska 213, Wrocław, Poland,

Tel/Fax: 71 733 1010,

Email: wk@softstar.pl

Table 1. The patients baseline characteristics according to TST status

	With TST	Without TST
Primary diagnosis		
Ta	194 (47%)	106 (25.8%)
T1	167 (40.5%)	231 (56.2%)
HG	170 (41.3%)	239 (58.2%)
LG	242 (58.7%)	172 (41.8%)
TaLG	166 (40%)	66 (16%)
TaHG	28 (6.7%)	40 (9.7%)
T1HG	91 (22%)	199 (48.4%)
T1LG	76 (18.4%)	32 (7.8%)
CIS	51 (12.5%)	74 (18%)
Gender		
Male	332 (80.5%)	330 (80.3%)
Female	80 (19.5%)	81 (19.7%)
Median age (mean; range; median)	64.3; 27-85; 65	65.4. 28-89. 66
Toxicity	181 (44%)	196 (47%)
Observation time (mean; range; median)	102.4; 12-242; 94	43.8; 12-257; 34
Muscle in primary TURB specimen (y/n/missing)	317 (76.9%)/50 (12.1%)/45 (11%)	328 (79.8%)/76 (18.5%)/7 (1.7%)
Concomitant CIS (y/n)	60 (14.6%)/301 (73%)	66 (16.1%)/271 (65.9%)
Number of tumours (solitary/multiple/cis/missing data)	111 (26.9%)/246 (59.7%)/51 (12.4%)/4 (1%)	142 (34.5%)/188 (45.8%)/74 (18%)/7 (1.7%)
Tumour size (<3cm/≥3cm)	214 (51.9%)/147 (35.6%)	216 (52.5%)/121 (29.4%)
Newly diagnosed/recurrent tumours	177 (43%)/235 (57%)	223 (54.3%)/188 (45.7%)
TST result negative/positive/excessively positive	130 (31.6%)/77 (18.7%)/205 (49.7%)	-

Table 2 Mantel Cox analysis of survival according to TST status.

	Overall survival			Cancer specific survival			Recurrence free survival			Progression free survival		
TST Performed vs. not performed	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value
Total study population	1.1	0,8-1.5	0,548	1.0	0,6-1.6	0,993	1.0	0.8-1.3	0,949	1.0	0,8-1.3	0,949
CIS	1.0	0.5-2.0	0.925	0.9	0.3-2.5	0,770	1.1	0.4-3.0	0.827	1.1	0.5-2.4	0.753
T1HG	0.7	0.5-1.2	0.227	0.8	0.4-1.5	0,471	1.0	0.4-2.5	0.910	0.7	0.4-1.1	0.131
T1LG	1.3	0.5-3.3	0.646	0.7	0.11-4.5	0,709	1.2	0.5-2.9	0.672	0.8	0.3-2.6	0.735
TaHG	0.6	0.2-1.4	0.216	0.6	0.2-2.0	0,408	0.5	0.1-2.1	0.349	0.9	0.2-2.0	0.735
TaLG	0.6	0.3-1.2	0.1292	0.4	0.1-1.5	0,181	0.7	0.3-1.4	0.332	0.6	0.3-1.4	0.268

Bold values ($P < 0.05$) are statistically significant. Abbreviations: CI, confidence interval.

Table 3. Mantel Cox analysis of survival according to TST result (negative/positive).

	Overall survival			Cancer specific survival			Recurrence free survival			Progression free survival		
TST result positive vs. negative	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value	Hazard Ratio	95% CI	P value
Total study population	1.0	0.7-1.4	0.987	1.0	0.7-1.4	0.883	1.2	0.9-1.6	0.333	0.9	0.6-1.5	0.756
CIS	1.0	0.5-2.1	0.951	1.0	0.3-3.0	0.958	1.0	0.3-2.9	0.959	0.9	0.3-2.6	0.871
T1HG	1.1	0.6-2.2	0.662	1.3	0.5-3.2	0.606	1.0	0.4-2.5	0.910	1.0	0.5-2.2	0.876
T1LG	1.2	0.5-2.5	0.706	1.0	0.2-4.3	0.975	1.2	0.5-2.9	0.672	1.2	0.4-3.4	0.739
TaHG	2.4	0.5-11.8	0.292	3.2	0.4-27.0	0.277	1.6	0.1-18.0	0.725	2.4	0.5-11.8	0.292
TaLG	0.8	0.5-1.3	0.315	0.6	0.2-1.6	0.328	0.8	0.5-1.5	0.551	0.8	0.4-1.6	0.474

Bold values ($P < 0.05$) are statistically significant. Abbreviations: CI, confidence interval.

Table 4 Mantel Cox analysis of survival according to TST result: negative/positive/excessively positive (P-values).

TST result negative/positive/excessively positive	Overall survival	Cancer specific survival	Recurrence free survival	Progression free survival
CIS	0.256	0.202	0.838	0.347
T1HG	0.894	0.835	0.993	0.866
T1LG	0.899	0.999	0.876	0.662
TaHG	0.578	0.558	0.936	0.688
TaLG	0.337	0.479	0.481	0.677

Bold values ($P < 0.05$) are statistically significant