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Retrospective Study

Neutrophile-to-lymphocyte, lymphocyte-to-monocyte and platelet-to-lymphocyte ratios as prognostic and response biomarkers for resectable locally advanced gastric cancer

Tomás TC *et al.* Blood ratios as biomarkers in LAGC

Abstract

BACKGROUND

Perioperative FLOT improved prognosis in locally advanced gastric cancer (LAGC). Neutrophil-to-lymphocyte (NLR), lymphocyte-to-monocyte (LMR) and platelet-to-lymphocyte (PLR) ratios are biomarkers known as independent prognostic factors, but not as predictive factors.

AIM

To assess blood ratios' (NLR, LMR and PLR) potential predicting response to FLOT and survival outcomes in resectable LAGC patients.

METHODS

Multicentric retrospective study investigating NLR, LMR and PLR clinical potential in resectable LAGC patients, treated with at least one preoperative FLOT cycle, from 12 Portuguese hospitals. Means were compared through non-parametric Mann-Whitney tests. Receiver operating characteristics curves' analysis defined cut-off values: High PLR > 141 for progression and > 144 for mortality; high LMR > 3.56 for T stage regression (TSR). Poisson/Cox regressions calculated relative risks/hazard ratios, with NLR, pathologic complete response, TSR and tumor regression grade (TRG) as independent variables, and overall survival (OS) the dependent variable.

RESULTS

In total, 295 patients included (mean age of 63.7 years, 59.7% males). NLR was correlated with time of survival ($r = 0.143$, $P = 0.014$). PLR was associated with systemic progression during FLOT ($P = 0.022$) and mortality ($P = 0.013$), with high PLR patients having 2.2 times higher risk of progression [95% confidence interval (CI): 0.89-5.26] and 1.5 times higher risk of mortality (95%CI: 0.92-2.55). LMR was associated with TSR and high LMR patients have 1.4 times higher risk of achieving TSR (95%CI: 1.01-1.99). OS benefit was found with TSR ($P = 0.015$) and partial/complete TRG ($P < 0.001$). Patients

without TSR as well as no evidence of pathological response have 2.1 (95%CI: 1.14-3.96) and 2.8 (95%CI: 1.6-5) times higher risk of death.

CONCLUSION

Higher NLR correlated with longer survival time. High LMR patients had higher risk of decreasing T stage, while high PLR patients have higher odds of progressing under FLOT and dying. Patients with TSR have better OS and lower risk of dying, as well as those with pathological response.

Key Words: Gastric cancer; Perioperative FLOT; Neutrophil-to-lymphocyte; Lymphocyte-to-monocyte; Platelet-to-lymphocyte; Tumor regression grade

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Core Tip: FLOT perioperative regimen became the standard of care in resectable locally advanced gastric cancer, but there is still a need for prognostic and predictive response biomarkers. Blood ratios as neutrophil-to-lymphocyte (NLR), lymphocyte-to-monocyte (LMR) and platelet-to-lymphocyte (PLR) ratios have already been considered useful in solid tumors, and we performed an exploratory analysis regarding pathological response prediction and survival outcomes: NLR is weakly correlated to overall survival; high LMR patients have higher risk of T stage regression; high PLR patients have higher odds of progressing under FLOT and higher risk of mortality; and patients without T stage or pathological regression have higher risk of dying.

INTRODUCTION

Locally advanced gastric cancer (LAGC) are ¹ common gastrointestinal malignancies, responsible for the third leading cause of cancer-related mortality worldwide^[1]. Patients with stage IIC and IV gastric cancer have reported 5-year survival rate of 20.2% and 8.8%, respectively, with most patients diagnosed at an advanced stage, given the absence of signs and symptoms in early stage disease^[2]. Recent developments in chemotherapy (CT) improved prognosis, even in patients with advanced gastric cancer. There has been a focus on neoadjuvant CT and, despite lacking prognostic and treatment response biomarkers, FLOT perioperative CT showed improved prognosis in patients with LAGC^[3-6]. Available evidence suggests that a favorable histopathological response to treatment may be a useful positive predictive marker in LAGC. Nonetheless, tumor response and prognosis still are difficult to predict before treatment initiation, making potential biomarkers required and crucial to predict patient prognosis and individualize treatment and follow-up. Many serum biomarkers for early and advanced gastric cancer have already been reported: Carcinoembryonic antigen is a frequently used biomarker, as well as carbohydrate antigen (CA) 19-9, CA 72-4, CA 125 and alpha-fetoprotein, that ¹ have additionally been reported to be helpful for prognosis and monitoring gastric cancer recurrence^[7-9]; however, these biomarkers are not recognized to predict responses and survival outcomes because of their limited specificity and sensitivity in LAGC. Although plenty of other serum biomarkers are under investigation (small noncoding microRNAs, fibroblast growth factor 2 amplification, e-cadherin CDH1 mutation, PIK3CA and PTEN mutations, MET amplification/overexpression, TP53 mutation, microsatellite instability, among others), their cost-effectiveness ratio is also high, being expensive to routinely analyse in the clinical practice^[10]. Lately, there has been an effort to validate blood ratios ³ [neutrophil-to-lymphocyte (NLR), lymphocyte-to-monocyte (LMR) and platelet-to-lymphocyte (PLR)] as possible biomarkers, as they are routinely analysed, cheap and with known pathophysiological mechanism associated to cancer development: Inflammation impacts carcinogenesis, including tumor initiation, promotion and metastization for solid and haematological malignancies; blood immune and inflammatory cells

(neutrophils, lymphocytes, platelets and monocytes), as well as cytokines, play an important role influencing tumor micro-environment, invasion and metastization and are potential biomarkers worthy of being explored in the context of LAGC^[11-14].

Lymphocytes seem to reflect the systemic inflammatory status and some parameters using these cells have been reported to predict survival outcomes in cancer patients^[15-19]. With lymphocytes playing an important role in cancer immune monitoring and prevention, it appears a pro-inflammatory micro-environment leads to impaired T-cell response, mediated by cytokines, compromising cell-mediated immunity^[20]. Depending on the inflammatory cytokines mediating the environment, neutrophils may exert either antitumoral or protumoral activities^[21]. Under acute inflammatory stimulus, activated neutrophils can undertake antitumoral activity, while under chronic inflammation these neutrophils can induce a tumor growth and metastization process. Cytokines such as interleukin-6, which has already been reported to be elevated in gastric cancer patients, can induce protumoral neutrophil activity^[22]. This behavior might explain why there is a correlation between neutrophil count and prognosis. The effect of inflammatory response mediated by cytokines also influences tumor-associated monocytes, another main regulator of cancer inflammation. Tumor cells induce monocyte differentiation into tumor-associated macrophages, weakening antitumor immune response and stimulating migration and metastatic spread^[23-26]. Peripheral monocyte serum levels were negatively associated with prognosis, already known in different cancer types^[27-29].

NLR, LMR and PLR are inflammation biomarkers already reported as independent prognostic factors for survival in several solid tumors, being their assessment inexpensive^[30,31]. It has been previously reported that preoperative assessment of NLR has clinical potential to predict tumor progression and prognosis in patients with resectable gastric cancer and oesophageal squamous cell carcinoma^[32,33]. In recent studies, high PLR has been associated with tumor aggressiveness in patients with several malignancies, as well as poor overall survival (OS) and disease-free survival (DFS)^[34-38]. Combined NLR and PLR have also been studied to predict CT response and

prognosis in patients with advanced gastric cancer^[39]. Lower LMR has been independently associated with worse OS of several malignancies in advanced setting, and more aggressive tumor behavior^[40-42]. However, NLR, LMR and PLR as predictors of response to CT have not been assessed in patients with resectable LAGC cancer.

¹ Tumor regression grade (TRG) is a system used to evaluate residual tumor in patients submitted to preoperative therapies. TRG focuses on the quantity rather than location of the tumor, providing information on the response to therapies and predicting prognosis^[43]. There are many different grading systems without global consensus^[44-47]. Among various scores, the College of American Pathology (CAP) TRG score system, based on the relative amount between fibrosis and residual tumor, is widely applied in gastrointestinal cancers^[48]. Some studies suggest that patients with lower TRG score (1-2 or 1-3) have better survival than those with higher TRG score (3-5 or 4-5)^[44,49,50]. ¹ Based on the MAGIC trial, Smyth *et al*^[51] found that patients in TRG 3-5 and node positive group had worse OS than others, while patients with TRG 1-2 and node negative had better OS. This study is an exploratory analysis to assess NLR, LMR and PLR potential as biomarkers predicting response and survival outcomes in resectable LAGC patients, determined before preoperative CT. To our knowledge, this is the first study analysing the clinical potential of these ratios in a Portuguese population in a preoperative setting.

MATERIALS AND METHODS

Study design

This is a multicentric retrospective study including 295 patients from 12 Portuguese oncological centres, diagnosed with resectable LAGC [histologically-confirmed advanced clinical stage cT2 or higher, or nodal positive stage (cN+), or both] and submitted to curative intent surgery after at least one preoperative FLOT (preFLOT) cycle, since its initial use in the institutions until 31st December 2020. Data collection ended in April 2021, with a minimum follow-up time of 1 mo after surgery.

Participant oncological centres by region: South & Centre Region - Hospital Professor Doutor Fernando Fonseca, Centro Hospitalar Barreiro-Montijo, Centro Hospitalar

Lisboa Ocidental, Centro Hospitalar Lisboa Central, Hospital da Luz de Lisboa, Hospital Beatriz Ângelo, Hospital Garcia de Orta, Hospital Distrital de Santarém, Hospital do Espírito Santo de Évora, Centro Hospitalar Universitário do Algarve; North Region - Centro Hospitalar de Trás-Os-Montes e Alto Douro, Hospital da Senhora da Oliveira de Guimarães. The exclusion criteria were as follows: (1) Age inferior to 18 years; (2) Synchronous or metachronous cancer in other organs; and (3) Absent detailed therapeutic information and unknown laboratory values for NLR, LMR or PLR.

Variables

The data base included demographic, histological, clinical, surgical and pathological variables. Regarding age, patients were divided into elder (≥ 75 years) and non-elder (< 75 years). This division was made due to the growing ageing society in western countries, where it is considered that increased frailty risk occurs above 75 years^[52]. Neutrophils, lymphocytes, monocytes and platelets were considered as absolute cell counts, based on the last blood sample drawn previously to initiation of preFLOT. NLR was determined by dividing neutrophil absolute count by lymphocyte absolute count, LMR was determined by dividing lymphocyte absolute count by monocyte absolute count and PLR was determined by dividing platelet absolute count by lymphocyte absolute count^[15-20]. Histological diagnosis and grade were considered, as well as tumor site and TNM stage, which was evaluated according to the 8th edition of the American Joint Committee on Cancer staging manual. Patients were staged before the initiation of the preoperative therapy. All patients were clinically staged with thorax, abdomen and pelvic computerized tomography scans; upper endoscopic ultrasound was not requested for additional staging, but if the information was available, it would be considered for local staging; patients were submitted to exploratory laparoscopy for staging purposes, being considered for local staging and for exclusion of macroscopic peritoneal metastization. Surgical variables contemplated type of resection, type of lymphadenectomy and margins. Regarding systemic treatment, number of cycles, toxicity and time until surgery were considered. Pathological tumor response was

assessed considering TRG: As previously stated, there are several grade scoring systems used, the CAP score being the most used in the participant institutions; in need of standardization for analysis, we simplified the different scores in 3 categories - "Complete Tumor Regression" (pathological complete response), "Partial/Incomplete Tumor Regression" (pathological partial/incomplete response) and "No Evidence of Regression" (absence of pathological response). Complete tumor regression was considered also as pathological complete response (pCR). Clinical responses were compared to pathological response obtained with surgery, and tumor downstaging was considered comparing clinical and pathological staging, as at least one of the following: T stage reduction and/or N stage reduction (downstaging). Patients with maintenance or increase in either T or N were considered as not having downstaged. Patients' period of survival was considered as time since histological diagnosis until death or last time seen alive, in months.

Statistical analysis

Data was described using relative and absolute frequencies, central tendency measures and dispersion. Means regarding all ratios were compared using *t*-tests, and in case of non-normality through non-parametric Mann-Whitney tests, specifically for gender, age group (< 75 years *vs* \geq 75 years), preFLOT suspension, progression under preFLOT, tumor downstaging, T and N regression, TRG, OS and mortality. Analysis of receiver operating characteristics (ROC) curve defined a cut-off value for group stratification, to facilitate patient stratification in the clinical practice. The areas under the curves were calculated to evaluate the predictive abilities of PLR to discriminate patients' progressive systemic disease and mortality and of LMR to discriminate patients with T downstaging. High PLR was defined as > 141 for progression and > 144 for mortality; high LMR was defined as > 3.56 for T stage regression (TSR). Relative risk was calculated using poisson regression, with the progression under preFLOT, mortality and TSR the dependent variables and PLR and LMR groups the independent variables. Pearson's correlation coefficient was used to assess the association between NLR and

OS. Kaplan-Meier estimator was used to compare survival curves considering several variables (pCR, TSR and TRG) and log-rank tests were used to assess statistical significance. Cox regression was used to calculate hazard ratios, with NLR, pCR, TSR and TRG being the independent variables, and OS the dependent variable. Significance level was established at 0.05. All statistical analyses were performed using SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). This retrospective observational study was approved in May 19th 2021 by the Ethics Committee of Hospital Professor Doutor Fernando Fonseca (approval number: 045/2021).

RESULTS

Patients' demographics are described in detail in Table 1. Two-hundred and ninety-five patients were included, with a mean age of 63.7 years (range: 31-84 years), and 59.7% males ($n = 176$). Mostly adenocarcinomas (98.3%), while only three patients had non-specified carcinoma and one patient with squamous cell carcinoma. Poorly differentiated histology was identified in 40.9% ($n = 121$), while 25.3% ($n = 75$) were moderately differentiated and 8.8% ($n = 26$) well differentiated. Regarding Lauren classification, 51.4% ($n = 152$) were intestinal or mixed subtypes and 20.6% ($n = 61$) were diffuse subtype. Most were located in the stomach ($n = 272$; body: 47%; antrum: 33.8%; cardia: 11.1%), 6.8% ($n = 20$) in the gastroesophageal junction and only 1% ($n = 3$) in the lower oesophagus. Peritoneal lavage cytology was performed, previously to therapy initiation, in 253 patients (85.8%), with 42 missing data; of these, 6 patients had a suspicious cytology for neoplastic cells and 3 patients had a positive cytology, but all were considered for surgery in the absence of macroscopic peritoneal metastasis.

Among 295 patients, the descriptive analysis revealed: Absolute neutrophile counts ranged from 1170 to 21290, with a mean \pm SD of 6103 ± 3354 ; absolute lymphocyte count ranged from 200 to 9110, with a mean \pm SD of 1890 ± 1014 ; absolute monocytes count ranged from 600 to 4440, with a mean \pm SD of 585 ± 407 ; absolute platelets count ranged from 25600 to 658000, with a mean \pm SD of 268658 ± 92373 . NLR ranged from 0.48 to

99.5 and mean \pm SD was 4.66 ± 7.1 ; LMR ranged from 0.33 to 143.33 and mean \pm SD was 5.29 ± 9.2 ; PLR ranged from 15.79 to 1260 and mean \pm SD was 182.91 ± 148.19 .

Median number of preFLOT cycles was 4 (range: 1-12). Suspension of treatment due to toxicity occurred in 22 patients (7.5%). Twenty-four patients (8.1%) progressed systemically under preFLOT. Median waiting time to surgery was 12 wk (range: 6-33). Regarding patients with curative intent ($n = 271$, 8 missing data): Total gastrectomy was performed in 49% of patients ($n = 129$), while 46% ($n = 121$) had subtotal gastrectomy, 4.1% ($n = 11$) had Ivory-Lewis esophagectomy and 0.8% ($n = 2$) had distal gastrectomy; complete standard lymphadenectomies were performed in 96% (D2: 85.4%, $n = 193$; D1+: 10.6%, $n = 24$), with 45 missing data; R0 resection was achieved in 91.9% patients ($n = 249$), with 9 missing data. TSR occurred in 46.5% ($n = 126$; 17 missing data), and N stage regression occurred in 57.6% ($n = 156$; 9 missing data). Tumor downstaging (TD) defined as TSR and/or N stage regression in comparison of imagiological/clinical *vs* pathological staging, was observed in 61% patients ($n = 161$), while 39% didn't respond ($n = 53$) or progressed ($n = 50$), with 7 missing data.

Of the 239 surgical specimens evaluated for TRG, using our standardized classification, 46.4% ($n = 111$) showed partial/incomplete response, 33.5% ($n = 80$) no response, and 20.1% ($n = 48$) pCR. Mean OS of general population was 35.1 ± 1.2 mo, with a mean follow-up time of 19.4 ± 0.7 mo. At the end of study time, 233 patients (79%) were alive.

NLR, LMR and PLR analysis

A statistically significant association between NLR and age group (< 75 *vs* ≥ 75 years) and gender was not found. However, the difference in means was considerable for both variables (NLR mean in male patients = 4.18 *vs* female patients = 5.37, $P = 0.073$; mean in patients ≥ 75 years = 4.21 *vs* patients < 75 years = 4.72, $P = 0.37$) in Table 2. No evidence of an association was found between NLR and early suspension of treatment due to toxicity. However, difference in means was clinically significant (NLR mean in patients with suspension = 5.32 *vs* without suspension = 4.61, $P = 0.336$). Although TD

was not associated with any ratio, difference in means regarding NLR was considerable (mean in patients with TD = 4.93 *vs* without TD = 3.57; $P = 0.081$). NLR was not associated with T or N stage regression, although difference in means was considerable regarding N stage regression (NLR mean in patients with N regression = 5.02 *vs* without N regression = 4.06, $P = 0.119$).

LMR was not associated with gender and age group, but showed a considerable difference between age groups (LMR mean in patients ≥ 75 years = 4.68 *vs* patients < 75 years = 5.37, $P = 0.335$). An association between LMR and TSR was found ($P = 0.021$). Among 264 patients, LMR ranged from 0.33 to 143.33, with mean \pm SD LMR value in patients with T regression ($n = 126$) of 4.38 ± 3.64 and patients without TSR ($n = 138$) of 6.5 ± 12.84 . According to ROC analysis, optimal cut-off of LMR was established at 3.56, with a sensitivity/specificity of 0.594 and 0.421. ¹ Patients were divided into the following groups according to the cut-off values for LMR: High (> 3.56 ; $n = 134$) and low LMR (≤ 3.56 ; $n = 130$). High LMR patients have 1.4 times higher relative risk of achieving TSR than low LMR patients [95% confidence interval (CI): 1.01-1.99, $P = 0.043$] in Table 3.

PLR did not reveal a statistically significant association between genders and age groups, but showed a considerable difference between age groups (PLR mean in patients ≥ 75 years = 214.98 *vs* patients < 75 years = 178.88, $P = 0.323$). Considering early suspension of treatment due to toxicity, PLR did not show to be associated, although difference in means was considerable (PLR mean in patients with suspension = 208.26 *vs* without suspension = 180.87, $P = 0.144$). We also found PLR to be associated with systemic progression during preFLOT ($P = 0.022$). PLR ranged from 15.79 to 1260, with mean \pm SD PLR value in patients with systemic progression during preFLOT ($n = 24$) of 251.94 ± 173.3 and in patients without progression ($n = 271$) of 176.8 ± 144.54 . According to ROC analysis, optimal cut-off of PLR was established at 141 for progression, with a sensitivity/specificity for the PLR of 0.667/0.5. ¹ Patients were divided into the following groups according to the cut-off values for PLR: High (> 141 ; $n = 152$) and low PLR (\leq

141; $n = 143$). High PLR patients have 2.2 times higher relative risk of progressing under preFLOT (95%CI: 0.89-5.26, $P = 0.088$).

PLR was not associated with T and/or N stage regression, although difference in means was considerable regarding N stage regression (PLR mean in patients with N regression = 184.45 *vs* without N regression = 166.13, $P = 0.157$). Even though this ratio was not associated with TRG evaluation, difference in means regarding PLR was considerable (PLR mean in patients with partial/incomplete or complete tumour regression to treatment = 180.4 *vs* no evidence of regression to treatment = 189.73, $P = 0.305$). This ratio was also associated with mortality ($P = 0.013$), regardless of surgery. The mean \pm SD PLR value in patients dead at the end of the study time analysis ($n = 62$) was 210.27 ± 142.87 and in patients alive at the end of the study time ($n = 233$) was 175.64 ± 149.48 . According to ROC analysis, optimal cut-off of PLR was established at 144 for survival status, with a sensitivity/specificity of 0.613/0.481. Patients were divided into the following groups according to the cut-off values for PLR: High (> 144 ; $n = 152$) and low PLR (≤ 144 ; $n = 143$). High PLR patients have 1.5 times higher relative risk of dying than low PLR patients, although not statistically significant but the 95%CI lower limit is near 1 (95%CI: 0.92-2.55, $P = 0.103$).

Survival outcomes

NLR was found to be associated with OS time, with a weak positive correlation ($r = 0.143$, $P = 0.014$): For every one unit increase in NLR, there is 0.1 mo increase in mean OS. Nevertheless, through Cox regression, there was no evidence of survival benefit between OS and NLR in Table 3. Neither LMR or PLR were associated with OS. Regarding pCR, the three ratios did not show a statistically significant association nor reveal considerable difference in means. We also did not find an association between pCR and OS; however, difference of means was considerable (mean OS without pCR of 14.9 mo *vs* 18.4 mo with pCR; $P = 0.428$). Although not statistically significant, patients without pCR seem to have higher risk (2.1 times) of dying than patients with pCR (95%CI: 0.8-5.2, $P = 0.129$) in Table 3. With TRG categories as partial/incomplete or

complete tumor regression *vs* no evidence of regression to treatment, there is an association with OS ($P = 0.009$), with a survival of 39 mo *vs* 28.5 mo, respectively (95%CI: 33.6-38.4, $P < 0.001$) in Figure 1. The univariate Cox regression revealed that patients without evidence of tumor regression have 2.8 times higher risk of dying than patients with partial or complete tumor regression (95%CI: 1.6-5, $P < 0.001$). OS was found to be associated with TSR ($P = 0.03$). Patients with T regression had better OS than those without T regression (39.9 *vs* 32.7 mo, respectively, $P = 0.015$) in Figure 2. The univariate Cox regression revealed that patients without TSR have 2.1 times higher risk of dying than patients with TSR (95%CI: 1.14-3.96, $P = 0.017$).

DISCUSSION

In summary, our statistically significant results: (1) NLR was weakly (positive) correlated with OS time, but there is no evidence of survival benefit; (2) LMR was associated with TSR; (3) High LMR patients had 1.42 times relative risk of TSR; (4) PLR was associated with systemic progression under preFLOT, as well as mortality; (5) Patients without TSR had 2.1 times risk of dying; and (6) Patients without pathological tumour regression had 2.8 times higher risk of dying.

Regarding cancer-related mortality and morbidity, gastric cancer is still one of the main causes, despite the development of new approaches, surgery and systemic treatment-wise^[53]. It is known that survival outcomes vary considering patients in different conditions, degrees of invasion and differentiation, as well as TNM stages. The importance of searching for new treatment strategies and their validation, with the objective of improving survival outcomes, relies on the considerable number of patients with advanced disease at diagnosis. The perioperative FLOT strategy, studied by Al-Batran *et al*^[3] demonstrated a palpable benefit providing better outcomes. Nonetheless, it is still difficult to predict tumor response to systemic treatment and identify predictive factors to stratify patients into risk groups, which would be important to optimize individualized treatment and follow-up.

Several studies describe a correlation between elevated neutrophil count or high NLR and worse prognosis, as well as progression of disease^[39,54]. Our findings seem to slightly contradict the literature, as we found NLR to have a weak positive correlation with OS, revealing that the higher the calculated NLR is, a better OS is expected, but Cox regression did not find an association between NLR and death status. Some studies published reference values and prognostic correlations for NLR. However, these studies were conducted using small cohort of patients with different races, mainly Asiatic patients. It is possible differences could be found regarding other populations, specifically European patients. Also, it seems that in the literature there is a consistent difference in gender and age group, which our study supports despite not reaching statistical significance (women and younger patients have a higher NLR mean than men and older patients), but this needs to be replicated in further investigation^[55]. This reveals the need for more validation studies in the European population.

LMR has been suggested to be an important factor for predicting prognosis in patients with hematologic and solid malignancies, such as lung cancer or colon cancer^[41,42,57,58]. Lower LMR was independently associated with poor OS in gastric cancer patients undergoing radical gastrectomy, and low LMR patients had a higher hazard ratio for both OS and DFS than high LMR^[58]. This suggests that lower LMR might be associated with a more aggressive tumor behavior, higher surgical mortality rates, and worse long-term survival^[59,60].

Although we did not find evidence of an association between LMR and prognosis, we showed an association between LMR and TSR, with high LMR patients having higher risk of achieving T stage reduction than low LMR patients. Also, our data showed an association between OS and TSR, documenting that patients without TSR had 2.1 times risk of dying, which we could indirectly interpret that patients with high LMR could have a better prognosis. We know that higher TNM staging, specifically T staging, is associated with less survival probability, crosswise solid and haematological tumors, so we could indirectly interpret that patients with low LMR are less likely to have TSR and, consequently, lower survival odds, but this needs to be further investigated.

N stage regression was not found to be associated with the three ratios, but NLR and PLR values seem to be clinically considerable, as patients with N reduction present higher ratio values. Further studies are needed to understand whether advanced TNM staging is associated with higher NLR and PLR ratio values^[15,61]. Combining T and N stages to assess TD did not reveal a statistically significant association, despite patients that achieved TD tend to have higher NLR values.

PLR has been widely associated with different stages of tumor development, systemic treatment response and prognostic survival outcomes of cancer patients, namely in LAGC^[61-63]. Nonetheless, specific physiopathology is complex and remains unclear. One plausible hypothesis is that a decreased PLR could reflect tumor disadvantage status, such as inflammatory status, immune disorders, malnutrition and thrombosis^[64-66]. Low PLR has been associated with less aggressive clinicopathological features, including decreased depth of invasion, less lymph node metastasis, and early tumor stage, while higher risk of lymph node metastasis, increased serosa invasion risk, and advanced stage disease is associated with elevated PLR in patients with gastric cancer^[15,61]. It has also been considered as a prognostic factor, with high PLR patients being associated with worse OS^[39].

Our study found an association between PLR and disease progression during preFLOT, showing that patients with high PLR have higher risk of progressing under CT. We also found a correlation between death and PLR, with high PLR patients having higher risk of dying than low PLR patients. Regarding tumoral response to CT, none of the ratios was associated with pCR achievement, albeit in TRG evaluation, PLR showed a considerable difference in means despite not being statistically significant, with patients without pathological response to CT presenting higher PLR values. Despite not finding an association between pCR and OS, TRG evaluation revealed an association with OS, documenting that patients without pathological tumor regression had 2.8 times higher risk of dying. Although not statistically significant, we could infer that patients with high PLR did not respond so well to CT and could have worse prognosis, despite further analysis is needed. We can extrapolate that these data show a more

biologically aggressive tumor and that this might be a reasonable motive for a more active surveillance in these higher risk patients, to detect early progression.

Finally, treatment-associated toxicity is an important clinical factor to be considered in patients, with more emphasis in the elderly population. Although we did not find a correlation between the 3 ratios and early suspension of treatment due to toxicity, we demonstrate that patients in need of treatment suspension tend to have higher NLR and PLR values. The association with treatment toxicity could be an aspect worthy of future investigation.

This study gathered information suggesting that these biomarkers could be suited to stratify patients by risk to preoperative CT. Knowing the difficulty to predict tumor response before initiation of systemic treatment using clinical and pathological data, we focused on the potential of NLR, LMR and PLR, already reported as systemic inflammatory and immune cancer environment important pieces. Our study stands out from others due to the analysis of these already known blood ratios in a curative setting of gastric cancer, with an approach to the CT response prediction. It has a good representation of Portugal population, being also one of the few studies undergone in a European population: We should have in mind that possible genetic differences regarding gastric cancer and also inflammation/immune mechanisms should be considered as influencers of the blood ratios analysis (*vs* Asian or Latino-American patients). Due to lack of information regarding treatment prediction in resectable gastric cancer (even less information regarding the European population in this setting), it would be interesting to retrospectively analyse these blood ratios in the phase II/III FLOT4-AIO trial.

Nonetheless, the present study had several limitations. Firstly, this exploratory study involved a retrospective analysis in a relatively small population ($n = 295$); consequently, larger validation studies are needed to confirm the potential of these ratios. Secondly, with NLR, LMR and PLR known to be associated with the inflammatory response, patients under anti-inflammatory effects from other medications could potentially bias the results. Thirdly, we did not control the process

and timing of blood collection and analysis; expectedly, blood analysis were undertaken before initiation of systemic treatment, but the period of time since blood results until treatment initiation can widely vary, and patients inflammatory state can be also volatile; depending on the length of time between blood collection and analysis, the composition of blood cells could be altered or destroyed, compromising correct estimation of the different ratios. Fourthly, clinical and pathological staging were observational dependent: Every participant hospital was responsible for the evaluation process of its patients, and even different gastroenterologists, radiologists and pathologists evaluated these patients, with possible subjective assessments that could bias the results. Fifthly, our median waiting time to surgery was 12 wk, being the recommended interval 4-6 wk (used in the FLOT4-AIO trial); this could possibly be a confounding variable and our results should be replicated prospectively in order to be confirmed.

CONCLUSION

We believe that the NLR, LMR and PLR could be important biomarkers, and that further prospective investigation is need in order to validate these ratios and possibly develop a score that could help in the decision-making process in the clinical management of patients with resectable LAGC.

ARTICLE HIGHLIGHTS

Research background

FLOT perioperative regimen became the standard of care in resectable locally advanced gastric cancer (LAGC), but there is still a need for prognostic and predictive response biomarkers. Blood ratios, such as neutrophil-to-lymphocyte (NLR), lymphocyte-to-monocyte (LMR) and platelet-to-lymphocyte (PLR) ratios, are known prognostic biomarkers in several solid tumors. Tumor regression grade (TRG) is a system used to evaluate residual tumor in patients submitted to preoperative therapies, providing information on the response to therapies and predicting prognosis.

Research motivation

This study investigated the prognostic and predictive significance of pre-treatment blood ratios in resectable LAGC.

Research objectives

This study aimed to assess the potential value of NLR, LMR and PLR in predicting survival outcomes and response to preoperative FLOT (preFLOT) regimen in resectable LAGC.

Research methods

We retrospectively analysed patients with resectable LAGC treated with at least one preFLOT cycle, from 12 Portuguese hospitals. NLR, LMR and PLR pre-treatment values were exploratory correlated with different variables, and with those statistically significant pre-treatment values were divided according to into high or low groups, determined by receiver operating characteristic curve, and evaluated regarding association to survival outcomes and response prediction. Relative risks and hazard ratios were calculated, with NLR, pathological complete response, T stage regression (TSR) and TRG as independent variables, and overall survival (OS) the dependent variable.

Research results

We included 295 patients in this study. NLR was correlated with time of survival ($r = 0.143$, $P = 0.014$). High PLR was defined as > 141 for progression and > 144 for mortality; high LMR was defined as > 3.56 for TSR. PLR was associated with systemic progression during FLOT ($P = 0.022$) and mortality ($P = 0.013$), with high PLR patients having 2.2 times higher risk of progression [95% confidence interval (CI): 0.89-5.26] and 1.5 times higher risk of mortality (95%CI: 0.92-2.55). LMR was associated with TSR and high LMR patients have 1.4 times higher risk of achieving TSR (95%CI: 1.01-1.99). OS

benefit was found with TSR ($P = 0.015$) and partial/complete TRG ($P < 0.001$). Patients without TSR as well as no evidence of pathological response have 2.1 (95%CI: 1.14-3.96) and 2.8 (95%CI: 1.6-5) times higher risk of death.

Research conclusions

NLR, LMR and PLR are significant biomarkers that are potential indicators for prognosis and treatment response prediction.

Research perspectives

In further investigation, validation of these blood ratios is important, as well as integration into risk scores that could help clinicians in the decision-making strategy in the clinical management of patients with resectable LAGC.

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