

Original Article



Potential Utility of FDG PET-CT as a Non-invasive Tool for Monitoring Local Immune Responses

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ABSTRACT

Purpose: The tumor microenvironment is known to be associated with the metabolic activity of cancer cells and local immune reactions. We hypothesized that glucose metabolism measured by 2-deoxy-2-(¹⁸F)fluoro-D-glucose (¹⁸F-FDG) positron emission tomography (PET)-computed tomography (CT) (¹⁸F-FDG PET-CT) would be associated with local immune responses evaluated according to the presence of tumor infiltrating lymphocytes (TILs).

Materials and Methods: We retrospectively reviewed 56 patients who underwent ¹⁸F-FDG PET-CT prior to gastrectomy. In resected tumor specimens, TIL subsets, including cluster of differentiation (CD) 3, CD4, CD8, Forkhead box P3 (Foxp3), and granzyme B, were subjected to immunohistochemical analysis. The prognostic nutritional index (PNI) was calculated as: (10×serum albumin value)+(0.005×peripheral lymphocyte counts). Additionally, the maximum standard uptake value (SUV_{max}) was calculated to evaluate the metabolic activity of cancer cells.

Results: The SUV_{max} was positively correlated with larger tumor size (R=0.293; P=0.029) and negatively correlated with PNI (R=-0.407; P=0.002). A higher SUV_{max} showed a marginal association with higher CD3 (+) T lymphocyte counts (R=0.227; P=0.092) and a significant association with higher Foxp3 (+) T lymphocyte counts (R=0.431; P=0.009). No other clinicopathological characteristics were associated with SUV_{max} or TILs. Survival analysis, however, indicated that neither SUV_{max} nor Foxp3 held prognostic significance.

Conclusions: FDG uptake on PET-CT could be associated with TILs, especially regulatory T cells, in gastric cancer. This finding may suggest that PET-CT could be of use as a non-invasive tool for monitoring the tumor microenvironment in patients with gastric cancer.

Keywords: Fluorodeoxyglucose F18; PET-CT; Tumor infiltrating lymphocytes; Regulatory T-cells; Tumor microenvironment

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide, and the third leading cause of cancer-related death [1]. Despite improvements in diagnostic methods and therapeutic strategies, the prognosis of gastric cancer is still determined by cancer stage alone [2]. However, accurate tumor staging can only be achieved after surgical resection.

Author Contributions

Conceptualization: Y.M.J., K.H.I.; Data curation: L.S., C.S., K.S.Y.; Formal analysis: L.S., K.H.I.; Funding acquisition: K.H.I.; Investigation: Y.M.J., K.H.I.; Methodology: L.S., C.S., K.S.Y.; Project administration: K.H.I.; Supervision: Y.M.J., K.H.I.; Writing - original draft: L.S.; Writing - review & editing: K.H.I.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

To predict prognosis in patients with cancer, 2-deoxy-2-(¹⁸F)fluoro-D-glucose (¹⁸F-FDG)-positron emission tomography (PET)-computed tomography (CT) (¹⁸F-FDG PET-CT) has been widely used [3-5]. In PET-CT analysis, increased cell metabolic activity is reflected by increased FDG uptake. This mechanism of action allows PET-CT to be used in diagnosing cancer severity [3,6] and in predicting response to preoperative chemotherapy [7]. In this respect, the metabolic activity of cancers can be considered an important factor affecting tumor biology and patient prognosis. In gastric cancer, however, the sensitivity and specificity of ¹⁸F-FDG PET-CT have been found to vary according to histologic type [8], limiting the role of this modality in the detection of primary tumors [9]. Notwithstanding, several studies have reported that increased FDG uptake by primary tumors and metastatic lymph nodes is associated with poor prognosis in gastric cancer [10-12].

Recently, the tumor microenvironment has emerged as another aspect important in the further understanding of tumor biology [13]. In particular, the tumor microenvironment has been found to play an essential role in the metabolic activity of cancers [14]. In this respect, the potential utility of ¹⁸F-FDG PET-CT as an indirect tool for monitoring the tumor microenvironment has been suggested [14-17]. Additional factors associated with the tumor microenvironment are tumor infiltrating lymphocytes (TILs) [18-20]. TILs are considered prognostic factors in local anti-tumor immunity and oncologic outcomes [21-24]. Indeed, in gastric cancer, several studies have reported a relationship between subsets of TILs and oncologic outcomes [22,25,26]. Interestingly, regulation of TIL subsets and the function of T cells have been shown to be influenced by the tumor microenvironment [18,19,27,28].

We hypothesized that the tumor microenvironment would be associated with the metabolic activity of cancer cells and would contribute to local immune responses in gastric cancer. Accordingly, we explored associations between FDG uptake on PET-CT and TILs in patients with gastric cancer.

MATERIALS AND METHODS

Patients

The present study included 56 patients with gastric cancer who underwent surgical resection and ¹⁸F-FDG PET-CT for staging workup at Severance Hospital, Yonsei University College of Medicine between June 2005 and December 2010. The medical records of these patients were retrospectively reviewed, and clinicopathological data were collected, including age, sex, tumor size, histologic type, pathologic T classification, and N classification. Tumor staging and pathologic grading were based on the American Joint Committee on Cancer (AJCC), seventh edition [29]. We obtained PET-CT workup and laboratory data, including serum albumin levels and lymphocyte counts, from baseline workup conducted within 2 months prior to surgery. The prognostic nutritional index (PNI) was calculated as follows [30]:

$$\text{PNI}=(10\times\text{serum albumin value [g/dL]})+(0.005\times\text{peripheral lymphocyte count [number/mm}^3\text{]})$$

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (4-2017-0824).

¹⁸F-FDG PET-CT imaging

¹⁸F-FDG PET-CT scans were performed with a PET-CT scanner (Discovery STe; GE Healthcare, Little Chalfont, UK; or Biograph TruePoint 40; Siemens Healthcare, Erlangen, Germany). All

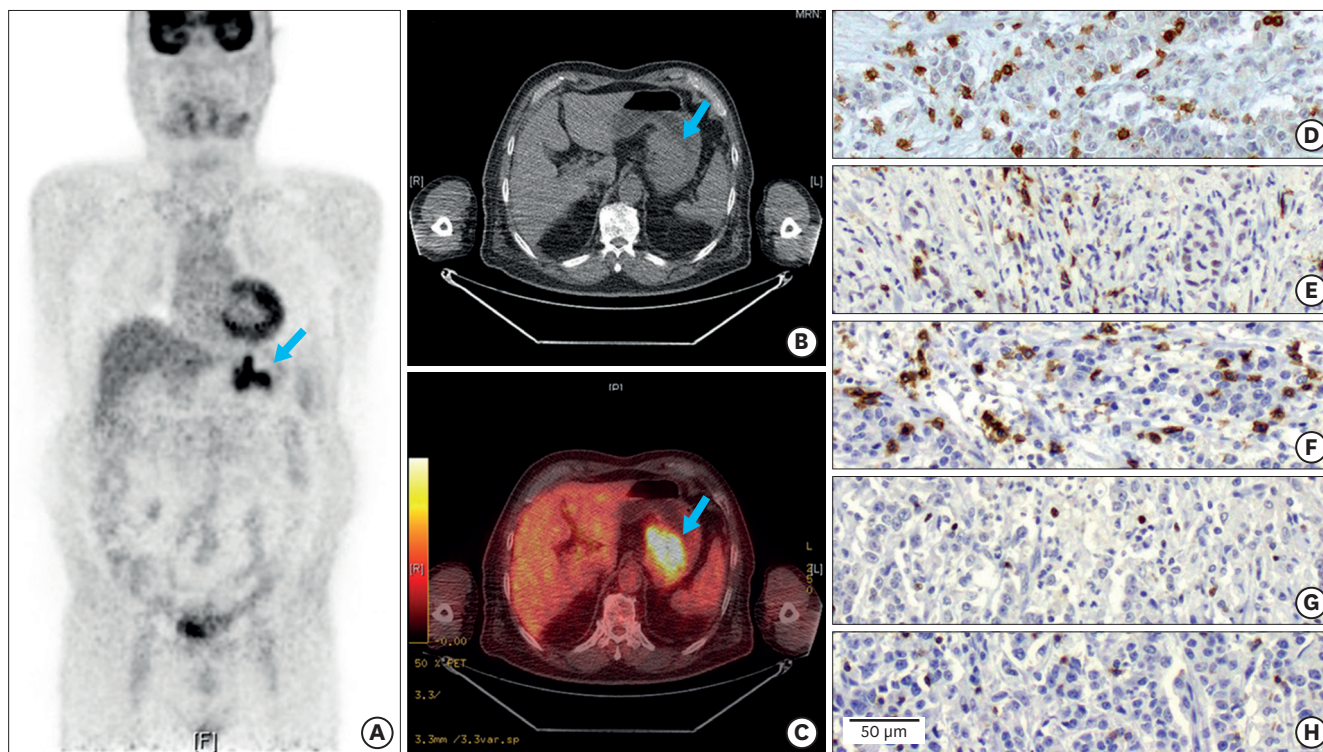


Fig. 1. Representative images of ¹⁸F-FDG PET-CT and immunohistochemical staining for TILs. (A) Whole-body ¹⁸F-FDG PET image demonstrates increased FDG uptake in stomach. (B, C) Axial CT and fused images indicate primary gastric cancer. (D-H) Immunohistochemical analysis of TILs, including CD3, CD4, CD8, Foxp3, and granzyme B.

¹⁸F-FDG = 2-deoxy-2-(¹⁸F)fluoro-D-glucose; PET = positron emitting tomography; CT = computed tomography; TIL = tumor infiltrating lymphocyte; CD = cluster of differentiation; Foxp3 = Forkhead box P3.

patients fasted for at least 6 hours before undergoing PET-CT scan, and a dose of 5.5 MBq/kg of ¹⁸F-FDG was intravenously injected 60 minutes prior to PET-CT. CT scans were initially performed at 30 mA and 130 kVp without contrast enhancement. After the CT scan was complete, a PET scan was performed with an acquisition time of 3 minutes per bed position in 3-dimensional mode. PET images were reconstructed using ordered subset expectation maximization with an attenuation correction. ¹⁸F-FDG PET-CT images were reviewed by nuclear medicine physicians (**Fig. 1A**). The maximum standard uptake value (SUV_{max}) on PET images was measured using volume viewer software (MIM-6.4; MIM software Inc., Cleveland, OH, USA). Each tumor was examined with a spherical-shaped volume of interest (VOI) that included the entire lesion in the axial, sagittal, and coronal planes. Using CT images, ¹⁸F-FDG uptake of normal organs, such as the brain, heart, liver, kidney, and small bowel was not included in the VOI. The SUV_{max} of the VOI was calculated as:

$$\text{SUV}_{\text{max}} \text{ of the VOI} = (\text{decay corrected activity/tissue volume}) / (\text{injected dose/body weight})$$

Immunohistochemistry of TILs

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded gastric cancer tissue sections. We used primary monoclonal antibodies for TIL subsets, including cluster of differentiation (CD) 3 (T lymphocytes, 1:100; Lab Vision Corp., Fremont, CA, USA; **Fig. 1B**), CD4 (helper T lymphocytes, 1:100; Novocastra Laboratories Ltd., Newcastle Upon Tyne, UK; **Fig. 1C**), CD8 (cytotoxic T lymphocytes, 1:100 Novocastra Laboratories Ltd.; **Fig. 1D**), Forkhead box P3 (Foxp3) (regulatory T lymphocytes, 1:100, ab20034; Abcam,

Cambridge, UK; **Fig. 1E**), and granzyme B (activated cytotoxic T lymphocytes, 1:100; Lab Vision Corp.; **Fig. 1F**). Stained slides were reviewed by an experienced pathologist who was blinded to patient data. Precise immunohistochemical staining methods and quantification of TILs were described in our previous study [22].

Statistical analysis

Categorical data are expressed as numbers with percentages, and continuous variables are expressed as means±standard deviations. Continuous variables were analyzed with analysis of variance (ANOVA). Correlation analyses were performed with Spearman's correlation analysis test. P-values less than 0.05 were considered statistically significant. Overall survival (OS) was compared with Kaplan-Meier analysis and the log-rank test. All statistical analyses were performed with SPSS software, version 23 (IBM Corporation, Chicago, IL, USA).

RESULTS

Patients

Clinicopathologic characteristics of the patients are shown in **Table 1**. Fifty-six patients with gastric cancer who underwent radical resection were included, including 36 men and 20 women. The mean tumor size was 57.6 mm. The majority of patients had poorly differentiated cancer (44.6%), T3 (30.4%), and T4 (32.1%) tumors, N0 tumors (33.9%), and stage III (37.5%) tumors. The mean SUV_{max} was 7.59, and the mean numbers of patients with CD3, CD4, CD8, Foxp3, and granzyme B TILs were 173.1, 104.2, 84.4, 18.0, and 19.9, respectively.

SUV_{max} on PET-CT and associated characteristics

In correlation analysis of clinicopathological characteristics, SUV_{max} was not associated with age, sex, histologic subtype, N classification, or final stage. A high SUV_{max} value was associated with larger tumor size (**Fig. 2A**, R=0.293; P=0.029) and advanced T classification (**Fig. 2B**, P=0.039), and showed a negative correlation with PNI (**Fig. 2C**, R=-0.407; P=0.002). In correlation analysis of TILs (**Fig. 2D-H**), SUV_{max} showed a marginal association with CD3 (+) lymphocytes (**Fig. 2D**, R=0.227; P<0.092) and a significant association with Foxp3 (+) regulatory T cell counts (**Fig. 2G**, R=0.431; P<0.001). CD4, CD8, and granzyme B were not associated with SUV_{max}.

Foxp3 (+)TILs and associated characteristics

Because only Foxp3 was correlated with SUV_{max}, we performed further investigation of Foxp3 and clinicopathological characteristics. Although Foxp3 and SUV_{max} were significantly correlated, Foxp3 counts showed poor correlation with clinicopathological characteristics, as shown in **Fig. 3**. Notably, however, Foxp3 counts were highest in patients with T3 disease and lowest in those with T4 disease (**Fig. 3C**, mean Foxp3 count=23.27 and 14.65, respectively, P=0.033).

Survival analysis

No OS differences were found between the groups with high and low SUV_{max} (**Fig. 4A**) or between the high and low Foxp3 (+) T cell groups (**Fig. 4B**).

DISCUSSION

In this study, we used SUV_{max} on ¹⁸F-FDG PET-CT to evaluate the metabolic activity of gastric cancer tumors and TIL subsets as a reflection of local immune responses. SUV_{max} showed positive

Table 1. Patient characteristics

Characteristics	Value
Age (yr)	59.3±12.5
Sex	
Male	36 (64.3)
Female	20 (35.7)
Tumor size (mm)	57.6±31.7
Histologic subtype	
AWD	8 (14.3)
AMD	14 (25.0)
APD	25 (44.6)
Other*	9 (16.1)
Pathologic T classification	
T1	13 (23.2)
T2	8 (14.3)
T3	17 (30.4)
T4	18 (32.1)
Pathologic N classification	
N0	19 (33.9)
N1	13 (23.2)
N2	9 (16.1)
N3	15 (26.8)
TNM stage	
I	13 (23.2)
II	17 (30.4)
III	21 (37.5)
IV	5 (8.9)
PNI	51.4±6.9
SUV _{max}	7.6±6.7
TIL subset	
CD3	173.1±52.0
CD4	104.2±59.5
CD8	84.4±28.5
Foxp3	18.0±9.5
Granzyme B	19.9±19.1
Foxp3/CD4 (%)	21.9±16.6

Data are shown as mean±standard deviation or number (%).

AWD = well-differentiated; AMD = moderately differentiated; APD = poorly differentiated; TNM = tumor, node, and metastasis; PNI = prognostic nutritional index; SUV_{max} = maximum standard uptake value; TIL = tumor infiltrating lymphocyte; CD = cluster of differentiation; Foxp3 = Forkhead box P3.

*Mucinous-1, signet-ring cell cancer-1, and undifferentiated-7.

correlations with tumor size and regulatory T lymphocytes, and a negative correlation with PNI. We noted no prognostic significance for SUV_{max} and regulatory T lymphocytes in the present study.

Standard uptake value is the ratio between ¹⁸F-FDG concentrations in a tumor and those throughout the entire body, and SUV_{max} is the maximum concentration of ¹⁸F-FDG in an organ of interest. High SUV_{max} generally indicates increased cancer metabolism, and has been shown to be associated with aggressive behavior, advanced disease status, and poor oncologic outcomes [5,11,31,32]. In the present study, we identified associations among tumor size, SUV_{max}, and regulatory T lymphocytes. The observed association between large tumor size and high SUV_{max} corroborates the findings of a previous report on gastric cancer [16]. To the best of our knowledge, however, we are the first to report a strong association between SUV_{max} and TILs: this relationship had never previously been explored in gastric cancer, and has rarely been studied in other solid cancers [33].

Hypothetically, increased glucose uptake in cancerous tissue would likely be associated with changes in the tumor microenvironment, reflecting tumor proliferation and increased

FDG Uptake in PET-CT and TILs

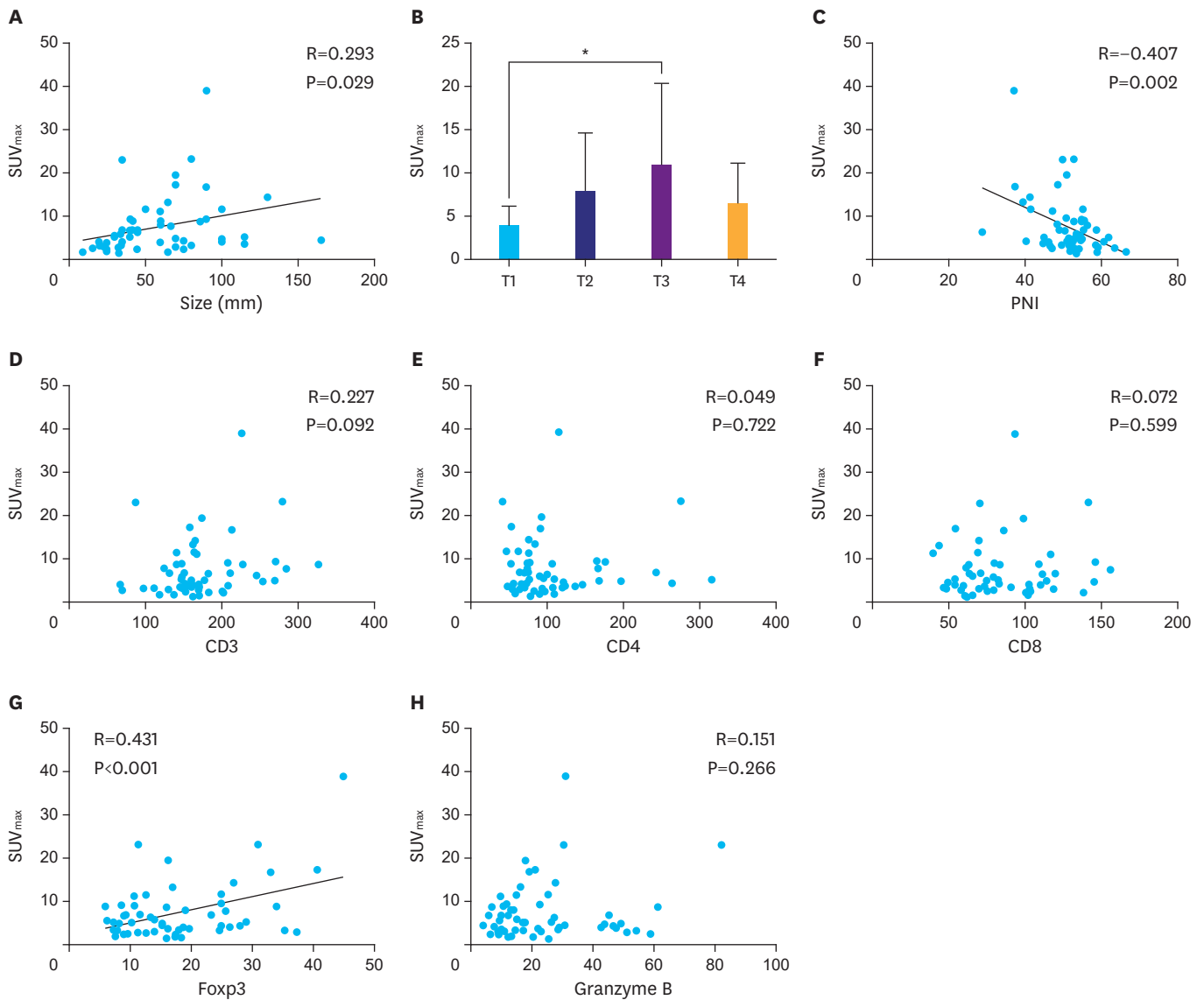


Fig. 2. SUV_{max} on PET-CT and associated characteristics. (A) Correlation analysis of SUV_{max} and tumor size. (B) SUV_{max} in comparison to T classification. (C-H) Correlation analysis of SUV_{max} and TIL subsets, including CD3, CD4, CD8, Foxp3, and granzyme B. Significant correlation shown only between Foxp3 and SUV_{max} (R=0.431, P<0.001).

SUV_{max} = maximum standard uptake value; PET = positron emitting tomography; CT = computed tomography; TIL = tumor infiltrating lymphocyte; CD = cluster of differentiation; Foxp3 = Forkhead box P3.

*P<0.05.

glucose metabolism [34,35]. These changes are also known to influence regulation of TIL subsets and the function of T cells [18,19,27,28]. Among the many factors in the tumor microenvironment that modulate the differentiation and function of TILs [36-39], the most relevant connection between SUV_{max} and Foxp3 could be hypoxia and acidic conditions. Regarding the former, FDG uptake is upregulated in hypoxic conditions [17,40,41], and it is well-known that hypoxia modulates immune responses in the tumor microenvironment [36,42] and promotes proliferation of regulatory T cells [18,43]. In relation to the latter, high lactate concentrations in the tumor microenvironment have been found to block lactate export in T cells, thereby perturbing the metabolism and function of these cells [27]. Although our study did not explore the mechanism linking high FDG uptake and high

regulatory T lymphocyte infiltration, our results suggest that ¹⁸F-FDG PET-CT could be of use as a potential diagnostic modality for assessing the tumor-immune microenvironment and as a predictive tool for identifying patients who might benefit from regulatory T cell depleting immunotherapy.

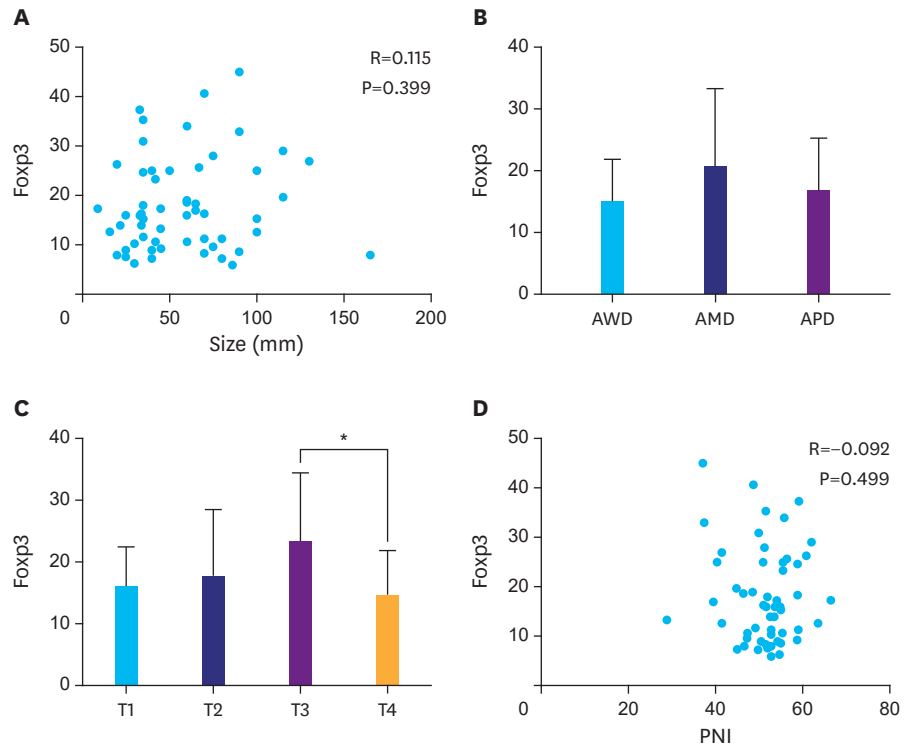


Fig. 3. Fxp3 (+) TILs and associated characteristics. (A) Correlation analysis of tumor size and Fxp3. (B, C) Comparison of Fxp3 according to histologic grade of gastric cancer and T classification. No significant associations among histologic grade and T classification were noted, except between T3 and T4. (D) Correlation analysis of PNI and Fxp3. Fxp3 = Forkhead box P3; TIL = tumor infiltrating lymphocyte; PNI = prognostic nutritional index; AWD = well-differentiated; AMD = moderately differentiated; APD = poorly differentiated. * $P < 0.05$.

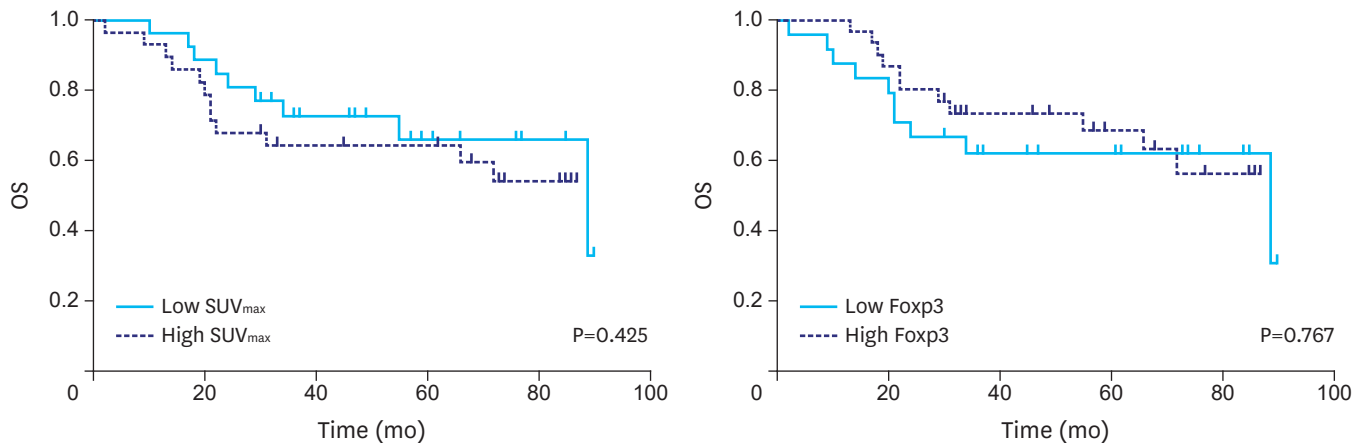


Fig. 4. Kaplan-Meier analysis according to SUV_{max} and Fxp3 positivity. (A) OS for the high SUV_{max} and low SUV_{max} groups. A median OS of 89 months was recorded in the low SUV_{max} group. However, a median OS was not defined in the high SUV_{max} group ($P = 0.425$). (B) OS between high Fxp3 and low Fxp3 groups. The median OS in the low Fxp3 group was 89 months, while the median OS in the high Fxp3 group was undefined ($P = 0.767$). The differences between the groups were calculated using the log-rank test. SUV_{max} = maximum standard uptake value; Fxp3 = Forkhead box P3; OS = overall survival.

The present study has several limitations. First, this was a retrospective observational study of a small number of cases. As a potential result thereof, our study identified no survival differences between the groups with high and low SUV_{max} and between the high and low Foxp3 (+) T cell groups, despite the fact that SUV_{max} and Foxp3 are well-known prognostic factors in gastric cancer [11,22]. Second, the biologic mechanisms underlying associations between higher numbers of regulatory TILs and high FDG uptake were not assessed. Nonetheless, the strength of the present study is that it is the first to evaluate associations among ¹⁸F-FDG uptake on PET-CT, clinicopathological characteristics, and TILs in gastric cancer.

In conclusion, the present study highlights a novel association between SUV_{max} on ¹⁸F-FDG PET-CT and regulatory T cells in gastric cancer, suggesting that the metabolic activity of gastric cancer cells could be related to local immune responses. Accordingly, this study provides the rationale for further studies of the role of ¹⁸F-FDG PET-CT as a non-invasive tool for monitoring cancer metabolism and immune responses in the tumor microenvironment of gastric and other cancers.

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