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RESEARCH ARTICLE

Prognostic value of hemogram parameters in osteosarcoma: The French OS2006 experience

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Abstract

Background: Previous studies have shown that neutrophil-to-lymphocyte (NLR) ratio at diagnosis and early lymphocytes recovery on doxorubicin-based chemotherapy, may impact the outcome in patients with osteosarcoma (OST). This study aimed to evaluate the prognostic value of hemogram parameters in patients with OST treated with high-dose methotrexate and etoposide/ifosfamide (M-EI) chemotherapy.

Materials and methods: We retrospectively analyzed the prognostic value of various hemogram parameters at diagnosis and during therapy in a large consecutive cohort of patients with OST included in the French OS2006 trial and treated with M-EI chemotherapy.

Results: A total of 164 patients were analyzed. The median age was 14.7 years (interquartile range [IQR]: 11.7–17). Median follow-up was 5.6 years (IQR: 3.3–7.7 years). Three-year event-free survival (EFS) and overall survival (OS) were 71.5% (95% confidence interval [CI]: 64%–78%) and 86.4% (95% CI: 80%–91%), respectively. In univariate analysis, blood count parameters at diagnosis and early lymphocyte recovery at Day 14 were not found prognostic of survival outcomes. By contrast, an increase of NLR ratio at Day 1 of the first EI chemotherapy (NLR-W4) was associated with reduced OS in univariate ($p = .0044$) and multivariate analysis (hazards ratio [HR] = 1.3, 95% CI: 1.1–1.5; $p = .002$), although not with EFS. After adjustment on histological response and metastatic status, an increase of the ratio NLR-W4 of 1 was associated with an increased risk of death of 30%.

Conclusions: We identified NLR-W4 as a potential early biomarker for survival in patients with OST treated with M-EI chemotherapy. Further studies are required to

Abbreviations: ALC-14, absolute lymphocyte count; EFS, event-free survival; FOXP3, forkhead box P3; HDM, high-dose methotrexate; HR, hazards ratio; LMR, lymphocyte-to-monocyte ratio; M-EI, methotrexate and etoposide/ifosfamide; NLR, neutrophil-to-lymphocyte ratio; NLR-W4, neutrophil-to-lymphocyte ratio, Week 4; OS, overall survival; OST, osteosarcoma.

Paul Bastard and Nathalie Cozic had equal participation (co-first authors).

confirm the prognostic value of NLR and better identify immune mechanisms involved in disease surveillance.

KEYWORDS

neutrophil-to-lymphocyte ratio, osteosarcoma, pediatric, prognostic factor

1 | INTRODUCTION

The osteosarcoma (OST) bone microenvironment is heterogeneous, and consists of bone marrow and mineralized extracellular matrix. Bone marrow contains hematopoietic stem cells with hematopoietic and immunologic functions and bone marrow mesenchymal stem cells, which are responsible for differentiating into non-blood cell components in bone, including mesenchymal stem cells, osteoblasts, osteoclasts, and so forth. Immune infiltration including intra-tumoral accumulation of forkhead box P3 (FOXP3⁺) regulatory T cells,¹ but also macrophage polarization dysregulation, have been associated with metastatic progression in OST.^{2,3} Moreover, various groups have suggested that hemogram parameters, reflecting systemic but also local inflammation, may have prognostic value. Indeed, early lymphocyte recovery, evaluated by absolute lymphocyte count on Day 14 (ALC-14) has been associated with a favorable outcome in some small cohorts of patients with OST treated with cisplatin and doxorubicin.^{4–6} Similarly, other studies have shown a prognostic role of various hemogram ratios. Elevated pretreatment neutrophil-to-lymphocyte ratio (NLR) was associated with a poorer outcome in pediatric patients with sarcomas, including OST.⁴ Furthermore, it has been shown that pretreatment NLR is an independent prognostic factor in a broad range of other adult tumors.^{4,7–10}

Treatment of patients with OST includes neoadjuvant chemotherapy and surgery followed by adjuvant chemotherapy. In France, pediatric patients usually undergo high-dose methotrexate and etoposide–ifosfamide combination (M-EI).^{11,12} Survival of patients with localized OST is about 70%, but patients with metastatic disease and relapse cases have still a very poor outcome, with survival rates lower than 30%. Major prognostic factors in OST include the metastatic status and histologic response, but it is still needed to identify patients early at high risk of treatment failure, to better stratify therapy and introduce new drugs. Given that the chemotherapy regimen used for pediatric OST in France has substantial differences in comparison with the populations previously reported, we hypothesized that OST patients undergoing M-EI chemotherapy might have other hemogram prognostic factors and/or at different time points. We thus performed a multicentric retrospective study in patients with OST, included in the OS2006 trial, and treated with M-EI regimen. The main objective of the study was to evaluate the prognostic value of several parameters of the hemogram measured at diagnosis and during therapy.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

Patients were included from three large French pediatric oncology units (Gustave Roussy, Institut Curie, and Nantes Hospital). They were eligible for the analysis if they had diagnosis of OST (localized or metastatic), registered between January 1, 2004 and January 1, 2016 in the phase 3 randomized OS2006 trial (NCT00470223; <http://www.unicancer.fr/protocole-sarcome-09>), and treated with M-EI regimen. All participants had been prospectively registered in the OS2006 database. The protocol was approved by an independent ethics committee and the institutional review boards. Written informed consent was obtained from patients and/or their parents/guardians before enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and with Good Clinical Practice guidelines.

2.2 | Treatment

Preoperative chemotherapy combined seven courses of methotrexate (12 g/m²) and two courses of etoposide (300 mg/m²) and ifosfamide (12 g/m²) in all patients, and postoperative chemotherapy 12 courses of methotrexate, two courses of etoposide and ifosfamide, and one course of ifosfamide (12 g/m²) in patients with good histological response and no metastasis, and five cycles of methotrexate and doxorubicin 75 mg/m² and cisplatin 120 mg/m² in patients with poor histological response, initial metastases, unresectable primary, or both (Figure 1).¹³ Patients assigned to zoledronate arm received additional 10 monthly intravenous infusions during the pre- and postoperative treatment phases.

2.3 | Data collection

Data were prospectively collected in the OS2006 trial, except for patient's hemograms (count of hemoglobin, platelets, leukocytes, neutrophils, basophils, monocytes, eosinophils, and lymphocytes) that were filled retrospectively at six time points for 46 patients from Gustave Roussy: at Day 0 (baseline), Day 14 (after two high-dose methotrexate [HDM] courses), Day 1 of Week 4 (after three HDM

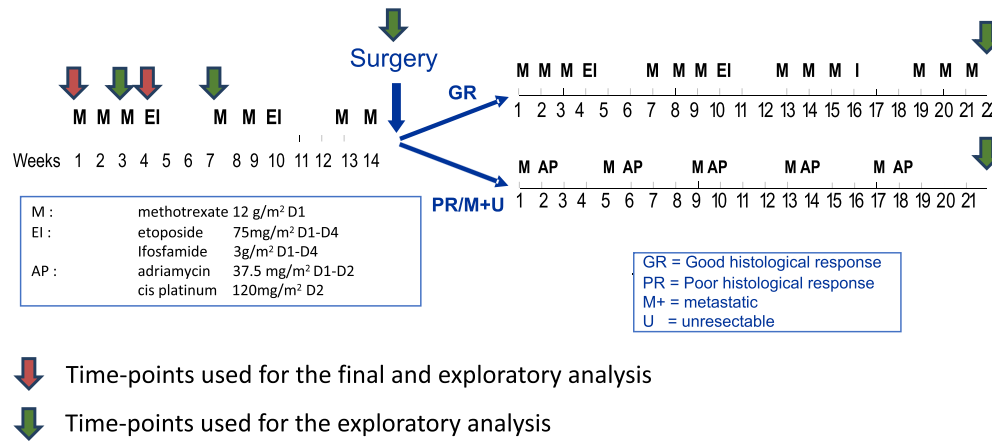


FIGURE 1 Backbone OS2006/M-EI (methotrexate and etoposide/ifosfamide) chemotherapy scheme. Preoperative chemotherapy was based on M-EI regimen administered over a period of 13 weeks, surgery of the primary was planned at Week 14, and postoperative chemotherapy adapted to risk factors (histological response, metastatic status, resectability of the primary tumor). Surgery of lung metastasis was performed after surgery of the primary when residual lung lesions persisted after surgery of the primary. The modalities of chemotherapy administration were as follows and previously described in the OS94 trial. High-dose methotrexate (HD-MTX) (12 g/m^2) was administered in 1.0 L of 5% dextrose in water with 1 mEq/kg of sodium bicarbonate administered as a 4-hour infusion. Oral or intravenous rescue with leucovorin began 20 hours after each methotrexate infusion at a dose of 15 mg up to a total of at least 11 doses given every 6 h, or methotrexate level less than $0.15 \mu\text{M/L}$. Hydration and alkalinization were performed orally or intravenously (IV) to achieve urine output of 1.6 L/m^2 for the first 24 hours and 2.0 L/m^2 for the next 48 hours with urine pH ≥ 7.0 . Serum methotrexate levels and renal function were monitored daily. Etoposide (75 mg/m^2 IV) over 1 hour in 250–500 mL saline serum and ifosfamide ($3 \text{ g/m}^2/\text{day}$ IV) over 3 hours in 250–500 mL saline serum were given daily for 4 days, with Mesna ($3.6 \text{ g/m}^2/\text{day}$) given as a continuous infusion for 4 days, with hydration up to $2.0 \text{ L/m}^2/\text{day}$.

courses), at Week 6 (21 days after the first etoposide-ifosfamide, i.e., before HDM no. 4), at Week 14 (before surgery), and 2–4 weeks after the completion of treatment (Figure 1). After preliminary analyses on these 46 patients, two time points (Day 0 and Day 1 of Week 4) were selected for which hemogram parameters showed promising prognostic value on overall survival (OS) and event-free survival (EFS) (Table S1). Hemogram information was available for 164 patients including those for the initial 46 patients. The prognostic analysis covered the following hemogram parameters: absolute lymphocyte count (ALC) and absolute monocyte count (AMC), NLR, platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR).

2.4 | Statistical analysis

All analyses were conducted in the Gustave Roussy's biostatistics department. EFS was defined as the time between randomization and the time of the first event (relapse, progression, secondary cancer, or death). OS was defined as the time from the randomization to the time of death (whatever the cause of death), or last information on status available for patients lost to follow-up. Survival rates at 3 years (EFS and OS) were estimated using Kaplan–Meier method with Rothman's 95% confidence intervals (CIs). Median follow-up was estimated using the reverse Kaplan–Meier method.

All hemogram parameters were introduced into univariate Cox models estimating hazards ratio (HR) on OS and EFS, those with Wald chi-square p -value under the .1 threshold were introduced into multivariate Cox models adjusted on clinical factors. The two clinical factors

considered were: metastases at inclusion and histological response to chemotherapy at surgery. These clinical factors were known to be prognostic factors of survival in OST. Metastasis at inclusion was considered through two categories: definite metastases and no metastases (including localized OST and possible presence of metastases but not validated). Histological response was considered through two categories: poor histological response (i.e., $\geq 10\%$ viable cells on surgical piece after neoadjuvant chemotherapy) or good histological response ($< 10\%$ viable cells). The analysis considered five hemogram parameters (NLR, PLR, LMR, ALC, and AMC) at two time points (baseline and Week 4).

To evaluate the predictive ability of previous multivariate Cox models to discriminate patients who died and those who did not, the time-dependent area under the curve (AUC; the higher the better discrimination) was computed at four time points (at 1, 2, 3, and 4 years since registration) using the nearest neighbors technique of Heagerty, Lumley, and Pepe (2000).

Robustness analyses on final multivariate models were conducted through a bootstrap method. Random sampling of 1000 samples including 164 patients with replacement was realized. Multivariate Cox models were performed on each of these 1000 samples to calculate a mean HR for hemogram factor with a 95% bootstrap percentile interval. Robustness of the multivariate model was evaluated through the proportion of models selecting the hemogram factor among the 1000 bootstrap iterations at a 5% significant level. For hemogram parameters selected in multivariate analyses, the Youden index (sensitivity + specificity - 1) was used to determine a threshold value for clinical use at different time points. The threshold which maximized the

Youden index was selected and the predictive values were reported. Several thresholds published for hemogram factors^{4,5,8,14} were tested as prognostic factors of OS, and sensitivity and specificity associated were provided.

Estimates are provided with 95% CI or median with interquartile range (IQR). All tests were two-sided with a 5% level of significance. All analyses were performed using SAS 9.4 software (SAS Institute).

2.5 | Exploratory analysis of biological data

Biological samples have been collected prospectively in parallel with the therapeutic protocol approved for the OS2006 trial. A specific informed consent for blood and tumor samples was obtained from patients or their parents or guardians if patients were under 18 years of age upon enrollment. Immunostainings were performed with antibodies directed against CD68, CD163, CMAF, pSTAT1, CD8, and PD1, as described previously.² In brief, immunoreactivity was considered positive if detected in greater than 1% of cells per core of 1 mm, irrespective of staining intensity. Anti-CD68 and -CD163 were used to identify macrophages in tissue sections. Their staining was considered "high" when greater than 50% positive cells per core were present. The macrophage polarization was determined in situ by pSTAT1 and CMAF staining, respectively, for the characterization of M1 and M2 subpopulations. Osteoclastic cells (also known as giant cells) were evaluated independently as giant multinucleated cells by CD68 staining. The presence of CD8 (lymphocyte) checkpoint markers was analyzed with PD1 and PDL-1 antibodies. Tonsils and lymphoid nodes were used as positive controls for the CD8, PD1, and PDL-1 antibodies, giant cell tumors for the CD68 and CD163 antibodies, and lymphoma samples were used for pSTAT1 and CMAF antibodies. RNA seq analyses were performed as previously described. Cytokines were assessed as previously described.¹⁵

3 | RESULTS

3.1 | Patients' initial characteristics and outcome

Between January 1 2004 and January 1 2016, 164 patients were included in the analysis. Their baseline characteristics are presented in Table 1. Median age at diagnosis was 14.7 years (range: 4.7–24.5). Most patients had appendicular tumor locations (84%) and most had localized OST (83%), defined as the absence of definite metastasis. At the time of analysis, the median follow-up was 5.6 years (IQR: 3.3–7.7 years).

Of the 164 patients included, 64 (39%) patients had one of the following events: a metastatic progression for 47 patients (including 44 of them had lung metastases), local and metastatic progression for eight patients, local progression only for three patients, secondary malignancy for four patients, and death without prior event for two patients. A total of 127 (77%) patients were alive, of whom 100 patients were alive without any event, four patients had secondary malignancy, and

TABLE 1 Main clinical characteristics of 164 patients with osteosarcoma treated with M-EI chemotherapy in French centers, at baseline and during treatment and follow-up (whole cohort).

	Overall population (N = 164)
Gender (female), N (%)	74 (45%)
Age at study entry: median [IQR] (range)	14.7 years [11.6–17 years] (4.7–24.5)
Primary tumor site (N = 163)	
Limb, N (%)	137 (84%)
Axial (trunk, head and neck), N (%)	26 (16%)
Primary tumor size (N = 144)	
<10 cm, N (%)	130 (90%)
≥10 cm, N (%)	14 (10%)
Initial staging^a (N = 163)	
Localized, N (%)	135 (83%)
Definite metastasis, N (%)	28 (17%)
Histological subtype (N = 163)	
Common form, N (%)	145 (89%)
Other, N (%)	17 (11%)
Histological response (N = 156)	
Good response (<10% cells), N (%)	106 (68%)
Poor response (>10% cells), N (%)	50 (32%)
Median follow-up [IQR]	5.6 years [3.3–7.7 years]
EFS	
Events (relapse, progression, secondary cancer, or death), N (%)	64 (39%)
Death without prior event, N	2
Secondary malignancy, N	4
Local progression, N	3
Local and metastatic progression, N	8
Metastatic progression, N	47
3-year EFS [95% CI]	71.5% [64%–78%]
OS	
Deaths from any cause, N (%)	37 (22.6%)
3-year OS [95% CI]	86.4% [80%–91%]

Note: Data are n (%) or median (range).

Abbreviations: CI, confidence interval; EFS, event-free survival; IQR, interquartile range; M-EI, methotrexate and etoposide/ifosfamide; OS, overall survival.

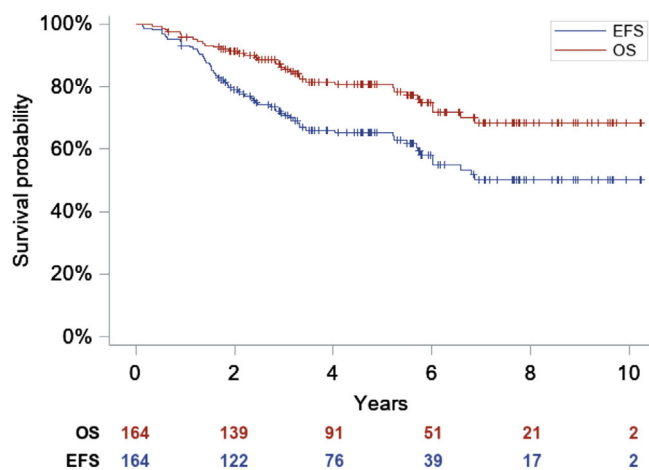
^aLung metastases were considered definite if there were at least a lesion 10 mm or greater, two or more lesions 5 mm or greater, or five or more lesions, whatever the size.

23 patients had previous progression (20 metastatic progression, two local and metastatic progression, and one local progression). The 3-year EFS and OS were, respectively, 71.5% (95% CI: 64%–78%) and 86.4% (95% CI: 80%–91%) (Figure 2). As expected, in univariate analysis, a prognostic impact was found for metastatic status and histological response on EFS and OS (Table 2).

TABLE 2 Prognostic univariate analyses of clinical factors and hemogram parameters at baseline at Day 0, and Day 1 at Week 4 on survival outcomes EFS and OS (whole cohort).

Description	EFS					OS				
	N	HR	95% Wald confidence limits		p-Value	N	HR	95% Wald confidence limits		p-Value
Poor response vs. good response	156	1.93	1.16	3.21	.011	156	2.48	1.28	4.81	.0074
Metastases vs. no metastases	163	3.21	1.88	5.46	<.0001	163	3.29	1.67	6.46	.0006
NLR-d0	162	0.93	0.76	1.14	.46	162	0.99	0.79	1.27	.99
LMR-d0 (unit = 10)	161	0.64	0.27	1.53	.32	161	0.50	0.13	1.99	.33
PLR-d0 (unit = 100)	162	0.92	0.62	1.35	.66	162	0.81	0.46	1.40	.45
Lymphocytes-d0 (unit = 1000)	162	1.17	0.83	1.64	.37	162	0.99	0.62	1.60	.98
Monocytes-d0 (unit = 1000)	162	1.02	0.70	1.47	.93	162	0.97	0.55	1.70	.91
NLR-W4 (unit = 1)	149	1.12	0.97	1.30	.13	149	1.27	1.08	1.49	.0044
LMR-W4 (unit = 10)	148	1.09	0.95	1.27	.20	148	1.16	0.99	1.37	.07
PLR-W4 (unit = 100)	149	1.22	0.97	1.54	.09	149	1.15	0.84	1.57	.37
Lymphocytes-W4 (unit = 1000)	149	0.96	0.69	1.34	.8	149	0.94	0.60	1.49	.79
Monocytes-W4 (unit = 1000)	149	0.46	0.17	1.21	.12	149	0.79	0.23	2.76	.71

Abbreviations: EFS, event-free survival; HR, hazards ratio; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PLR, platelet-to-lymphocyte ratio; W4, Week 4.

**FIGURE 2** Overall survival (OS) and event-free survival (EFS) of the 164 patients.

3.2 | Prognostic value of hemogram parameters at baseline and Week 4

Despite the fact we observed prolonged and durable lymphopenia during treatment from the first chemotherapy with EI course at Week 3 up to the end of therapy (median ALC <750, range: 50–1200) (Figure S1), no effect of ALC as a prognostic factor on OS or EFS was observed at the two time points (Table 2).

Median NLR, PLR, and LMR at 4 weeks of treatment initiation were 1.6 (IQR: 1.1–2.8) (NLR, Week 4 [W4]), 150 (IQR: 113–214) (PLR-W4), and 4.4 (IQR: 3.1–7.1) (LMR-W4), respectively (Table S2). None of these hemogram parameters had a significant impact on EFS in univari-

ate analysis. By contrast, an increase of NLR-W4 was associated with lower OS ($p = .0044$), and was selected for multivariate analysis, along with LMR-W4, at a .10 level (Table 2). In multivariate analysis, after adjustment on other prognostic factors (i.e., presence or absence of metastasis and good vs. poor histological response), HR of death associated with NLR-W4 was 1.30 (95% CI: 1.11–1.54). Thus, the increase of one point of NLR is associated with a higher risk of death of 30%. NLR-W4 were distributed in a wide range across patients (Figure S2). The factor NLR-W4 is significantly associated with the risk of death in patients who have already relapsed (p -value .0384, HR = 1.219, 95% CI: 1.011–1.471).

The robustness analysis found that a higher NLR-W4 was significantly associated with a higher risk of death in more than 75% bootstrap replications. The bootstrap mean HR was 1.30 with 95% CI: 1.05–1.52. At 1 year, the optimal cutoff according to the Youden index for the NLR-W4 was 1.51 leading to a sensitivity of 100% and a specificity of 88.7%. For this model, the time-dependent AUC was highest at 1 year (AUC = 0.93). At 3 years, the cutoff for the NLR-W4 was 3.14 leading to a specificity of 60.5% and sensitivity of 89.7% (Figure S3). OS according to NLR-W4 dichotomized (<1.51 vs. ≥ 1.51) is shown in Figure S4, and the distribution of NLR based on status in Figure S5. After adjustment on clinical factors, LMR-W4 was not found to be a prognostic factor of OS at a 5% level (Table 3).

No other prognostic value was found in our series of hemogram factors using thresholds reported in five other main publications with different ratios or lymphocytes levels (LMR = 3.43 Liu et al.,¹⁶ NLR = 4 Templeton et al.,⁸ NLR = 2.5 and PLR = 133.9 Que et al.,¹⁷ NLR = 2 Vasquez et al.,⁴ and lymphocytes = 800 Moore et al.⁵) (Table S3), but OS2006 chemotherapy backbone was different.

TABLE 3 Multivariate analyses of NLR at Week 4 and LMR at Week 4 as prognostic factors of OS after adjustment on known prognostic factors (histological response and metastatic status).

Description	OS			
	N	HR	95% CI	p-Value
NLR at Week 4 (unit = 1)	141	1.30	1.10 1.53	.0016
LMR at Week 4 (unit = 10)	140	1.17	0.99 1.38	.07

Abbreviations: CI, confidence interval; HR, hazards ratio; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival.

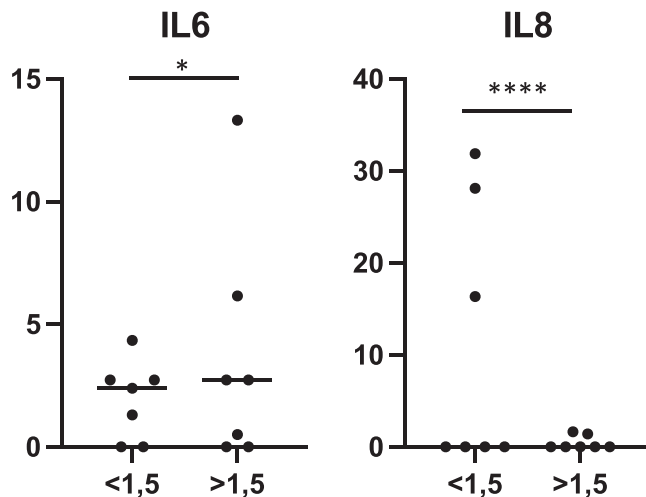


FIGURE 3 Cytokine levels in patients with neutrophil-to-lymphocyte ratio at Week 4 (NLR-W4) higher or lower than 1.5.

3.3 | Correlation between NLR at baseline or Week 4 and biological findings

We first compared NLR at baseline and at Week 4 with immunohistological findings for the 30 patients (18%) with available PD1/PDL1/CD8/CD163/CD68/CSF1R immunohistochemistry (IHC) data on surgical piece (after neoadjuvant M-EI chemotherapy). However, no correlation between IHC and NLR were observed. Second, high-quality RNA seq data were available for expression analysis of selected immune genes in 11 and 13 patients with NLR at baseline and at Week 4, respectively (Figure S6). Although the number of patients was limited, we observed an inverse correlation between NLR baseline and CCL17 RNA expression, but no other specific correlation was observed (Table S4). Finally, we analyzed the correlation with a panel of cytokines that has been shown to impact prognosis.¹⁵ We were able to evaluate the correlation between NLR and cytokines panel for 14 patients for which data were available. Interestingly, IL-6 were significantly higher in patients with a higher NLR at Week 4, while IL-8 levels were significantly higher in those with a lower NLR at Week 4 (Figure 3), further suggesting that NLR can be an easily accessible marker of inflammation, although most other markers were not significant (Figure S7).

4 | DISCUSSION

Our results indicate that NLR-W4 may be useful as an early prognostic factor of survival in pediatric and adolescent patients suffering from OST treated with chemotherapy regimen based on the alternation of M-IE. Indeed, an increase of NLR ratio at Day 1 of the first M-EI chemotherapy (NLR-W4) was associated with reduced OS in univariate ($p = .0044$) and multivariate analysis (HR = 1.3, 95% CI: 1.1–1.5; $p = .002$). After adjustment on histological response and metastatic status, a 1-point increase of the ratio NLR-W4 was associated with an increased risk of death of 30%. This exploratory result should be confirmed in an external cohort to validate this finding. Indeed, in univariate and multivariate analysis, an increase of NLR-W4 was associated with a higher risk of death, although not associated with EFS.

The NLR has been widely studied in adult malignancies and shown to be an important prognostic and predictive factor in a broad range of cancers at diagnosis and during therapy.^{7–9} Nevertheless, it had not been reported as a relevant prognostic factor in pediatric OST. Our results showed that the best cutoff was 1.51 for the NLR at 1 year, as described in several other studies.⁸ Interestingly, ALC is shown to be a significant prognostic factor in publications on pediatric patients with OST undergoing a chemotherapy regimen based on doxorubicin and cisplatin,^{5,6} but was not found significantly associated with survival outcomes in our patients undergoing M-EI-based chemotherapy regimen. In our cohort, patients first undergo 3 weeks of HDM on a weekly basis (which does not usually lead to a decrease in lymphocyte count) before undergoing a more myelotoxic chemotherapy.¹⁸ As expected, lymphopenia only appeared after the first EI chemotherapy, but was persistent until the end of the treatment. We did not find a clear correlation between the NLR at Week 4 and histological or RNA-seq findings, but the small number of patients for which data were available makes it difficult to conclude. Studying only the NLR might seem too simplistic, given today's knowledge about the impact of the different actors of the immune system in the pathogenesis of tumors, but it could be the reflection of a deleterious inflammation. Nevertheless, if confirmed, it could be an easy, fast, and non-costly way of having an early assessment of the prognosis for a given patient.

Several limitations can be identified in our study. First, the parameters of the hemogram were collected in a retrospective matter, although done without selection and exhaustively of all consecutive patients, and although there should not be any change in the values, we observed missing data (especially at later time points). Second, our study only took place in France, but this might be balanced by the multicentric nature of the study, given that three independent centers recruited the 164 patients. Third, although 164 patients are a relatively important number of patients given the frequency of pediatric OST, patients included in the initial analysis were also analyzed in the final one. Fourth, while we observed an association of NLR-W4 with OS both on univariate and multivariate analysis, NLR-W4 was not significantly associated with EFS ($p = .13$). Although there is no clear explanation, we can hypothesize a lower effect of NLR-W4 on

EFS requiring a larger sample size to be detected. Another possibility is also that progression/relapse is more aggressive in patients with high NLR-W4, as NLR-W4 is significantly associated with an increased risk of death in patients who have already relapsed ($p = .0384$). Finally, it would have been interesting to study the ALC 14 days after the first chemotherapy with IE, but unfortunately, we did not have hemograms for this time point (and 3 weeks after the first IE, we found no statistical difference). Studies on other chemotherapy regimens would be informative.

Further studies should be conducted to address the questions of the correlation between the NLR and parameters of the tumor microenvironment, such as tumor infiltrating lymphocytes and myeloid cells. Although NLR has been validated in several cohorts of patients, including those receiving immunotherapy, reasons why NLR is associated with poor survival are scarcely known. NLR could be the reflection of inflammation, myeloid dysfunction, and T-cell immune suppression. Neutrophils of the tumor microenvironment secrete growth factors such as interleukine-6 (IL-6), IL-8, and vascular endothelial growth factor. Interestingly, our study has shown that patients with a higher NLR have a higher level of IL-6, which suggests that NLR could be used as a surrogate marker of inflammation. Moreover, studies have shown that an elevated NLR was associated with an increase in the peritumoral infiltration of macrophages and an increase in IL-17.¹⁹ OSTs have been shown to have a higher percentage rate of CD8⁺ infiltrating lymphocytes than other sarcoma subtypes,²⁰ and it has recently been shown that the degree of infiltration of CD8⁺-positive cytotoxic lymphocytes and CD163-positive tumor-associated macrophages correlates positively with survival.² Macrophages are involved in bone homeostasis and immunity and have central functions in osteoimmunology. The NLR-W4 is a biomarker that is easy to collect in routine practice, and this study showed that it might also be an early prognostic factor for patients with OST. If confirmed, these results will mark a significant advancement that can spread rapidly in the global medical community for the management of this daunting disease. Future studies are needed to confirm this finding along with additional immune monitoring to better understand the immune surveillance of the disease. Indeed, we hope that by using the NLR-W4 prospectively and validating its prognostic value in OST, it could be easily implemented in the clinic to stratify patients early during therapy, and intensify therapeutics in patients with high NLR, either with chemotherapy or more interestingly with new immune therapies targeting inflammation and neutrophils, or boosting lymphoid immunity.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare in regards to the contents of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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