African Journal of Urology

ORIGINAL RESEARCH

Open Access

Pre-therapeutic lymphocytopenia: a new prognostic factor for failure of endovesical BCG-immunotherapy in non-muscle invasive bladder cancer

Idriss Ziani[1,2*], Ahmed Ibrahimi[1,2], Omar Bellouki[1,2], Hachem Elsayegh[1,2], Redouane Abouqal[2,3,4], Yassine Nouini[1,2] and Amal Bouziane[2,4,5]

Abstract

Background Inflammation plays a key role in the initiation and development of cancers. The prognostic value of inflammation biomarkers is proven in several urological and non-urological cancers. Knowing that the mechanism of action of endovesical BCG-immunotherapy in the treatment of non-muscle-invasive bladder cancer (NMIBC) is based on inflammation; lymphocytes have a key role in this reaction, particularly in the cytotoxic phase and can be predictive biomarkers of the response to BCG-therapy. The main objective of our work is therefore to study the impact of the number of lymphocytes on the response to endovesical BCG-immunotherapy, and more specifically lymphocytopenia (Lp) as a prognostic factor for BCG-failure.

Methods Our study is a monocentric retrospective cohort carried for prognostic purposes, including 200 patients neodiagnosed with non-muscle-invasive bladder cancer (Ta -T1 stages), who required adjuvant treatment to TURB by BCG-immunotherapy, over a period of 5 years from January 2012 to December 2016. The cutoff value chosen was 1.67×10^9/L using maximized Log-Rank test. Survival analysis was studied using a Kaplan–Meier model. The comparison between the thresholds ($L \leq Vs > 1.67 \times 10^9$/L) concerning the recurrence and progression rates was carried out using the Log-Rank test. The association between lymphocytopenia and BCG-therapy failure was assessed in univariate and multivariate analysis by the Cox model. Statistical analysis was performed using Jamovi statistical software.

Results One hundred and eight patients had a lymphocyte count $> 1.67 \times 10^9$/L while 92 had a lymphocyte count $\leq 1.67 \times 10^9$/L. The median lymphocyte value was 1.64 (1.19; 2.4). The median survival without failure of BCG treatment was significantly better in the high lymphocyte-count group, with median of 22 months in the $> 1.67 \times 10^9$/L group versus 11 months until failure in the $\leq 1.67 \times 10^9$/L group. A lymphocyte count $\leq 1.67 \times 10^9$/L was associated with failure of BCG-therapy in univariate (HR = 4.80, $P \leq 0.001$) and multivariate (HR = 1.88, $P = 0.025$) studies. Other factors associated in the univariate study were found: T1 stage ($P = 0.001$), high-grade urothelial carcinoma ($P = 0.001$), multifocal tumor ($P = 0.001$), tumor size > 3 cm ($P = 0.001$), concomitant carcinoma in situ (Cis) ($P = 0.001$) and vascular emboli ($P = 0.001$). Multivariate study showed significant factors that are, in addition to lymphocytopenia, the presence of T1 stage ($P = 0.011$) and vascular emboli ($P = 0.013$).

*Correspondence:
Idriss Ziani
idrisszianI20@gmail.com; idriss_ziani@um5.ac.ma
Full list of author information is available at the end of the article

Conclusion Our study has shown an association between lymphocytes count and NMIBC progression. Patients with lymphocytopenia carry an increased risk of endovesical BCG-immunotherapy failure. These results should be further validated.

Keywords Lymphocytopenia, BCG-immunotherapy, Recurrence, Progression

1 Background

Bladder cancer is the 7th most common cancer in men worldwide, and the 11th in both sexes [1]. The age-standardized incidence rate worldwide is 9.0 per 100,000 people/year for males and 2.2 for females [1]. In Europe, this rate is 19.1 for men and 4.0 for women [1]. In 2012, the age-standardized death rate was 3.2 per 100,000 people/year for men compared to 0.9 for women worldwide [1].

Bladder cancer incidence and death rates vary from country to country due to differences in risk factors, detection practices, diagnosis and also the availability of treatments. These variations can also be due to different methodologies of data collection [2].

At the time of diagnosis, 75–85% of bladder cancers are non-muscle-invasive (NMIBC) and 15–25% are muscle-invasive (MIBC) [3].

The management of NMIBC is represented by a transurethral resection of the bladder (TURB) with, if necessary, the so-called second-look re-resection, followed or not by an adjuvant treatment by endovesical chemotherapy or BCG-immunotherapy following the risk stratification determined by the AFU and EAU guidelines [4, 5].

Despite the multitude of treatment options, the progression of bladder cancer is marked by a high recurrence rate, approaching 70%, and by a significant progression rate exceeding 30% [5]. This raises the interest of studying possible predictive factors of failure of adjuvant therapies, in particular endovesical immunotherapy using bacillus calmetus guerin (BCG).

Knowing that the mechanism of action of endovesical BCG-immunotherapy is based on inflammatory responses that are mainly composed of complex cascade of interactions, its biomarkers may be predictive of the BCG-efficacy and failure [6]. The lymphocyte (L) count is one of inflammation biomarkers and is also involved in tumor development, but remains one of the least studied cancer biomarkers in the international literature, even less in urologic oncology [6].

Lymphocytes have a central action in the cascade of inflammatory reactions to BCG-therapy, especially in the cytotoxic phase. One of the main mechanisms of action of BCG is based on the production of cytokines [5, 6]. These cytokines may be involved in inflammation, such as interleukins 1, 8 and 6, TNFα and GM-CSF. They may also be involved in the Th1 lymphocyte immune response, such as interferon-gamma and interleukin-2 (IL2), which induce cytotoxic lymphocyte activation and may therefore provide information on the BCG response. During this effector phase, Th1 lymphocytes perform cytotoxic activity and Th2 lymphocytes perform suppressive activity [7].

The lymphocyte count has also been reported as a predictive factor in other non-urological cancers (ovary, esophagus, etc.)[6], and the association between lymphocytopenia (Lp) and the failure of some therapies has been described in the literature. This is already the case for chemotherapy in muscle-invasive bladder cancer [8]. This biomarker has the advantage of being easily reproducible, and of low cost to the health system.

The main objective of our work is therefore to study the impact of lymphocytopenia as a factor associated with BCG-failure in NMIBC.

2 Methods

This is a retrospective, monocentric, observational study of the cohort type with a prognostic aim. Our study was spread over a period of 5 years, from January 2012 to December 2016 with a follow-up until December 2019. We included a series of patients admitted for primary diagnosis with intermediate or high risk NMIBC (Ta-T1) who required adjuvant treatment to TURB by BCG-immunotherapy. We excluded from this study patients with low risk NMIBC, patients with malignant hemopathy, anemia with hemoglobin < 10 g / dL, HIV positive patients, patients with isolated carcinoma in situ (cis), NMIBC intermediate risk patients who underwent adjuvant treatment with doxorobucin, and patients with a history of autoimmune disease.

The anamnestic data (age, sex, risk factors, etc.) were collected from the hospital medical records of each patient in the study. Lymphocyte counts were collected from the preoperative blood count performed during the week prior to surgery. Operative data were collected from the operative report registers, and included the following data: macroscopic description of the tumor, size (> 3 cm vs. < 3 cm), number of lesions, and a bladder mapping of all lesions. Pathological data were retrieved from the archives of the pathology department. A careful analysis of the

pathology report of each patient was carried out, this analysis included T stage according to the TNM 2009 classification (Ta, T1), tumor grade according to the WHO 73 system [10], presence or not of concomitant carcinoma in situ (cis), or vascular emboli.

The management of our patients has been adapted to the risks. All of our patients underwent an initial TURB followed by a second-look resection (when indicated) within 2 to 6 weeks, and adjuvant treatment with BCG-immunotherapy including an induction therapy of 6 cycles followed by a maintenance therapy of 12 or 36 months, following the risk stratification according to AFU and EAU guidelines [4, 5]. Then a calculation of the probability of recurrence and progression was carried out according to the EORTC model (European Organization for Research and Treatment of Cancer) including all the previous data collected. The follow-up was carried out following the AFU and EAU guidelines. A cystoscopy was performed to make sure there are no visible papillary tumors before endovesical instillation of BCG.

Our main endpoint was BCG-immunotherapy failure, defined according to the EAU guidelines any high-grade disease occurring during or after BCG-immunotherapy, excluding late high-grade recurrences (> 12 months) if second-look resection does not show residual high-grade lesion [5]. It can also be defined by disease progression to an advanced stage (including MIBC) during follow-up. Any papillary tumor detected on control cystoscopy was considered as cancer recurrence, indicating a TURB and an update of the risk stratification.

In terms of statistical analysis, the qualitative variables are expressed in number and percentage, and quantitative variables in mean and standard deviation or median and interquartile range depending on the type of distribution. In order to specify a sensitive and specific cutoff of lymphocytopenia in our sample, the cutoff was specified by a survival threshold analysis using the Maximized Log-Rank test. A survival analysis has been performed to investigate the association between lymphocytopenia and BCG-therapy failure. The failure-free survival curve was performed using the Kaplan–Meier model. The comparison of the probability of BCG-therapy failure between the between the two groups determined by the lymphocytopenia threshold was performed by a Log-Rank model. The study of factors associated with the failure of BCG-therapy, including lymphocytopenia, was performed by univariate and multivariate analysis using COX regression. The lymphocytopenia, as well as other variables with a threshold of $P < 0.2$ in the univariate model, were included in the multivariate model. Statistical analysis of our data was performed by the statistical software Jamovi. Statistical significance is retained when $P < 0.05$.

3 Results

3.1 General characteristic of the study population

Our cohort included 200 patients, 166 males (83%) and 34 females (17%), with a mean age of 58.95 (±9.87). The median lymphocyte value was 1.64×10^9 /L (1.19; 2.4). Using the maximized log-rank test; we determined the cutoff value for lymphocytopenia set at a lymphocyte count $\leq 1.67 \times 10^9$/L (Fig. 1).

108 (54%) patients had L > 1.67×10^9/L while 92 (46%) had L ≤ 1.67×10^9/L. A total of 80 (40%) recurred, 41 (20%) progressed, and 26 (13%) patients underwent radical cystoprostatectomy at the end of follow-up. In total, 81 (40%) patients had BCG-therapy failure during follow-up, of which 40 (50%) as recurrence meeting the criteria for failure and 41 (50%) as progression (Table 1).

3.2 Survival analyses

Survival analysis using the Kaplan–Meier model showed a median BCG-failure-free survival of 27 months (Fig. 2). The median recurrence-free survival in the study population was 15 months (Fig. 3).

Median BCG-failure-free survival was significantly better in the group without lymphocytopenia, with a median survival of 22 months in the L > 1.67×10^9/L group versus a median BCG-failure-free survival of 11 months in the L ≤ 1.67×10^9/L group (< 0.001) (Fig. 4).

The median recurrence-free survival was 12 months in the L ≤ 1.67×10^9/L group, compared with a median survival of 20 months in the L > 1.67×10^9/L group. This difference was statistically significant ($P < 0.001$) (Fig. 5).

3.3 Factors associated with BCG-therapy failure

In univariate study, factors associated with BCG-failure were lymphocytopenia (HR = 4.80, CI 95% = 3.18–7.27, $P < 0.001$),T1 stage (HR = 4.42, CI95% = 2.70–7.21, $P < 0.001$,), high tumor grade (HR = 4.08, CI 95% = 2.43–6.84, $P < 0.001$), multifocal tumors (HR = 4.05, CI 95% = 2.34–7.03, $P < 0.001$), tumor size > 3 cm (HR = 3.45, CI 95% = 2.06–5.79, $P < 0.001$), presence of concomitant carcinoma in situ (HR = 4.52, CI 95% = 2.82–7.26, $P < 0.001$) and, finally, the presence of vascular emboli (HR = 6.59, CI 95% = 4.10–10.61, $P < 0.001$).

In the multivariate study, after adjustment for significant variables in the univariate study, the factors associated with failure were lymphocytopenia (HR = 1.88, CI 95% = 1.08–3.27, $P = 0.025$), T1 stage (HR = 2.09, CI 95% = 1.18–3.68, $P = 0.011$), and the presence of vascular emboli (HR = 2.06, CI 95% = 1.16–3.64, $P = 0.013$) (Table 2).

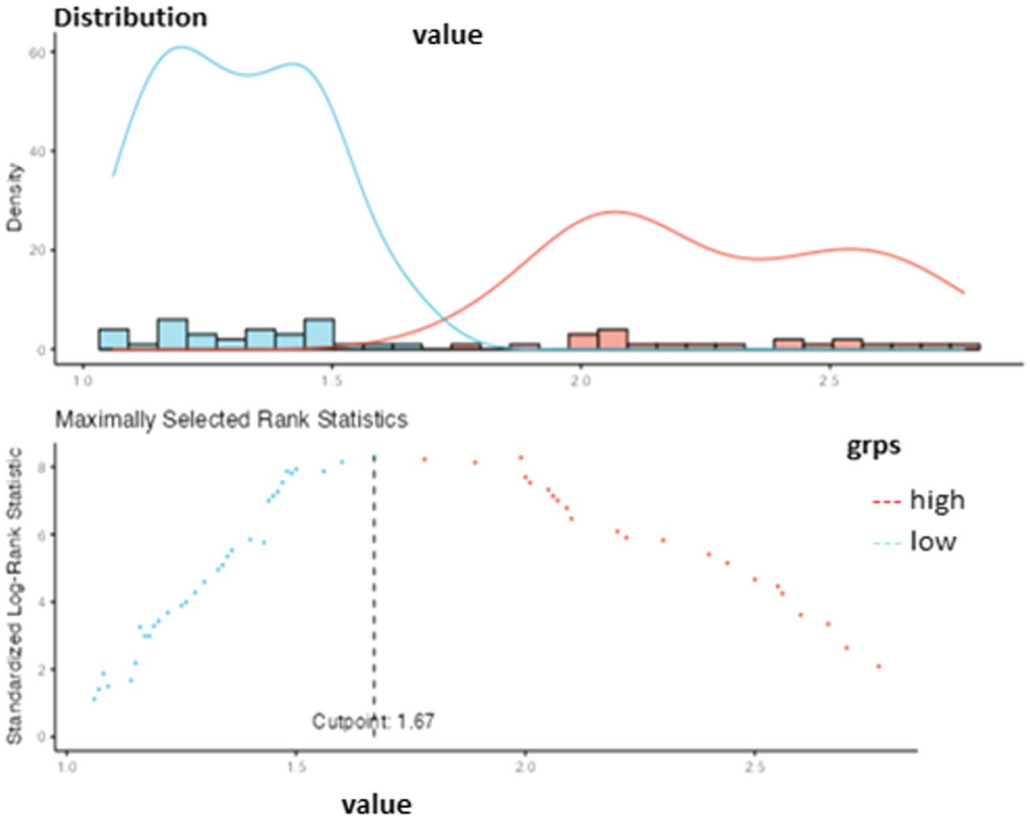

Fig. 1 Cutoff survival analysis using maximized Log-Rank test

3.4 Factors associated with tumor recurrence

In univariate study, factors associated with recurrence in our series were lymphocytopenia (HR = 1.93, CI 95% = 1.45–2.57, $P < 0.001$),T1 stage (HR = 2.34, CI 95% = 1.69–3. 25, $P < 0.001$,), high tumor grade (HR = 2.42, CI 95% = 1.69–3.46, $P < 0.001$), multifocal tumors (HR = 1.98, 95% CI = 1.38–2.84, $P < 0.001$), tumor size > 3 cm (HR = 2.02, CI 95% = 1.41–2.90, $P < 0.001$), the presence of concomitant carcinoma in situ (cis) (HR = 2.53, CI 95% = 1.54–3.38, $P < 0.001$) and the presence of vascular emboli (HR = 2.53, CI 95% = 1.72–3.73, $P = 0.001$). In multivariate study after adjustment for significant variables only lymphocytopenia (HR = 1.69, CI 95% = 1.07–2.86, $P < 0.02$), and multifocal tumors (HR = 1.71, CI 95% = 1.16–2.53, $P < 0.001$), remained associated with recurrence (Table 2).

4 Discussion

A statistically significant association was observed between the lymphocytopenia and failure of adjuvant BCG-immunotherapy. Low lymphocyte count was associated with failure of BCG-immunotherapy in univariate and multivariate studies. Also, a significant difference in BCG-immunotherapy failure-free survival in favor of the lymphocyte count > 1.67×10^9/L group. Lymphocytopenia was also associated with recurrence. These findings reflect the role of lymphocytes and inflammation in the immune responses to BCG and in the process of carcinogenesis. The inflammatory response plays a key role in cancer initiation and development [6]. Early in the carcinogenesis process, carcinoma cells and stromal cells lead to the generation and release of neutrophil cells from the bone marrow in the tumor microenvironment [8]. Carcinoma cells generate phenotypic and functional polarization of neutrophils that are able to stimulate tumor growth. Locally and systemically in the target organs of metastasis, neutrophils and cancer cells produce regulatory enzymes, such as matrix metalloproteinases (MMPs), lysyl oxidase (LOX) and urokinase (uPA), which modify the tumor extracellular matrix [9]. This "modified" extracellular matrix mediates myeloid and cancer cell function [10]. Furthermore, through the secretion of MMPs, neutrophils inhibit the activity of natural killer (NK)

Table 1 Patients' characteristics

Variable	Descriptif $N=200$ (%)
Age[a]	58,95 ± 9,87
Tobacco smoking	135 (90)
Gender	
Male	166 (83)
Female	34 (17)
Lymphocyte count	
> 1,67 10^9/L	108 (54)
≤ 1,67 10^9/L	92 (46)
T stage	
PTa	80 (40)
PT1	120 (60)
Grade	
High grade	131 (34,5)
Low grade	69(65,5)
BCG-failure/side effects	81 (40)
CIS	90 (45)
Tumor	136 (68)
Multifocal	64 (32)
Solitary	59 (29,5)
Size	
> 3 cm	141 (70,5)
< 3 cm	26(13)
Radical cystoprostatectomy	
Recurrence	80 (40)
Progression	41 (20)

BCG Calmette-Guerin bacillus, *CIS* carcinoma in situ, *PTa* variable grade papillary carcinoma without lamina propria invasion, *PT1* variable grade papillary carcinoma with lamina propria invasion but no muscle invasion

a = mean variable expressed + / − standard deviation

lymphocytes and stimulate tumor cell extravasation [11, 12]. There is also a complex interaction between tumor-associated neutrophils (TANs) and tumor-infiltrating lymphocytes (TILs) [13, 14]. Suppressor cells derived from myeloid lineages develop during inflammation and suppress T cell responses [15, 16]. Increased levels of CD8 + TILs have been associated with a better prognosis in different histological types of cancers [17, 18]. Studies have confirmed a negative correlation between the number of NATs and the number of CD8 + TILs [19, 20].

Lymphocytes play a key role in the immune response to BCG especially in the cytotoxic phase. One of the main mechanisms of action of BCG is based on the production of cytokines [5–8]. These cytokines may be related to inflammation, such as interleukins 1, 8 and 6, TNFα and GM-CSF. They can also be linked to the immune response of Th1 lymphocytes, which is the case of interferon-gamma and interleukin 2 (IL2). These interleukins stimulate the activation of cytotoxic lymphocytes and have prognostic value in the response to BCG. During this effector phase, Th1 lymphocytes have cytotoxic activity and Th2 lymphocytes have suppressive activity [21].

For these reasons, inflammation biomarkers of inflammation such as neutrophil/lymphocyte ratio, CRP, mean platelet volume etc...., are currently well studied and have proven their prognostic value in several urological or non-urological cancers [6].

Lymphocyte count is one of the least studied biomarkers of inflammation and tumor development in the literature, especially in urological cancers; few studies have been found in the international literature [6].

Yong Jae Lee et al. studied the prognostic value of lymphocytopenia in advanced ovarian cancer. In a retrospective cohort of 506 patients with advanced ovarian cancer at Yonsei Cancer Hospital, multivariate analysis showed that lymphocytopenia was an independent prognostic factor for poor progression-free survival (PFS) (HR, 1.73; 95% CI 1.20–2.49) and overall survival (OS) (HR, 1.87, 95% CI 1.27–2.75) in the PDS (primary debulking surgery) group. Absolute lymphocyte count was a significant factor when analyzed as a continuous variable in the NAC (neoadjuvant chemotherapy) and PDS groups [22].

N. Joseph et al. [8], studied the relevance of lymphocytopenia in muscle-invasive bladder cancer. An absolute lymphocyte count of 1.5×10^9/L was determined as a cut-off on ROC curve analysis. Lymphocytopenia (HR, 1.6; 95%, CI 1.1–2.4, $P=0.02$) and performance status index (HR 1.7, 95% CI 1.0–2.7, $P=0.047$) were poor prognostic factors in the multivariate binary-variate model. Absolute lymphocyte count was the only significant factor when analyzed as a continuous variable (HR 0.66, 95% CI 0.5–0.87, $P=0.003$) [8].

In a meta-analysis, Jiawen Zhao et al. [6], evaluated the prognostic value of pre-treatment lymphocyte count, regarding clinical outcomes in patients with solid tumors. A total of 42 studies involving 13,272 patients were included in this systematic review and meta-analysis. Low pre-treatment lymphocyte counts were associated with poor OS (HR = 1.27, 95% CI 1.16–1.39, $P<0.001$, I 2 = 58.5%) and PFS (HR = 1.27, 95% CI 1.15 to 1.40, $P<0.001$, I 2 = 25.7%). Subgroup analysis, based on the cancer type, showed that a low pre-treatment lymphocyte count was most closely associated with poor OS in colorectal cancer, followed by breast cancer and kidney cancer [6].

Arguably, our study remains the first study to demonstrate the prognostic value of lymphocytopenia in NMIBC and also to assess the impact of this biomarker

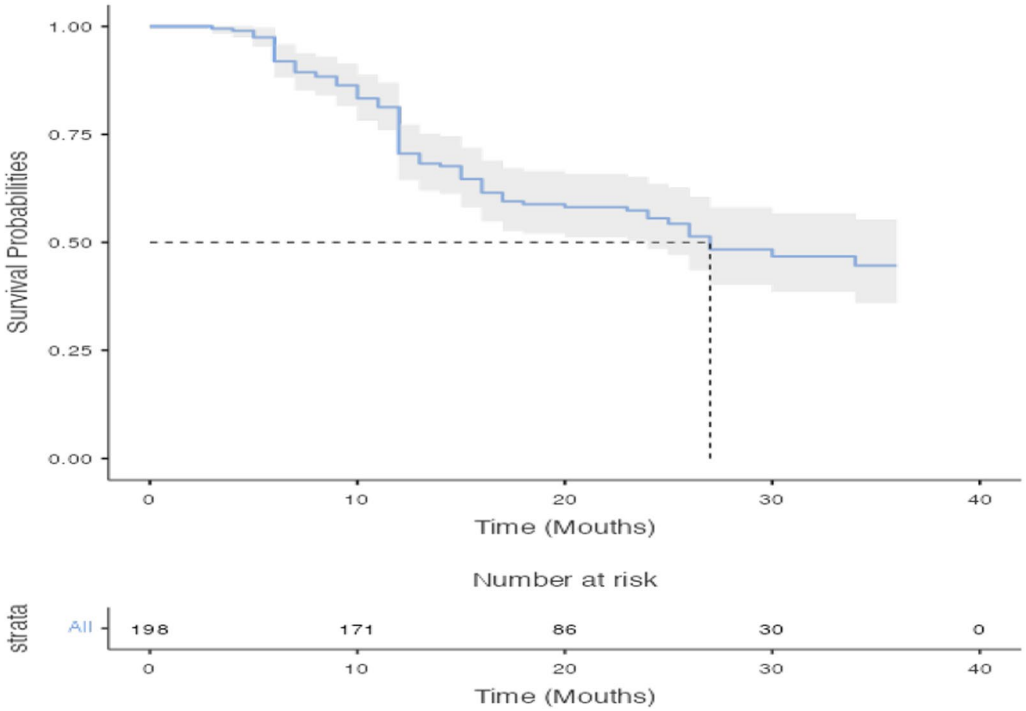

Fig. 2 The probability of BCG-therapy failure according to lymphocyte count by Kaplan–Meier model

on the response to endovesical BCG-immunotherapy, which currently remains a gold standard in adjuvant treatments of NMIBC. Our study can lead to predicting potentially BCG-immunotherapy resistant patients in order to optimize the therapeutic outcomes.

These results must be validated by large-scale studies in order to validate this biomarker and this cutoff in order to integrate it as predictive factors allowing the selection of patients potentially resistant to BCG-therapy.

The retrospective and monocentric nature constitutes the main limitation of our cohort study. The small number of samples due to the strict inclusion criteria also represents a limitation.

5 Conclusion

Our study showed an association between lymphocytopenia and BCG-immunotherapy failure in NMIBC. Patients with lymphocytopenia have an increased risk of BCG-immunotherapy failure; lymphocytopenia also has a significant impact on recurrence in our study. These results still need to be validated.

Table 2 The factors associated of failure BCG-therapy and recurrence of NMIBC in multivariate study using COX regression model

Variable	*HR*	**Failure BCG-therapy** *IC 95%*	*p*	*HR*	**Recurrence of NMIBC** *IC95%*	*p*
L ≤ 1.67 10^9/L	1.88	1.08–3.27	**0.025**	1,69	1.07–2.86	**0.02**
pT1 stage	2.09	1.18–3.68	**0.011**	0.95	0.65–1.39	0.80
High grade	1.50	0.79–2.85	0.220	1.08	0.69–1.69	0.113
CIS	0.97	0.55 -1.73	0.927	0.97	0.58–1.63	0.90
Lympho-vascular emboli	2.06	1.16–3.64	**0.013**	1.33	0,80–2.20	0,27
Multifocal tumors	1.45	0.69–3.03	0.323	1.71	1.16–2.53	**0.007**
Size > 3 cm	1.10	0.52–2.34	0.800	1.20	0.76–1.91	0.43

bold values indicates the significant p values

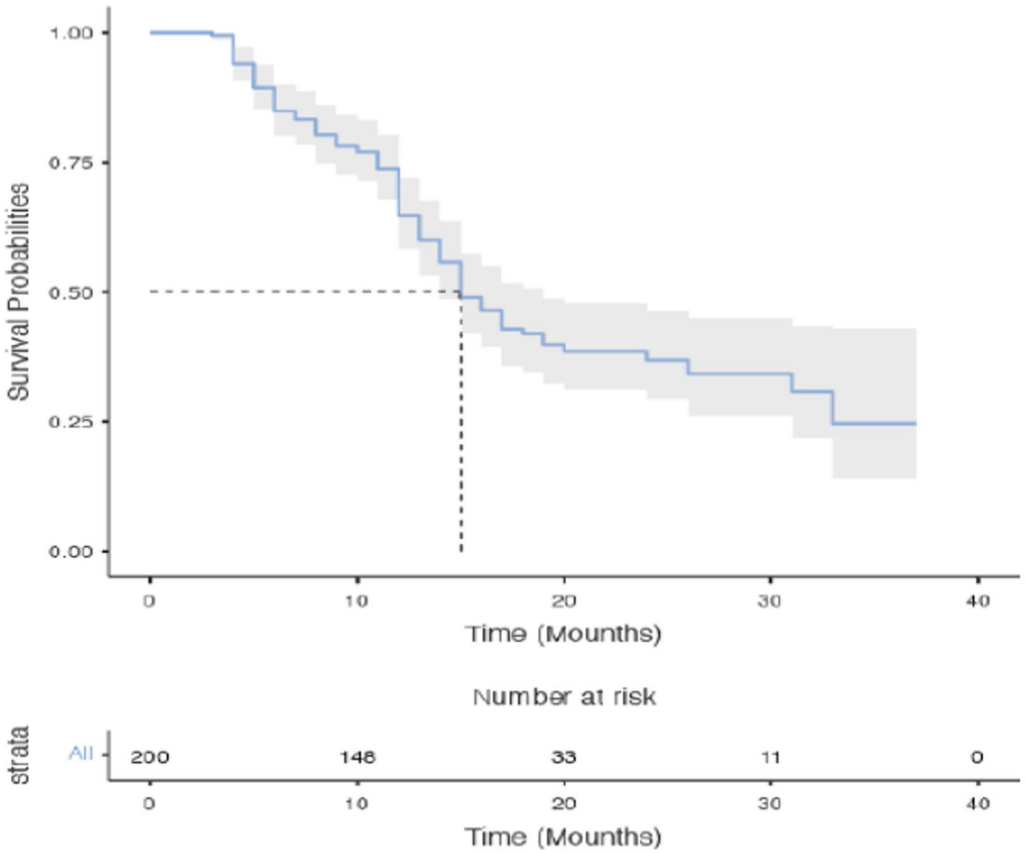

Fig. 3 The probability of recurrence according to lymphocyte count by Kaplan–Meier model

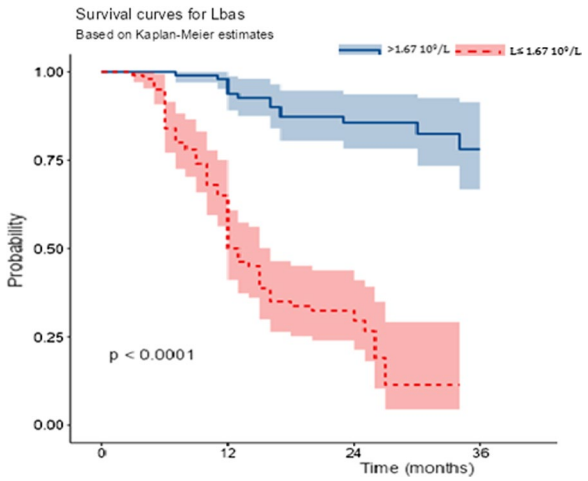

Fig. 4 BCG-therapy resistance-free survival in the Lymphocyte > 1.67×10^9/L group vs. the Lymphocyte ≤ 1.67×10^9/L group by a Log-Rank model

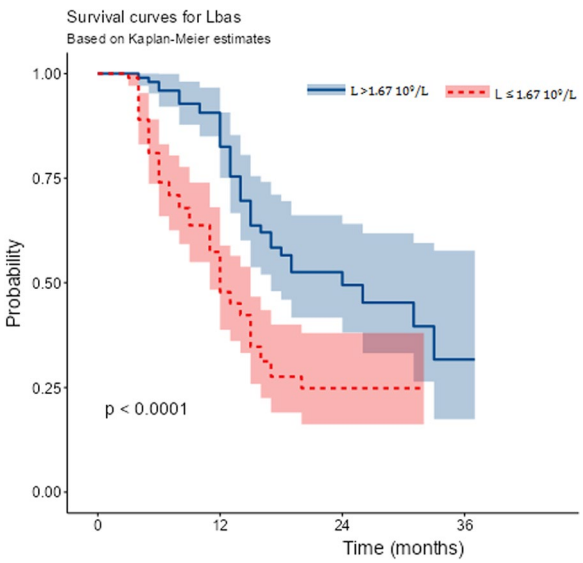

Fig. 5 Recurrence-free survival in the Lymphocyte > 1.67×10^9/L group Vs the Lymphocyte ≤ 1.67×10^9/L group by a Log-Rank model

Abbreviations

MIBC	Muscle-invasive bladder cancer
NMIBC	Non-muscle-invasive bladder cancer
L	Lymphocytes
Lp	Lymphocytopenia
AFU	French Urology Association
EAU	European Association of Urology guidelines
TURB	Transurethral bladder resection
EORTC	European Organization for Research and Treatment of Cancer
WHO	World Health Organization
Cis	Carcinoma in situ
BCG	Calmette-Guerin bacillus

Author contributions

IZ, AI, OB took care of the preparation of the material, the collection and the analysis of the data. Histopathological information was provided by IZ. The first draft of the manuscript was written by IZ, AI, OB. The statistical analysis carried out by AB, RA; proofreading and editing were contributed by YN, HE, RA, AB. All authors were included in the design of the study. All authors have read and approved the final manuscript.

Funding

Authors did not receive any funding for the conduct of this project.

Availability of data and materials

All data used in this study are available from the corresponding author.

Declarations

Ethics approval and consent to participate

This study obtained the favorable opinion of the ethics commission at the biotechnology laboratory of the Faculty of Medicine of Rabat University under reference: CERB 24-22.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Urological Surgery "A", Ibn Sina University Hospital of Rabat, Rabat, Morocco. [2]Faculty of Medicine and pharmacy, Mohammed V University in Rabat, Rabat, Morocco. [3]Acute Medical Unit, Ibn Sina University Hospital of Rabat, Rabat, Morocco. [4]Laboratory of Biostatistics, Clinical Research and Epidemiology, Mohammed V University in Rabat, Rabat, Morocco. [5]Department of Periodontology, Faculty of Dental Medicine, Mohammed V University in Rabat, Rabat, Morocco.

Received: 20 December 2022 Accepted: 9 March 2023
Published online: 29 March 2023

References

1. Ferlay J et al. (2015) GLOBOCAN 2012 v1.0: estimated cancer incidence, mortality and prevalence worldwide in 2012
2. Burger M et al (2013) Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63:234
3. Comperat E et al (2015) Clinicopathological characteristics of urothelial bladder cancer in patients less than 40 years old. Virchows Arch 466:589
4. Rouprêt M, Neuzillet Y, Pignot G, Compérat E, Audenet F, Houédé N, Larré S, Masson-Lecomte A, Colin P, Brunelle S, Xylinas E, Roumiguié M, Méjean A (2018) Recommandations françaises du Comité de Cancérologie de l'AFU-Actualisation 2018—2020: tumeurs de la vessie Progrès en Urologie Volume 28, Issue 12, Supplement, November 2018, pp S46–S78
5. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM et al (2017) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71:447–461
6. Zhao J, Huang W, Wu Y, Luo Y, Wu B, Cheng J, Chen J, Liu D, Li C (2020) Prognostic role of pretreatment blood lymphocyte count in patients with solid tumors: a systematic review and meta-analysis. Cancer Cell Int 20:15. https://doi.org/10.1186/s12935-020-1094-5
7. Mantovani A, Romero P, Palucka AK, Marincola FM (2008) Tumour immunity: effector response to tumour and role of the microenvironment. Lancet Lond Engl 371(9614):771–83
8. Joseph N, Dovedi SJ, Thompson C, Lyons J, Kennedy J, Elliott T, West CM, Choudhury A (2016) Pre-treatment lymphocytopaenia is an adverse prognostic biomarker in muscle-invasive and advanced bladder cancer. Ann Oncol 27(2):294–9. https://doi.org/10.1093/annonc/mdv546
9. Nicolás-Ávila JÁ, Adrover JM, Hidalgo A (2017) Neutrophils in homeostasis, immunity, and cancer. Immunity 46(1):15–28
10. Coffelt SB, Wellenstein MD, de Visser KE (2016) Neutrophils in cancer: neutral no more. Nat Rev Cancer 16(7):431–46
11. Jiang D, Lim SY (2016) Influence of immune myeloid cells on the extracellular matrix during cancer metastasis. Cancer Microenviron 9(1):45–61
12. Liang W, Ferrara N (2016) The complex role of neutrophils in tumor angiogenesis and metastasis. Cancer Immunol Res 4(2):83–91
13. Spiegel A, Brooks MW, Houshyar S, Reinhardt F, Ardolino M, Fessler E et al (2016) Neutrophils suppress intraluminal NK cell-mediated tumor cell clear ance and enhance extravasation of disseminated carcinoma cells. Cancer Discov 6(6):630–49
14. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L et al (2009) Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN. Cancer Cell 16(3):183–194
15. Fridlender ZG, Albelda SM (2012) Tumor-associated neutrophils: friend or foe? Carcinogenesis 33(5):949–55
16. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9(3):162–174
17. Hu W-H, Miyai K, Cajas-Monson LC, Luo L, Liu L, Ramamoorthy SL (2015) Tumor infiltrating CD8(+) T lymphocytes associated with clinical outcome in anal squamous cell carcinoma. J SurgOncol 112(4):421–426
18. Han S, Zhang C, Li Q, Dong J, Liu Y, Huang Y et al (2014) Tumour-infiltrating CD^{4+} andCD^{8+} lymphocytes as predictors of clinical outcome in glioma. Br J Cancer 110(10):2560–8
19. Uribe-Querol E, Rosales C (2015) Neutrophils in cancer: two sides of the same coin. J Immunol Res 2015:1–21
20. Wang J, Jia Y, Wang N, Zhang X, Tan B, Zhang G et al (2014) The clinical significance oftumor-infiltrating neutrophils and neutrophil-to-CD^{8+} lymphocyte ratio in patients withresectable esophageal squamous cell carcinoma. J Transl Med 12(1):7
21. Saint F (2008) Immunothérapie par Bacille de Calmette-Guérin: quel protocole? [Bacillus of Calmette-Guérin immunotherapy: which protocol?]. Prog Urol 185:599–104. https://doi.org/10.1016/S1166-7087(08)72485-0
22. Lee YJ, Chung YS, Lee JY, Nam EJ, Kim SW, Kim S, Kim YT (2019) Pretreatment lymphocytopenia is an adverse prognostic biomarker in advanced-stage ovarian cancer. Cancer Med 8(2):564–571

Publisher's Note