

Preoperative PD-1⁺CD4⁺ lymphocytes reflect immunological and physical frailty and are associated with postoperative infectious complications following gastrectomy for gastric cancer

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Abstract. Postoperative infectious complications (PICs) are major adverse events following gastrectomy for gastric cancer. The present study investigated whether preoperative programmed cell death 1 (PD-1)⁺CD4⁺ lymphocytes reflect immunological and physical frailty and predict PICs. A total of 85 patients who underwent gastrectomy for gastric cancer were retrospectively enrolled. Blood samples were collected preoperatively, and PD-1⁺CD4⁺ cells were analyzed using flow cytometry. Patients were divided into PD-1^{high} (N=43) and PD-1^{low} (N=42) groups based on the median value of preoperative PD-1⁺CD4⁺/CD4⁺ cells (cutoff, 22.3%). Preoperative immune-inflammatory markers and nutritional indices included the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein-to-albumin ratio (CAR), prognostic nutrition index (PNI) and controlling nutritional status (CONUT) score. Physical vulnerability was assessed using the fall risk assessment score (FRAS) and Charlson comorbidity index (CCI). The PD-1^{high} group was older than the PD-1^{low} group, with no significant differences in sex, comorbidities, or surgical and pathological factors. The PD-1^{high} group had a significantly higher incidence of the PICs (42 vs. 19%; P<0.05). In addition, the PD-1^{high} group exhibited higher C-reactive protein levels, and lower total lymphocyte counts and albumin levels compared with the PD-1^{low} group (P<0.05, respectively). The two groups had significant differences in preoperative NLR, CAR, PNI, CONUT score and FRAS. Notably, there were significant associations between PD-1⁺CD4⁺/CD4⁺ cells and NLR, PLR, CONUT score, PNI,

FRAS and CCI (all P<0.05). Preoperative PD-1⁺CD4⁺/CD4⁺ cells were significantly associated with increased PIC risk and markers of immunological and physical frailty. This biomarker may be useful for identifying vulnerable patients requiring tailored perioperative care.

Introduction

Gastric cancer is one of the most common cancers worldwide, and was the fifth leading cause of cancer-related deaths in 2022 (1,2). Although considerable recent advances in chemotherapy, including immune checkpoint inhibitors, have improved the prognosis of gastric cancer (3,4), gastrectomy remains the most effective treatment strategy, even for elderly patients (5). However, surgical resection often leads to functional disorders and postoperative infectious complications (PICs) (6-8). Previous studies have reported that PICs result in prolonged hospital stays, increased treatment costs, and a lack of effective adjuvant therapy (9-11). Additionally, PICs are reportedly associated with unfavorable outcomes after radical surgery for gastrointestinal cancers (7,12-14). Therefore, the ability to predict PICs preoperatively is crucial for optimizing surgical procedures, tailoring perioperative management, and refining treatment strategies to improve patient prognosis.

The American Society of Anesthesiologists Physical Status (ASA-PS) classification (15), frailty (16,17), sarcopenia (18,19), nutritional status (20,21), and comorbidities (22) are reportedly useful physical predictors of PICs. However, the mechanisms by which these indicators influence PICs remain unclear. Furthermore, the relationship between preoperative immunosuppression and PICs is not well understood.

The programmed cell death-1 (PD-1)/programmed cell death ligand (PD-L) signaling pathway has recently been implicated as a potential immune escape mechanism in several malignancies (23), and several anti-PD-L1 antibodies have been applied as standard treatments for various malignancies (24,25). Additionally, PD-1 is a critical costimulatory molecule and a pivotal immune checkpoint receptor that inhibits T-cell activation. Studies have shown that PD-1⁺CD4⁺ cells increase with age and that PD-1⁺CD4⁺ cells derived

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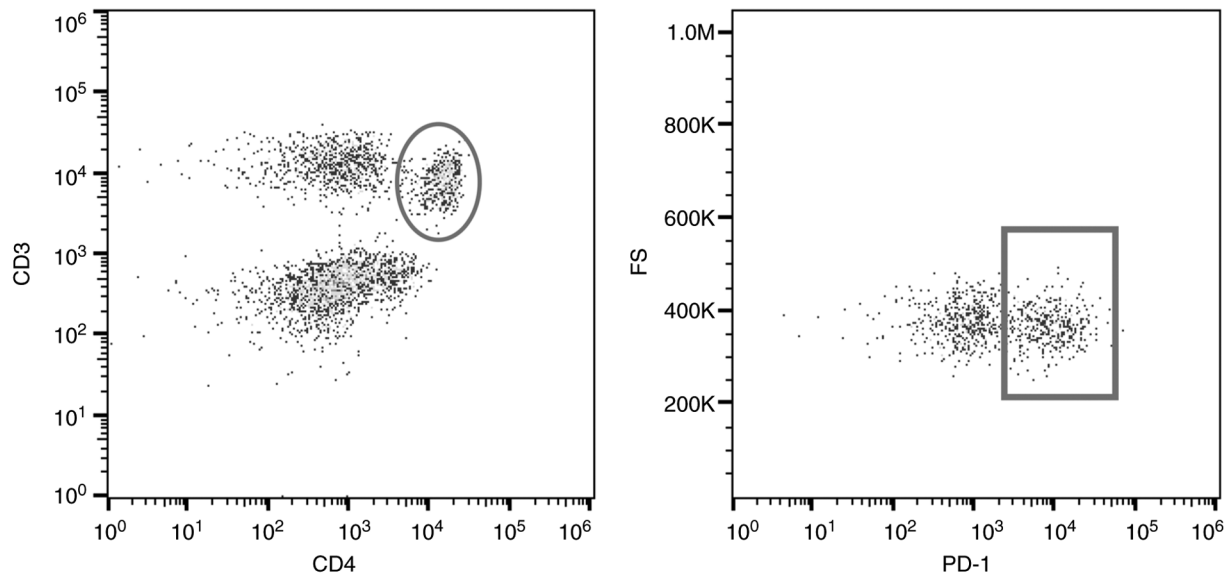


Figure 1. Gating strategy for flow cytometric analysis. Blood samples were labeled with fluorescent dye-conjugated monoclonal antibodies against PD-1-PE, CD4-PC7 and CD3-FITC. PD-1⁺CD4⁺ cells were defined as PD-1 positive cells (square) with double positive CD3 and CD4 cells (circle). PD-1, programmed cell death 1.

from aged mice have a reduced capacity to respond to antigen stimulation, demonstrating that PD-1⁺CD4⁺ cells are associated with immunosenescence (26). However, the relationship between PD-1⁺CD4⁺ cells and PICs has not been reported.

In this study, we aimed to investigate the predictive value of preoperative PD-1⁺CD4⁺ cells for the development of PICs and their association with preoperative immune-inflammatory markers, nutritional indices, and physical vulnerability.

Materials and methods

Patients' selection. We retrospectively analyzed the data of 85 patients (67 men, 18 women; median age 72, range 46-92) who underwent curative gastrectomy for gastric cancer at the National Defense Medical College Hospital between January 2014 and December 2020. Patients who had undergone preoperative chemotherapy, which may have affected preoperative lymphocyte counts, were excluded. Patients were divided into two groups; PD-1^{high} (N=43) and PD-1^{low} (N=42) groups, based on the median value of preoperative PD-1⁺CD4⁺/CD4⁺ cells (cutoff value: 22.3%).

We collected the patients' clinical records at admission for gastrectomy and pathological records. Pathological findings of the specimens were recorded according to the third English edition of the Japanese Classification of Gastric Carcinoma published by the Japanese Gastric Cancer Association (27).

Definition of postoperative infectious complications. PICs were defined as Clavien-Dindo Grade ≥ 2 within 30 days postoperatively (28). In this study, PICs referred to pneumonia, pancreatic fistula, anastomotic leakage, intraabdominal abscess, cholecystitis, cholangitis, pneumonia, pyothorax, intestinal ischemia, and sepsis. Superficial wound infections were excluded because they have minimal impact on the systemic immune response. All surgeries were performed by expert surgeons with more than 10 years of experience.

Flow cytometric analysis of PD-1 expression on lymphocytes. Ethylenediaminetetraacetic acid-anticoagulated blood samples were collected preoperatively (immediately before gastrectomy) and stored at 4°C; the analysis was performed within 48 h of sample collection. Blood samples were incubated for 30 min. at 4°C with fluorescent dye-conjugated monoclonal antibodies against PD-1 (CD179)-PE (clone: eBioJ105, Thermo Fisher Scientific, Inc.), CD4-PC7 (clone: SFC112T4D11, Beckman Coulter), and CD3-FITC (clone: UCTH1, Beckman Coulter). After incubation, samples were washed twice with cold calcium and magnesium-free phosphate-buffered saline (PBS) supplemented with 2% fetal bovine serum (FBS) and 0.1% sodium azide (2% flow buffer). Red blood cells were lysed using a lysis buffer, and the remaining cells were suspended in IsoFlow (Beckman Coulter). Following two additional washes with 2% flow buffer and centrifugation at 1200 rpm for 2 min, the cell pellets were resuspended in 500 μ l of IsoFlow, kept on ice, and analyzed within 6 h using a flow cytometry (Cytomics FC500, Beckman Coulter). Appropriate isotype-matched negative controls were used for each antibody. Flow cytometric data were analyzed using FlowJo software (Tree Star Inc.). CD4⁺CD3⁺ cells were gated, and PD-1 expression on these cells was evaluated (Fig. 1) (29).

Immune-inflammatory markers, nutritional indices, fall risk assessment score, and Charlson comorbidity index. We assessed preoperative neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP)-to-albumin ratio (CAR), and platelet-to-lymphocyte ratio (PLR) as immune-inflammatory markers, and prognostic nutrition index (PNI) and controlling nutritional status (CONUT) as nutritional indexes (30-32). ASA-PS was described from the anesthesia chart. Carlson comorbidity index (CCI) (22) and the fall risk assessment score (FRAS) (16) was used to determine physical vulnerability. The FRAS was evaluated by nursing staff upon admission (Table SI), and was calculated as the sum of the

Table I. Clinicopathological characteristics according to the PD-1⁺CD4⁺/CD4⁺ cells.

Factors	Groups	PD1 ⁺ CD4 ⁺ /CD4 ⁺ cells (%)		P-value
		PD-1 ^{high} (N=43)	PD-1 ^{low} (N=42)	
Clinical factors				
Mean age (range), years		74.2 (46-89)	70.0 (47-88)	0.04
Sex, n (%)	Male	33 (76.7)	34 (81.0)	0.63
	Female	10 (23.3)	8 (19.0)	
BMI (range), kg/m ²		22.7 (15.7-31.1)	22.6 (13.7-35.3)	0.94
Comorbidity, n (%)	Yes	38 (88.4)	31 (73.8)	0.08
	No	5 (11.6)	11 (26.2)	
Cardiovascular, n (%)	Yes	27 (62.8)	23 (54.8)	0.45
	No	16 (37.2)	19 (45.2)	
Diabetes, n (%)	Yes	9 (20.9)	8 (19.0)	0.82
	No	34 (79.1)	34 (81.0)	
Respiratory, n (%)	Yes	4 (9.3)	2 (4.8)	0.41
	No	39 (90.7)	40 (95.2)	
Previous laparotomy, n (%)	Yes	13 (30.2)	15 (35.7)	0.59
	No	30 (69.8)	27 (64.3)	
Postoperative hemoglobin ± SD, g/dl		11.7±2.4	12.3±1.9	0.16
Postoperative hospital stays (range), days median		11 (6-255)	9 (6-138)	0.46
Surgical factors				
Time ± SD, min		268±96	257±76	0.74
Bleeding ± SD, g		481±601	319±348	0.23
Surgical approach, n (%)	Laparotomy	19 (44.2)	18 (42.9)	0.90
	Laparoscopy	24 (55.8)	24 (57.1)	
Blood transfusion, n (%)	Yes	14 (32.6)	6 (14.3)	0.04 ^a
	No	29 (67.4)	36 (85.7)	
Pathological factors				
Localization, n (%)	U	13 (30.2)	13 (31.0)	0.92
	M	12 (27.9)	13 (31.0)	
	L	18 (41.9)	16 (38.0)	
Diameter ± SD, mm		60±40	46±30	0.08
Pathological type (Lauren classification), n (%)	Diffuse	22 (51.2)	16 (38.1)	0.20
	Intestinal	21 (48.8)	24 (57.1)	
	Others ^a	0 (0)	2 (4.8)	
Tumor depth, n (%)	T1	13 (30.2)	19 (45.2)	0.42
	T2	7 (16.3)	7 (16.7)	
	T3	11 (25.6)	6 (14.3)	
	T4	12 (27.9)	10 (23.8)	
Nodal metastasis, n (%)	N0	22 (51.2)	24 (57.1)	0.16
	N1	9 (20.9)	6 (14.3)	
	N2	7 (16.3)	2 (4.8)	
	N3	5 (11.6)	10 (23.8)	
Pathological stage, n (%)	Stage I	18 (41.9)	23 (54.7)	0.46
	Stage II	8 (18.6)	7 (16.7)	
	Stage III	17 (39.5)	12 (28.6)	
Postoperative infectious complications ^b , n (%)	Yes	18 (41.9)	8 (19.0)	0.02 ^a
	No	25 (58.1)	34 (81.0)	
Pancreatic fistula, n (%)		8 (18.6)	4 (9.5)	0.22
Anastomotic leakage, n (%)		6 (14.0)	0 (0.0)	0.01 ^a
Intraabdominal abscess, n (%)		5 (11.6)	4 (9.5)	0.75

Table I. Continued.

Factors	Groups	PD1 ⁺ CD4 ⁺ /CD4 ⁺ cells (%)		P-value
		PD-1 ^{high} (N=43)	PD-1 ^{low} (N=42)	
Sepsis, n (%)		4 (9.3)	0 (0.0)	0.04 ^a
Cholecystitis/choolangitis, n (%)		2 (4.6)	1 (2.4)	0.57
Pneumonia, n (%)		5 (11.6)	0 (0.0)	0.02 ^a
Intestinal ischemia/necrosis, n (%)		3 (7.0)	0 (0.0)	0.08
Pyothorax, n (%)		2 (4.6)	0 (0.0)	0.15
Catheter-related bloodstream infection, n (%)		1 (2.3)	0 (0.0)	0.32

^aCarcinoma with lymphoid stroma and endocrine cell carcinoma. ^bOf all patients, 9 cases multiple PICs. BMI, body mass index; U, the upper third of the stomach; M, the middle third of the stomach; L, the lower third of the stomach; PD-1, programmed cell death-1.

scores for all items. It includes seven categories: age, history of falls or syncope, physical dysfunction, activity status, mental dysfunction, medicines, and toileting needs. In total, these categories comprise 46 individual fall risk items. The CCI, developed by Charlson *et al* (22), predicts the mortality by accounting for a range of comorbid conditions, such as renal, hepatic, and cardiac diseases, acquired immunodeficiency syndrome, and cancer-spanning 17 categories in total.

Definition of immune-inflammatory markers and nutritional indices. The markers and indices based on preoperative laboratory data were calculated as follows using preoperative laboratory data: NLR=neutrophil counts/lymphocyte counts, CAR=C-reactive protein levels/albumin levels, PLR=platelet counts/lymphocyte counts, PNI=10×serum albumin level (g/dl) + 0.005×total lymphocyte count (/μl) (31). The CONUT score was assessed using serum albumin, total cholesterol, and total lymphocyte count levels (32).

Ethics. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. All protocols were approved by the Institutional Review Board of the National Defense Medical College (Approval number: 5070) and written informed consent was obtained prior to the study.

Statistical analysis. Data are expressed as mean ± standard deviation, unless otherwise stated. Welch's t-test, Mann-Whitney U-test, and one-way analysis of variance were conducted, with post hoc Tukey procedures employed to adjust for multiple comparisons, when appropriate. To evaluate multicollinearity, we calculated the Variance Inflation Factor (VIF) for all explanatory variables, with VIF >5 indicating high collinearity. All statistical analyses were performed using JMP Pro 17.0.0 (SAS Institute Inc.), and statistical significance was set at P<0.05.

Results

Clinicopathologic characteristics of patients who underwent gastrectomy are summarized in Table I. The PD-1^{high} group

was significantly older (74.2±9.2 vs. 70.0±10.3) and had a higher frequency of perioperative blood transfusion (32.6% vs. 14.3%) compared to the PD-1^{low} group. There were no significant differences in sex, body mass index, comorbidities, postoperative hospital stays, and pathological factors, such as tumor depth, nodal metastasis, and pathological stage. The PD-1^{high} group demonstrated a significantly higher overall incidence of PICs (41.9% vs. 19.0%), with notably increased rates of anastomotic leakage (14.0% vs. 0%), pneumonia (11.6% vs. 0%), and sepsis (9.3% vs. 0%) (all P<0.05). Fig. S1 shows the various types of complications and the ratio of PD-1⁺CD4⁺/CD4⁺ cells.

There were no significant differences in preoperative white blood cell, neutrophil, and platelet counts between the two groups (Table II). However, the PD-1^{high} group had significantly lower preoperative total lymphocyte counts (1476±534/μl vs. 1780±536/μl) and serum albumin (3.5±0.5 g/dl vs. 3.9±0.6g/dl) and had a significantly higher preoperative CRP level (1.0±1.4 mg/dl vs. 0.1±1.1mg/dl). In addition, the PD-1^{high} group had significantly higher preoperative NLR (3.3±1.6 vs. 2.3±0.8), CAR (0.4±0.6 vs. 0.2±0.7), CONUT score (2.9±2.6 vs. 1.5±1.8), and FRAS (5.5±3.6 vs. 3.9±3.1) as well as significantly lower preoperative PNI (42.6±6.7 vs. 47.0±9.5) (all P<0.05).

Regarding the association of preoperative PD-1⁺CD4⁺/CD4⁺ cells and each preoperative immune-inflammatory marker, there were significant positive correlations between PD-1⁺CD4⁺/CD4⁺ cells and NLR, PLR, and CONUT score (all P<0.05) (Fig. 2A, C, D), whereas a significant negative correlation was observed between PD-1⁺CD4⁺/CD4⁺ cells and PNI (P<0.01) (Fig. 2E). In addition, PD-1⁺CD4⁺/CD4⁺ cells significantly increased as ASA-PS increased (P<0.05; Fig. 3A) and significantly positively correlated with age, FRAS, and CCI (all P<0.05) (Fig. 3B-D).

The results of univariate and multivariate analyses that may affect the incidence of PICs are shown in Table III and Fig. S2. Five variables (preoperative FRAS, CAR, PNI, CCI, and PD-1⁺CD4⁺/CD4⁺ cells) with P<0.05 in the univariate analysis were selected as explanatory variables for the multivariate analysis. Multivariate analysis demonstrated that only CCI was identified as independent factors for the development of PICs. No evidence of problematic multicollinearity was found, as all VIF values were below 2.0. These findings are

Table II. Preoperative laboratory data, immune-inflammatory markers, nutritional status and fall risk assessment score according to the PD-1⁺CD4⁺/CD4⁺ cells.

Factors	Groups	PD1 ⁺ CD4 ⁺ /CD4 ⁺ cells		P-value
		PD-1 ^{high} (N=43)	PD-1 ^{low} (N=42)	
ASA-PS, n (%)	I	6 (13.9)	8 (19.0)	0.08
	II	30 (69.8)	33 (78.6)	
	III	7 (16.3)	1 (2.4)	
FRAS, median (range)		4 (1-16)	3 (0-14)	0.02
	I (1-5), n (%)	25 (58.2)	31 (73.8)	0.23
	II (6-15), n (%)	17 (39.5)	11 (26.2)	
	III (-16), n (%)	1 (2.3)	0 (0.0)	
CCI ± SD		6.7±1.5	6.1±1.4	0.08
CONUT score, median (range)		2 (0-11)	1 (0-8)	<0.01
	Normal (0-1), n (%)	16 (37.2)	27 (64.3)	0.07
	Mild (2-4), n (%)	18 (41.9)	11 (26.2)	
	Moderate (5-8), n (%)	8 (18.6)	4 (9.5)	
	Severe (9-12), n (%)	1 (2.3)	0 (0.0)	
White blood cell counts ± SD, /μl		6165±1810	6104±2043	0.77
Neutrophil counts ± SD, /μl		4068±1487	3778±1658	0.28
Lymphocyte counts ± SD, /μl		1476±534	1780±536	0.02
Platelet counts ± SD, x10 ³ /μl		246±100	254±108	0.81
Hemoglobin ± SD, g/dl		11.7±2.4	12.3±1.9	0.16
Albumin ± SD, g/dl		3.5±0.5	3.9±0.6	<0.01
CRP ± SD, mg/dl		1.0±1.4	0.5±1.1	0.01
NLR ± SD		3.32±1.56	2.33±0.84	<0.01
CAR ± SD		0.37±0.56	0.24±0.66	0.03
PLR ± SD		185.6±101.1	148.3±62.1	0.15
PNI ± SD		42.6±6.7	47.0±9.5	<0.01
CD4 ⁺ cell counts ± SD, /μl		544±439	733±574	0.06
PD1 ⁺ CD4 ⁺ cell counts ± SD, /μl		596±373	459±408	0.04

CRP, C-reactive protein; ASA-PS, American Society of Anesthesiologists physical status; FRAS, fall risk assessment score; CCI, Charlson comorbidity index; CONUT, controlling nutrition status; NLR, neutrophil-to-lymphocyte ratio; CAR, C-reactive protein-to-albumin ratio; PLR, platelet-to-lymphocyte ratio; PNI, prognostic nutritional index; GNRI, geriatric nutritional risk index; PD-1, programmed cell death-1.

in agreement with the results of the primary multivariate analysis.

Discussion

In this study, we investigated the predictive value of preoperative PD-1⁺CD4⁺ cell counts for the development of PICs and their association with preoperative immune-inflammatory markers, nutritional indices, and physical vulnerability. The results showed that patients who underwent gastrectomy and developed PICs had significantly higher preoperative PD-1⁺CD4⁺/CD4⁺ cells. We also found significant associations between preoperative PD-1⁺CD4⁺/CD4⁺ cells, immune-inflammatory markers, nutritional indices, CCI, and the FRAS on admission.

Although there are increasing reports regarding the association of unfavorable long-term outcomes with PICs in several malignancies (7,12-14,33), the precise mechanism underlying

this association remains unclear (34). Various factors can lead to the development of PICs after gastrectomy, including the patients' physical condition, cancer stage, and surgical technique (35,36).

In their systematic review, Joharatnam-Hogan *et al* (37) demonstrated that elderly patients can benefit from curative treatment to a similar extent as younger patients. However, they emphasized that improving outcomes in physically frail populations requires an individualized approach to treatment approach, with greater reliance on indicators of functional age and frailty rather than chronological age when determining gastric cancer treatment (37). In this study, we focused on PD-1⁺CD4⁺ cells to evaluate their immunological vulnerability to PICs. T-cell function, which is essential for defense against infection, is regulated not only by the T-cell receptor but also by costimulatory molecules, such as PD-1 and cytotoxic T-lymphocyte-associated antigen-4 (38). Notably, PD-1⁺CD4⁺ cells are characterized by proliferative hyporesponsiveness

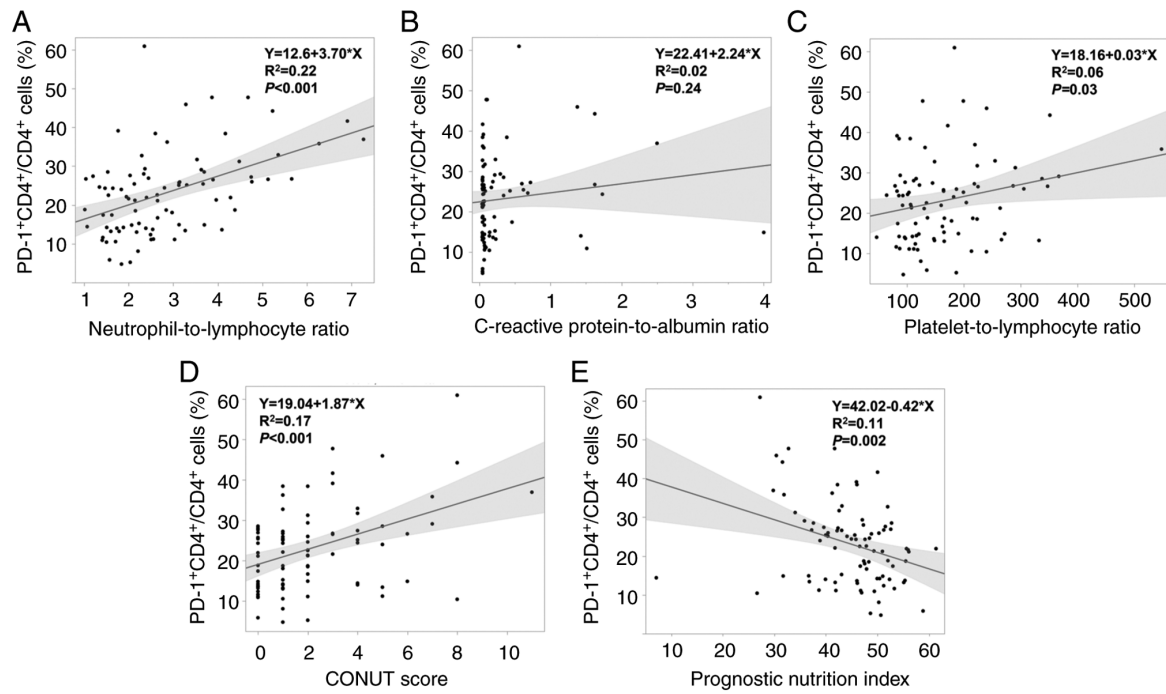


Figure 2. Correlation between PD-1⁺CD4⁺/CD4⁺ cells and immune-inflammatory markers and nutritional indices. (A) There are significant positive correlations between PD-1⁺CD4⁺/CD4⁺ cells and NLR, (C) PLR and (D) CONUT score ($P<0.05$), (E) whereas a significant negative correlation is observed between PD-1⁺CD4⁺/CD4⁺ cells and PNI ($P<0.05$). (B) There is no correlation between PD-1⁺CD4⁺/CD4⁺ cells and CAR ($P=0.23$). The gray zone indicates the 95% confidence interval. The regression equations are inserted. NLR, neutrophil-to-lymphocyte ratio; CAR, C-reactive protein-to-albumin ratio; PLR, platelet-to-lymphocyte ratio; CONUT, controlling nutrition status; PNI, prognostic nutritional index; PD-1, programmed cell death-1.

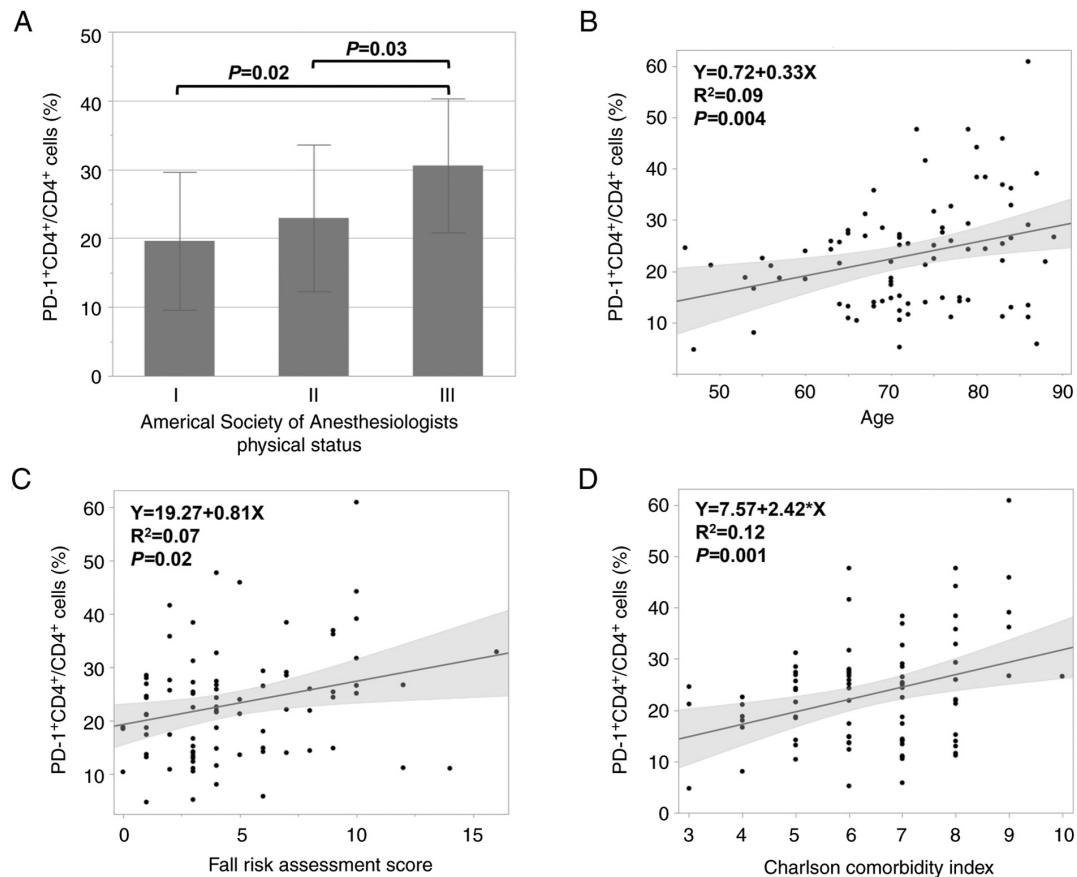


Figure 3. Correlation between the PD-1⁺CD4⁺/CD4⁺ cells and physical status. (A) The PD-1⁺CD4⁺/CD4⁺ cells are significantly increased as the ASA-PS increases ($P<0.05$). (B) There are significant positive correlations between PD-1⁺CD4⁺/CD4⁺ cells, age ($P<0.01$), (C) the fall risk assessment score ($P<0.05$) and (D) the Charlson comorbidity index ($P<0.01$). The gray zone indicates the 95% confidence interval. The regression equations are inserted. ASA-PS, American Society of Anesthesiologists physical status; FRAS, fall risk assessment score; CCI, Charlson comorbidity index; PD-1, programmed cell death-1.

Table III. Univariate and multivariate analyses that may affect the incidence of postoperative infectious complications.

Factor	Groups	Univariate analysis		Multivariate analysis		VIF
		OR (95% CI)	P-value	OR (95% CI)	P-value	
ASA-PS	I-II	1	0.053			1.35
	III	4.44 (0.97-20.2)				
FRAS	I	1	0.04	1	0.8	1.43
	II-III	2.68 (1.02-7.01)		0.85 (0.24-3.00)		
CONUT score	Normal, mild	1	0.98			1.55
	Median, severe	1.01 (0.28-3.63)				
NLR (median)	<2.44	1	0.09			1.38
	≥2.44	2.23 (0.86-5.82)				
CAR (median)	<0.06	1	0.01	1	0.08	1.24
	≥0.06	3.69 (1.29-10.5)		2.83 (0.87-9.19)		
PLR (median)	<145.5	1	0.31			1.42
	≥145.5	1.61 (0.63-4.10)				
PNI (median)	<46.1	1	<0.01	1	0.36	1.82
	≥46.1	0.21 (0.07-0.60)		0.54 (0.15-2.03)		
CCI (median)	<7	1	<0.01	1	<0.01	1.37
	≥7	7.06 (2.33-21.4)		6.05 (1.71-21.3)		
PD-1+CD4+/CD4+ cells (%, median)	<22.3	1	0.02	1	0.15	1.23
	≥22.3	3.15 (1.14-8.15)		2.41 (0.73-7.95)		

ASA-PS, American Society of Anesthesiologists physical status; FRAS, fall risk assessment score; CONUT, controlling nutrition status; NLR, neutrophil-to-lymphocyte ratio; CAR, C-reactive protein-to-albumin ratio; PLR, platelet-to-lymphocyte ratio; PNI, prognostic nutritional index; CCI, Charlson comorbidity index; PD-1, programmed cell death-1; OR, odds ratio; CI, confidence interval; VIF, Variance inflation factor.

and are incapable of responding to antigenic stimulation (26). These findings prompted us to investigate preoperative PD-1⁺CD4⁺ cells in patients with PICs.

We demonstrated that PD-1⁺CD4⁺/CD4⁺ cells were significantly associated with age, which supports and extends previous reports in mice (39). Foldi *et al* demonstrated that human immunodeficiency virus (HIV)-infected children not receiving antiretroviral therapy (ART) had a higher proportion of PD-1⁺CD4⁺/CD4⁺ cells than healthy and HIV-infected children on ART. They also indicated that PD-1⁺CD4⁺ cells preferentially produce Th1 (interferon- γ) and Th17 cytokines, despite weak proliferative potential (40). In humans, the process of aging upregulates PD-1 in natural killer cells and enhances pro-inflammatory cytokines, similar to that of HIV-infected children. Thus, patients who develop PICs may already be immunosuppressed, similar to older and HIV-infected individuals (41,42). Our previous research has reported that patients who developed PIC after surgery for gastric or esophageal cancer had a high preoperative level of MDSCs, which have a strong immunosuppressive effect (29). In addition, there was no correlation between the disease stage and PD-1⁺CD4⁺/CD4⁺ cells, suggesting that the elevated PD-1⁺CD4⁺/CD4⁺ cells in patients with PICs were not affected by tumor progression.

This study also revealed that preoperative PD-1⁺CD4⁺/CD4⁺ cells are associated with the FRAS and CCI, which reflects the patients' activity, comorbidity, and frailty. Several FRAS have been widely used in hospital-specific formats, including age,

medical history, physical dysfunction, activity status, mental dysfunction, medications, and assistance required for toileting, all of which may reflect patient physical frailty. We previously reported that patients with higher FRAS had longer hospital stays and poorer overall and recurrence-free survival than those with lower FRAS (16). Our findings regarding the association between preoperative PD-1⁺CD4⁺/CD4⁺ cells, FRAS and CCI implied an association between immunological frailty and physical frailty. In a previous study, Wang *et al* (43) demonstrated that a decline in CD4⁺ cells is associated with worse outcomes in older and frail patients with severe community-acquired pneumonia. Notably, although frailty is thought to be one of the causes of PICs immunity, to our knowledge, no studies on the association between PD-1⁺CD4⁺ cells and susceptibility to PICs have been conducted.

This study has some limitations. This retrospective and single-institutional study included a relatively small number of patients, which is associated with the potential limitations arising from heterogeneity in our retrospective cohort, including patient background, tumor stage, and perioperative management. We used the median value of PD-1⁺CD4⁺/CD4⁺ cells as an exploratory, hypothesis-generating cutoff value because no clinically validated biologically relevant threshold exists; however, the median split may not represent the optimal biologically relevant threshold, and future studies should aim to determine validated clinical cutoffs, ideally from larger prospective cohorts. Furthermore, CD4⁺T cells, particularly the Th17 subset, are known to secrete pro-inflammatory cytokines such as IL-17. We are currently planning prospective study based on this research,

and we hope to publish further findings on the relationship between preoperative PD-1⁺CD4⁺/CD4⁺ cells, the Th17 subset, and IL-17. In this study, physical frailty was evaluated using only ASA-PS, FRAS, and CCI; thus, it is necessary to assess physical frailty as sarcopenia and kinesiological evaluations in the future.

In conclusion, elevated preoperative PD-1⁺CD4⁺/CD4⁺ cells were associated with the development of PICs after gastrectomy, although multivariate analysis did not identify them as an independent predictor; rather, our findings highlight their associative value with immunological and physical frailty, suggesting that preoperative PD-1⁺CD4⁺/CD4⁺ cell measurement may have potential clinical relevance as part of a screening tool or multiparameter predictive model. Importantly, although our results did not demonstrate superiority over conventional clinical parameters such as immuno-inflammatory, nutritional, and frailty indices, PD-1⁺CD4⁺/CD4⁺ cell evaluation offers unique mechanistic insight into host immune competence by reflecting T-cell functional exhaustion mediated through immune checkpoint pathways—an aspect not directly captured by standard measures. Such immune profiling may provide complementary information to conventional indices and could, when integrated into composite prediction models, improve perioperative risk assessment and individualized management strategies. Future prospective studies are warranted to validate this potential additive value.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

NU, HT, NI, TK, HM, and HU conceived and designed the study. NU, NI, TK and HM conducted the experiments. NU, HH, KK, SF, TS, YY, RK, AI and HT interpreted the data. NU, HT, and HU prepared the manuscript. HT and HU supervised the study. NU and HT confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the Institutional Review Board of the National Defense Medical College on human experimentation and with the Helsinki Declaration of 1964 and later versions. The Institutional Review Board of the National Defense Medical College approved the study (permission no. 5070), and written informed consent was obtained prior to its commencement.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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