The Prognostic Value of Prognostic Nutritional Index in Patients With Advanced or Metastatic Gastric Cancer Treated With Immunotherapy

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Research Article

Keywords: prognostic nutritional index, immunotherapy, advanced gastric cancer, immunotherapy, prognostic factor, survival outcome

Posted Date: February 28th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2503613/v1

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Abstract

Background

In recent years, the therapeutic effect of monoclonal antibody against programmed cell death protein-1 (PD-1) monotherapy or combined chemotherapy in patients with locally advanced or metastatic gastric or gastroesophageal junction (G/GEJ) cancer has been confirmed by many studies. A number of clinical studies of anti-PD-1 antibody combined with chemotherapy in the perioperative treatment of locally advanced and resectable G/GEJ cancer are underway. The exploration and discovery of new biomarker combinations based on tumor characteristics and tumor microenvironment, especially tumor immune microenvironment, are helpful to screen superior patients and realize precise immunotherapy.

Methods

We selected 268 consecutive AGC patients who were treated with ICI therapy from December 2014 to May 2021. We measured their pretreatment the prognostic nutritional index (PNI) levels and performed univariate and multivariate Cox regression analyses of progression-free survival (PFS) or overall survival (OS) after ICI therapy.

Results

The low pretreatment PNI level of AGC patients was significantly correlated with shorter PFS ($P<0.001$) and OS ($P<0.001$) after ICI treatment. In univariate and multivariate analyses of the associations between neutrophil lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) or PNI, and OS or PFS, PNI (HR=1.483; 95% CI, 1.129-1.947; $P=0.005$) and NLR (HR=1.531; 95% CI, 1.164-2.014; $P=0.002$), but not LMR (HR=1.130; 95% CI, 0.829-1.538; $P=0.440$) are independent prognostic factors for PFS. Moreover, PNI (HR=1.385; 95% CI, 1.004-1.911; $P=0.048$) and LMR (HR=1.433; 95% CI, 1.039-1.977; $P=0.028$), but not NLR (HR=1.277; 95% CI, 0.896-1.820; $P=0.176$) are independent prognostic factors for OS.

Conclusion

The pretreatment PNI might help to identify AGC patients who will obtain a survival benefit from ICI therapy. The low pretreatment PNI is related to unsatisfactory survival outcomes.

1. Introduction

Gastric cancer (GC) is a worldwide clinically aggressive gastrointestinal tumor. It is the fifth most common cancer and the fourth leading cause of death from cancers in the world[1,2]. In China, the morbidity and mortality of GC rank second and third respectively[2], seriously endangering the public health. Despite the decline in morbidity and mortality over the past few decades, GC is still one of the major global health challenges[3]. The median overall survival (mOS) of advanced GC (AGC) is only about 8 months[4]. So far, both chemotherapy and targeted drugs are facing a bottleneck, and the mOS can barely exceed 2 years. Globally, the 5-year overall survival (OS) rate for AGC is only 10–15%[5].
As one of the breakthroughs in cancer treatment, immunotherapy has become an effective approach after surgery, chemotherapy, radiotherapy and targeted therapy[4]. This treatment involves stimulating the immune system to control tumor growth, and it specifically targets at tumor cells rather than normal ones. At present, therapeutic strategies utilizing the immune system involve checkpoint inhibitors, chimeric antigen receptor T cells (CAR T cells), monoclonal antibodies, cancer vaccines, cytokines, radiation immunotherapy and oncolytic virus therapy[6], among which immune checkpoint inhibitors (ICIs) have always been the research focus[7]. As one of the representative drugs, pembrolizumab demonstrated its potential value in antitumor therapy in the phase I Keynote-012 study, Keynote-059 cohort 1, Keynote-659(NCT03382600), and Keynote-811(NCT03615326), but Keynote-061 and KEYNOTE062 were not so good[5, 8–12]. Another drug, nivolumab, has also shown promising antitumor effects in attract-2 studies, CheckMate 649 Phase 3 trials and Attract-4 (NCT02746796) [13–15].

Although immunotherapy offers hope to patients with AGC, good results can only be achieved in specific populations. Predictive biomarkers can play a crucial role in screening patients who may benefit from selected or targeted therapies[7]. However, clinical evaluation of serum markers such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA19-9) and gastric cancer antigen (CA724) to immunotherapy is limited. There is no definitive biomarker that can accurately evaluate the therapeutic effect of ICIs[5]. Recent studies have indicated that self-expressed programmed death ligand-1 (PD-L1) and microsatellite steady-state (MSI) can be used to effectively assess the treatment results of immunotherapy in patients with AGC[16–20]. Peripheral blood inflammation composite indicators such as lung immune prognostic index (LIPI), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and hemoglobin (Hb) level have been proved to be convenient and promising biomarkers for GC prognosis[21–27].

Among numerous biomarkers that have been reported recently, peripheral blood biomarkers stand out because of their convenient and quick detection methods. In addition, due to the high heterogeneity of tumor tissue in GC, the accuracy of tumor biomarkers to evaluate treatment prognosis is not ideal, which will inevitably hinder the precise diagnosis of patients and the selection of targeted therapy[28].

Fumihiro Shoji et al. studied that the relationship between pretreatment c-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR) and the prognostic nutritional index (PNI) and the efficacy and prognosis of immunotherapy in 102 patients with non-small cell lung cancer (NSCLC)[29]. They found that progression free survival (PFS) and overall survival (OS) in low PNI group were significantly shorter than those in high PNI group, and PNI was still an independent prognostic factor of PFS after multivariate analysis[29]. Therefore, they believe that the pretreatment PNI is helpful to identify patients with AGC who may benefit from immunotherapy[29]. Based on this, we retrospectively studied the relationship between pretreatment PNI and the prognosis of patients with advanced gastric cancer treated with immunotherapy.

2. Materials And Methods
2.1 Research Subjects

A total of 268 patients with stage GC-IV who received ICIs treatment in the Advanced Oncology Department of the General Hospital of the Chinese People's Liberation Army from December 2014 to May 2021 were selected. Inclusion criteria: 1) The patient was diagnosed with AGC by comprehensive examination including imaging examination, serum tumor marker examination and histopathological biopsy; 2) Received at least two cycles of ICIs therapy; 3) Completed at least one radiographic efficacy evaluation based on RECIST1.1 criteria during treatment; 4) Complete blood routine and blood biochemical results within one week before the first use of ICIs; 5) < 80 years old; 6) Expected survival ≥ 3 months; 7) There were no obvious abnormalities in blood routine, coagulation function, liver and kidney function. Exclusion criteria: 1) patients who could not provide imaging data before and after ICIs treatment for comparison; 2) Complicated with other types of malignant tumors; 3) Complicated with other diseases that may affect peripheral blood albumin and neutrophils, such as hematological diseases, infections, viral hepatitis, cirrhosis, etc.; 4) Clinical, pathological and laboratory results and other relevant data are incomplete; 5) Non-tumor causes of death. The clinical features and tumor features of patients were collected by means of medical record inquiry, including their smoking history, smoking exposure, sex, age, tumor type, the status of HER-2 expression, the status of liver metastasis, response to line before immunotherapy, the status of pleural fluid, the status of ascites, the number of metastatic sites, lines of treatment with ICIs, ICIs agent, immunotherapy scheme, and eastern cooperative oncology group performance status scores (ECOG PS). At the same time, albumin and neutrophil values from the blood routine parameters obtained 7 days before immunotherapy were collected.

2.2 Treatment Regimens

According to the difference of treatment regimen, the patients were divided into immune monotherapy group and combined chemotherapy group. The types and doses of ICIs used in immunotherapy are as follows: 1) The recommended intravenous infusion of sintilimab is 200mg, once every 3 weeks; 2) The recommended intravenous infusion of riprilizumab is 3mg/kg, once every 2 weeks; 3) The recommended intravenous infusion of Pembrolizumab is 2 mg/kg, over 30 minutes at one time, once for every 3 weeks; 4) The recommended intravenous injection of nivolumab is 3 mg/kg or at the fixed dose of 240 mg, once every 2 weeks. The first imaging assessment of nivolumab was performed 2–4 weeks after the 3rd intravenous injection, while the assessment of toripalimab, sintilimab, and pembrolizumab were conducted 3–5 weeks after the 2nd intravenous injection. The 3-week dosing regimen for trastuzumab is: initial loading dose of 8mg/kg, followed by 6mg/kg, once every 3 weeks. The infusion time was about 90 minutes when the 6mg/kg dose was repeated once every three weeks. If the patient showed good tolerance at the initial infusion, the subsequent infusion might be changed to 30 minutes. The recommended dose of Ipilimumab is 1 mg/kg, once every 6 weeks for 30 minutes intravenous infusion; 30 minutes intravenous infusion of combined 360 mg nivolumab once every 3 weeks, or 3 mg/kg nivolumab once every 2 weeks. Chemotherapy regimen included: 1) XELOX regimen: 130mg/m² oxaliplatin on the first day; from day 1 to day 14, capecitabine (850-1250mg/m²) was taken twice a day.
after breakfast and dinner, and the regimen was repeated every 3 weeks. 2) SOX regimen: intravenous drip of 130mg/m2 oxaliplatin on the first day; tiggiu capsule (40-60mg) was taken twice a day after breakfast and dinner for 2 weeks, and then suspended for 7 days, forming a 21-days treatment course; 3) DCF regimen: intravenous injection of 75mg/m² docetaxel on the first day of each course, which should be finished in 1 hour; continuous intravenous drip of 75mg/m² cisplatin on the first day of each course; continuous intravenous drip of 750mg/m² 5-fluorouracil on the 1st to 5th day. One treatment course was repeated every 21 days. 4) PF regimen: intravenous drip of 25mg/m2 cisplatin on the 1st to 3rd day; continuous intravenous infusion of 500mg/m2 5-fluorouracil on the 1st to 5th day, one chemotherapy cycle every 4 weeks; 5) FOLFOX regimen: intravenous infusion of 85 mg/m2 oxaliplatin for 2 hours on the first day; intravenous infusion of 200 mg/m2 leucovorin for 2 hours on the 1st and 2nd days; intravenous injection of 400 mg/m² 5-fluorouracil on the 1st and 2nd days, together with the drip of 600 mg/m² fluorouracil for 22 hours. The treatment was suspended for 1 week after 14 days; 6) Combination of irinotecan and oxaliplatin: intravenous injection of irinotecan (180 mg/m²) and oxaliplatin (130mg/m²) on the first day of each 14-day session. 7) Combination of irinotecan and raltitrexed: intravenous injection of irinotecan (180 mg/m²) and raltitrexed (3 mg/m²) on the first day of each 14-day session. 8) Others. Each patient was assigned a treatment plan according to the stage of the case and general health status.

2.3 Assessment

RECIST1.1 criteria were adopted to assess the efficacy, including complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The short-term efficacy was evaluated by disease control rate (DCR) = (CR + PR + SD)/total cases×100%, and overall response rate (ORR) = (CR + PR)/total cases ×100%. For long-term efficacy evaluation, progression free survival (PFS) was defined as the time from the first treatment to the confirmation of PD, death, or the last follow-up, and OS was defined as the time from the start of immunotherapy to death.

2.4 Pretreatment calculation of the PNI, NLR, and LMR and the PNI, NLR, and LMR cut-off value

NLR, PNI and lymphocyte-to-monocyte ratio (LMR) were calculated using the following formula: absolute neutrophil count (ANC)/ absolute lymphocyte count (ALC), 1×serum albumin level (g/L) + 5×ALC (10⁹ /L) and absolute lymphocyte count (ALC) / absolute monocyte count (AMC) in the peripheral blood within one week before the first use of ICIs, respectively[30]. We determined to take the average 44.11 as the best cut-off value of PNI, and take the median 3.14 and 2.87 as the best cut-off value of NLR and LMR.

2.5 Statistical analysis

All data were processed by SPSS26.0. The data were summarized as the min–max range and median for non-normally distributed continuous variables. The data were reported in the form of percentage and count of categorical variables. With  =0.005 and β = 0.2 is the standard to predict the survival period of two groups of patients with low-PNI and high-PNI. The analysis shows that the OS of low-PNI and high-
PNI group is 17 months and 11 months, respectively. (Supplementary Document 1). Kaplan-Meier method was used to describe the survival curve. To evaluate the value of PNI to OS and PFS, Cox regression model was used to analyze the influencing factors. Values lower than 0.05 ($P<0.05$) were considered statistically significant.

3. Results

3.1 Baseline Characteristics

A total of 268 AGC patients who have received ICIs participated in this study. The patients were clinically characterized by the following features shown in Table 1. Among the patients, one hundred and ninety-nine (74.3%) are male, and 69 (25.7%) are female. More than half had no history of smoking (62.3%), most had short history of smoking exposure (82.1% less than or equal to 30 years); the ECOG PS was 0–1 (94%), the tumors were located at Body/Fundus (41%), there was no hepatic metastasis (55.6%), no negative expression of HER-2 (66%), no pleural fluid (92.9%) and no ascites (75.4%). After grouping according to the best cut off value of PNI, NLR and NLR, one hundred and thirty-one patients (48.9%) had PNI levels $\geq 44.11$ (high-PNI) and the remaining 137 (51.1%) patients had PNI $< 44.11$ (low-PNI), 134 (50%) had LMR levels $\geq 2.87$ (high-LMR) and the remaining 134 (50%) patients had PNI $< 2.87$ (low-LMR), 134 (50%) had LMR levels $\geq 2.87$ (high-LMR) and the remaining 134 (50%) patients had PNI $< 2.87$ (low-LMR), and 134 (50%) had NLR levels $\geq 3.14$ (high-NLR) and the remaining 134 (50%) patients had NLR $< 3.14$ (low-NLR).

3.2 Treatment Characteristics

175 of the 268 patients (65.3%) progressed before ICIs and 93 (34.7%) did not progressed before ICIs; 88 patients (32.8%) were treated with nivolumab, 36 (13.4%) with pembrolizumab and 144 (53.7%) with other immunotherapy drugs, 125 patients (46.6%) with first-line ICIs and 143 (53.4) the multi-line ICIs; 43 patients (16%) with the combined regimen of immunization and chemotherapy, 43 (16%) with the combined regimen of immunization and target therapy, 130 (48.5%) with the combined regimen of immunization, target therapy and chemotherapy and 52 (19.4%) with the immunization without additional therapy; 175 (65.3%) with dosage of immunotherapy $\geq 200$ mg and 93 (34.7%) with dosage of immunotherapy $< 200$ mg (Table 1).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of Patients</th>
<th>Characteristics</th>
<th>Number of Patients</th>
</tr>
</thead>
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<tr>
<td><strong>Overall (n = 268)</strong></td>
<td></td>
<td><strong>Overall (n = 268)</strong></td>
<td></td>
</tr>
<tr>
<td>Median age (range), years</td>
<td>59 (18–86)</td>
<td>Sex</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Female</td>
<td>69 (25.7)</td>
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<tr>
<td></td>
<td></td>
<td>Male</td>
<td>199 (74.3)</td>
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<td></td>
<td>Smoking exposure</td>
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<td>Yes</td>
<td>101 (37.7)</td>
<td>&lt; 30 packs per year</td>
<td>48 (17.9)</td>
</tr>
<tr>
<td>No</td>
<td>167 (62.3)</td>
<td>≥ 30 packs per year</td>
<td>220 (82.1)</td>
</tr>
<tr>
<td>Response to line before immunotherapy</td>
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<td>Family history</td>
<td></td>
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<tr>
<td>PD</td>
<td>175 (65.3)</td>
<td>Yes</td>
<td>101 (37.7)</td>
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<tr>
<td>Others</td>
<td>93 (34.7)</td>
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<td>167 (62.3)</td>
</tr>
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<td>Pleural fluid</td>
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<td>Present</td>
<td>19 (7.1)</td>
<td>Present</td>
<td>66 (24.6)</td>
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<td>Absent</td>
<td>249 (92.9)</td>
<td>Absent</td>
<td>202 (75.4)</td>
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<td>Dosage of immunotherapy</td>
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<td>HER-2</td>
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<td>≥ 200 mg</td>
<td>175 (65.3)</td>
<td>Present</td>
<td>34 (12.7)</td>
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<tr>
<td>&lt; 200 mg</td>
<td>93 (34.7)</td>
<td>Absent</td>
<td>177 (66)</td>
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<tr>
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<td></td>
<td>Unknown</td>
<td>57 (21.3)</td>
</tr>
<tr>
<td>Lines of immunotherapy</td>
<td></td>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>≥ 2</td>
<td>143 (53.4)</td>
<td>≥ 2</td>
<td>16 (6)</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>125 (46.6)</td>
<td>0–1</td>
<td>252 (94)</td>
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<tr>
<td>Tumor_location</td>
<td></td>
<td>PD-1 inhibitor Combined with other therapie</td>
<td></td>
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<tr>
<td>Cardia</td>
<td>73 (27.2)</td>
<td>Monotherapy</td>
<td>52 (19.4)</td>
</tr>
<tr>
<td>Body/Fundus</td>
<td>110 (41)</td>
<td>Chemotherapy</td>
<td>43 (16)</td>
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<tr>
<td>Pylorus</td>
<td>83 (31)</td>
<td>Target therapy</td>
<td>43 (16.0)</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Number of Patients</td>
<td>Characteristics</td>
<td>Number of Patients</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------</td>
<td>--------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Overall (n = 268)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (0.7)</td>
<td>Chemotherapy and target therapy</td>
<td>130 (48.5)</td>
</tr>
<tr>
<td>NLR, median (range)</td>
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<td>LMR, median (range)</td>
<td></td>
</tr>
<tr>
<td>&lt; 3.14</td>
<td>134 (50)</td>
<td>≥ 2.87</td>
<td>134 (50)</td>
</tr>
<tr>
<td>≥ 3.14</td>
<td>134 (50)</td>
<td>&lt; 2.87</td>
<td>134 (50)</td>
</tr>
<tr>
<td>PNI, mean (range)</td>
<td></td>
<td>Liver metastasis</td>
<td></td>
</tr>
<tr>
<td>≥ 44.11</td>
<td>131 (48.9)</td>
<td>Present</td>
<td>119 (44.4)</td>
</tr>
<tr>
<td>&lt; 44.11</td>
<td>137 (51.1)</td>
<td>Absent</td>
<td>149 (55.6)</td>
</tr>
</tbody>
</table>

PD: progressive disease; HER-2: human epidermal growth factor receptor-2; PD-1: programmed cell death-1; ECOG PS: eastern cooperative oncology group performance status scores; ICIs: immune checkpoint inhibitors; LMR: lymphocyte-to-monocyte ratio; NLR: neutrophil-lymphocyte ratio; PNI: the prognostic nutritional index; PD-1: programmed cell death-1.

### 3.3 Association between PNI and Efficacy

The study evaluated the optimal efficacy of all AGC patients, the results of which were as follows: PD accounted for 41.4% (111 patients), CR 1.5% (4 patients), PR 29.1% (78 patients), and SD 28% (75 patients). The ORR was 30.6% and DCR was 58.6% (Table 2). The low-PNI and high-PNI groups did not show clear difference in DCR (53.3% vs 64.1%, \(P = 0.072\)), or in ORR (29.2% vs 32.1%; \(P = 0.611\)) (Table 2).
Table 2
Relationship between PNI groups and response to anti-PD-1 treatment

<table>
<thead>
<tr>
<th>Best Overall Response</th>
<th>Number of Patients (%)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>the low- PNI group</td>
</tr>
<tr>
<td>CR</td>
<td>n = 268</td>
<td>n = 137</td>
</tr>
<tr>
<td></td>
<td>4 (1.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PR</td>
<td>78 (29.1)</td>
<td>40 (29.2)</td>
</tr>
<tr>
<td>SD</td>
<td>75 (28)</td>
<td>33 (24.1)</td>
</tr>
<tr>
<td>PD</td>
<td>111 (41.4)</td>
<td>64 (46.7)</td>
</tr>
<tr>
<td>Objective response</td>
<td>82 (30.6)</td>
<td>40 (29.2)</td>
</tr>
<tr>
<td>Disease control rate</td>
<td>157 (58.6)</td>
<td>73 (53.3)</td>
</tr>
</tbody>
</table>

PNI: the prognostic nutritional index; PD-1 :programmed cell death-1; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

3.4 Association between PNI, NLR and LMR and PFS

Regarding the last follow-up on July 1, 2021, 220 of the 268 AGC patients (82.1%) showed tumor progression. Compared with the patients in the low- LMR or low- PNI group, those in the high- LMR or high- PNI group showed a strong association with longer PFS (6.7 months vs 3.7 months; \(P<0.001\) and 5.8 months vs 3.9 months; \(P=0.001\)) (Fig. 1a and Fig. 1c). However, compared with the patients in the high- NLR group, those in the low- NLR group showed a strong association with longer PFS (6.3 months vs 3.6 months; \(P<0.001\)) (Fig. 1e). Table 3 summarized the results of univariate and multivariate analyses related to PFS. As for the univariate analysis, PFS improvement was detected on the patients with good PS (ECOG PS 0–1) and no ascites or pleural effusion. Among patients who did not achieve PD prior to immunotherapy, those who received with dosage of immunotherapy \(\geq 200\) mg, and 1st line ICIs treatment also showed improvements in PFS.

According to the multivariate analysis, patients in the low- PNI group were independently associated with more than 1.4-fold increased risk of disease progression (HR = 1.483; 95% CI, 1.129–1.947; \(P=0.005\)) higher than in the high- PNI group. Patients in the high- NLR group were independently associated with more than 1.5-fold increased risk of disease progression (HR = 1.531; 95% CI, 1.164–2.014; \(P=0.002\)) higher than in the low- NLR group. However, there was no statistical difference in PFS between patients in the high- LMR group and in the low- LMR group after multivariate analysis.

Apart from the above, patients receiving ICIs after 1st line were independently associated with more than 1.9 times the risk of disease progression (HR = 1.975; 95% CI, 1.500-2.601; \(P<0.001\)), higher than the 1st
line ICIs group. Patients without pleural fluid were independently associated with 1.7 times the risk of disease progression (HR = 1.780; 95% CI, 1.085–2.918; P = 0.022), higher than patients with pleural fluid.

### 3.5 Association between PNI, NLR and LMR and OS

At the last follow-up on July 1, 2021, 175 of 268 AGC patients (65.3%) passed away. Compared with patients in the low- LMR or low- PNI group group, those in the high- LMR or high- PNI one were closely associated with longer OS (19.7 months vs 9.1 months; P < 0.001 and 16.1 months vs 9.5 months; P < 0.001) (Fig. 1b and Fig. 1d). However, compared with the patients in the high- NLR group, those in the low- NLR group showed a strong association with longer OS (15.8 months vs 9.4 months; P < 0.001) (Fig. 1f).

Univariate and multivariate analyses relevant to OS are shown in Table 3. The results of univariate analysis of OS are similar to those of PFS indicated that improvement in OS was detected on patients with good PS (ECOG PS 0–1) and no ascites or pleural effusion. Among patients who did not achieve PD prior to immunotherapy, those who received with dosage of immunotherapy ≥ 200 mg, and 1st line ICIs treatment also showed improvements in OS.

Multivariate analysis revealed that the low- PNI group were associated with more than 1.3 times risk of the risk of death (HR = 1.398; 95% CI, 1.013–1.931; P = 0.042) than high- PNI group. The low- LMR group were associated with more than 1.4-fold increased risk of disease progression (HR = 1.433; 95% CI, 1.039–1.977; P = 0.028) higher than high- LMR group. However, there was no statistical difference in OS between patients in the high- NLR group and in the low- NLR group after multivariate analysis.

In addition, patients with no pleural effusion and good PS (ECOG PS 0–1) were independently in association with OS improvement. Patients receiving 1st line ICIs and dosage of immunotherapy ≥ 200 mg were also independently associated with OS improvement. Patients with pleural fluid were independently associated with over 2.3 times the risk of death (HR = 2.385; 95% CI, 1.406–4.046; P = 0.001), higher than those without pleural fluid. Patients with good PS (ECOG PS 0–1) were independently associated with 1.9 times the risk of death (HR = 1.837; 95% CI, 1.018–3.316; P = 0.044), higher than those with poor PS (ECOG PS ≥ 2). Patients receiving ICIs after 1st line treatment were independently associated with 1.8 times the risk of death (HR = 1.819; 95% CI, 1.321–2.503; P < 0.001), higher than the first-line ICIs group. Patients treated with dosage of immunotherapy < 200 mg were independently associated with 1.5 times the risk of death (HR = 1.693; 95% CI, 1.226–2.338; P = 0.001), higher than dosage of immunotherapy ≥ 200 mg treatment group.

### 3.6 Association between PNI and Outcomes of One or Multiple (≥ 2) Immunotherapy Lines: Subgroup Analysis

According to the results of the multivariate analysis, the patients treated with first-line ICIs were independently associated with improved OS and PFS. The subgroup analysis was conducted based on different immunotherapy lines. Among the 143 patients with subsequent ICIs, 67 (46.9%) were in the high-
PNI group and 76 (53.1%) in the low-PNI group. Improvement in OS was seen in the good group, in comparison with the moderate and the poor group (10.2 months vs 7.6 months, \(P = 0.008\)). However, there was no statistical difference in PFS between the high-PNI and low-PNI groups (3.3 months vs 2.6 months, \(P = 0.184\)) (Fig. 2a, 2b).

Among the 125 patients receiving the 1st line ICIs treatment, 64 (51.2%) were in the high-PNI group and 61 (48.8%) in the low-PNI group. In comparison with PFS and OS of the high-PNI and low-PNI groups, that of the high-PNI group improved (12.3 months vs 5.5 months, \(P<0.001\); 35.2 months vs 11.8 months, \(P<0.001\)) (Fig. 3a, 3b).

### 3.7 Association between PNI and Outcomes of ICIs with or without additional therapy: Subgroup Analysis

In order to reduce the sample error caused by using different immunotherapy schemes in different treatment lines, we also performed a subgroup analysis on the basis of different immunotherapy regiments. Among the 52 patients who received ICIs without additional therapy, 20 (38.5%) were in the high-PNI group and 32 (61.5%) in the low-PNI group. In comparison with PFS and OS of the high-PNI and low-PNI groups, that of the high-PNI group improved (5.7 months vs 1.8 months, \(P = 0.003\); 12.7 months vs 4.9 months, \(P = 0.032\)) (Fig. 4a, 4b).

Among the 216 patients treated with the ICIs combined with additional therapy, 111 (51.4%) were in the high-PNI group and 105 (48.6%) in the low-PNI group.

In comparison with PFS and OS of the high-PNI and low-PNI groups, that of the high-PNI group improved (6.7 months vs 4.1 months, \(P = 0.012\); 24.4 months vs 10.3 months, \(P = 0.002\)) (Fig. 5a, 5b).

### 4. Discussion

Immunotherapy is emerging as a promising anticancer strategy for many tumors[31]. Immunotherapy for GC has achieved fruitful results and changed treatment procedures[32], showing great potential to improve the prognosis of patients[33]. Recent breakthrough studies on ICIs represented by programmed death-1 (PD-1) and PD-L1 have opened new avenues for immunotherapy of GC[18, 34]. However, although anti-PD-1 antibody is a promising approach for AGC patients, its efficiency is still limited[18]. Furthermore, immunotherapy drugs are expensive, and easy to develop drug resistance and hyper-progressive disease, which limits their wide application in clinical practice[35–37].

In order to optimize the comprehensive anti-cancer regimen of immunotherapy, it is urgent to study how biomarkers accurately identify the population with immunotherapy advantages, and thus achieve as much precision and predictability as possible[38]. However, existing prediction biomarkers are still insufficient, and prediction methods need to be improved[37]. Peripheral blood inflammation composite indicators such as LIPI, NLR, PLR and Hb level have proved to be convenient and promising biomarkers for GC prognosis[21–27]. Considering the high heterogeneity of tumor tissue in GC, the accuracy of tumor biomarkers to evaluate treatment prognosis is not high, which will inevitably hinder the precise diagnosis
of patients and the selection of targeted therapy[28]. In contrast, the accuracy of the composite index, which combines multiple indicators, in screening beneficiaries of immunotherapy improved significantly.

However, the mechanism of correlation between these peripheral blood inflammatory complex indicators and tumor prognosis is complex and needs to be further explored through basic experiments and clinical trials. Recent studies reported that chronic inflammation may be a trigger for gastrointestinal cancer, which may be one of the reasons for the correlation between peripheral blood inflammation indicators and tumor prognosis[39]. Apart from the direct immune response to kill tumor inflammation indicators, these biomarkers are also related to tumor immunostimulatory signals and the activation of effector cells.

Neutrophils are traditionally defined as short-lived myeloid cells with unique crack. As a type of white blood cells, they exist in the form of nuclear, and rank the top in terms of importance and quantity in the blood circulating. They are usually the first responders under autoimmune physiological or pathological conditions of sterile injury, infection and inflammation, and act as the first line of defense to protect the host from tissue injury and infection[40, 41]. Tumor-associated neutrophils (TAN) accumulate in local areas and can be triggered by external stimuli in the tumor microenvironment (TME), switching between an antitumorigenic phenotype and a pro-tumorigenic phenotype[42]. Neutrophils that promote tumor cell growth and metastasis have the following functions: direct cytotoxicity, secretion of reactive oxygen species (ROS), nitric oxide (NO) and proteases, regulation of reticulocytosis, autophagy and other immune cells[43]. These neutrophils can activate CD8+ T cells and DCS, and may even present tumor antigens[44]. Antitumor neutrophils kill tumor cells through direct cytotoxic effects as well as indirect effects by the activation of adaptive immune responses[45]. Moreover, increased numbers of neutrophils can inhibit the immune effect ability of lymphocytes[46]. Lymphocytes are important cells in the body's immune response, especially responsible for adaptive immunity, providing antigen-specific responses regulated by class I major histocompatibility complex (MHC)[46,47]. Studies have shown that lymphocytes and their subsets (CD8+ T cells and CD3+ T cells) are related to the good prognosis of some tumors [48–50]. Lymphocytes infiltrated in TME are usually an indicator of the body's immune state, which can prevent the proliferation and migration of tumor cells [51].

The occurrence of tumor-related inflammation can inhibit the synthesis of albumin[52]. Meanwhile, with the decrease of serum albumin, tumor patients will suffer from decreased immunity and malnutrition[52,53]. Due to the lack of sufficient serum albumin combined with drugs, if patients with cancer develop hypoproteinemia, Chemotherapy drugs may have high residues in the blood and have high toxicity [53].

Studies have reported that low albumin concentration reflects cancer-induced malnutrition, and may have negative impact on prognosis[54]. In recent years, more and more researchers have paid attention to the combination of inflammatory cells representing systemic inflammatory state and albumin reflecting nutritional status.
Glasgow Prognostic Score (GPS), a composite biomarker of albumin and c-reactive protein (CRP), is reported to be a sensitive prognostic marker for GC[55]. Zhang J et al. found that a composite biomarker of serum CEA and fibrinogen/albumin ratio could also be used as a positive indicator to predict tumor progression and prognosis for GC patients[56]. As mentioned above, higher neutrophil levels and lower albumin levels are likely to cause a poor prognosis for cancer patients.

PNI, a composite index formed by ALC and albumin, has been proved to be related to the prognosis of advanced head and neck cancer treated with ICI[57]. PNI was initially established to evaluate the relationship between baseline nutritional status of tumor patients undergoing gastrointestinal surgery and postoperative complications[58]. However, the correlation between the prognosis of PNI and ICI treatment is unclear. Ul M et al. retrospectively analyzed 99 patients with advanced stage IV head and neck tumors who received ICI treatment in Johns Hopkins Hospital from 2014 to 2020, and found that PNI and BMI were related to the prognosis of ICI treatment [58]. According to the optimal cut-off value of PNI of 45, patients were divided into low PNI(< 45) group and normal PNI(≥ 45) group. Compared with normal PNI, lower PNI is significantly correlated with shorter OS ($P = 0.014$) and PFS ($P = 0.016$). After multivariate adjustment, PNI was still an independent prognostic factor of OS ($P = 0.041$) and PFS ($P = 0.011$)[58]. A retrospective study by Shoji et al. on 102 consecutive patients with NSCLC treated with ICI showed that the baseline PNI level was significantly correlated with PFS ($P = 0.0013$) and OS ($P = 0.0053$) [29]. The PFS and OS of low- PNI group (< 45.5) were significantly shorter than those of high- PNI group (≥ 45.5)[29]. In addition, Peng et al. also proved that PNI is related to the prognosis of NSCLC patients receiving ICI treatment[59].

In our study, the average PNI of 44.11 is taken as the best cut-off value, which is close to the best cut-off value taken by Ul M et al., Shoji et al. and Peng et al[29, 58, 59]. According to the optimal cut-off value determined by PNI, patients were divided into high-level group and low-level group. Our study shows that the prognosis of PNI treated with ICI in AGC patients is basically consistent with Ul M et al., Shoji et al. and Peng et al. The PFS and OS of high-level group were longer than those of low-level PNI group. Due to our included subjects used different immunotherapy regimens in different therapeutic lines, we further conducted subgroup analysis to explore the influence of PNI on OS and PFS in different lines and different immunotherapy regimens. Subgroup analysis showed that patients with high-PNI could benefit from OS whether they used immunotherapy in the 1st line or the multi-line, but PFS could only benefit from immunotherapy in the 1st line. Moreover, patients in the high- PNI group can benefit from OS and PFS, both in immunotherapy alone and in combination with other regimens. Our research also found that AGC patients with pleural fluid and subsequent ICIs can not benefit from PFS and OS after immunotherapy and with dosage of immunotherapy ≥ 200 mg can not benefit from OS.

There are some limitations in this study, including but not limited to: 1) the accuracy of the results may be undermined by retrospective bias such as selection, recall and measurement; 2) Patients included in the criteria received different drugs in different treatment lines; 3) The study subjects were confined to the same hospital; 4) The collected peripheral blood results could not reflect the actual dynamic changes; 5) The exploration scope of this study was limited to peripheral blood, which was widely used in clinical
practice and easy to operate, and did not involve genomics and radiomics, which might provide more valuable information and enrich the contents.

5. Conclusions

This study demonstrated that a composite biomarker of PNI was independently associated with the survival of AGC patients receiving immunotherapy. Compared to patients with malignant tumors with strong immunity, those with GC benefit less from immunotherapy. Although PD-1/PD-L1 expression level, microsatellite instability level, tumor mutation load, EBV and other indicators significantly affect the efficacy of immunotherapy, these indicators cannot fulfil the criteria for accurate screening. Whether the peripheral blood composite index of PNI can be used as an effective and economical prognostic biomarker needs to be further investigated in future studies.

Immunotherapy is a new option for the treatment of GC. In future scientific studies, effective immunotherapy markers will be explored in multiple aspects combined with the results of genetic testing or immunohistochemistry so as to achieve accurate treatment of GC. It is expected that more patients with AGC will benefit from the new ICIS-based treatment strategy.

Abbreviations

Gastric cancer (GC); median overall survival (mOS); advanced GC (AGC); overall survival (OS); chimeric antigen receptor T cells (CAR T cells); immune checkpoint inhibitors (ICIs); carcinoembryonic antigen (CEA); carbohydrate antigen (CA19-9); gastric cancer antigen (CA724); programmed death ligand-1 (PD-L1); microsatellite steady-state (MSI); lung immune prognostic index (LIPi); neutrophil-lymphocyte ratio (NLR); lymphocyte-to-monocyte ratio (LMR): the prognostic nutritional index (PNI); platelet-lymphocyte ratio (PLR); hemoglobin (Hb); non-small cell lung cancer (NSCLC); eastern cooperative oncology group performance status scores (ECOG PS); complete response (CR); partial response (PR); stable disease (SD); progressive disease (PD); disease control rate (DCR); overall response rate (ORR); progression free survival (PFS); programmed death-1 (PD-1); Tumor-associated neutrophils (TAN); tumor microenvironment (TME); reactive oxygen species (ROS); nitric oxide (NO); major histocompatibility complex (MHC); glasgow prognostic score (GPS); c-reactive protein (CRP);

Declarations

Ethics approval and consent to participate: all methods were carried out in accordance with relevant guidelines and regulations and all experimental protocols were approved by the Ethics Committee of Chinese PLA General Hospital. Besides, the ethical approval, accordance Declaration of Helsinki and waiver of Informed consent from participants.

Consent for publication: Not applicable.
Availability of data and materials: Yuting Pan of co-author should be contacted, if someone wants to request the data from this study.

Competing interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding: Not applicable.

Author Contributions: YP was in charge of writing and analysis. GD provided the guidance and idea. All authors contributed to the completion of the article and approved the submitted version.

Acknowledgments: The author gratefully acknowledges the advice and inspiration from Dr. Guanghai Dai, and the support by the Department of Medical Oncology, Chinese PLA General Hospital.

References


**Table**

Table 3 is available in the Supplementary Files section.

** Figures**
Figure 1

PFS (A, C, E) and OS (B, D, F) of the NLR, LMR and PNI of patients with AGC receiving ICIs cohort.

PFS: progression free survival; OS: overall survival; LMR: lymphocyte-to-monocyte ratio; NLR: neutrophil-lymphocyte ratio; PNI: the prognostic nutritional index; AGC: advanced gastric cancer.
Figure 2

PFS (A) and OS (B) of the multi-line of patients with AGC, receiving PD-1 inhibitor cohort.

Abbreviations: PFS: progression free survival; OS: overall survival; PD-1: programmed cell death-1; PNI: the prognostic nutritional index.

Figure 3

PFS (A) and OS (B) of the 1st line of patients with AGC, receiving PD-1 inhibitor cohort.

Abbreviations: PFS: progression free survival; OS: overall survival; PD-1: programmed cell death-1.
Figure 4

PFS (a) and (b) OS of AGC patients treated with PD-1 inhibitor combined without additional therapy.

Abbreviations: PFS: progression free survival; OS: overall survival; PD-1: programmed cell death-1.

Figure 5

PFS (A) and (B) OS of AGC patients treated with PD-1 inhibitor combined with additional therapy.

Abbreviations: PFS: progression free survival; OS: overall survival; PD-1: programmed cell death-1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.docx
- file.pdf