Original Article

Retrospective Evaluation of Neutrophil to Lymphocyte Ratio in patients with Metastatic Testicular Cancer

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ABSTRACT

Objectives: Our aim was to evaluate the impact of neutrophil to lymphocyte ratio (NLR) in patients with metastatic testicular cancer (tCa).
Design: Retrospective view of prospective recorded data
Setting: Clinical study was conducted at multicentre between May 2010 and September 2016
Subjects: Patients with tCa who underwent radical orchiectomy were enrolled. Similar surgical methods, laboratory analyses, and radiologic examinations were performed and all patients were divided into 2 groups. Group 1 (n = 108) consisted of patients with non-metastatic testicular cancer; Group 2 (n = 38) consisted of patients with metastatic (solid organ-lymph node metastasis) tCa.
Intervention: Radical orchiectomy, blood sample
Main outcome measures: Demographic, preoperative, and postoperative data were noted. Postoperative complications were interpreted according to modified Clavien classifications. Statistical significant p was $p \leq 0.05$. Receiver operating curves (ROC) were obtained for determining cut-off value NLR in terms of tCa metastases.
Results: Mean follow-up was 36.4 (4 - 72) months. Mean age was 39 years (19 – 71 years). There were significant differences between groups in preoperative NLR, tCa markers and diameter of tumour ($p = 0.03$, $p < 0.001$, $p = 0.01$, respectively). Besides, invasion to lymphovascular, rete testis, cord, epididymis, and surgical margin positivity, postoperative tCa markers were significantly higher in group 2 than group 1. Area under ROC curve was 0.69, ($p < 0.001$) and cut-off value for NLR was 3.11 in terms of any metastasis. There was no serious complication after operation. Five patients experienced wound infections (Clavien 1).
Conclusions: Preoperative NLR could help us to predict lymph node and solid organ metastasis in patients with tCa. If the NLR is over 3.1, clinicians should be aware of metastasis.

KEY WORDS: inflammation, metastasis, testicular neoplasms

INTRODUCTION

Testicular cancer (tCa) is seen in 1% of all male cancers[1]. Besides, 3/100,000 new cases are consisted per year worldwide[1,2]. Incidence of tCa has also been increasing in developed countries[3,4].

Germ cell tumours are the most common type of tCa (90 - 95%) and bilateral ones are rare[1]. The peak incidence is observed in the 3rd decade of life for non-seminoma, and in the 4th decade for pure seminoma. The tCa has excellent response to chemotherapy, specifically to cisplatin[5]. It is very important to make accurate staging at time of diagnosis. Thus, adequate and early treatments can be performed. In the course of diagnosis, clinicians usually use traditional tCa markers and radiologic examinations such as abdominal and thorax computed tomography(CT). The lower stage of tCa can be treated surgically by radical orchiectomy in peripheral hospitals[6]. However, the metastases of tCa can be misdiagnosed and relapses in metastatic sites can be annoying[7]. The advanced diagnostic modalities such as positron emission tomography (PET/CT) can show metastasis of tCa. However, the peripheral hospital might not have CT and PET/CT devices. Besides, the frequency of post-chemotherapy residual tumour resection has been associated with perioperative mortality and overall survival[8,9]. In view of these lines, accurate diagnosis of metastases should be performed.

On the other hand, involvement of systemic inflammation and progression was reported in tCa during the cancer development[10]. Increases in neutrophils with decrease of lymphocytes were

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Table 1: Comparison of mean data in groups

Parameters	Group 1 (n = 108)	Group 2 (n = 38)	p-value
Age	39.3 (19 - 71)	36.9 (20 - 55)	0.9
Preoperative AFP	483.3 (0.7 - 8802)	6650 (1.3 - 54260)	0.001*
Preoperative bHCG	2597.8 (0 - 181999)	132592.5 (1.35 - 1164157)	0.001*
Preoperative LDH	322.5 (114 - 2779)	890.5 (188 - 2782)	0.001*
Preoperative NLR	3.3 (1.04 - 21.9)	4.9 (1.5 - 14.5)	0.003*
Tumour size	4.4 (0.3 - 17)	6.1 (0.9 - 13)	0.25
Lymphovascular invasion	0.3 (0 - 1)	0.5 (0 - 1)	0.13
Rate of invasion to rete testicle	0.06 (0 - 1)	0.3 (0 - 1)	0.001*
Rate of invasion to cord	0.06 (0 - 1)	0.2 (0 - 1)	0.001*
Rate of invasion to epididymis	0.05 (0 - 1)	0.2-0.4 (0 - 1)	0.001*
Rate of surgical margin positivity	0.009 (0 - 1)	0.1 (0 - 1)	0.001*
Rate of lymphadenopathy in retroperitoneum	0.2 (0 - 1)	0.9 (0 - 1)	0.001*
Postoperative AFP	3.1 (0 - 25)	11.9 (0 - 107)	0.001*
Postoperative bHCG	1.9 (0 - 2.8)	7600 (0 - 145276)	0.001*
Postoperative LDH	170.8 (0 - 276)	379.6 (0 - 1635)	0.001*
Rate of receiving chemotherapy	0.6 (0 - 1)	0.7 (0 - 1)	0.28
Rate of receiving radiotherapy	0.2 (0 - 1)	0.2 (0 - 1)	0.72
Rate of RPLND	0.07 (0 - 1)	0.5 (0 - 1)	0.43
Alive	0.9 (0 - 1)	0.4 (0 - 1)	0.001*
Living in months	45.5 (3 - 124)	25.2 (1 - 84)	0.27

AFP: alpha-fetoprotein; bHCG: beta human chorionic gonadotropin; LDH: lactate dehydrogenase; NLR: neutrophile to lymphocyte ratio; RPLND: retroperitoneal lymphnode dissection
*Statistical significant p-value.
One-way ANOVA test was used.

shown in literature that was related with systemic inflammatory response development against the tumour. The neutrophil to lymphocyte ratio (NLR) has been used as an indicator of systemic inflammatory response[11]. The mechanism includes the increased supply of factors that promote carcinogenesis and tumour progression by cells of the innate immune systems and decreased anti-tumour response by immune cells of the adaptive system[12].

The NLR, which can easily be calculated from routine complete blood count (CBC) with differentials, is an emerging marker of host inflammation[13]. This was also shown to be an independent prognostic factor for a variety of solid malignancies[14,15]. There are some emerging studies on impact of NLR in usage of secondary marker of tCa[16]. However, there has not been a published study on comparison of NLR between metastatic and non-metastatic tCa in literature.

We here investigated the impact of NLR in metastasis of tCa to lymph nodes and solid organs. We also determined cut-off value of NLR in terms of metastasis to these tissues. Our hypothesis was NLR could increase more in tCa patients than in non-metastatic ones.

SUBJECTS AND METHODS

Patient data

This is a multicentre study including retrospective view of prospective collected data. Additionally, the present study is an on-going study. Institutional review board and ethical committee approved the study. Signed consent forms were obtained from all patients. Between May 2010 and September 2016, all patients with tCa who underwent surgery (radical orchiectomy)[17], enrolled in the study. Exclusion criteria were metastasis to brain and irregular follow-up.

All patients were divided into 2 groups as group 1 (n = 108) consisting of non-metastatic tCa and group 2 (n = 38) consisting of metastatic (solid organ and lymph node metastasis) tCa.

Data collection

Demographic, preoperative, and postoperative data of patients were collected. The collected data was noted on Microsoft Excel Data Sheet. Laboratory analyses included alpha-fetoprotein (AFP), beta human chorionic gonadotropin (HCG), lactate dehydrogenase (LDH), CBC, scrotal ultrasound (USG), abdominal USG (when needed), abdomen-pelvic CT and thorax CT. The NLR was calculated from CBC. Similar surgical technique was performed in all cases for radical orchiectomy[17].

Metastatic sites were decided according to pathology reports, CT, and PET/CT slides. Additionally, if the patient had neurologic symptoms, cranial enhanced CT was performed for detecting brain metastasis of tCa. Besides, some of the metastatic tissues occurred in the follow-up.

Postoperative complications were classified according to modified Clavien classification[18].

Statistical analyses

Statistical Package for the Social Sciences (SPSS, V16.0, Chicago, IL) was used for statistical analyses. One-way ANOVA tests were used for comparing mean values in groups. The receiver operating curves (ROC) were drawn and cut-off value for NLR was determined in terms of tCa metastasis. The significant p-value was $p \leq 0.05$.

RESULTS

The mean follow-up was 36.4 months (range: 4 - 43). The mean age was 39 years old (range: 19-71). There were statistically significant higher elevations in group 2 than group 1 in terms of preoperative beta HCG, LDH, NLR ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.003$, respectively). Mean age, tumour size and the rate of lymphovascular invasion were similar in both groups.

Besides, statistically significant elevated ratio of invasion to rete testis-cord-epididymis, surgical margin, and lymph node in retroperitoneum were obtained in group 2 than group 1 (for all parameters; $p < 0.001$).

The mean values of postoperative AFP, beta HCG, and LDH were higher in group 2 than group 1 in follow-up (for all parameters; $p<0.001$). The rate of used chemotherapy and radiotherapy were comparable in both groups. Patients with stage 1 seminoma and/or non-seminoma tCa were rarely administered chemotherapy. All these were summarized in Table 1.

Table 2 shows the comparable chancing pathologic diagnosis of tCa in both groups. Group 1 consisted of stage 1 patients. Besides, there were 27 stage 2 and 11 stage 3 patients in group 2.

Table 2: Pathology data of patients in groups

Parameters	Group 1 (n = 108) n (%)	Group 2 (n = 38) n (%)	p-value
Seminoma	43 (39.8)	10 (26.3)	
Germ cell tumour	4 (3.7)	11 (28.9)	
Mixt type	47 (43.5)	10 (26.3)	0.7
Embryonal cell carcinoma	6 (5.5)	2 (5.2)	
Teratocarcinoma	4 (3.7)	1 (2.6)	
Teratoma	2 (1.8)	3 (7.8)	
Lymphoma	2 (1.8)	1 (2.6)	

We drew the ROC curve for determining cut-off value of NLR in terms of tCa metastases. The area under ROC curve was 0.69 ($p < 0.001$). The cut-off value for NLR was 3.11 (Fig. 1).

The most common complication was wound infection (Clavien 1) that was treated with medication. We additionally performed wound dressing regularly during follow-up. There were no Clavien 3b, 4, and 5 complications.

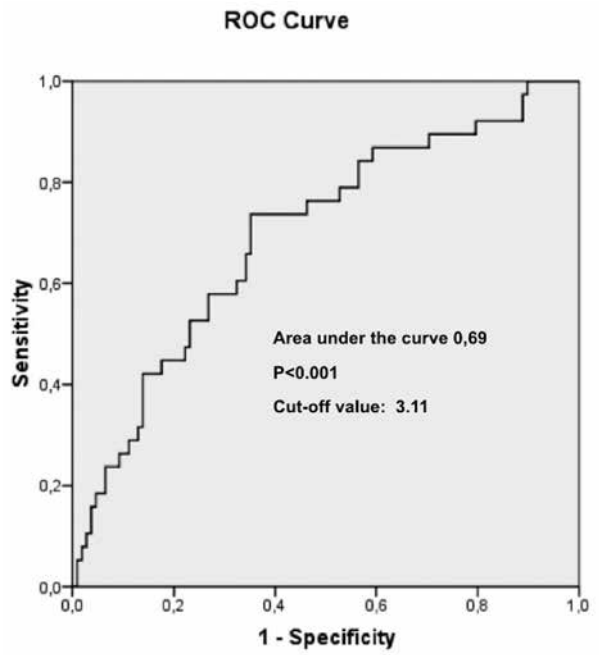

Fig. 1: Receiver operating curves was used to determine cut-off value of neutrophil to lymphocyte ratio in terms of metastasis.

DISCUSSION

In the present study, we found significantly higher NLR in patients with metastatic tCa than non-metastatic ones. The blood tests and radiological evaluations are performed for diagnosing tCa. We determined the cut-off NLR for predicting tCa metastases by using simple laboratory tests. Thus, our hypotheses were proved with these findings. If the tCa patient's NLR is over 3.11, clinicians should be aware of metastases.

One of the common points of tCa and NLR is the inflammation that can easily be put forward[16]. Our hypothesis was based on these. We traditionally use tCa laboratory tests and radiological examinations as AFP, beta HCG, LDH and thorax, abdomen, and pelvic CT, respectively. Additionally, PET/CT can be used in suspicious cases for detecting metastasises of tCA. However, PET/CT could mostly determine tCa metastases to retroperitoneal lymph nodes easily. Since these radiological diagnostic tools may not be available in peripheral hospitals, metastases can be misdiagnosed. At this point, NLR value may warn clinicians. Besides, it is very easy to access CBC for detecting NLR. To our best knowledge, this is the first study in which impact of NLR was presented in patients with metastatic tCa. Additionally, the cut-off NLR value for tCa metastasis was determined.

We found significantly higher preoperative NLR in group 2. Additionally, preoperative AFP, beta HCG, and LDH were significantly higher in group 2. Yuksel *et al* reported that higher NLR in tCa can be used as a secondary marker for tCa[16]. In their study, there were

36 tCa patients with mean NLR at 2.58[16]. Higher mean preoperative NLR was determined in the present study than study of Yuksel *et al*. Besides, we determined the cut off value for NLR in ROC curves as 3.11, for metastatic tCa. These may relate to higher numbers in groups, in our study. We determined metastases according to pathology in retroperitoneal lymph node dissections and CT, PET/CT slides. Zhao *et al* reported use of PET in determining tCa metastasis[19]. Höltl *et al* reported spread of tCa by vessels[20]. In view of these lines, we accepted lymph node involvement as metastasis. In the light of the above data, it is clear that metastases of tCa increase inflammation. Therefore, all those caused to increase NLR. Besides, if the NLR is higher than 3.11, in patients with tCa, clinicians should be aware of metastasis.

Postoperative pathology results supported higher clinical stages of tCa in our study. The invasion to rete testis-cord-epididymis, surgical margin, and lymph nodes in retroperitoneum were significantly higher in group 2. Additionally, these were parallel to postoperative mean serum tCa markers such as significantly higher postoperative AFP, beta HCG, and LDH in group 2. All these are similar to published literature on tCa and its metastases[21]. Wu and Zhou showed molecular pathways that linked inflammation in the way of tumour metastasis[22]. Rajput and Wilber published similar findings with them[23]. Porta *et al* also concluded that inflammation showed progression of the tumour[24]. However, we did not perform any molecular based analyses. Our findings in terms of higher NLR in metastatic tCa could support those literatures above. It is inevitable that molecular basis of higher inflammation in metastatic tCa should be investigated in detail with molecular studies.

On the other hand, Takashi *et al* reported that increased preoperative NLR was strongly associated with poor prognosis in patients with upper urinary tract urothelial carcinoma[25]. We showed significantly higher survival rates in group 1 than group 2. This is also similar to published literature on this topic.

We have some limitations in this study. There was unequal distribution of patients in the 2 groups and low number of participants in the groups. Additionally, we retrospectively evaluated patient files and follow-up database. We also used CT and PET/CT slides for determining metastases, knowing this examination could easily detect retroperitoneal lymph nodes. We did not have the opportunity to perform biopsy for tissue diagnosis in most cases. However, we focused on higher NLR in metastatic tCa.

To the best of our knowledge, this is the first study that showed higher NLR was obtained in metastatic NLR than non-metastatic ones. Thus, higher preoperative NLR (>3.11) may not be an independent marker for metastasis of tCa, but it could show poor prognosis in tCa patients. The molecular evidence of these findings should be investigated in detail in future studies.

CONCLUSION

The preoperative NLR can be higher in metastatic tCa than non-metastatic ones. The NLR can be easily calculated from CBC and could be a pathfinder for clinicians in tCa patients. If the NLR is higher than 3.11, clinicians should be aware of metastases of tCa. Thus, misdiagnosis could be overcome in metastasis of tCa. More molecular based studies are needed on this topic for showing details of inflammation modalities for tCa metastasis.

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Conflicts of interest: The authors declare no conflicts of interest.

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