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ORIGINAL PAPER

# Haematology panel biomarkers for humeral, femoral, and tibial diaphyseal fractures

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## Abstract

**Purpose** The neutrophil to lymphocyte ratio (NLR) is a simple predictor used in oncology and cardiology. We aimed to analyze the NLR profile of patients with diaphyseal fractures of the humerus, femur, and tibia.

**Methods** We performed a cross-sectional, consecutive-case population-based study including 148 patients (41.9% men respectively 58.1% women) with humeral (23.0%), femoral (30.4%), and tibial (46.6%) diaphyseal fractures, admitted for surgical treatment in our level 1 trauma centre over two years.

**Results** The differences in NLR between the studied subgroups were not significant ($p = 0.067$), the highest value being observed in patients with femoral fracture (5.6) in contrast to patients with humeral fracture (4). In the global cohort, there was a significantly positive correlation between NLR and PLR (platelet to lymphocyte ratio; Spearman's $r = 0.595$; $p < 0.001$). The stratified subgroup analysis found significant association between NLR and duration of admission only for patients with femoral fracture (Spearman's $r = -0.308$; $p < 0.001$). When compared with controls, all three fracture types had significantly higher neutrophil numbers and NLR and lower thrombocyte numbers.

**Conclusions** NLR are elevated in femur diaphyseal fractures compared with tibia and humerus, up to cut-off values with negative prediction of outcome in malignancy and cardiovascular patients. Increased NLR are predictive of longer hospital admissions for femur fractures.

**Keywords** Neutrophil to lymphocyte ratio · Inflammation · Fracture · Femur · Tibia · Humerus

## Introduction

Long bone diaphyseal fractures account for a small percentage of all fractures (1% humerus, and 3% for femur and tibia) and are mostly associated with high-energy trauma [1–3]. Nevertheless, they lead to severe functional impairment, and as a consequence, virtually all require surgical treatment [4, 5].

Neutrophil to lymphocyte ratio (NLR) is a simple haematology panel biomarker of inflammatory response that can be predictable of the outcome and severity in tumours, cardiovascular disease, hip fracture, and polytrauma [6–12]. However, there is no data regarding the profile of NLR in patients with diaphyseal fractures.

We aimed to analyze the NLR profile of patients with diaphyseal fractures of the humerus, femur, and tibia.

## Material and method

### Study design and patients

We performed a retrospective analysis of our level 1 trauma centre electronic data base over two years. We searched for patients discharged with the main diagnosis of any of the

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following ICD-10 codes: S42.3, S72.3, S82.21, and S82.28, representing diaphyseal fractures of the humerus, femur, and tibia. The search retrieved 172 records that were individually reviewed for data consistency. As a result, 24 patients were excluded for coding errors or missing data. The haematology panel at admittance was performed using either Nihon Kohden Celltac 6500, Sysmex XT-4000i, or ADVIA 2120 analyzers. We manually extracted haemoglobin value and neutrophil, lymphocyte, and platelet counts, from which we computed NLR and PLR (platelet to lymphocyte ratio) as markers of inflammation. A control group was extracted from healthy patients undergoing routine blood tests required by the annual medical exam. Out of 1260 value sets collected in the hospital during the same time frame, we manually age (approximately) and gender (exact) matched at a 1:2 ratio tested versus controls.

We then performed a cross-sectional, consecutive-case population-based study including 148 patients with diaphyseal fractures (41.9% men respectively 58.1% women). The study group was stratified for analysis purposes in three cohorts in respect to the type of the fracture: humeral (23.0%), femoral (30.4%), and tibial (46.6%). The dimensions of the cohort were calculated prior to enrolment to provide statistical power (1-$\beta = 0.8$) with a type I statistical error probability of $\alpha = 0.05$.

## Statistical analysis

Data was collected with a dedicated, customized case report form based on a Microsoft Office 365 Access database (Microsoft INC, Redmond, CA, USA) and was analyzed using SPSS v.17 (SPSS INC, Chicago, IL, USA). Prior to any analysis, the normality of the distribution of the variables was assessed according to Shapiro-Wilk's method. The homoscedasticity of the distributions was evaluated with Levene's test. Since the distributions were not Gaussian distributed, the results are presented as medians and interquartile range (in case of numerical variables) respectively number of cases and percentage from the subgroup's total in case of categorical variables. To assess the significance of differences between central tendency indicators found in the analyzed subgroups, the Mann-Whitney *U* test (two subgroups) and Kruskal-Wallis tests (more than two groups) were used. In case of categorical variables, the statistical significance of differences between proportions was assessed using chi-square test, performed according to Pearson's method. The continuous association between numerical variables was evaluated using the Spearman's correlation coefficient, and the *p* value for the correlation was calculated using the *t*-score distribution test. A comparison between tested and the age- and gender-matched controls was performed using *t* test (GraphPad Software Inc., CA, USA, QuickCalcs free online calculator). In this study, the threshold for statistical significance was considered a *p* value lower than 0.05.

## Results

In the studied group, there were significant differences between the types of fractures regarding the patient's age ($p < 0.001$), duration of admission ($p < 0.001$), haemoglobin value (0.002), and lymphocytes ($p = 0.030$). Femoral fracture was associated with a higher age and a longer admission duration, in contrast with patients with tibial fracture, cohort in which we observed the lowest median age (41 years). Patients with tibial fracture had the highest haemoglobin and lymphocyte value. The differences in NLR between the three studied subgroups were not significant ($p = 0.067$), the highest value being observed in patients with femoral fracture (5.6) in contrast to patients with humeral fracture (4). The detailed results of the studied parameters between the three groups are presented in Table 1.

**Table 1** Comparison of studied parameters stratified in respect to the type of fracture

| | Type of fracture | | | |
|---|---|---|---|---|
| | Humeral | Femoral | Tibial | *p* value |
| Age (years) | 55 [44–63] | 67 [48–78] | 41 [30–61] | *< 0.001* |
| Admission duration (days) | 6 [4–8] | 15 [11–19] | 6 [4–11] | *< 0.001* |
| Haemoglobin (mg/dL) | 13.5 [11.6–15.3] | 12.8 [11.8–13.6] | 13.9 [13.2–14.8] | *0.002* |
| Neutrophils (×1000/mm$^3$) | 7.6 [5.8–9.6] | 7.9 [6.4–12.1] | 9.1 [6.2–11.5] | 0.304 |
| Lymphocytes (×1000/mm$^3$) | 2.0 [1.6–2.5] | 1.7 [1.3–2.2] | 2.1 [1.6–2.8] | *0.030* |
| Thrombocytes (×1000/mm$^3$) | 200 [177–242] | 202 [181–234] | 214 [190–255] | 0.406 |
| NLR | 4 [2.3–5.9] | 5.6 [3.9–7.9] | 4.3 [2.6–6.4] | 0.067 |
| PLR | 103.4 [77.0–143.3] | 126.4 [75.4–163.8] | 99.6 [76.2–127.1] | 0.103 |

All studied variables had a non-parametric distribution ($p < 0.05$ is considered statistically significant; Shapiro-Wilk test)

Results are presented as medians and [interquartile range]. The *p* value of the differences between the groups was calculated using Kruskal-Wallis test

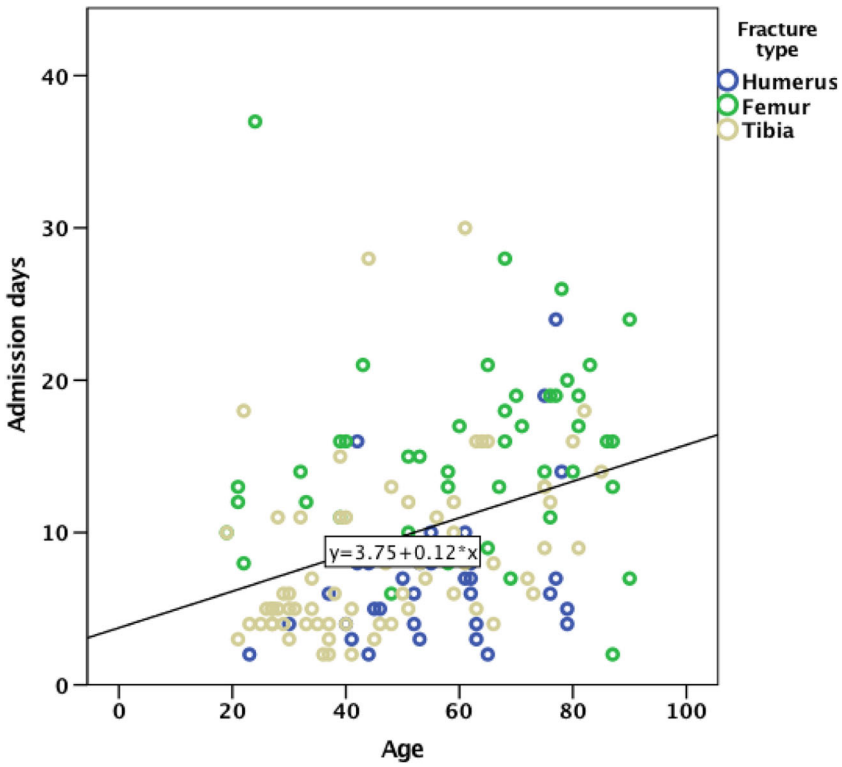


**Fig. 1** Associations between patient's age and the duration of admission

We found a significant association between gender and the type of fracture: 52.9% of patients with humeral fracture and 57.8% of patients with femoral fracture being women in contrast to only 26.1% of the patients with tibial fracture ($p = 0.001$; Pearson's chi-square test).

In the global cohort, we found significantly positive correlations between patient's age and admission duration (Spearman's $r = 0.420$; $p < 0.001$; Fig. 1) and between NLR and PLR (Spearman's $r = 0.595$; $p < 0.001$).

Significantly negative correlations were found between patient's age and haemoglobin (Spearman's $r = -0.490$; $p < 0.001$), haemoglobin and duration of admission (Spearman's $r = -0.345$; $p < 0.001$; Fig. 2) and between haemoglobin and PLR (Spearman's $r = -0.351$; $p < 0.001$), respectively.

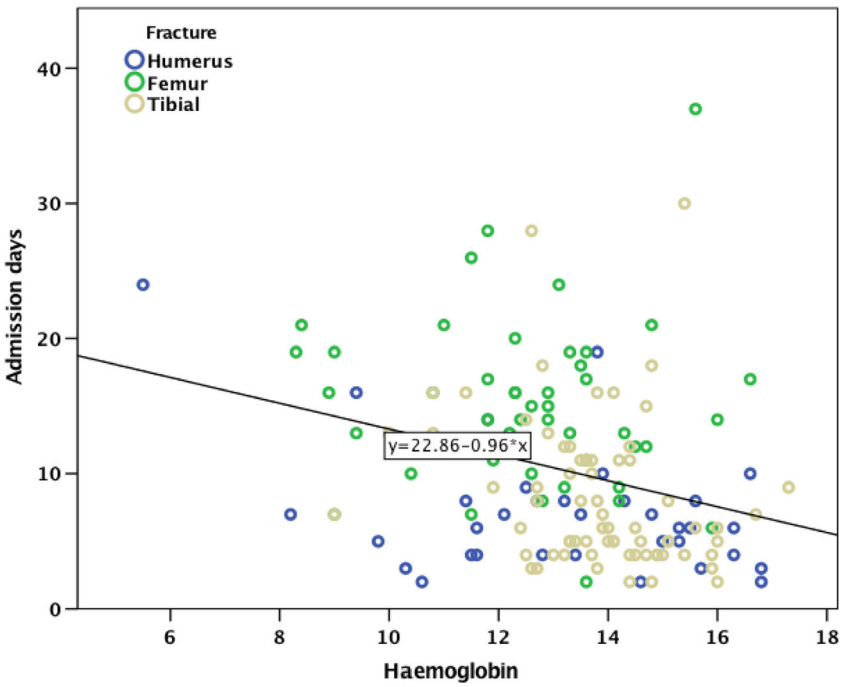


**Fig. 2** Associations between haemoglobin and duration of admission

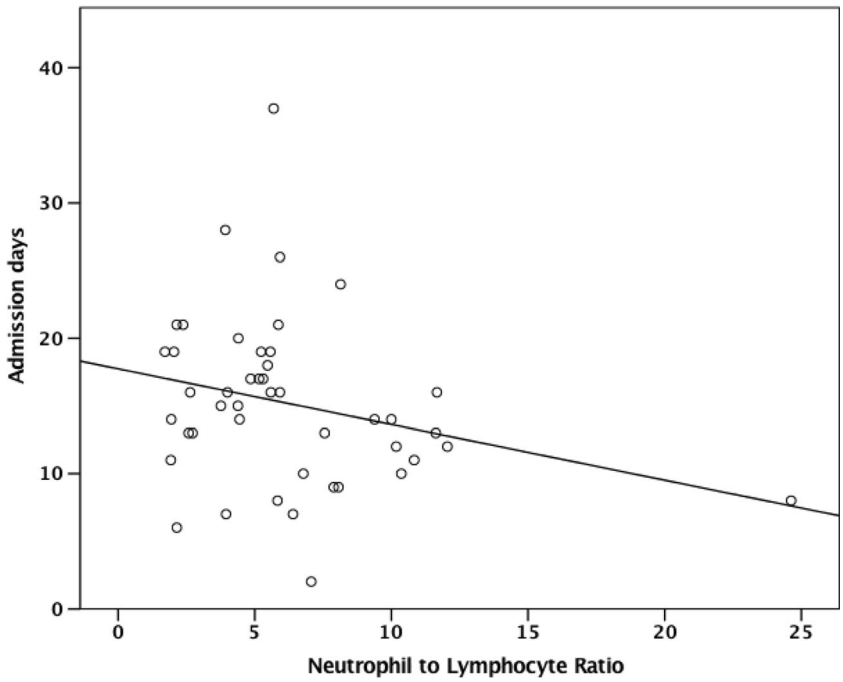


**Fig. 3** Association between neutrophil to lymphocyte ratio and duration of admission

Regarding the stratified subgroup analysis, the only significant association found was between NLR and duration of admission in patients with femoral fracture (Spearman's $r = -0.308$; $p < 0.001$; Fig. 3). The association between the duration of admissions and NLR and PLR respectively stratified according the fracture type is presented in Table 2.

When compared with controls, all three fracture types had significantly higher neutrophil numbers and NLR and lower thrombocyte numbers (Tables 3, 4, and 5). For the femur fractures, in order to mitigate the age discrepancy between the tested and control groups, we excluded patients over 80 years old.

## Discussion

To the best of our knowledge, this is the first analysis of NLR and PLR in patients with humeral, femoral, and tibial diaphyseal fractures. The differences in NLR between the three studied subgroups were not significant ($p = 0.067$), the highest value being observed in patients with femoral fracture (5.6) in contrast to patients with humeral fracture (4). When compared with controls, all three fracture types had significantly higher neutrophil numbers and NLR and lower thrombocyte numbers. We believe this can be explained by the impact of the fracture on the inflammation status.

Cut-off values of 4–5 are considered to predict a worse outcome in malignancy, hip fracture patients, with higher cut-off values (8) for critically ill trauma surgical patients [6, 7, 10, 11]. Even lower values such as 3.3 have been reported to increase myocardial strain in patients with coronary disease [8, 9]. This is especially important in the management of diaphyseal fractures in the elderly, where higher NLR may expose patients to cardiac events.

In the global cohort, the significantly positive correlation between NLR and PLR (Spearman's $r = 0.595$; $p < 0.001$)

**Table 2** The associations between NLR and PLR stratified according to the type of fracture

| Admission days | *r* (NLR) | *p* (NLR) | *r* (PLR) | *p* (PLR) |
|---|---|---|---|---|
| Humeral | 0.262 | 0.135 | 0.181 | 0.304 |
| Femoral | − 0.308 | *0.039* | − 0.200 | 0.187 |
| Tibial | − 0.004 | 0.975 | − 0.058 | 0.485 |

**Table 3** Comparative values for the humerus fractures and controls

| | Humeral | Controls | *p* value |
|---|---|---|---|
| *N* | 34 | 68 | – |
| Age (years) | 55.8 (14.7) | 55.7 (11.8) | 0.965 |
| Gender (females to males) | 18/34 | 36/68 | – |
| Neutrophils (×1000/mm$^3$) | 8.19 (3.4) | 3.91 (1.37) | *< 0.001* |
| Lymphocytes (×1000/mm$^3$) | 2.06 (0.68) | 2.4 (0.65) | *0.010* |
| Thrombocytes (×1000/mm$^3$) | 209.7 (49.2) | 249.5 (60.4) | *0.001* |
| NLR | 4.67 (2.87) | 1.69 (0.59) | *< 0.001* |
| TLR | 112.7 (40.4) | 108.9 (33.6) | 0.619 |

Results presented as means (*SD*, standard deviation) with a two-tailed *p* value significant if < 0.005

**Table 4** Comparative values for the femur fractures and controls

| | Femur | Controls | *p* value |
|---|---|---|---|
| *N* | 35 | 70 | – |
| Age (years) | 54.7 (19.2) | 54.3 (15.7) | 0.928 |
| Gender (females to males) | 18/35 | 36/70 | – |
| Neutrophils (×1000/mm$^3$) | 11.04 (5.73) | 4.06 (1.41) | *< 0.001* |
| Lymphocytes (×1000/mm$^3$) | 1.98 (0.76) | 2.27 (0.72) | *0.012* |
| Thrombocytes (×1000/mm$^3$) | 214.7 (51.3) | 259 (58.5) | *< 0.001* |
| NLR | 6.45 (4.45) | 1.84 (0.88) | *< 0.001* |
| TLR | 126.8 (62.6) | 118 (45.2) | 0.41 |

Results presented as means (*SD*, standard deviation) with a two-tailed *p* value significant if < 0.005; patients in the study group over 80 years old were excluded

shows that both these markers are elevated in association with the presence of a major fracture. The stratified subgroup analysis' significant association between NLR and duration of admission in patients with femoral fracture may explain the impact of the fracture on recovery and may well be used as a predictor lengthier return to pre-injury activities of daily living.

The differences between the types of fractures regarding the patient's age and gender may have been caused by the higher prevalence of osteoporotic fractures in the humeral and femoral groups compared with tibial. These gender differences and size of fracture haematoma and recovery particularities may consequently explain the differences in hospitalization duration and haemoglobin value.

There are several weaknesses in our study. Firstly, our primary data search methodology did not include diaphyseal fractures inappropriately listed under different codes; we only excluded miscoded non-diaphyseal fractures. In addition, some humeral diaphyseal fractures that were treated conservatively were not admitted to hospital and therefore were not included, leaving a predominance of relatively younger and rather unstable humeral fractures for analysis.

**Table 5** Comparative values for the tibia fractures and controls

| | Tibia | Controls | *p* value |
|---|---|---|---|
| *N* | 69 | 138 | – |
| Age (years) | 46.5 (18.1) | 46.54 (15.5) | 0.988 |
| Gender (females to males) | 18/69 | 36/138 | – |
| Neutrophils (×1000/mm$^3$) | 9.41 (4.05) | 4.12 (1.51) | *< 0.001* |
| Lymphocytes (×1000/mm$^3$) | 2.27 (0.91) | 2.36 (0.67) | 0.454 |
| Thrombocytes (×1000/mm$^3$) | 223.2 (58) | 249.8 (56.8) | *0.002* |
| NLR | 4.87 (2.97) | 1.85 (0.84) | *< 0.001* |
| TLR | 113.6 (60.5) | 111.9 (34.7) | *0.796* |

Results presented as means (*SD*, standard deviation) with a two-tailed *p* value significant if < 0.005

In our analysis, we did not take into account several factors that may influence NLR such as the delay between fracture occurrence and hospital admittance, presence of comorbidities (malignancy with or without pathologic fracture, cardiovascular disease, peripheral artery disease, deep vein thrombosis, infection), low- or high-energy trauma, and type of fracture (open or closed, AO classification) [6–15].

We only used NLR at hospital admittance, whereas postoperative day two to five values may have higher predictive values in fracture patients [10, 11]. For outcome, we only used the indirect measure of hospitalization duration, with no data regarding functional results, fracture fixation technique, and healing or survival [16, 17]. Furthermore, we cannot explain the mechanism behind NLR elevation, nor its impact on measures of outcome [18].

NLR are elevated in femur diaphyseal fractures compared with tibia and humerus, up to cut-off values with negative prediction of outcome in malignant and cardiovascular patients. PLR follows a similar trend. Increased NLR are predictive of longer hospital admissions for femur fractures.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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