Prognostic Significance of Immunologic Factors and Metabolic Parameters in Triple-negative Breast Cancer

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Prognostic Significance of Immunologic Factors and Metabolic Parameters in Triple-negative Breast Cancer

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ABSTRACT

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Purpose: Triple negative breast cancers (TNBCs) are particularly aggressive tumors, however emerging data clearly indicate that TNBC is a heterogeneous class with variable prognosis according to clinical, pathologic, and genetic factors. We investigated the prognostic impacts of metabolic and immunologic signatures in TNBC.

Materials and Methods: Between February 2004 and December 2011, 145 patients with TNBC who had undergone preoperative ^{18}F-FDG PET/CT were included. The

metabolic standardized uptake value (SUV_{max}), metabolic tumor volume (MTV) as well as the total lesion glycolysis (TLG) of primary tumor were measured, and the expression of programmed death ligand-1 (PD-L1), programmed cell death 1 (PD-1), and Ki-67 proteins were evaluated in 117 tumor samples. The prognostic impact and relevance among parameters were assessed.

Results: At a median follow up of 53 months after surgery, 5-year progression-free survival (PFS) was 76% and 5-year overall survival (OS) was 82%. With a cut-off value of 6.06, high SUV_{max} group had significantly worse PFS (5-year: 88% vs. 74%, $p = 0.021$). High MTV (≥ 2.54) and high TLG group (≥ 5.57) also showed significantly worse PFS. High SUV_{max} group had younger patients (< 50), more advanced T stage (≥ T3), higher N stage (N3), and higher grade (grade 3) than low SUV_{max} group. Strong positive staining of PD-L1 (> 70%) was noted in 37 patients (32%). PD-L1 expression was significantly correlated with larger tumor size and PD-L1 strong positive group showed significantly poor prognosis (5-year PFS 59%, 5-year OS 70%), especially high rate of systemic recurrence. High PD-L1 expression and N3 stage were significant in multivariate analysis for both PFS and OS.

Conclusion: Increased ^{18}F-FDG uptake on PET/CT and PD-L1 expression was associated with significantly inferior clinical outcome, and it was correlated with several unfavorable prognostic factors including larger tumor size.

Key words: triple-negative breast cancer, ^{18}F-FDG PET/CT, standardized uptake value (SUV_{max}), PD-L1, progression-free survival

Prognostic significance of immunologic factors and metabolic parameters in Triple-negative Breast Cancer

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I. INTRODUCTION

Breast cancer is the second most common cancer in Korean women [1]. The management and prognosis of breast cancer depend on the size, histologic grade of the tumor, and hormonal receptor status. The positivity of the estrogen receptor (ER) or progesterone receptor (PR) is a predictive factor in a good prognosis and response to hormonal therapy. Four major molecular classes of breast cancer (luminal A and B, basal-like, and human epidermal growth factor receptor 2 (HER2) overexpressing) are identified by comprehensive gene expression profile analyses and are used as powerful predictive prognostic tools [2].

Triple negative breast cancers (TNBCs), defined as breast cancers that do not express the genes for ER, PR and HER2, are particularly aggressive with a poor

prognosis and higher recurrence rate than other subtypes of breast cancer and do not respond to receptor targeted treatments. TNBC makes up about 15% of all subtypes of breast cancer and the relative rarity of special subtypes within cases of TNBC render them difficult to study. Recently, emerging data clearly indicate that TNBC is a heterogeneous class with variable prognosis according to clinical, pathologic, and genetic factors [3-5]. Many studies to identify subgroups with distinct characteristics in TNBC to reliably select high and low- risk subsets of patients more exactly and apply tailored treatment options are in progress.

Positron emission tomography (PET)/computed tomography (CT) is one of well-established oncologic imaging tools to quantify functional tumor biology using ^{18}F-fluorodeoxyglucose (FDG) radiotracer which targets tumor cells that exhibit increased glucose metabolism. The clinical role of ^{18}F-FDG PET/CT also has increased in breast cancer for tumor detection and diagnosis, staging of loco-regional and distant metastasis, and monitoring the treatment response [6-8]. Previous studies in breast cancer have correlated high FDG uptake with tumor size, histological grade or hormonal receptor expression status (negativity of the hormonal receptor, HER2 overexpression, or triple negativity) [9-11], all of which are validated prognostic indicators, and reported that high FDG uptake in primary breast tumors correlated with disease progression [12] and/or shorter overall survival [13]. Especially, TNBC would be an excellent candidate for PET/CT staging because this subtype is more ^{18}F FDG-avid [14,15] than other phenotypes and also associated with a relatively poor prognosis. However, there is limited data upon utility of ^{18}F-FDG PET/CT in TNBC subtype

than other subtypes.

Recently, many immune pathways have been also studied in breast cancer and one of the important pathways is the interaction between programmed death ligand-1 (PD-L1) and programmed cell death 1 (PD-1). Some TNBC cells and tumor infiltrating immune cells express high levels of PD-L1, allowing cancer cells to weaken and escape immune surveillance [16,17]. Although the frequency of PD-L1 expression in breast cancer varies considerably as there is no standard immunohistochemical technique, there have been several reports that suggest PD-L1 expression was strongly associated with poor prognostic factors including younger age, larger tumor size, higher histologic grade, high Ki-67 expression and HER-2 expression, and the absence of ER and PR expression [17-20].

The aim of this study was to assess the prognostic impact of several prognostic factors including ^{18}F-FDG PET/CT parameters and immunologic signature, PD-L1 in this specific subtype as previously reported in other studies. Overall treatment outcome and patterns of failure in TNBC patients of our institution was also analyzed.

II. MATERIALS AND METHODS

1. Patient selection

Between January 2004 and December 2011, a consecutive series of 539

patients with newly diagnosed TNBC underwent surgery (breast conserving surgery or mastectomy) in our institution. Among these patients, 154 patients underwent whole-body ^{18}F-FDG PET/CT for initial staging before surgical treatment. After excluding 9 patients with unanalyzable ^{18}F-FDG PET/CT, we reviewed the medical records and pathology reports of 145 patients during the study period. This study was approved by the institutional review board of Severance Hospital, Yonsei University, Seoul, Republic of Korea in accordance with good clinical practice guidelines and the Declaration of Helsinki.

2. ^{18}F-FDG PET/CT method

^{18}F-FDG PET/CT scans were performed using a dedicated PET/CT scanner (Discovery STE, GE Healthcare, Little Chalfont, UK, or Biograph TruePoint 40, Siemens Healthcare, Erlangen, Germany). The detailed protocols for measurement of blood glucose concentration, determination of injected ^{18}F-FDG quantity, low-dose and contrast enhanced CT and PET scans, and PET data reconstruction have all been described previously [21]. Semiquantitative and volumetric measurements of maximum standardized uptake value (SUV_{max}), mean SUV (SUV_{mean}), metabolic tumor volume (MTV), and total lesion glycolysis (TLG) of whole body tumors were performed with the PETedge tool that is available in MIMvista software (MIMvista Corp., Cleveland, OH), according to the protocol of Liao et al.[22] After contouring the tumor using the PETedge tool, volumes of interest (VOIs) were automatically produced by spatial derivatives to locate the tumor surface. The estimated VOIs were manually adjusted

using a 2-D “ball” contouring tool. In this study, SUV_{max} was the maximum SUV_{max} of all primary breast tumors. TLG was calculated as follows: TLG = SUV_{mean}×MTV. MTV and TLG were computed after summing the corresponding values of all primary tumors. ^{18}F-FDG/PET was administered at the clinical discretion of the treating physician during the study period. The median values of SUV_{max}, MTV, as well as TLG of primary tumor measured in our study were as follows: 9.42 (range, 1.26-37.71), 7.88 (range, 0.44-515.32), and 37.73 (range, 1.34-3292.89), respectively.

3. Immunohistochemistry

The available tumor samples were collected from only 117 patients before the initiation of adjuvant chemotherapy and/or hormone therapy. The expression of PD-1, PD-L1, and Ki-67 were evaluated with formalin-fixed, paraffin-embedded tissue. Tissue sections of 4 um in thickness were deparaffinized, rehydrated, and washed two times in buffer. To reduce nonspecific background staining due to endogenous peroxidase, the slides were incubated in Hydrogen Peroxide Block for 10 minutes, and washed 4 times in buffer. The primary antibodies Anti-PD1 antibody (1:100, abcam, UK), and Anti-PD-L1 antibody (1:2000, EMD Millipore, Temecula, CA), Ki-67 antibody (1:200, Fremont, CA) were applied and incubated according to the manufacturers’ recommended protocols, and the slides were washed 4 times in buffer. The slides were then applied with Primary Antibody Enhancer, incubated for 20 minutes at room temperature, and then washed 4 times in buffer. Afterwards, HRP Polymer was applied to the slides, and the slides were incubated for 30 minutes at

room temperature and washed 4 times in buffer. They were then incubated with hematoxylin for chromogen, washed 4 times in deionized water, and counterstained.

Immunohistochemically stained slides from each subject were reviewed by an experienced pathologist who was blinded to all clinical data. PD-L1 expression was mainly confirmed in tumor cells and PD-1 expression was confirmed in lymphocytes. Since PD-L1 is expressed on the cell membrane as well as the endomembrane system, membranous as well as cytoplasmic staining was considered positive. We evaluated tumors as "PD-L1 positive" if ≥ 5% of the tumor cells or tumor infiltrating lymphocytes displayed at least moderate staining, and recorded the percentage of PD-L1 positive cells. Because there is no established standard regarding PD-L1, we made a cut-off value of 70% for PD-L1 to categorize groups as either strong positive (> 70%), weak positive (≤ 70%), or negative. In this study, 37 patients (32%) were included in strong positive group and 60 patients (51%) were included in weak positive group, and the rest 20 patients were negative for PD-L1 staining. Fig. 1 shows a representative example of PD-L1 immunohistochemical expression.

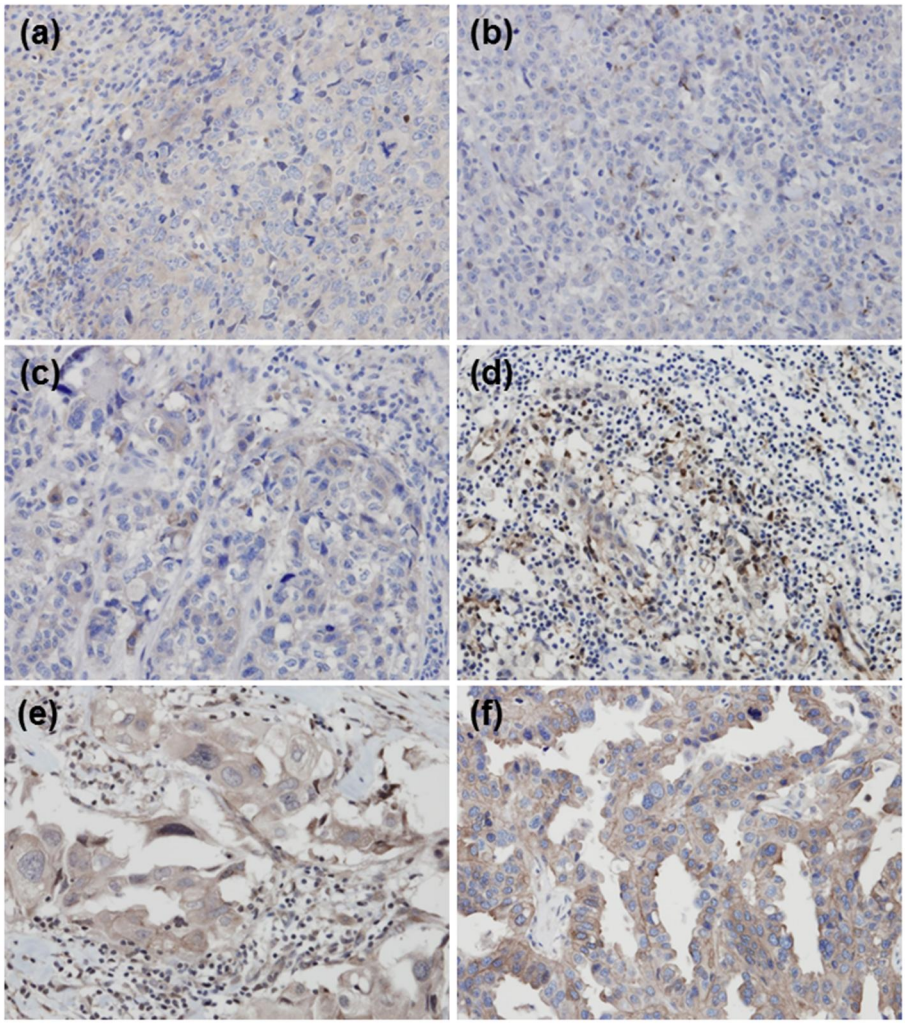

Fig. 1. Representative example of PD-L1 immunohistochemical expression (Magnification x 200)

(a) was classified as negative staining (< 5 %, only weak intensity).

(b) ~ (d) were classified as "weak positive" ≤ 70% ((b) 10%, (c) 20%, (d) 50%)

(e) and (f) were classified as "strong positive" ((e) 80%, (f) 100%)

Tumor was evaluated as PD-1 positive if ≥ 5% of the lymphocytes displayed PD-1 staining. Forty-six patients (39%) showed positive PD-1 staining and 71 patients (61%) showed negative PD-1 staining. Most patients (93%, n = 43) with positive PD-1 staining also showed positivity for PD-L1 expression. The Ki-67 of each case was evaluated based on the percentage of Ki-67-positive cells among at least 200 tumor cells. "High Ki-67 group" was defined as a tumor that > 20% of tumor cells were stained and Ki-67 expression was considered as negative if ≤ 20% of tumor cells were stained. Forty-six patients (39%) were included in low Ki-67 group and 81 patients (69%) were included in high Ki-67 group.

4. Statistical analysis

All cases were assigned to one of two groups according to the prognostic variables: SUV_{max} (high vs. low), Ki-67 (high > 20% vs. low ≤ 20%), PD-L1 (high > 70% vs. low ≤ 70%). Characteristics of the subgroups were compared using the Pearson's Chi-square test, Fisher's exact test, and Student T-test. The correlations among SUV_{max}, PD-L1 (%), and Ki-67 (%) were assessed using Spearman correlation analysis. For survival analysis, progression-free survival (PFS) and overall survival (OS) were defined as the time from the date of surgery to any recurrence or last follow-up, and to death from any cause or last follow-up, respectively. These rates were calculated using the Kaplan-Meier method and prognostic impacts of clinical factors were analyzed with the log-rank test (for categorical variables) and the logistic

regression analysis (for continuous variables). Variables with $p < 0.05$ in the univariate analysis were applied to a multivariate analysis to determine which variables were independently associated with PFS and OS. The data were analyzed using statistical software (SPSS ver. 20 (IBM, Armonk, NY, USA)). Statistical significance was defined as $p < 0.05$.

III. RESULTS

1. Patient, tumor, and treatment characteristics

Data on patient and tumor characteristics are summarized in Table 1. The median age of patients was 50 years (range, 24 to 79 years). American Joint Committee on Cancer (AJCC) stage I, II, and III TNBCs were diagnosed in 28 patients (19%), 70 patients (48%), and 47 patients (32%), respectively. Most patients (n = 131, 90%) were pathologically diagnosed with invasive/infiltrating ductal carcinoma. Breast-conserving surgery was done in 63 patients (43%), and 82 patients (57%) received mastectomy. Neoadjuvant chemotherapy was administered in about half of patients (n = 73, 50%), radiotherapy was delivered to 79% of patients, and adjuvant chemotherapy was administered in 76% of patients. We determined the cut-off point of PET parameters using the time-dependent Receiver-operating characteristic (ROC) curve in relation to PFS and OS. Youden's index was highest at the cut-off 6.06 (PFS) and 9.43 (OS) for SUV_{max}. Other highest cut-offs were 2.54

(PFS) and 5.55 (OS) for MTV, and 5.57 (PFS, OS) for TLG.

Table 1. Patient characteristics

Characteristics	N (145)	%
Age (year)	Median 50 (range, 24-79)	
< 50	68	46.9
≥ 50	77	53.1
BMI		
< 18.5	2	1.4
18.5~22.9	80	55.2
23~24.9	24	16.5
≥ 25	35	24.1
Unknown	4	2.8
Pathologic tumor size (cm)		
≤ 1	13	9.0
1~2	29	20.0
2~5	86	59.3
> 5	17	11.7
T stage		
T1	40	27.6
T2	88	60.7
T3	12	8.3
T4	5	3.4
N stage		
N0	60	41.4
N1	43	29.7
N2	23	15.8
N3	18	12.4
Nx	1	0.7
Numbers of positive pLNs		
None	90	62.1

1~3	36	24.8
4~9	11	7.6
≥ 10	7	4.8
pNx	1	0.7
AJCC stage		
I	28	19.3
II	70	48.3
III	47	32.4
Histologic grade		
G1	6	4.1
G2	39	26.9
G3	87	60.0
Unknown	13	9.0
Histologic subtype		
Invasive/Infiltrating ductal ca	131	90.3
Metaplastic ca	12	8.3
Others	2	1.4

Abbreviations: BMI, body mass index; AJCC, American Joint Committee on Cancer

2. Correlation among prognostic factors (PET parameters, Ki-67, and PD-L1)

Young age < 50 years, high grade tumors, and advanced tumors (N3, AJCC stage III) were associated with high SUV uptake ($SUV_{max} \geq 6.06$) (all p values < 0.05) (Table 2). Similar association was observed in high MTV group. In Spearman correlation analysis, there were also positive correlations between tumor size and SUV_{max} (rho 0.256, p = 0.002) and between tumor size and MTV (rho 0.309, p <0.001). Regarding Ki-67 labelling index, there were more high grade tumors (grade 3) (p <0.001) and N3 or AJCC stage III tumors (p = 0.061, 0.077) in high Ki-67 group.

There was no significant difference between high PD-L1 group and low PD-L1 group in Chi-square test (Table 3). However, pathologic tumor size was larger in high PD-L1 group by Student's t-test (mean 1.94 vs. 2.93, p = 0.035). To find any relevance among these prognostic factors (SUV_{max}, Ki-67, PD-L1), we did additional Spearman correlation analysis and positive correlation between Ki-67 and SUV_{max} (rho 0.224, p = 0.011) was observed while others were insignificant.

Table 2. Characteristics of high SUV_{max} (≥ 6.06) group and low SUV_{max} (< 6.06) group

Characteristics	SUV_{max} < 6.06 (N = 39)	SUV_{max} ≥ 6.06 (N = 106)	
	N (%)		p value
Age (year)			0.002
≥ 50	29 (74)	48 (45)	
< 50	10 (26)	58 (55)	
BMI			0.204
High	20 (51)	39 (37)	
Low	19 (49)	63 (59)	
Unknown	0 (0)	4 (4)	
Histol grade			<0.001
1	5 (13)	1 (1)	
2	19 (49)	20 (19)	
3	14 (36)	73 (69)	
Unknown	1 (2)	12 (11)	
Tumor size (cm)			0.561
≤ 5	36 (92)	92 (87)	
> 5	3 (8)	14 (13)	
T stage			<0.001

T1	23 (59)	17 (16)	
T2	13 (33)	75 (71)	
T3	3 (8)	9 (8)	
T4	0 (0)	5 (5)	
No. of +pLN			0.208
< 10	38 (97)	99 (93)	
≥ 10	1 (3)	7 (7)	
N stage			0.012
N0	23 (59)	37 (35)	
N1	10 (25)	33 (31)	
N2	4 (10)	19 (18)	
N3	1 (3)	17 (16)	
Nx	1 (3)	0 (0)	
AJCC stage			<0.001
1	17 (44)	11 (10)	
2	15 (38)	55 (52)	
3	7 (18)	40 (38)	

Abbreviations: SUV, standardized uptake value; BMI, body mass index; AJCC, American Joint Committee on Cancer

Table 3. Characteristics of high PD-L1 (> 70%) group and low PD-L1 (≤ 70%) group

Characteristics	High PD-L1 (N = 37)	Low PD-L1 (N = 80)	
	N (%)		p value
Age (year)			0.651
< 50	15 (41)	36 (45)	
≥ 50	22 (59)	44 (55)	
Histol grade			0.914
1	1 (3)	4 (5)	

2	10 (27)	23 (29)	
3	24 (65)	50 (63)	
Unknown	2 (5)	3 (4)	
$SUV_{max} \geq 6.06$			0.643
Yes	27 (73)	55 (69)	
No	10 (27)	25 (31)	
MTV ≥ 2.54			0.159
Yes	32 (86)	60 (75)	
No	5 (14)	20 (25)	
TLG ≥ 5.57			0.351
Yes	33 (89)	66 (82)	
No	4 (11)	14 (18)	
Tumor size (cm)			0.086
≤ 5	31 (84)	75 (94)	
> 5	6 (16)	5 (6)	
T stage			0.276
T1	9 (24)	28 (35)	
T2	22 (60)	47 (59)	
T3	4 (11)	4 (5)	
T4	2 (5)	1 (1)	
No. of +pLN			0.560
< 10	36 (97)	75 (95)	
≥ 10	1 (3)	4 (5)	
N stage			0.974
N0	18 (49)	38 (47)	
N1	11 (30)	23 (29)	
N2	5 (13)	11 (14)	
N3	3 (8)	7 (9)	
Nx	0 (0)	1 (1)	
AJCC stage			0.488
1	6 (16)	21 (26)	
2	20 (54)	38 (48)	
3	11 (30)	21 (26)	

Abbreviations: PD-L1, programmed death ligand-1; SUV, standardized uptake value;

MTV, metabolic tumor volume; TLG, total lesion glycolysis; AJCC, American Joint Committee on Cancer

3. Survival outcome

The median follow-up period was 53 months (range, 4 to 135 months). There were 30 recurrences (21%) and 27 deaths (17%), which consisted of 5 disease-unrelated events (3 unknown causes, 2 other medical condition). The 3-, 5-year PFS and OS were 78%, 76% and 86%, 82%, as shown in Fig. 2.

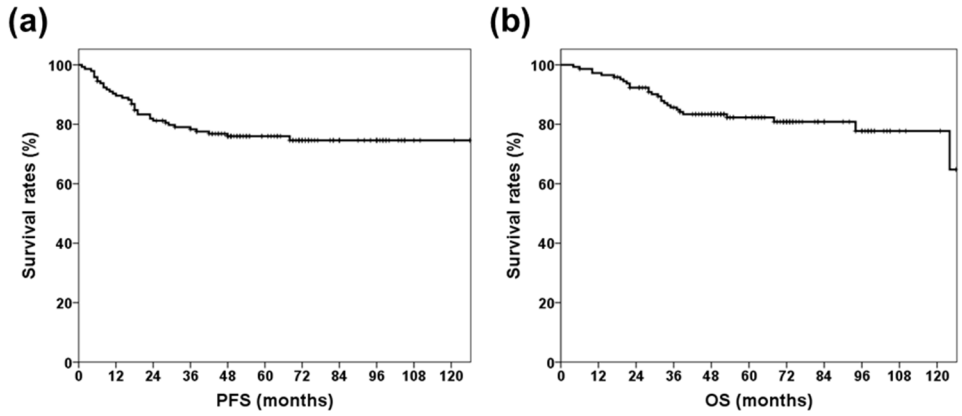

Fig. 2. Kaplan–Meier survival curves for (a) progression-free survival (PFS) and (b) overall survival (OS) in all patients (n = 145)

In univariate analysis, surgery type (mastectomy), number of pathologic LNs ≥ 10, N3 stage, advanced AJCC stage, sentinel lymph node biopsy (-), SUV_{max} ≥ 6.06, MTV ≥ 2.54, TLG ≥ 5.57, and PD-L1 > 70% were significant factors for poor PFS.

The number of pathologic LNs ≥ 10, N3 stage, advanced AJCC stage, sentinel lymph node biopsy (-), and PD-L1 > 70% were also significant factors for poor OS. With a cut-off value of 6.06, high SUV_{max} group had significantly worse PFS (5-year: 88% vs. 71%, p = 0.047). With a cut-off value of 2.54, high MTV group had significantly worse PFS (5-year: 93% vs. 72%, p = 0.026). High TLG group (≥ 5.57) also showed significantly worse PFS (5-year: 100% vs. 73%, p = 0.015) and OS (5-year: 100% vs. 80%, p = 0.048).

Among metabolic or immunologic factors, SUV_{max} ≥ 6.06 and PD-L1 > 70% were the most powerful prognostic factors and these variables could predict prognosis better with combined use. Patients with both SUV_{max} ≥ 6.06 and PD-L1 > 70% showed significantly worse outcome, and there was no recurrence or death in patients with both SUV_{max} < 6.06 and PD-L1 ≤ 70% (5-year PFS: 60% vs. 100%, p <0.001, 5-year OS: 68% vs. 100%, p = 0.005). Survival outcome of patients with only SUV_{max} ≥ 6.06 did not show significant difference with that of patients with only PD-L1 > 70%. (Fig. 3)

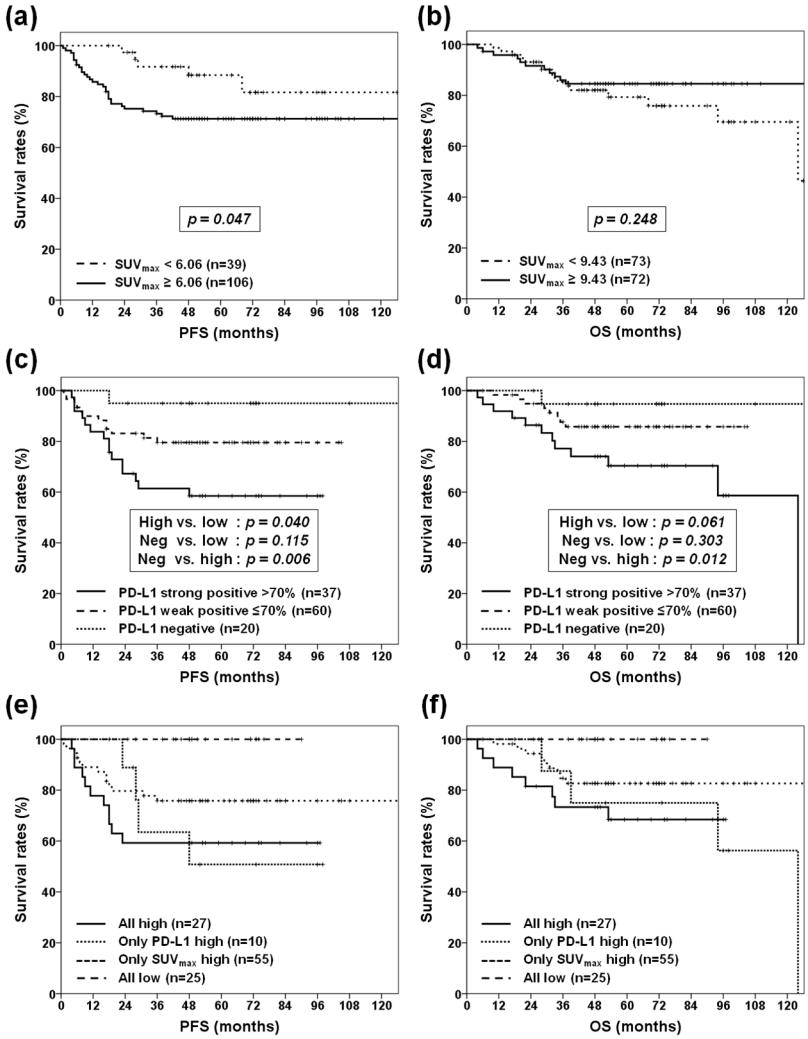

Fig. 3. Kaplan–Meier survival curves for progression-free survival (PFS) and overall survival (OS) according to each prognostic factor

(a) PFS and (b) OS according to high SUV uptake in all patients (n = 145).

I PFS and (d) OS according to positivity of PD-L1 staining in available patients (n = 117).

(e) PFS and (f) OS according to high SUV uptake and positivity of PD-L1 staining.

P values for PFS:

All high vs. only PD-L1 high: 0.749, All high vs. only SUV high: 0.122,

Only PD-L1 high vs. only SUV high: 0.360, All high vs. All low: <0.001*,

Only PD-L1 high vs. all low: <0.001*, Only SUV high vs. all low: 0.009*

P values for OS:

All high vs. only PD-L1 high: 0.778, All high vs. only SUV high: 0.144,

Only PD-L1 high vs. only SUV high: 0.158, All high vs. All low: 0.005*,

Only PD-L1 high vs. all low: 0.013*, Only SUV high vs. all low: 0.034*

In multivariate analysis, surgery (mastectomy), N3 stage, and PD-L1 > 70% were significant for PFS (p = 0.002, 0.004, 0.007, respectively), and N3 stage and PD-L1 > 70% were significant for OS (p = 0.019, 0.009, respectively). (Table 4)

Table 4. Univariate and multivariate analysis for PFS and OS

Characteristics	PFS			OS		
	Univariate	Multivariate		Univariate	Multivariate	
Categorical variables*	P value	HR (95% CI)	P value	P value	HR (95% CI)	P value
Age ≥ 50	0.850			0.155		
BMI < 23	0.749			0.924		
BCS* vs. mastectomy	0.004	5.4 (1.822-16.000)	0.002	0.008		
Tumor size (≤ 2 cm)	0.121			0.253		
≥ T3 vs. others (< T3)	0.666			0.319		
N3 vs. others (< N3)*	<0.001	5.079 (1.673-15.421)	0.004	<0.001	4.695 (1.292-17.057)	0.019
No. of +pLN ≥ 10 vs. others*	<0.001			<0.001		
AJCC stage (low)	0.001			0.002		
Histologic grade (low)	0.822			0.317		
Neoadjuvant chemotherapy	0.089			0.091		
Adjuvant chemotherapy	0.307			0.584		
SLNB (yes)	0.002	1.366 (0.510-3.659)	0.536	0.013	1.062 (0.336-3.359)	0.918
SUV_{max} < 6.06	0.021	2.297 (0.753-7.007)	0.144	0.14		
MTV < 2.54	0.064			0.255		

TLG < 5.57	0.028			0.078	280626 (0.000-α)	0.971
Ki-67 > 20%	0.388			0.537		
PD-L1 ≤ 70%	0.005	2.839 (1.339-6.019)	0.007	0.006	3.207 (1.331-7.723)	0.009
PD-1 (negative)	0.434			0.714		
PD-L1 ≤ 70% and PD-1 (-)	0.009			0.006		
Continuous variables¶						
SUV_{max}	0.359			0.417		
MTV	0.672			0.833		
TLG	0.695			0.886		
Ki-67	0.972			0.603		
PD-L1	0.021			0.016		

Better prognostic factors are demonstrated and are marked with *.

¶ For continuous variables, logistic regression analysis method was used.

Abbreviations: PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; BMI, body mass index; BCS, breast conserving surgery; AJCC, American Joint Committee on Cancer; SLNB, sentinel lymph node biopsy; SUV, standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; PD-L1, programmed death ligand-1; PD-1, programmed cell death 1

4. Patterns of failure

Systemic recurrence was the most common type of failure (26/30, 87%) (Local only: 3/30, 10%, regional only: 1/30, 3%, systemic only: 19/30, 63%, regional + systemic: 4/30, 13%, local + systemic: 3/30, 10%). In patients without neoadjuvant chemotherapy (n = 73), 11 recurrences were noted and systemic recurrence was most common (9/11, 82%). It was similar to 89% (17/19) in patients with neoadjuvant chemotherapy. In chi-square test to find prognostic factor that can predict systemic recurrence rate, there was no significant difference between systemic recurrence (+) group and systemic recurrence (-) group. Otherwise, strong positivity of PD-L1 expression (> 70%) was the only significant factor for systemic recurrence (p = 0.05), and 13 recurrences in PD-L1 strong positive group were all systemic recurrences.

IV. DISCUSSION

In this study, we investigated prognostic impacts of clinical, metabolic, and immunologic signatures and whether any correlation existed among variables in 145 operable, stage I-III TNBCs. We observed that high PET uptake of primary tumor and high PD-L1 expressions were significantly correlated with prognosis as other important clinical factor including N3 stage. High SUV_{max} group had younger patients (< 50), more advanced T stage (≥ T3), higher N stage (N3), and higher grade (grade 3). In addition, there was a positive correlation between Ki-67 and SUV_{max} variables. PD-

L1 expression was significantly correlated with larger tumor size and high rate of systemic recurrence in PD-L1 strong-positive group. The poorest prognosis was expected when these two were combined in use (high SUV_{max} and PD-L1 strong-positive expression).

In several reports of breast cancer, ^{18}F-FDG uptake expressed as SUV_{max} was significantly correlated with tumor size, histological grade or hormonal receptor expression status, all of which are important prognostic indicators for long-term survival in breast cancer patients. TNBCs are generally PET-avid and tumors with aggressive biology are more associated with higher SUV_{max} than ER-positive or HER2-positive breast cancers. Tchou et al. [23] and Koo et al. [24] suggested that SUV_{max} was higher in TNBCs with larger tumor size and/or higher Ki-67 scores. ^{18}F-FDG PET/CT in TNBC has been also regarded as a tool to help predict a patient's response to chemotherapy and a risk of early relapse, and high SUV_{max} was even the only significant independent prognostic factor in recent series. Our results, consistent with those of previous studies, support the idea that ^{18}F-FDG PET/CT has the potential to be used as a noninvasive tool to assess the Ki-67 proliferation index in TNBCs and to select TNBCs with more aggressive biological feature. Furthermore, our study included large numbers of patients (n =145) and correlated this finding with prognosis using adequate follow-up data. In particular, it is the first study that investigated relevance to other immunologic factors as PD-1 and PD-L1.

PD-1 is constitutively expressed on the surface of T-cells and controls immune reactions. PD-1 has two ligands (PD-L1 and PD-L2) [25] and both are

expressed in tumor cells and tumor-infiltrating lymphocytes (TIL). This immune pathway has been studied in several tumor types including breast, lung, kidney, and malignant melanomas [26]. In breast cancer, PD-L1 expression is known to be significantly higher in TNBC than non-TNBC patients ($p < 0.001$) [27]. Ghebeh et al. [17] and Muenst et al. [18] reported an association between PD-L1 expression and larger tumor size, higher tumor grade, HER2 expression, and absence of ER expression, positive lymph node status and the association with high Ki-67 expression was also noted in larger patient cohort [18,20]. These findings could be translated into the hypothesis that the activation of the PD-1/PD-L1 pathway may help these tumors evade antitumor immune responses and consequently proliferate and spread more rapidly. Our study also demonstrated larger tumor size in PD-L1 strong positive group (mean 1.94 vs. 2.93 cm, $p = 0.035$) although we could not find any other significant association with clinical, metabolic parameters in our study cohort.

However, the prognostic value of PD-L1 is still to be defined. PD-L1 expression was associated with poor prognosis in pancreatic and renal cell cancers [28] and several series have suggested that the same trend also existed in breast cancers after first study by Muenst et al. [18,29]. Consistent with these studies, we also demonstrated that PD-L1 expression could translate into a strong negative prognostic biomarker for PFS especially in TNBCs. However, recent reports found quite the opposite. PD-L1 expression was associated with better outcomes in different cancer types [30-32] as well as breast cancers [19,33]. The large cohort analyzed 3916 breast tumors and found a significant association between PD-L1 expression and longer disease-

specific survival in ER-negative disease. The survival relationship between PD-L1 expression in breast cancer and better outcomes could be explained by the presence of a strong antitumor immune response leading to PD-L1 up-regulation. CD8-positive T cells and interferon γ were suggested as one of related immune pathways [27,34]. Another hypothesis is that the expression of PD-L1 might represent antigen-induced antitumor immune pressure by resulting in recruitment of TIL to the tumor site, where they induce a partial antitumor effect[35]. However, there is still conflict on the prognostic value of PD-L1 and the study performed in specific subtype as TNBC is limited until now.

The frequency of PD-L1 expression in breast cancer varies considerably as there is no standard immunohistochemical technique. In breast cancer series, the expression rates of PD-L1 were reported from 23.4% to 58% and different antibody clones and different scoring systems were used. Muenst et al.[29] used the modified Histo-score (H-score) (both frequency and intensity were scored) and cut-off score of ≥ 100 for positive expression. Higher rates even larger than 50% were reported by recent studies in which PD-L1 mRNA expression level was evaluated [17,36]. By other authors, tumors were evaluated as PD-L1 positive if ≥ 5% of the tumor cells displayed at least moderate staining in non-small lung cancers [37]. In gastric cancer, distribution was graded according to the percentage of PD-L1 positive cancer cells and then divided into quartiles as follows: no staining, 0~5 % staining; 1+, 6~25 % staining; 2+,26~50 % staining; 3+, 51~75 % staining; and 4+, 76~100 % staining. A total score of more than 3+ (> 25%) was defined as PD-L1-positive expression [38]. We performed

an arbitrarily determined dichotomous classification of the PD-1, PD-L1 proteins for statistical purpose by referring other studies because no standard cutoff points have been described so far. As we wanted to clarify the prognosis of “the strong PD-L1 positive group”, not just positive PD-L1 expression considering the uncertainty of scoring method, we used cut-off of 70% for PD-L1 and divided patients in 3 subgroups (PD-L1 strong positive vs. PD-L1 weak positive vs. PD-L1 negative). The number of patients with both PD-1 positivity and PD-L1 strong positivity was 21 (57% in PD-L1 strong-positive group).

Our study has several limitations. First, patients who received neoadjuvant chemotherapy were included. In this study, staging was done clinically with preoperative image findings in patients with neoadjuvant chemotherapy while pathologic findings were used in patients with upfront surgery. It is true that there is limitation to analyze these patients in the same manner, however, we used pre-chemotherapy PET-CT and biopsied tumor samples before the influence of chemotherapy for these patients trying to minimize the bias. The same trends were found after performing analysis only in patients without neoadjuvant chemotherapy (PD-L1 as a poor prognostic factor, not included in this manuscript). Second, only Small numbers of tumor samples (n = 115) were included in analysis because we wanted to identify whether there is any relevance among PET parameters, immunogenic proteins, and prognosis, therefore, we initially included only patients with preoperative PET/CT. It might also unavoidably introduce selection bias. Even with these limitations, this study would be worthy to note for that statistically

significant results were consistently found in small numbers of cohort, only composed of TNBCs. The analysis of PD-L1 expressions can reveal important implications for breast cancer treatment, especially in TNBC which is known to company with high rate of PD-L1 expression.

V. CONCLUSION

We investigate the correlation between PD-L1 protein expressions with other clinical and metabolic features and progression-free survival and overall survival in TNBCs for the first time. Although we could not find any significant correlation between metabolic and immunologic factors, PD-L1 was suggested as a strong unfavorable factor, more powerful with SUV_{max}. Further prospective study with larger patient population is warranted to define the role of these prognostic factors in the treatment decision for TNBC patients more exactly.

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ABSTRACT (IN KOREAN)

삼중 음성 유방암 환자에서 여러 면역학적, 대사적 인자들의 예후적 중요성

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최 서 희

목적: 삼중 음성 유방암은 특히 예후가 나쁜 종양으로 알려져 있으나, 많은 최근 연구 결과에 따르면 임상, 병리, 유전 인자들에 따라 예후가 그 안에서도 서로 다를 것으로 여겨지고 있다. 따라서 본 연구에서는 삼중 음성 유방암에서 대사, 면역학적 인자들의 예후적 중요성을 확인해보고자 하였다.

대상 및 방법: 2004년 2월부터 2011년 12월까지 수술 전 ^{18}F-FDG PET/CT 가 시행되었던 145명의 삼중 음성 유방암 환자들을 포함하였다. 해당 환자들의 ^{18}F-FDG PET/CT 에서는 원발성 종양의 standardized uptake value (SUV_{max}), metabolic tumor volume (MTV), the total lesion glycolysis (TLG) 를 포함한 대사 인자들을 측정 하였고 그 외에도 117명의 종양 조직에서 programmed death ligand-1 (PD-L1), programmed cell death 1 (PD-1), Ki-67 단백질 발현 정도를 측정

하였으며 예후와의 관련성 및 인자들간의 연관성을 평가하였다.

결과: 대상 환자의 중앙추적조사기간은 수술로부터 53개월이었으며, 5년 무질병생존율은 76%, 5년 생존율은 82% 였다. SUV_{max} 에 대해 6.06의 절단점을 기준으로 하였을 때, 고 섭취를 보인 환자들에서 유의하게 나쁜 예후를 보였으며 (5년 무질병생존율: 88% 대 75%) MTV 혹은 TLG 가 높은 그룹에서도 동일한 결과를 보였다. 고 섭취 환자군 (SUV_{max}) 에서는 젊은 환자 (50세 미만), 진행 병기 (T3, N3 이상), 고등급 (grade 3) 종양이 유의하게 많은 것으로 나타났다. PD-L1 의 강한 발현(< 70%)은 전체 중 37명의 환자 (32%) 에서 보였으며 PD-L1 의 발현성은 유의하게 큰 종양 크기와 관련이 있었다. 또한 PD-L1 의 강한 발현을 보이는 환자들은 유의하게 나쁜 예후를 보였고 (5년 무질병생존율: 59%, 5년 생존율: 70%) 다변량 분석 시 예후과 유의하게 연관 있는 인자로 확인 되었다.

결론: PET/CT 상 ^{18}F-FDG 의 강한 섭취 및 PD-L1 단백의 강한 발현은 삼중 음성 유방암에서 나쁜 예후 인자였으며, 종양 크기를 포함한 여러 다른 임상적 인자들과 연관 관계를 보였다.

핵심되는 말: 삼중 음성 유방암, ^{18}F-FDG PET/CT, 표준화된 섭취값 (SUV_{max}), PD-L1 단백, 무질병생존율