Pre-operative lymphocyte to monocyte ratio predicts clinical outcome in resected transitional cell carcinoma of the bladder

Sally Temraz\textsuperscript{1§*}, Deborah Mukherji\textsuperscript{1*}, Zein Al Abideen Farhat\textsuperscript{1}, Rami Nasr\textsuperscript{2}, Maya Charafeddine\textsuperscript{1}, Mohammed Shahait\textsuperscript{3}, Mohammad Rachad Wehbe\textsuperscript{2}, Rami Abou Ghaida\textsuperscript{3}, Ibrahim Abu Gheida\textsuperscript{4}, Ali Shamseddine\textsuperscript{1}

\textsuperscript{1}Department of Hematology-Oncology, American University of Beirut Medical Center, Riad El Solh, Beirut, Lebanon
\textsuperscript{2}Department of General Surgery, American University of Beirut Medical Center, Riad El Solh, Beirut, Lebanon
\textsuperscript{3}Department of Urology, American University of Beirut Medical Center, Riad El Solh, Beirut, Lebanon
\textsuperscript{4}Department of Radiation Oncology, American University of Beirut Medical Center, Riad El Solh, Beirut, Lebanon

*These authors contributed equally to the work

§Corresponding author

Email addresses:

ST: st29@aub.edu.lb
DM: dm25@aub.edu.lb
ZF: zf08@aub.edu.lb
RN: rn05@aub.edu.lb
MC: mc16@aub.edu.lb
MS: ms189@aub.edu.lb
Abstract

Background

Inflammation is a critical component of tumorigenesis with many cancers arising from sites of infection, chronic irritation and inflammation. Studies have suggested that the peripheral blood lymphocyte to monocyte ratio (LMR) could be a prognostic marker in different types of cancer. The aim of this study was to investigate the effect of the preoperative LMR on overall survival (OS) and time to treatment recurrence (TTR) in a cohort of patients with muscle invasive bladder cancer.

Methods

This study involved a retrospective review of patients undergoing cystectomy for transitional cell carcinoma of the bladder at our institution between 1998 and 2007. The association between LMR with OS and TTR were analyzed using Kaplan–Meier curves and compared by the log-rank test.

Results

Sixty eight patients with transitional cell carcinoma of the bladder were included in this retrospective analysis. In our study cohort, elevated preoperative LMR was significantly associated with increased TTR (P=0.001) and OS (P=0.020). Patients with a LMR < 2.87 showed a median TTR of 2.0 years (24 months) with 95%CI
[0.27-3.73], whereas patients with a LMR $\geq 2.87$ had a median TTR of 11.1 years (133 months) with 95%CI [2.31-19.88] (P=0.001). Patients with a LMR $< 2.81$ showed a median OS of 2.7 years (32 months) with a 95%CI [0.63-4.70], whereas patients with a LMR $\geq 2.81$ had a median OS of 6.0 years (72 months) with a 95%CI [3.60-8.40] (P=0.020). Clinical stage at diagnosis was the only clinicopathologic feature affecting the LMR while tumor invasion depth was borderline significant.

**Conclusions**

The LMR is a prognostic marker that is feasible and cost effective and which has shown to be significantly correlated with OS and TTR in the present retrospective analysis. Larger prospective trials are warranted to confirm this as prognostic marker in bladder cancer.

**Keywords**

Bladder cancer; urothelial cancer; transitional cell; lymphocyte; monocyte; prognostic marker

**Background**

Bladder cancer is the most common malignancy involving the urinary system and is the ninth most common cancer worldwide[1]. Males have a 3 to 4-fold increased risk of bladder cancer compared to females. Transitional cell carcinoma is the predominant histologic type in the United States and Western Europe accounting for approximately 90 percent of bladder cancers. At the time of diagnosis, around 25–30% of bladder tumors are found to be muscle-invasive[2]. Radical cystectomy remains the definitive treatment for patients with muscle-invasive bladder cancer
However, muscle-invasive disease carries a 5-year survival rate of only 27–50% [3].

Inflammation is a critical component of tumourigenesis with many cancers arising from sites of infection, chronic irritation and inflammation. Many of the cell types active in chronic inflammation can be found in the surrounding tumor stroma and also within the neoplasm itself. Mast cells were reported as histological findings at the tumor periphery and many neoplasms, particularly those that are epithelial in origin, have a significant inflammatory cell component. This includes a diverse leukocyte infiltrate of macrophages, neutrophils, eosinophils and mast cells often in association with lymphocytes. The variation in systemic inflammatory cells might be a valuable pre-treatment prognostic maker for stratifying patients at risk for tumor recurrence in bladder cancer. This was shown for example in measuring the peripheral blood neutrophils to lymphocyte ratio or platelets to lymphocyte ratios in various types of tumors [4-9]. To our knowledge, no individual biomarker has shown an independent prognostic value in muscle invasive bladder cancer thus far.

Few data currently exist regarding the preoperative lymphocyte to monocyte ratio (LMR) as a prognostic marker in cancer. Results in hematologic malignancies suggest that a survival benefit is associated with an increased LMR [10, 11]. In stage III colon cancer patients, an increased LMR might be an independent prognostic marker for time to treatment recurrence (TTR) and overall survival (OS) [12]. Moreover, a decreased LMR represented a novel independent poor prognostic factor in soft tissue sarcoma patients [13]. Hence, LMR could be a good reflection of lymphopenia, a marker of weak immune response, and/or an increased monocyte count, a marker of high tumor burden.
Based on these findings and the biological plausibility of an impact in bladder cancer, the aim of the present study was to investigate the effect of the preoperative LMR on OS and TTR in a cohort of patients with muscle invasive bladder cancer undergoing cystectomy at our institution.

**Methods**

This study involved a retrospective review of clinical and pathological data from patients diagnosed with bladder cancer at our institution between 1998 and 2007. Before initiation of the study approval was obtained from the Institutional review Board (IRB). Inclusion criteria involved patients with transitional cell carcinoma undergoing cystectomy. Patients with squamous cell carcinoma, adenocarcinoma and clear cell carcinoma and not undergoing cystectomy were excluded from the analysis. Clinical, histopathological and demographical features were all retrospectively obtained from the patients’ medical records. The preoperative white blood cell count was obtained 3 days prior to surgery and performed for routine clinical practice. The LMR was calculated from this routinely performed preoperative blood cell count as the absolute count of lymphocytes divided by the absolute count of monocytes. Analysis of the white blood cell count was performed in the general routine laboratory of our hospital.

**Statistical Analysis**

To determine the optimal cut-off levels for the LMR as a predictor of OS and TTR, the receiver operating curve (ROC) analysis was applied for each of the OS and TTR events. OS being the time calculated between the date of diagnosis of the disease and the date of death of any cause, and TTR being the time between the date of diagnosis of bladder cancer and the date of tumor recurrence. Patients who are still alive or
disease free, were censored at the last follow up date. The Kaplan-Meier curves were applied to assess the correlation between LMR and the time-to-event for OS and TTR. To determine the presence of a statistical difference between the 2 groups of the LMR in survival or disease progression, the log-rank test was applied to the groups to be compared. Median time to survival or progression and their corresponding standard deviations were retrieved from the Kaplan-Meier curves. In order to check the correlation between survival or recurrence, as events only and clinical characteristics, chi square was calculated, and relative risk was obtained where applicable. Chi square was also calculated to test the link between the found LMR for survival (2.81) and clinical characteristics at diagnosis. All statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). A two-sided p-value < 0.05 was considered statistically significant.

**Results**

A total of 334 patients with histologically confirmed bladder cancer diagnosed at the American University of Beirut Medical Center (AUBMC) between 1998 and 2007 were included in this retrospective analysis. Median age at diagnosis of the entire cohort was 65 years (range between 43-88 years). The median follow up was 2 years. Of the 334 patients included in the primary analysis, only 68 patients met the inclusion criteria. Table 1 shows the baseline patient and tumor characteristics. The majority of these patients were males (88%). Sixty seven percent had a history of smoking. The majority of patients had lymph node density (LND) of ≤0.2 (82%). Seventeen patients received adjuvant chemotherapy and only 3 patients received neoadjuvant chemotherapy.
Of the 68 patients with TCC bladder cancer, 23 (34%) had disease recurrence and 37 (54%) died within the follow up period. Applying ROC analysis, the optimal cutoff levels for the LMR was 2.81 for OS and 2.87 for TTR. LMR was calculated for all patients. The tumor recurred in 12 patients out of 30 with LMR < 2.87 and in 11 patients out of 38 with LMR ≥ 2.87. Death occurred in 17 patients out of 29 with LMR < 2.81 and in 20 patients out of 39 with LMR ≥ 2.81. Clinical stage at diagnosis was the only clinocopathologic features affecting the LMR while tumor invasion depth was borderline significant (Table 2).

In our study cohort, elevated preoperative LMR was significantly associated with increased TTR (P=0.001) and OS (P=0.020). An elevated LMR was associated with a longer TTR (Figure 1). Patients with a LMR < 2.87 showed a median TTR of 2.0 years (24 months) with 95%CI [0.27-3.73], whereas patients with a LMR ≥ 2.87 had a median TTR of 11.1 years (133 months) with 95%CI [2.31-19.88] (P=0.001). Only muscle invasive disease had a significant effect on TTR.

Patients with a LMR < 2.81 showed a median OS of 2.7 years (32 months) with a 95%CI [0.63-4.70], whereas patients with a LMR ≥ 2.81 had a median OS of 6.0 years (72 months) with a 95%CI [3.60-8.40] (P=0.020) (Figure 2). Similarly, muscle invasive disease was the only clinical feature affecting OS while gender, smoking history, adjuvant chemotherapy, neoadjuvant chemotherapy and LND had no effect (Table 1).

**Discussion**

Bladder cancer is frequently associated with chronic or recurrent inflammation and a significant number of inflammatory cells are found at the tumor site. Monocytes represent a source of multiple chemokines/cytokines which may contribute to
inflammation and immune dysfunction[14]. Monocytes were found to promote
tumorigenesis and angiogenesis through local immune suppression and stimulation of
tumor neovasculogenesis[15]. Moreover, macrophages, which are more differentiated
monocytes, were shown to support tumor-associated angiogenesis as well as tumor
cell invasion, migration and intravasation and even lead to a suppression of anti-tumor
immune reaction[16, 17]. This could explain the reason why an elevated monocyte
count confers a negative prognosis in solid tumors[18, 19].

Lymphocytes, on the other hand, are essential in anti-tumor reaction of the immune
system through induction of tumor cell apoptosis and are mediators of antibody-
dependent cell-mediated cytotoxicity [20, 21]. Hence, a low lymphocyte amount
might be responsible for a weak, insufficient immunologic reaction to the tumor and
could incur a negative prognostic outcome[22]. In patients with invasive bladder
carcinoma, the numbers of T and natural killer (NK) cells were significantly lower
compared to patients with superficial carcinoma[23]. Moreover, in patients with
invasive disease, the CD4/CD8 ratio, lymphocyte reactivity to mitogens and NK cell
activity was significantly diminished when compared with the controls and with
patients with superficial carcinoma. Patients with high-grade tumors also had a
significantly lower CD4/CD8 ratio and lymphocyte activity to mitogens when
compared to patients with low-grade tumors[24]. These findings indicate gross
immunological abnormalities in TCC patients which correlate with stage and grade of
tumor. Hence, a depressed LMR is an indicator of lymphopenia and/or an elevated
monocyte count; both of which are associated with worse prognostic outcome. In our
study, the only factor that affected the LMR was the stage at diagnosis while the
tumor depth was only borderline significant. Tumor grade did not have a significant
effect on the LMR. Since advanced stage at diagnosis has been shown to incur a
negative clinical outcome in the majority of tumors and was the only feature associated with the LMR, it is possible that a depressed LMR could be a reflection of advanced stage at diagnosis. However, this conclusion needs further validation from other trials.

In the present study we assessed the hypothesis that an elevated LMR is positively correlated with OS and TTR in patients with TCC bladder cancer. To control for the inherent biases of a retrospective study, the following steps were taken. We included only patients with TCC bladder cancer and excluded patients with squamous cell carcinoma, adenocarcinoma and clear cell carcinoma. We only included patients undergoing cystectomy. To best of our knowledge, this is the first study to assess the influence of LMR on OS and TTR in TCC bladder cancer. A LMR $\geq 2.87$ was significantly associated with longer TTR while a LMR $\geq 2.81$ was correlated with better OS. Results of our study were consistent with other studies involving various malignancies[10-13].

The limitations of the study include the small sample size, short follow-up period and the selection bias imparted by the retrospective design of the study. Moreover, white blood cell count was obtained preoperatively and we did not perform specific quality control analysis. Also, neo-adjuvant chemotherapy treatment and lymph node dissection were not standardized. Finally, potential confounding factors such as infection and other disease states like ischemia, acute coronary syndrome, diabetes and renal and hepatic dysfunction have not been assessed and which might have affected the lymphocyte and monocyte counts.

The LMR constitutes a single, low cost and predictive biomarker for clinical outcome in patients with TCC bladder cancer related to a patient’s adaptive immune response. Classifying patients into high and low risk groups based on such predictive biomarker
could tailor treatment related choices. Moreover, since a significant correlation between TTR and LMR was achieved, this biomarker could be used to identify patients who are at high risk of tumor recurrence and those who are more likely to have poorer survival outcomes. However, our results need to be validated in larger patient cohorts involving patients with TCC bladder cancer.

Several trials are currently underway to identify single biomarkers that might affect clinical outcome in patients with TCC bladder cancer. One such trial is aiming to find urine and blood markers that could identify patients at risk of tumor recurrence (ClinicalTrials.gov Identifier: NCT02053662). Another trial is currently investigating the methylation status of 4-6 genes in urine and matching bladder tissue biopsies, in order to find methylation markers for use in a noninvasive test in monitoring patients with bladder tumors (ClinicalTrials.gov Identifier: NCT00244205). A third trial is assessing the existence and frequency of tumor specific T-cells and regulatory T cells in patients with invasive bladder cancer. The results of which will be correlated to clinical data such as the cancer-specific survival and the response to chemotherapy (ClinicalTrials.gov Identifier: NCT01198808).

Prognostic markers are clinically relevant in order to tailor treatment-related choices. Bladder cancer patients have limited treatment options particularly patients whose tumors recur after initial therapy. However, recent emerging genomic data has identified new potential treatment targets in bladder cancer. In a study of 131 chemo-naïve patients with invasive bladder cancer, the researchers identified potential drug targets in 69% of the tumors evaluated[25]. The investigators also showed that genes that regulate chromatin were more frequently mutated in bladder cancer than in any other common cancer studied to date[25]. These findings suggest the possibility of developing therapies to target alterations in chromatin remodeling. Future research
directed towards targeting these genes could pave the way to a new era in bladder cancer treatment.

Conclusions

A whole new epoch in bladder cancer diagnosis, prognosis and treatment is currently emerging. Invasive bladder cancer remains a challenge to urologists and oncologists and a burden on patients who have a 5-year survival rate of only 27–50% and high rate of tumor recurrence. The LMR is a prognostic marker that is feasible and cost effective and which has shown to be significantly correlated with OS and TTR in several cancers including bladder cancer in the present retrospective analysis. Future prospective clinical trials in bladder cancer are warranted to prove the validity of this marker.

Competing Interests

All authors declare that they have no competing interests.

Authors' contributions

ST conceived of the study and participated in its design and helped in drafting the paper. DM participated in the interpretation of the data and helped in drafting and editing the paper. MC and ZF carried out statistical analysis and participated in interpretation of the data. RN and MW coordination of the study and helped in reviewing the paper. MS, RA and IA helped in the acquisition of data and in reviewing and editing the paper. AS conceived of the study and participated in the interpretation of data. All authors read and approved the final manuscript.
Acknowledgements

None

References


Figures

Figure 1 - Kaplan Meier Curve: Preoperative LMR and OS in patients with TCC

Figure 2 - Kaplan Meier Curve: Preoperative LMR and TTR in patients with TCC

Tables

Table 1 - Baseline patient and tumor characteristics and their association with recurrence and time survival, RR with 95% CI (first cited category in reference)

<table>
<thead>
<tr>
<th>parameter</th>
<th>n</th>
<th>%</th>
<th>P value</th>
<th>RR [95%CI]</th>
<th>P value</th>
<th>RR [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>88.2</td>
<td>0.068</td>
<td>3.26 [0.85-12.50]</td>
<td>0.213</td>
<td>2.51 [0.55-11.6]</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>11.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>32.4</td>
<td>0.809</td>
<td>1.04 [0.74-1.46]</td>
<td>0.122</td>
<td>1.30 [0.92-1.85]</td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>67.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LND</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.2</td>
<td>56</td>
<td>82.4</td>
<td>0.968</td>
<td>0.98 [0.33-2.91]</td>
<td>0.115</td>
<td>2.50 [0.74-8.48]</td>
</tr>
<tr>
<td>≥ 0.2</td>
<td>12</td>
<td>17.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adjuvant Chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>51</td>
<td>75.0</td>
<td>0.882</td>
<td>1.07 [0.45-2.52]</td>
<td>0.122</td>
<td>2.01 [0.79-5.08]</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>25.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neo-adjuvant chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65</td>
<td>95.59</td>
<td>0.985</td>
<td>0.98 [0.09-10.23]</td>
<td>0.453</td>
<td>0.42 [0.04-4.41]</td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>4.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Muscle- invasion (4 missing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non- muscle invasive</td>
<td>13</td>
<td>20.3</td>
<td>0.005</td>
<td>4.0 [1.39-11.59]</td>
<td>0.003</td>
<td>1.46 [1.11-1.92]</td>
</tr>
<tr>
<td>Muscle invasive</td>
<td>51</td>
<td>79.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 - Correlation of clinical features with LMR 2.81 (Survival cut-off)

<table>
<thead>
<tr>
<th></th>
<th>LMR&lt;2.81 (%)</th>
<th>LMR ≥2.81 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>5 (39%)</td>
<td>8 (61%)</td>
<td>0.064</td>
</tr>
<tr>
<td>T2</td>
<td>5 (19%)</td>
<td>21 (81%)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>6 (38%)</td>
<td>10 (62%)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>6 (67%)</td>
<td>3 (33%)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (43%)</td>
<td>8 (57%)</td>
<td>0.024</td>
</tr>
<tr>
<td>II</td>
<td>4 (18%)</td>
<td>18 (82%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8 (67%)</td>
<td>4 (33%)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>4 (44%)</td>
<td>5 (56%)</td>
<td>0.943</td>
</tr>
<tr>
<td>G2</td>
<td>5 (39%)</td>
<td>8 (61%)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>20 (43%)</td>
<td>26 (57%)</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph node density</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.2</td>
<td>22 (38%)</td>
<td>34 (62%)</td>
<td>0.226</td>
</tr>
<tr>
<td>≥0.2</td>
<td>7 (58%)</td>
<td>5 (41%)</td>
<td></td>
</tr>
</tbody>
</table>