



Impact of systemic inflammatory markers on the response to Hyperthermic IntraVesical Chemotherapy (HIVEC) in patients with non-muscle-invasive bladder cancer after bacillus Calmette–Guérin failure

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ABSTRACT

Objectives: To evaluate the impact of pre- and post-treatment systemic inflammatory markers on the response to Hyperthermic IntraVesical Chemotherapy (HIVEC) treatment in a cohort of patients with high-grade non-muscle-invasive bladder cancer with bacillus Calmette–Guérin (BCG) failure or intolerance who were unsuitable or unwilling to undergo early radical cystectomy. As a secondary endpoint, we assessed the influence of some demographic, clinical and pathological factors on the response to chemo-hyperthermia.

Patients and methods: Between March 2017 and December 2019, 72 consecutive patients were retrospectively analysed. Patients with diseases or conditions that could interfere with systemic inflammatory status or full blood count were excluded. The HIVEC protocol consisted of six weekly intravesical treatments with 40 mg Mitomycin-C diluted in 50 mL distilled water. The drug was heated to a temperature of 43°C. Association of categorical variables with response to HIVEC was evaluated using Yates' chi-squared test and differences in continuous variable were analysed using the Mann–Whitney test. Logistic regression analysis was performed to define independent predictors of response to HIVEC.

Results: Patients who failed HIVEC were more likely to have multiple tumours ($P = 0.039$) at transurethral resection of bladder and a recurrence rate of $>1/\text{year}$ ($P = 0.046$). Lower post-HIVEC inflammatory indices [C-reactive protein ($P = 0.021$), erythrocyte sedimentation rate ($P = 0.027$)] and lower pre- ($P = 0.014$) and post-treatment ($P = 0.004$) neutrophil-to-lymphocyte ratio (NLR) values were significantly associated with the response to the HIVEC regimen (no bladder cancer recurrence or progression). In the multivariate analysis, patients with a recurrence rate of $>1/\text{year}$ were eight-times more likely to experience failure of HIVEC ($P = 0.007$). Higher pre- ($P = 0.023$) and post-treatment NLR values ($P = 0.046$) were associated with a worse response to the HIVEC regimen.

Conclusions: The recurrence rate and systemic inflammatory response markers could be useful tools to predict the likelihood of obtaining a response with the HIVEC regimen. These markers might help to guide patients about the behaviour of the tumour after BCG failure, predicting failure or success of a conservative treatment.

Abbreviations: CHT: chemo-hyperthermia; CIS: carcinoma *in situ*; CRP: C-reactive protein; EAU: European Association of Urology; ESR: erythrocyte sedimentation rate; HG: high grade; HIVEC: Hyperthermic IntraVesical Chemotherapy; ICD: immunogenic cell death; IL: interleukin; MMC: Mitomycin-C; NK: natural killer; NLR: neutrophil-to-lymphocyte ratio; NMIBC: non-muscle-invasive bladder cancer; PLR: platelet-to-lymphocyte ratio; RC: radical cystectomy; SIR: systemic inflammatory response; TURB: transurethral resection of bladder

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Introduction

Intravesical BCG therapy fails in up to 40% of patients with non-muscle-invasive bladder cancer (NMIBC) [1]. Radical cystectomy (RC) represents the 'gold standard' in these patients, but carries significant morbidity [1,2]. Delayed RC is associated with a decreased disease-specific survival [3]. Despite this, many bladder preservation strategies have been proposed [4,5]. Hyperthermic IntraVesical Chemotherapy (HIVEC) is a novel and effective therapeutic choice for patients

with BCG-unresponsive NMIBC, showing a median disease-free survival of 17.7 months [6]. HIVEC with Mitomycin-C (MMC) increases the activity of the drug by ~1.4 times. Heated MMC at 43°C has a 10-times higher cytotoxic effect because heat causes instability of the phospholipid bilayer of the cancer cells and increases permeability to MMC [7]. The HIVEC regimen is a safe and efficient alternative also for patients with intermediate- and high-risk NMIBC who have contraindications or cannot tolerate BCG therapy or in cases of critical shortage of BCG. The purpose of the present

study was to identify factors that can influence the response to HIVEC in a cohort of patients with NMIBC with BCG failure or intolerance who were unsuitable or unwilling to undergo early RC.

Patients and methods

Study design

A total of 72 consecutive patients with NMIBC with BCG failure or intolerance who underwent HIVEC at our institution, between March 2017 and December 2019, were included in this study. The BCG protocol used at our Department consists of a BCG induction (6 weeks) course plus BCG maintenance (3 weeks) courses at 3, 6, 12, 18, 24, 30 and 36 months.

The benefit–risk balance of early and late RC was discussed with patients and written informed consent was obtained. The inclusion criteria were as follows: i) histologically confirmed papillary (Ta or T1) high-grade NMIBC (HG-NMIBC) (WHO 2014) potentially presenting with concomitant carcinoma *in situ* (CIS) detected by transurethral resection of bladder (TURB) after treatment with intravesical BCG and before starting the HIVEC regimen; ii) the criteria of ‘BCG intolerance’ and ‘BCG failure’ according to the European Association of Urology (EAU) Guidelines [1]: BCG refractory [A: T1G3/HG non-muscle-invasive papillary tumour is present at 3 months; B: TaG3/HG non-muscle-invasive papillary tumour or CIS is present at both 3 and 6 months (after a second induction course or the first maintenance course of BCG)], BCG relapsing (recurrence of G3/HG tumour after completion of BCG maintenance, despite an initial response), and BCG intolerance (severe side-effects that prevent further BCG instillation before completing treatment); (iii) patients who completed the HIVEC protocol.

Patients with diseases or conditions that could interfere with systemic inflammatory status or full blood count (e.g. thromboembolism, leukaemia, lymphoma, haematuria, presence of infection, chronic inflammatory diseases, autoimmune diseases, and consumption of steroids) were excluded.

The primary endpoint of the study was to determine the impact of pre- and post-treatment systemic inflammatory markers on the response to HIVEC. As a secondary endpoint, we assessed the influence of some demographic, clinical and pathological factors on the response to chemo-hyperthermia (CHT). Patients underwent a complete routine blood test 1 week before the first hyperthermic instillation and at 1 week after the last one. The neutrophil-to-lymphocyte ratio (NLR) was obtained by dividing the neutrophil count by the lymphocyte count [8]. The platelet-to-lymphocyte ratio (PLR) was obtained by dividing the platelet count by the lymphocyte count.

Treatment schedule

The induction HIVEC protocol consisted of six weekly intravesical treatments with 40 mg MMC diluted in 50 mL distilled water. The drug was heated to a temperature of 43°C and recirculated inside the bladder at 200 mL/min for 60 min [7]. All treatments were performed using the Combat BRS system v2.0 (Combat Medical, Wheathampstead, UK).

Response to HIVEC and follow-up

Response to HIVEC was assessed with urine cytology and cystoscopy performed 6 weeks after the last instillation and subsequent TURB of suspicious lesions. Moreover mapping biopsies (trigone, bladder dome, right, left, anterior and posterior bladder wall) from normal-looking mucosa were performed in all patients [1].

A total of 51 out of 72 patients (70.83%) were classified as a ‘HIVEC responder’ (Group A), while the remaining 21 (29.17%) were classified as ‘HIVEC non-responder’ (Group B). The patients in Group B were defined as all grades of bladder cancer recurrence or progression. Tumour recurrence was reported in 17 patients (five with associated CIS), while progression to muscle-invasive disease was found in four patients. Low-grade recurrent disease was managed with another six weekly HIVEC regimen, while HG recurrences were managed with RC. All patients with HG progression to muscle-invasive disease underwent consequent RC. All Group A patients underwent a maintenance course comprised of three monthly instillations. Subsequently, the disease-free patients underwent another six monthly maintenance course. A CT of the abdomen and pelvis was performed once a year.

Statistical analysis

All data were collected in a prospectively maintained database and retrospectively analysed. Association of categorical variables with response to HIVEC was evaluated using the Yates’ chi-squared test and differences in continuous variables were analysed using the Mann–Whitney test. Logistic regression model was performed to define independent predictors of response to HIVEC. Nagelkerke R^2 was used to understand how much variation in the dependent variable could be explained by the model. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS®), version 23.0 (IBM Corp., Armonk, NY, USA), using a significance level of 0.05.

Results

Baseline patient's characteristics

The baseline patients' characteristics are summarised in Table 1. The mean (SD) age of the patients was 67.54 (7.96) years. The mean (SD) pre- and post-treatment NLR in all patients was respectively 1.62 (0.45) and 1.39 (0.35).

Association of clinical and pathological characteristics with response to HIVEC

Patients who failed HIVEC were more likely to have multiple tumours ($P = 0.039$) at TURB and a recurrence rate of >1 /year ($P = 0.046$) (Table 2). In terms of blood sample variables, lower post-HIVEC inflammatory indices [C-reactive protein (CRP) ($P = 0.021$), erythrocyte sedimentation rate (ESR) ($P = 0.027$)], lower pre- ($P = 0.014$) and post-treatment NLR ($P = 0.004$) were significantly associated with a good response to HIVEC regimen (Table 2).

Table 1. Baseline characteristics of the patients who underwent HIVEC treatment.

Categorical variables	n/N (%)
Sex	
Male	47/72 (65.3)
Female	25/72 (34.7)
Smoking status	
No	30/72 (41.7)
Yes	27/72 (37.5)
Ex-smoker	15/72 (20.8)
Diabetes	13/72 (18.1)
Number of tumours	
Single	21/72 (29.2)
Multiple	51/72 (70.8)
Tumour size, cm	
<3	47/72 (65.3)
≥ 3	25/72 (34.7)
Recurrence rate	
≤ 1 year	57/72 (79.2)
> 1 year	15/72 (20.8)
Pathological stage	
TaG3	14/72 (19.4)
T1G3	58/72 (80.6)
Concomitant CIS	11/72 (15.3)
Tumour on second TURB	17/72 (23.6)
Prior history of upper tract urothelial carcinoma	7/72 (9.7)
Previously treated with MMC	16/72 (22.2)
BCG failure	
BCG intolerance	13/72 (18.1)
BCG refractory	39/72 (54.2)
BCG relapse	20/72 (2.8)
Continuous variables	Mean (SD)
Age, years	67.54 (7.96)
Body mass index, kg/m ²	27.35 (4.53)
Pre-HIVEC CRP, mg/L	4.75 (2.58)
Pre-HIVEC ESR, mm/h	7.68 (4.40)
Pre-HIVEC albumin, g/dL	3.77 (0.50)
Pre-HIVEC NLR	1.62 (0.45)
Pre-HIVEC PLR	147.63 (43.60)
Post-HIVEC CRP, mg/L	5.34 (3.93)
Post-HIVEC ESR, mm/h	11.38 (6.71)
Post-HIVEC albumin, g/dL	3.82 (0.53)
Post-HIVEC NLR	1.39 (0.35)
Post-HIVEC PLR	120.30 (27.24)

Independent predictive factors for response to HIVEC

The multivariate analysis considered all the factors that at chi-squared and Mann–Whitney testing were significantly associated with HIVEC response in order to better evaluate their role in relation to the primary endpoint (Table 3). The logistic regression model had statistical significance, $\chi^2(4) = 28.185$, $P < 0.001$. The model explained 46.2% (Nagelkerke R^2) of the variance in HIVEC response and properly classified 80.6% of cases. Patients with a recurrence rate of >1 /year were eight-times more likely to experience failure to HIVEC ($P = 0.007$). Higher pre- ($P = 0.023$) and post-treatment NLR values ($P = 0.046$) were associated with a worse response to the HIVEC regimen.

Discussion

BCG failure is a real challenge in everyday clinical practice because of the high probability of recurrence and progression. RC remains the 'gold standard' in these patients [1], even if several salvage therapeutic options have been described as alternatives to RC in the BCG-failure setting [4,5,9,10].

The use of clinical hyperthermia in bladder cancer treatment has a clear rationale. First of all, treatments at temperatures of 41–44°C are cytotoxic to cancer cells, which are unable to tolerate the heat as well as normal cells [11]. Yet, hyperthermia does not increase the toxicity to the patient [12]. Moreover hyperthermia inhibits angiogenesis [13] and increases the activation of natural killer (NK) cells [14].

According to the preliminary results of a randomised (1:1) clinical trial in which 50 patients with high-risk NMIBC were randomised to receive adjuvant BCG or HIVEC, CHT was not inferior to BCG in terms of efficacy as a primary treatment [15,16].

Identification of prognostic factors may help in the selection of specific subgroups of BCG-failure patients who could benefit from early RC. In our retrospective study, the number of tumours, prior recurrence rate, post-HIVEC inflammatory indices (CRP and ESR), pre- and post-treatment NLR may help to identify patients that may fail the HIVEC induction course.

Multivariate analysis showed that the recurrence rate and pre-treatment NLR values were independent factors of failure at first follow-up. Moreover, higher post-HIVEC NLR values could be considered a biomarker for poor response to CHT.

According to the EAU Guidelines [1], concomitant CIS, multiple, large and recurrent tumours are already considered characteristics of highest risk. Moreover, analysing a group of 1812 patients with intermediate- and high-risk NMIBC treated with BCG, the prior disease recurrence rate and numbers of tumours were the

Table 2. Comparison of categorical and continuous variables between Group A (HIVEC responder) and Group B (HIVEC non-responder).

Categorical Variables, n/N (%)	Group A (n = 51)	Group B (n = 21)	P	
Sex				
Male	34/51 (66.67)	13/21 (61.90)	0.910	
Female	17/51 (33.33)	8/21 (38.10)	0.910	
Smoking status				
No	21/51 (41.18)	9/21 (42.86)	0.895	
Yes	19/51 (37.25)	8/21 (38.10)	0.841	
Ex-smoker	11/51 (21.57)	4/21 (19.05)	0.936	
Diabetes	9/51 (17.65)	4/21 (19.05)	0.844	
Number of tumours				
Single	19/51 (37.25)	2/21 (9.52)	0.039	
Multiple	32/51 (62.75)	19/21 (90.48)	0.039	
Tumour size, cm				
<3	37/51 (72.55)	10/21 (47.62)	0.081	
≥3	14/51 (27.45)	11/21 (52.38)	0.081	
Recurrence rate				
≤1 year	44/51 (86.27)	13/21 (61.90)	0.046	
>1 year	7/51 (13.73)	8/21 (38.10)	0.046	
Pathological stage				
TaG3	10/51 (19.61)	4/21 (19.05)	0.785	
T1G3	41/51 (80.39)	17/21 (80.95)	0.785	
Concomitant CIS	7/51 (17.65)	4/21 (9.52)	0.610	
Tumour on second TURB	13/51 (13.73)	4/21 (19.05)	0.834	
Prior history of upper tract urothelial carcinoma	5/51 (9.80)	2/21 (9.52)	0.688	
Previously treated with MMC	14/51 (31.48)	2/21 (9.52)	0.095	
BCG failure				
BCG intolerance	11/51 (21.57)	2/21 (9.52)	0.384	
BCG refractory	25/51 (49.02)	14/21 (66.67)	0.269	
BCG relapse	15/51 (29.41)	5/21 (23.81)	0.847	
Continuous variables	Group (n)	Mean rank	Z	P
Age, years	A (51)	38.57	-1.309	0.190
	B (21)	31.48		
Body mass index, kg/m ²	A (51)	34.99	-0.955	0.339
	B (21)	40.17		
Pre-HIVEC CRP, mg/L	A (51)	34.87	-1.034	0.301
	B (21)	40.45		
Pre-HIVEC ESR, mm/h	A (51)	35.30	-0.766	0.444
	B (21)	39.40		
Pre-HIVEC albumin, g/dL	A (51)	35.20	-0.826	0.414
	B (21)	39.67		
Pre-HIVEC NLR	A (51)	32.61	-2.460	0.014
	B (21)	45.95		
Pre-HIVEC PLR	A (51)	34.48	-1.276	0.202
	B (21)	41.40		
Post-HIVEC CRP, mg/L	A (51)	32.87	-2.300	0.021
	B (21)	45.31		
Post-HIVEC ESR, mm/h	A (51)	33.01	-2.209	0.027
	B (21)	44.98		
Post-HIVEC albumin, g/dL	A (51)	35.82	-0.429	0.673
	B (21)	38.14		
Post-HIVEC NLR	A (51)	31.90	-2.906	0.004
	B (21)	47.67		
Post-HIVEC PLR	A (51)	34.62	-1.189	0.234
	B (21)	41.07		

Table 3. Logistic regression analysis for assessing the response to the HIVEC regimen.

Variables	OR [Exp(B)]	95% CI	P
Number of tumours			
Single (reference)	-	-	-
Multiple	1.925	0.382-9.700	0.427
Recurrence rate			
≤1/year (reference)	-	-	-
>1/year	8.002	1.770-36.171	0.007
Pre-HIVEC NLR	4.556	1.236-16.791	0.023
Post-HIVEC CRP	1.127	0.952-1.335	0.163
Post-HIVEC ESR	1.094	0.992-1.206	0.073
Post-HIVEC NLR	3.401	1.024-11.297	0.046

most important prognostic factors determining tumour recurrence [17].

Furthermore, the systemic inflammatory response (SIR) could provide some additional information

about the possible response to HIVEC. Few published studies have investigated the relationship between SIR markers and NMIBC. The NLR before BCG treatment was associated with both survival and oncological outcomes in patients with NMIBC [18].

Ferro et al. [19] demonstrated that NLR was a predictor of residual HG disease at re-TURB at univariate but not at multivariate analysis. Racioppi et al. [20] reported that higher preoperative NLR could be predictive of poor BCG response in multivariate analysis.

Several studies have evaluated the association between NLR and post-RC survival outcomes [21]. Interestingly, Kang et al. [22] showed that post-treatment NLR measured in the early post-RC period was an independent factor of poor oncological prognosis.

Some chemotherapeutic drugs (such as anthracyclines and oxyplatin) induce immunogenic cell death (ICD), resulting in increased immunity. However, many chemotherapeutic agents, including MMC, etoposide and cisplatin, do not cause ICD [23]. It is possible that CHT induces ICD or activates the immune system through heat shock proteins or other factors. Hyperthermia has an important impact on the immune system resulting in augmented activation of NK cells [13] that destroy heat-stressed cancer cells. Moreover, thermotherapy induces heat shock proteins expression on the cancer cell surface. As a consequence, cancer cells actively participate in their programmed death through the natural process of apoptosis [24].

The SIR activated by cancer induces a pro-tumour inflammatory state, facilitating tumour growth, recurrence, and progression. In particular, neutrophils and lymphocytes have an inhibitory and promoting action, respectively, on the immune system and they can reflect the inflammatory and immune response of the patients.

Inflammatory responses induce neutrophilia, lymphocytopenia and high production of pro-angiogenic, anti-apoptotic and growth factors, that can stimulate tumour growth, angiogenesis, and metastasis [25]. Moreover, peripheral neutrophils can inhibit the cytotoxic T lymphocytes and NK cells [26]. In addition, the increase in tumour-infiltrating lymphocytes has been associated with improved prognosis in various tumours [27].

Consequently, a high NLR value could indicate both an increased neutrophil-dependent inflammatory response and a diminished lymphocyte-dependent immune response [28], and might represent an interesting biomarker of the host–tumour interactions.

CRP is a non-specific marker of inflammation. It is considered an important prognostic biomarker in various malignancies including kidney and urothelial tumours.

Some cancer cells express CRP and secrete interleukin 6 (IL-6) and IL-8, which stimulate CRP synthesis in the liver. Moreover, CRP positivity creates a favourable microenvironment for the tumour cells through acute inflammatory cytokine network system maintenance [29].

The inflammatory state appears to increase the irritative and voiding symptoms during and after CHT. Bladder instillations with dexamethasone and sodium hyaluronate can control the local inflammatory state. Moreover, the oral administration of nuciferine may inhibit the production of lipopolysaccharide-induced inflammatory cytokine, IL-6 and TNF- α , with good control of irritative symptoms [30].

The small cohort of patients and the retrospective nature are some limitations of the present study. Moreover, the present study is limited by its short

follow-up period; this may have resulted in some patients being falsely deemed as ‘responders’ due to the short follow-up alone. Another limitation is that albumin, CRP, ESR, NLR and PLR do not characterise the whole SIR state. Nevertheless, we believe that the present study provides new evidence showing a possible association between SIR and risk of failure of HIVEC.

In conclusion, the recurrence rate and pre-treatment NLR could be useful tools to predict the likelihood of obtaining a response to the HIVEC regimen. Moreover, post-treatment NLR can be considered a biomarker for response to the induction course of CHT. These markers might help to guide patients about the behaviour of the tumour after BCG failure, giving a high probability of failure or success of a conservative treatment. Additional larger scale prospective trials are needed to validate these results.

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