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Inflammation Indexes Pretreatment Associated with Overall Survival and the Response to Nimotuzumab in Patients with Head and Neck Cancer Newly Diagnosed

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Abstract

Purpose: Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) are three inflammation indexes based on neutrophil (N), lymphocyte (L) and platelet (P) counts reported to be prognostic and predictive factors in several solid tumours. We aimed to investigate its association with overall survival (OS) and the response to treatment in head and neck squamous cell carcinoma (HNSCC).

Methods: A total of 427 newly diagnosed HNSCC patients treated with nimotuzumab (group 1) or radiotherapy (group 2) was enrolled. NLR (NLR=N/L), PLR (PLR=P/L) and SII (SII=N x P/L) were calculated based on the data obtained before treatment. The cut-off value for every index was determined using the Package 'bhm' in R 3.5.0 software. Kaplan–Meier method was used to analyse the association of three indexes with OS and the response to treatment. All statistical analyses were carried out using SPSS version 25 software. Differences were considered statistically significant when $p<0.05$.

Results: Kaplan Meier analyses revealed that higher NLR, PLR and SII correlated significantly with poorer OS ($p=0.002$; $p=0.001$; $p=0.000$ respectively) in nimotuzumab treated patients. Patients with low inflammation index before treatment that was subsequently treated with nimotuzumab had a longer survival advantage than those in the group treated with radiotherapy.

Keywords: Neutrophil lymphocyte ratio; Platelet lymphocyte ratio; Systemic immune inflammation index; Head and neck squamous cell carcinoma; Overall survival; Nimotuzumab

Introduction

Passive immunotherapy for malignant diseases associated to epidermal growth factor and its receptor (EGF/EGFR) using monoclonal antibody against EGFR, has been widely investigated [1,2]. Nimotuzumab is a humanized monoclonal antibody IgG1 isotype, which blocks the binding of EGFR to its ligands [3,4]. It has been registered in Cuba and other 28 countries for the treatment of different tumors overexpressing EGFR [5,6], including advanced head and neck cancer where overexpression of EGFR reaches 90% [7,8]. Head and neck cancer is within the ten tumors more common worldwide and affects to 1536031 people every year, with a 60-70% of mortality [9].

Head and neck tumors have a great inflammatory component that influences progression and prognosis of patients. Inflammation is driven by a balance between pro-inflammatory cells such as neutrophils (N) and platelets (P); and anti-inflammatory cells such as lymphocytes (L). Several investigations have evaluated the relationship between these cells in peripheral blood and survival, and response to treatment in different types of tumors such as castration resistant prostate cancer, colorectal, non-small cell lung cancer and head and neck cancer [10-14].

In the present paper, we evaluated three inflammation indexes in patients with HNSCC treated with Nimotuzumab or radiotherapy.

Patients and Methods

A retrospective study was carried out including 427 patients with head and neck squamous cell carcinoma (HNSCC) newly diagnosed. Data were collected from patients included in three clinical trials conducted by the Center of Molecular Immunology (CIM-Havana, Cuba).

Trial 055 was a phase II-III multicenter, randomized clinical trial, in patients with HNSCC stages III and IV, treatment-naïve who were randomly assigned to receive radiotherapy plus Nimotuzumab or radiotherapy plus placebo [15]. Trial named CIMAB 4 was a multicenter; open-label phase IV clinical trial in patients with HNSCC, newly diagnosed and treated with nimotuzumab in combination with chemotherapy (CT) and/or RT [16]. Finally, the trial 113 was also a phase IV, multicenter open-label clinical trial in patients with newly diagnosed HNSCC, who received nimotuzumab treatment in combination with CT and/or RT.

For our present analysis of inflammation indexes data from all patients who received Nimotuzumab were pooled in one group (group 1); and data from patients who did not receive Nimotuzumab were pooled in the other group (group 2 or control group).

Data from 427 patients were retrieved from the clinical trial database of the Center of Molecular Immunology. Data of the absolute count of neutrophils (N) and lymphocytes (L) allowed the calculation of the Neutrophil/Lymphocyte ratio (NLR) in all 427 patients. Platelet/Lymphocyte ratio (PLR) could be calculated in 416 patients, and all three cell population could be counted in 404 patients for the calculation of the Systemic Inmune-Inflammation Index SII=and platelets (P) were complete for 404 patients allowing the calculation of the SII (N*P/L) (Figure 1).

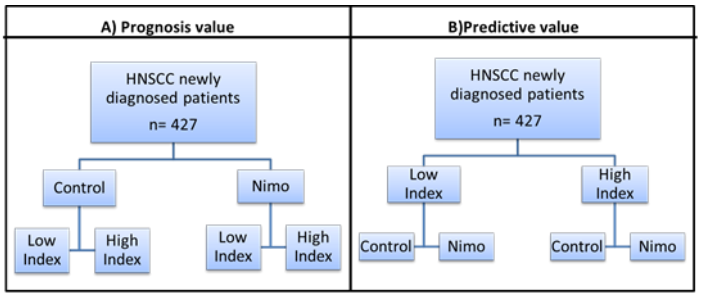

Figure 1 Stratification trees for the association of inflammation indexes with prognosis and with treatment response. A) Stratification according to inflammation indexes inside each treatment group. B) Stratification according to treatment inside each inflammatory index group.

For the stratification of patients, the cutoff value for every index was determined using the Package 'bhm' in R 3.5.0 software, that contains tools to fit both predictive and prognostic biomarker effects using biomarker threshold models and continuous threshold models. Patient's characteristics were analyzed with absolute and relative frequency. Median of overall survival (OS) was estimated by Kaplan Meier method and survival figures were compared among groups using the non-parametric log-rank test. All statistical analyses were carried out using SPSS version 25 software. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

The role of inflammation in cancer has attracted increasing interest, and inflammation is now considered the "seventh hallmark of cancer". This process includes elevated levels of circulating immune cells and infiltration of tumor environment, which facilitates tumor development through the production of factors that promote carcinogenesis and by enabling tumors to evade the host immune response. HNSCC is not an exception, neutrophils, platelets and other cells synthesize cytokines, chemokines, and growth factors that play key roles in both inflammation and cancer. Lymphocytes act as anti-inflammatory cells and are endowed with antitumor activity [17].

Inflammation indexes are easy to identify markers, based on the neutrophil, lymphocyte and platelet count in the peripheral blood. They could be prognostic markers and/or predictive markers of response to treatment in various pathologies related to inflammation such as cancer [10-12]. In the present study, the relevance of NLR, PLR and SII measurements was evaluated in patients with newly diagnosed head and neck carcinoma, treated with the monoclonal antibody against EGFR nimotuzumab or with radiotherapy.

Our set of patients treated with nimotuzumab who had high inflammation indexes prior to treatment, survived less than those with low inflammation indexes. These results suggest that NLR, PLR and SII could be inflammatory markers of prognostic value for the survival of patients with head and neck tumors.

Other researchers have studied the prognostic role of these markers. Increased NLR has been associated with a worse outcome in many early and advanced solid tumors [12], as well as head and neck cancer [18-20]. Here we ratify this association based in a larger series of patients. Bojaxhiu et al evaluated the prognostic value of pretreatment NLR and PLR in 186 HNSCC patients treated with first-line or adjuvant chemotherapy and/or radiotherapy, where high NLR levels were associated with lower survival, a result similar to ours; however, the PLR did not show significant associations with OS [14], which contrasts with our results. On the other hand, Rassouli et al have shown a significant impact of PLR and NLR on the survival of patients with HNSCC, with high levels of both inflammation indexes as a factor of poor prognosis [21]. SII has been less studied in head and neck tumors, Diao et al studied preoperative SII in 309 patients with primary oral squamous cell carcinoma after curative resection, obtaining that high levels were associated with larger tumor size and a decrease in overall and disease-free survival, behaving as an independent prognostic predictor for patient survival [22]. The prognostic value of NLR, PLR and SII was studied in 143 patients undergoing esophageal resection for esophageal cancer. Strong associations with pathological tumor depth were detected, PLR was significantly associated with tumor stage, and patients with higher SII and NLR values had significantly poorer OS compared with those with lower values; interestingly, there was no statistical difference between OS and PLR [23]. Our team in a larger sample of patients has verified these results.

In our set of patients with low inflammation index levels prior to treatment, nimotuzumab produced a bigger survival advantage over radiotherapy. These results suggest that low levels of NLR, PLR and SII are inflammatory markers predictive of good response to nimotuzumab.

Other researchers have also studied the value of these indexes as predictors of response to therapies for head and neck tumors. Yasumatsu et al. monitored the NLR before and after treatment with nivolumab in 49 patients with recurrent or metastatic HNSCC. They demonstrated an increase in the NLR in 76% of patients with progressive disease, and a decrease in 91% of patients with stable disease. A correlation was also evidenced between the high pretreatment NLR and shorter PFS and OS, thus suggesting that the NLR may be useful for predicting the anticancer effect of nivolumab in recurrent or metastatic head and neck cancer [24]. Ho et al obtained similar results in 34 patients with HNSCC, who received either nivolumab or pembrolizumab. High NLR was significantly associated with poorer clinical outcomes and survival with anti-PD1 therapy, which they attribute to the marked lymphopenia characteristic of this pathology. In this study the absolute lymphocyte count was the most reliable correlate for anti-PD1 therapy response [25]. A retrospective study with 40 patients with advanced head and neck cancers treated with docetaxel-combined induction chemotherapy, demonstrated that low PLR levels could predict a better overall response, and high NLR could predict more severe acute toxicity after induction chemotherapy with docetaxel [26]. SII has also been studied together with NLR and PLR in patients with esophageal squamous cell carcinoma, demonstrating its predictive value for survival and quality of life in response to surgery [27,28]. These results have been verified by our team in a wider sample of patients.

These results could be due to the role of these cells in the balance between the pro-inflammatory/anti-inflammatory response and cancer. Neutrophils granules contain a variety of enzymes, peptides, and proteins with anti-inflammatory function. Cytokines (IL-6, IL-1, TNF-α, IFN-γ), myeloperoxidase and matrix metalloproteinase 9 are pro-inflammatory factors released by neutrophils that enhance the angiogenesis, carcinogenesis, motility, migration, and invasion of tumor cells. Lymphocytic infiltration is a prominent feature in chronic inflammation of cancer. T lymphocytes may act as specific effectors of cytotoxicity or secret anti-inflammatory cytokines playing a predominant role in the regulation and resolution of the inflammatory response [29]. In a study realized with 114 head and neck cancer patients and 41 healthy volunteers as controls, an association was shown between high numbers of tumor-infiltrating neutrophils and advanced tumor stage and poor survival. The percentages of neutrophils in peripheral blood were higher in patient than controls. A significantly higher NLR in patients than in controls was detected because the total lymphocyte counts were similar in both groups [30].

In addition to its role in hemostasis, platelets also have inflammatory and antimicrobial functions such as the release of reactive oxygen species and pro-inflammatory cytokines (IL-2, IFN-γ, and TNF-α) that attract inflammatory cells, inducing to tissue damage, angiogenesis and carcinogenesis. Platelet-activating factor stimulates the release of inflammatory mediators from platelets, resulting in the activation of neutrophils. Thrombocytosis may manifest as a primary response to hemorrhage, acute inflammation, or infections or may develop as a secondary result of some chronic inflammatory conditions such as cancer [17,29].

In summary, our results confirmed that pretreatment NLR, PLR and SII are promising biomarkers associated with survival of patients with HNSCC and the response to nimotuzumab treatment. Patients treated with nimotuzumab, who had low levels of pretreatment NLR, PLR and SII, survive more than patients with higher inflammation indexes. Low pretreatment levels of NLR, PLR and SII, were associated with more survival advantage for patients treated with nimotuzumab as compared with patients who received only radiotherapy. On one hand, our study can help clinician better understand the associations of immunity, inflammation with cancer. On the other hand, our results can guide clinician to make suitable treatment strategies for individual patient.

Patient's characteristics

A total of 427 patients with HNSCC newly diagnosed, with a median age of 61 years old were included in this study. Primary tumor was in oral cavity, oropharynx or larynx in 81% of cases. Patients had III and IV stage of the disease to diagnosis in 76, 3%. A general condition according to Eastern Cooperative Oncology Group (ECOG) was 1 and 2 in 86, 1% of patients. Of the patients included, 89, 5% corresponded to group 1 of treatment (only nimotuzumab or nimotuzumab combined with RT, CT or RCT) (Table 1).

Table 1 Patient's characteristics

Characteristics	**Total (n=427) No. (%)**
Age	
Median (range)	61 (33-100)
Sex	
Female	82 (19,2)
Male	345 (80,8)
Race	
White	338 (79,2)
Mixed	50 (11,7)
Black	36 (8,4)
Missing	3 (0,7)
Smoking	
Smoker	157 (36,8)
Ex- Smoker	120 (28,1)
No-Smoker	55 (12,9)
Missing	95 (22,2)
Alcoholism	
Drink	168 (39,3)
No-Drink	163 (38,2)
Missing	96 (22,5)
Primary tumor localization	

Oral cavity	89 (20,8)
Nasopharynx	25 (5,9)
Oropharynx	164 (38,4)
Hypopharynx	24 (5,6)
Larynx	94 (22,0)
Paranasal sinus	4 (0,9)
Salivate glands	1 (0,2)
Missing	26 (0,1)
Stage	
I	39 (9,1)
II	60 (14,1)
III	114 (26,7)
IV	212 (49,6)
Missing	2 (0,5)
ECOG	
0	31 (7,3)
1	235 (55,0)
2	133 (31,1)
3	23 (5,4)
Missing	5 (1,2)
Received treatment	
Nimotuzumab	382 (89,5)
Radiotherapy	45 (10,5)

The optimal cut-off point that best separated the survival curves was 2.5 for NLR, 96.75 for PLR, and 727.64 for SII. These thresholds separate high vs low level for each index (Figure 2).

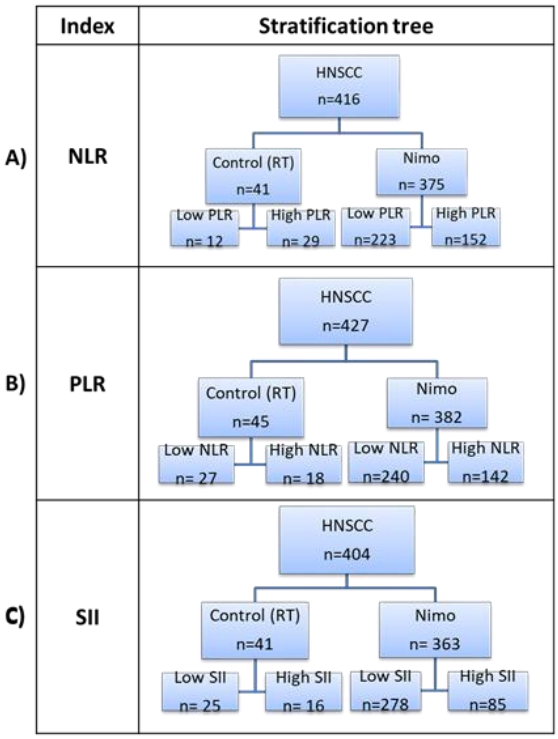

Figure 2 Stratification trees for the prognostic value of the three inflammation indexes. A) Stratification tree for NLR. B) Stratification tree for PLR. C) Stratification tree for SII.

Association between inflammation indexes and overall survival

The influence that each of the three indexes on OS was evaluated, comparing high vs low index groups inside each treatment group (Figure 3).

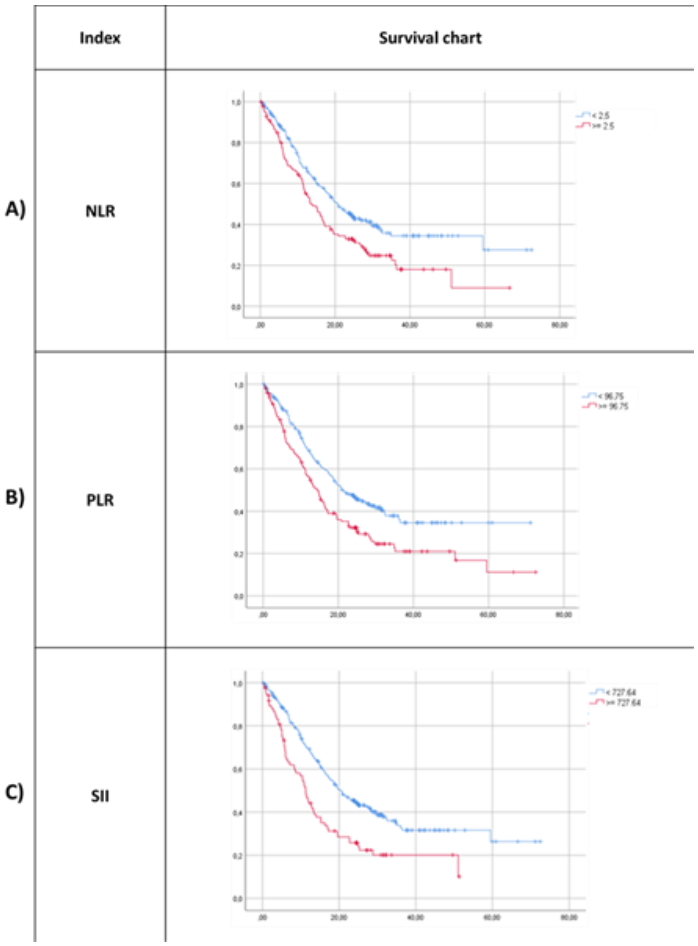

Figure 3 Kaplan Meier curves showing the association of NLR (A), PLR (B) and SII (C) with the survival inside nimotuzumab treated patients.

Overall, among nimotuzumab treated patients those who had high inflammation indexes prior to treatment survived less than those with low inflammation indexes. Patients with high NLR survived 7 months less than patients with low NLR (13.4 months vs 20.8 months) (p=0.002). Patients with high PLR survived 6 months less than patients with low PLR (14.5 months vs 20.8 months) (p=0.001). High SII patients prior to treatment survived 9 months less than those with low SII (11.3 months vs 20.6 months) (p=0.000). The Kaplan Meier survival curves for NLR, PLR and SII

are shown in Figure 4. This analysis was also done for patients who did not receive nimotuzumab (control group) but inside this group, there were no statistically significant with survival associations for any of the three indexes.

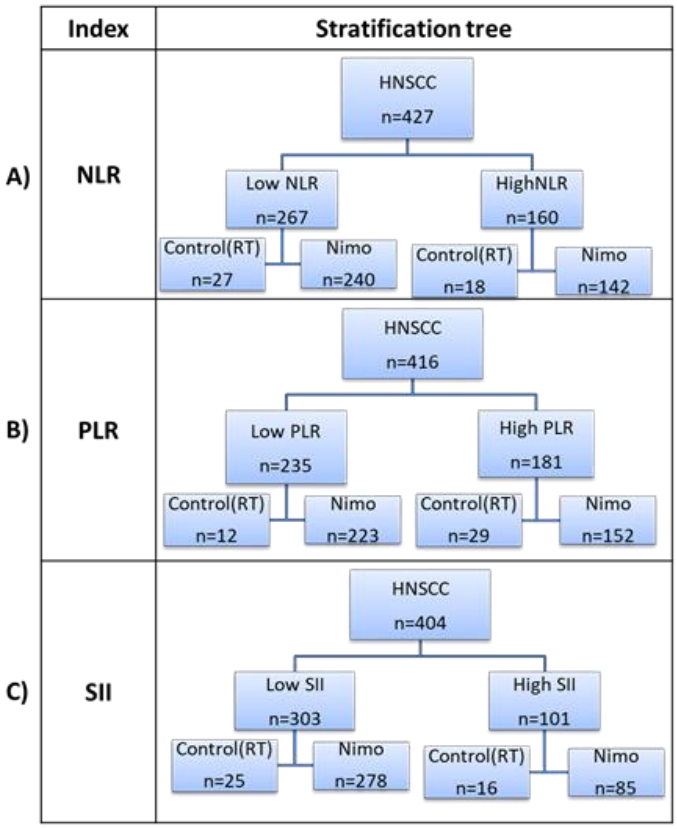

Figure 4 Stratification trees for the association of the three inflammation indexes with the response to treatment. A) Stratification tree for NLR. B) Stratification tree for PLR. C) Stratification tree for SII.

Association between inflammation index and treatment response

The influence of each treatment on OS was compared within the group of patients with low inflammation index. Likewise, the influence of each treatment on the OS of patients with high inflammation index was compared (Figure 5).

In general, patients with low inflammation index before treatment that was subsequently treated with nimotuzumab had a longer survival advantage than those in the group treated with RT. Of the patients with low NLR, the group that received nimotuzumab survived 12 months longer than the control group (20.8 months vs. 8.8 months) (p=0.002). Of the patients with low PLR, the group that received nimotuzumab survived 14 months longer than those that received radiation therapy alone (20.8 months vs 6.4 months) (p=0.000). Of the patients with low SII, the group that received nimotuzumab survived 12 months longer than those treated with radiotherapy alone (20.6 months vs 8.8 months) (p=0.002). This analysis was also done for high inflammation index patients but there were no statistically significant associations with treatment.

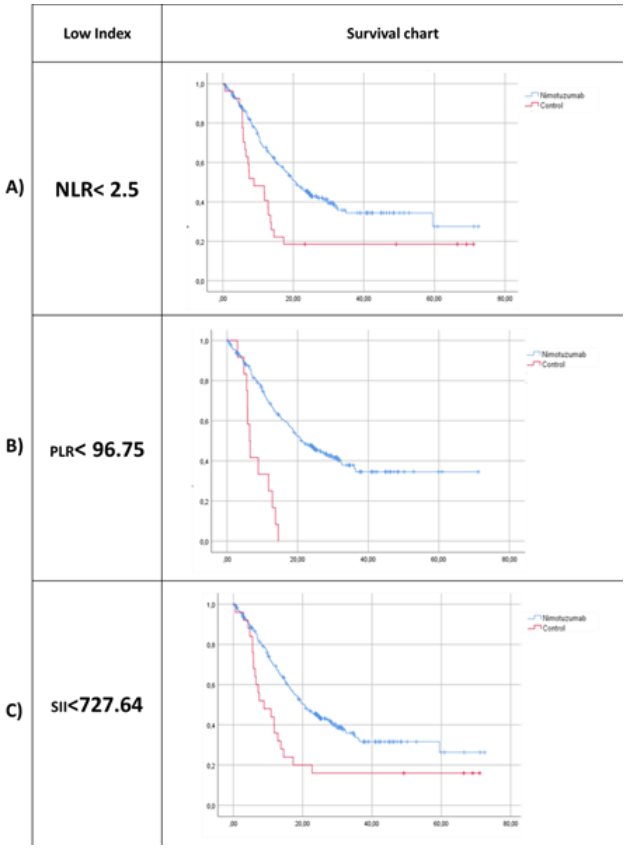

Figure 5 **Kaplan Meier curves showing the association of low NLR (A), PLR (B) and SII (C) with the survival advantage of patients treated with nimotuzumab or RT (control).**

Conclusion

The pretreatment NLR, PLR and SII are promising biomarkers associated with survival of patients with HNSCC and the response to nimotuzumab treatment. It may help to identify the high-risk patients for treatment strategy decisions.

Funding

This study was not funded.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional review board and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to Participate

Informed consent was obtained for all individual participants included in the in the three clinical trials that led to this study.

Consent for Publication

All authors have read the manuscript and approved the submission to your journal for publication.

Availability of Data and Material

Data sets during the current study are available from the corresponding author on reasonable request.

Code Availability

Not applicable

Authors' Contributions

Lisania Reyes searched the literature and designed the study. Lisania Reyes, Carmen Viada and Agustín Lage analyzed the data. All the authors wrote the manuscript. Agustin Lage revised the manuscript.

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