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RESEARCH ARTICLE

# Clinical implication of pretreatment neutrophil to lymphocyte ratio in soft tissue sarcoma

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**Abstract**

*Introduction*: Elevated neutrophil to lymphocyte ratio has been identified as a prognostic indicator in malignancies whereas; its association with extremity and trunk soft tissue sarcoma remain unclear. The aim of this study is to determine the utility of full blood neutrophil lymphocyte ratio (NLR) in preoperative diagnosis and its predictive value for survival in patients managed for soft tissue sarcoma of the trunk and extremities.

*Method*: 223 patients who presented with a soft tissue tumor were retrospectively reviewed. The study period was from January 2002–December 2009. Preoperative NLR as well as demographics, clinical and histopathological data were analysed.

*Results*: Full blood NLR was significantly higher in patient with a soft tissue sarcoma compared to benign soft tissue tumors ($p < 0.001$). Cox regression analysis demonstrated that elevated NLR >5 ($p < 0.05$) may be an adverse prognostic factor for Overall Survival.

*Conclusion*: The preoperative NLR is a simple, investigation predicting the preoperative diagnosis of a soft tissue sarcoma and a predictor of worse overall survival for patient with a soft tissue sarcoma.

**Keywords:** Biomarkers sarcoma, neutrophil lymphocyte ratio, inflammation in cancer, sarcoma diagnosis, soft tissue tumors



## Introduction

There is an increasing need for simple measures of diagnosis and prognosis determination in the management of extremity and trunk soft tissue tumors. Soft tissue lumps and bumps account for a large number of patients' visit to the Sarcoma units. The vast majority of these tumors turn out to be benign (Gustafson 1994; Fernebro et al. 2006; Grimer & Briggs 2010).

Cancer susceptibility and severity has been related to the activities of the inflammatory cells found at the local environment. This has triggered recent interest in inflammatory targets in the diagnosis and prognostication for cancers.(Mantovani et al. 2008; Sorbye et al. 2011; Sorbye et al. 2012; Gooden et al. 2011) Moreover, elevated pretreatment serum C-reactive protein has recently been reported as a poor prognostic factor for survival for extremity soft tissue sarcoma (Nakamura et al. 2012).

High neutrophil count as a surrogate marker of inflammation has been widely studied in various solid tumors. The clinical observations suggested that high neutrophil count suppresses host immunity, translating to poor clinical outcome. This has been supported by *in vitro* studies demonstrating correlation between degree of cytotoxic effector cell suppression and neutrophil count (Teramukai et al. 2009; Porrata & Markovic 2010).

The aim of this retrospective review was to: (i)determine the utility of pretreatment full blood neutrophil lymphocyte ratio (NLR) in preoperative diagnosis of soft tissue sarcoma (ii) determine the predictive value of pretreatment full blood NLR for survival in patients with soft tissue sarcoma of the trunk and extremities.

## Method

### Patients

The clinical database for patients treated surgically for extremity and trunk soft tissue tumors was reviewed.

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The study period was from January 2002–December 2009. The patients presenting with metastases at diagnosis were excluded. There were 223 patients available for review with complete hematological parameters (Figure 1).

The pretreatment investigations included full blood counts, ultrasound and or magnetic resonance imaging. Computed tomography scans of the lungs and abdomen were performed as staging procedures. The histopathological diagnosis and tumor grade were assigned using the French Federation of Cancer Centres Sarcoma Group system for all patients.

The total and differential white cell counts were measured at prebiopsy visit from the full blood count obtained from a peripheral blood sample in all patients. The full blood count was measured using Sarstedt Monovette EDTA-KE Haematology system by SARSTEDT Ltd. Leicester, United Kingdom. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. An NLR ≥5 was considered elevated in accordance with earlier reports (Walsh et al. 2005; An et al. 2010; Kim et al. 2010; Shimada et al. 2010). The preoperative neutrophil counts, lymphocyte counts, neutrophil lymphocyte ratio (NLR), as well as demographics, clinical and histopathological data were analysed. The institutional board review approval was obtained and informed consent was waived because the study was a retrospective medical records review.

### Statistical analysis

The differences in the NLR among malignant and benign soft tissue tumors were compared using Mann–Whitney *U*-test.

Demographic parameters (sex and age), anatomic location, and clinico-pathological variables (grade, size, neutrophil counts, lymphocyte counts, NLR, postoperative infection) were examined.

Investigated endpoints of the study were overall survival from the disease and recurrence free survival. Actuarial survival was estimated using the Kaplan-Meier method. The univariate and multivariate analysis were calculated by a Cox regression model. A p-value of <0.05 was considered significant. All calculations were made using SPSS Inc., Chicago, Illinois; version 13.

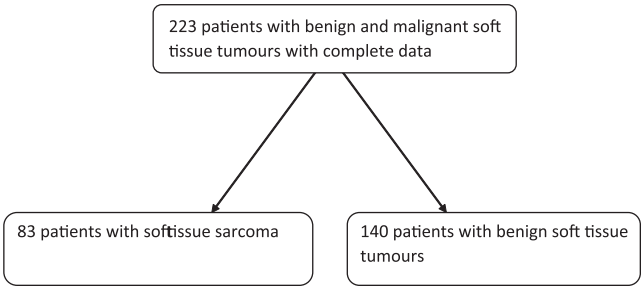


Figure 1. Process of patient's recruitment.

## Results

### Patient's characteristics

The age range of patients with benign soft tissue tumor was 24–88 years with a mean of 58 years. The age range of patients with soft tissue sarcoma was 21–90 years with a mean of 61 years (Table 1). The mean follow-up period for patients with soft tissue sarcoma was 28 months (range, 3–75 months). The mean tumor size for soft tissue sarcoma was 9.3 cm (range, 1.5–40 cm).

### Histological types

The benign soft tissue tumors diagnoses include, lipoma (n:87), schwannoma (n:11), neurofibroma (n:11), elastofibroma (n:6), and fibromatoses (n: 8). The less frequently occurring benign tumors include giant cell tumor, angiomyolipoma, angioleiomyoma, myoepithelioma.

The histological diagnoses in soft tissue sarcoma include liposarcoma(n:20), fibrosarcoma (n:24), leiomyosarcoma (n:17), and undifferentiated sarcoma (n:5). Malignant peripheral nerve sheath tumor, Ewing's sarcoma, epitheliod sarcoma, synovial sarcoma and chondrosarcoma were also recorded(n:17). Forty patients had grade 3 sarcomas according to the French Federation of Cancer Centres Sarcoma Group system, 12 patients were histologically classified as grade 2 and 31 as grade 1. The primary tumor sites were thighs ($n$ = 38), back ($n$ = 15), leg ($n$ = 9), upper arm ($n$ = 8), buttocks ($n$ = 6), forearm ($n$ = 4), popliteal fossa ($n$ = 3).

Eighty three patients underwent surgical excision of sarcoma, 28 patients received radiation therapy for primary site tumor. Six patients had adjuvant chemotherapy.

### Preoperative diagnosis

The mean NLR in benign tumors was 2.8 ± 2.1 which was significantly lower compared to soft tissue sarcoma group with mean NLR of 4.1 ± 3.1 ($p < 0.001$).

While high NLR ≥5 was recorded in 7.1% of patients with benign soft tissue tumors, 24.1% of patients with soft tissue sarcoma had elevated NLR ($p = 0.001$) (Table 2).

### Prognosis

Nine of the eighty three patients (10.8%) with a soft tissue sarcoma suffered a recurrence of the disease. Five year overall survival in patients with NLR <5 was 87% compared to 35% for patients with NLR ≥5, CI 95% HR 4.02 (1.16–13.96).

Moreover, 5 year recurrence free survival for patients with NLR <5 is 90%, while 5 year recurrence free survival for patients with NLR ≥5 is 70%, CI 95% HR 3.63 (1.04–12.63) (Figures 2 and 3). In multivariate analysis, grade

Table 1. Patients' characteristics.

| Histological type | Median age (range) | Gender (M:F) |
|---|---|---|
| Benign ($n$ = 140) | 58 (24–88) years | 56:84 (1:1.5) |
| Malignant ($n$ = 83) | 61 (21–90) years | 45:38 (1.2:1) |

Table 2. NLR ratio in benign vs. malignant groups.

| NLR | Benign ($n$ = 140) | Malignant ($n$ = 83) | $p$ value |
|---|---|---|---|
| Mean NLR (SD) | 2.8 ± 2.1 (range 0.2–18.2) | 4.1 ± 3.1 (range 2.8–23.5) | <0.001* |
| High NLR (≥5) | 7.1 % | 24.1% | 0.001** |
| Low NLR (<5) | 92.9% | 75.9% | 0.001** |

(AUROC = 0.66; CI: 0.584–0.735).
*Man–Whitney test.
**$Z$ test for difference in proportions.

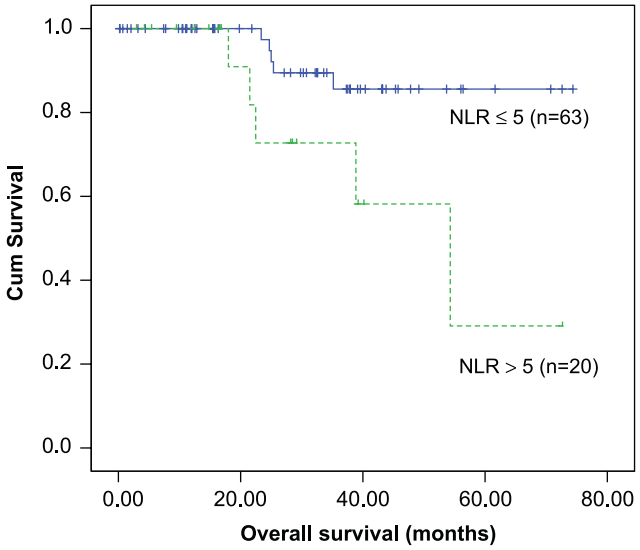


Figure 2. Kaplan–Meier curve showing OS for 83 patients with soft tissue sarcoma stratified by NLR status. OS was significantly worse in patients with a high NLR compared with patients with a normal NLR (Log-Rank = 5.782, $p$ = 0.016; Broken line = NLR ≥ 5).

and NLR were the significant factors for overall survival. (Tables 3–6) Cox regression analysis demonstrated that elevated NLR ≥5 ($p < 0.05$) was an adverse prognostic factor for Overall Survival.

## Discussion

This study represents the first report investigating NLR in the diagnosis and prognosis of extremity sarcoma. The study provided some evidence that elevated NLR was associated with decreased survival, and that it may be a useful screening measure. However, the limitations to this study include the retrospective single centre design with a mixture of various types of soft tissue sarcoma. Nevertheless soft tissue sarcomas are rare cancers with 75 different subtypes and most reports on prognosis of soft tissue sarcoma have adopted 'lumping' of various histological types and have been retrospective studies(Clark et al. 2005; Gronchi et al. 2010; Carneiro et al. 2011).

The neutrophil lymphocyte ratio (NLR) has been reported as cost effective tool for the preoperative diagnosis of uterine sarcoma. Moreover, elevated neutrophil to lymphocyte ratio and other inflammatory markers have been evaluated in the prognosis of cancers (Walsh et al. 2005; An et al. 2010; Kim et al. 2010; Shimada et al. 2010). The

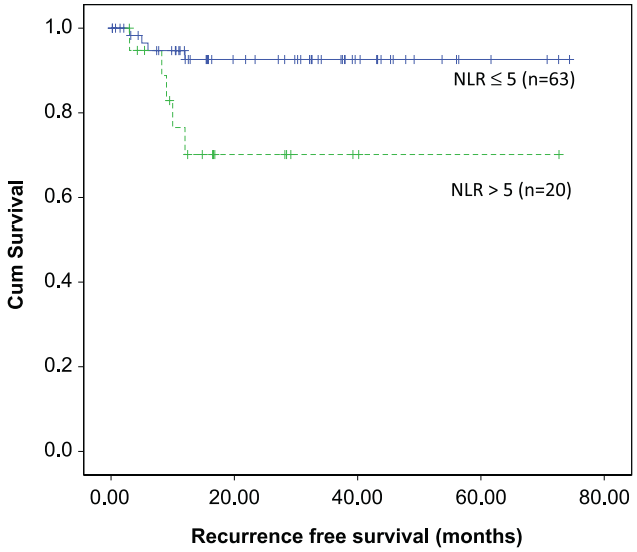


Figure 3. Kaplan–Meier curve showing RFS for 83 patients with soft tissue sarcoma stratified by NLR status. RFS was worse in patients with a high NLR compared with patients with a normal NLR (Log-Rank = 5.53, $p$ = 0.019; Broken line = NLR ≥ 5).

Table 3. Multivariate analysis of factors affecting overall survival.

| Risk factors | HR (95% CI) | $p$ value |
|---|---|---|
| Age (<65 and ≥65) | 4.152 (0.77–22.392) | 0.098 |
| Sex | 0.734 (0.129–4.19) | 0.728 |
| Anatomic location (lower, upper) | 0.937 (0.161–5.453) | 0.943 |
| Grade (high, intermediate, low) | 5.571 (0.888–34.954) | 0.017 |
| Tumor size (<5 cm and >5 cm) | 0.551 (0.123–2.479) | 0.438 |
| Surgical margin (marginal, wide) | 0.397 (0.063–2.494) | 0.042 |
| Neutrophil lymphocyte ratio (≥5 vs. <5) | 5.125 (1.245–21.086) | 0.024 |

Table 4. Univariate analysis of factors affecting overall survival.

| Risk factors | HR (95% CI) | $p$ value |
|---|---|---|
| Age (<65 and ≥65) | 3.16 (0.857–11.659) | 0.084 |
| Sex | 0.498 (0.14–1.772) | 0.282 |
| Anatomic location (lower, upper) | 0.771 (0.199–2.983) | 0.706 |
| Grade (high, intermediate, low) | 3.095 (0.872–10.986) | 0.041 |
| Tumor size (<5 cm and >5 cm) | 0.752 (0.202–2.807) | 0.672 |
| Surgical margin (marginal, wide) | 0.361 (0.093–1.399) | 0.014 |
| Neutrophil lymphocyte ratio (≥5 vs. <5) | 4.084 (1.179–14.148) | 0.026 |

increase in the numbers of neutrophils and or decreased numbers of lymphocytes may suppress lymphokine-activated killer cells. This is a plausible mechanism for decrease in the survival of patients with cancer (Shau & Kim 1988; Teramukai et al. 2009; Porrata & Markovic 2010).

Table 5. Multivariate analysis of factors affecting recurrence-free survival.

| Risk factors | HR (95% CI) | *p* value |
|---|---|---|
| Age (<65 and ≥65) | 3.236 (0.749–14.217) | 0.115 |
| Sex | 0.81 (0.191–3.434) | 0.775 |
| Anatomic location (lower, upper) | 0.838 (0.201–3.491) | 0.808 |
| Grade (high, intermediate, low) | 2.272 (0.505–10.219) | 0.028 |
| Tumor size (<5 cm and >5 cm) | 0.478 (0.101–2.26) | 0.351 |
| Surgical margin (marginal, wide) | 0.839 (0.151–4.653) | 0.841 |
| Neutrophil lymphocyte ratio (≥5 vs. <5) | 4.048 (0.964–16.989) | 0.056 |

Table 6. Univariate analysis of factors affecting recurrence-free survival.

| Risk factors | HR (95% CI) | *p* value |
|---|---|---|
| Age (<65 and ≥65) | 3.003 (0.75–12.024) | 0.12 |
| Sex | 0.676 (0.181–2.517) | 0.559 |
| Anatomic location (lower, upper) | 1.011 (0.253–4.044) | 0.988 |
| Grade (high, intermediate, low) | 2.982 (0.744–11.956) | 0.012 |
| Tumor size (<5 cm and >5 cm) | 0.842 (0.211–3.369) | 0.808 |
| Surgical margin (marginal, wide) | 0.887 (0.184–4.283) | 0.881 |
| Neutrophil lymphocyte ratio (≥5 vs. <5) | 4.243 (1.138–15.82) | 0.031 |

The poor prognosis of soft tissue sarcomas with elevated NLR may be attributable to a similar mechanism.

Adverse prognostic factors for soft tissue sarcoma include age, grade, size, vascular invasion, metastasis at diagnosis and depth. Moreover, there are a number of immuno-histochemical detected biomarkers associated with disease specific survival in soft tissue sarcoma. These include, GLUT-1, IRS2 and NF1 protein expression, alternative lengthening of telomeres, copy number alterations, expression of PI3K, TLE3, the polycomb transcription factor Yin Yang 1 (YY1), plasma D-dimer levels, c-Met pathway components like p-MEK and p-AKT, LGR5/GPR49, miR-34a activity, microRNA (miR-210) tumor-associated macrophages, Wilms tumor gene 1 (WT1), fibroblast growth factor 2 (FGF2), PAI-1 and uPAR-del4/5 mRNA levels, endothelin-1 (ET-1), Connexin 43, Cox2, TGF-β1, PDGF-B (Kilvaer et al. 2010; Valkov et al. 2011, Shon et al. 2011, de Nigris et al. 2011, Morii et al. 2011, Lahat et al. 2011, Rot et al. 2011, Fujiwara et al. 2011, Buddingh et al. 2011, Coosemans et al. 2011, Kilvaer et al. 2011, Kotzsch et al. 2011, Zhao et al. 2011, Bui et al. 2011, Lee et al. 2011, Valkov et al. 2011, Greither et al. 2012, Nakatani et al. 2012, Smeland et al. 2012; Shaw et al. 2012; Lee et al. 2012; Crago et al. 2012).

Previously reported serological markers of soft tissue sarcoma include vascular endothelial growth factors (VEGF), tissue inhibitors of metalloproteinase (TIMP 1, TIMP2), matrix metalloproteinase (MMP2, MMP9), and fibroblast growth factor beta (bFGF) (Graeven et al. 1999; Benassi et al. 2003; Hayes et al. 2004). Perhaps, the complexity of their measurement is responsible for the slow integration into clinical practice. These serological markers were not routinely measured in the current study. NLR is evaluated in routine clinical practice from the complete blood count which is a common laboratory test. Recently there has been some evidence supporting the inclusion of inflammatory prognostic scores such as the modified Glasgow prognostic score (mGPS) into daily oncological practice (Clarke et al. 2011; Proctor et al. 2011; Leung et al. 2012).

The study demonstrates that preoperative NLR can be useful in distinguishing benign and malignant soft tissue tumors. The study also demonstrates a relationship between NLR and disease specific outcome, i.e. patients with NLR ≥5 had significantly worse survival. The univariate and multivariate analysis revealed that elevated NLR was associated with decreased overall survival.

In conclusion, pre operative NLR appear to represent a simple investigation in the preoperative diagnosis of a soft tissue sarcoma and a predictor of worse OS for patients with soft tissue sarcoma. It may be a useful complement along with CT, MRI and PET in the management of soft tissue sarcoma. Further studies are necessary to confirm these results and evaluate NLR and other inflammatory markers as prognostic factors for soft tissue sarcoma.

## Declaration of interest

The authors report no conflicts of interest.

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