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Comparison of Programmed Cell Death Ligand 1 Status between Core Needle Biopsy and Surgical Specimens of Triple-Negative Breast Cancer

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Purpose: Pembrolizumab is currently used to treat advanced triple-negative breast cancer (TNBC) and high-risk early TNBC with neoadjuvant chemotherapy (NAC). The tumor-infiltrating lymphocyte (TIL) level and programmed cell death ligand 1 (PD-L1) status are predictors of response to NAC and immune checkpoint inhibitor treatment. We aimed to investigate whether the PD-L1 status in core needle biopsies (CNBs) could represent the whole tumor in TNBC.

Materials and Methods: A total of 49 patients diagnosed with TNBC who received upfront surgery without NAC between January 2018 and March 2021 were included. The PD-L1 expression (SP142 and 22C3 clones) and TIL were evaluated in paired CNBs and resected specimens. The concordance PD-L1 status and TIL levels between CNBs and resected specimens were analyzed.

Results: PD-L1 positivity was more frequently observed in resected specimens. The overall reliability of TIL level in the CNB was good [intraclass correlation coefficient (ICC)=0.847, p<0.001]. The agreements of PD-L1 status were good and fair, respectively (SP142, κ=0.503, p<0.001; 22C3, κ=0.380, p=0.010). As the core number of CNB increased, the reliability and agreement also improved, especially from five tumor cores (TIL, ICC=0.911, p<0.001; PD-L1 [22C3], κ=0.750, p=0.028). Regarding PD-L1 (SP142), no further improvement was observed with ≥5 tumor cores (κ=0.600, p=0.058).

Conclusion: CNBs with ≥5 tumor cores were sufficient to represent the TIL level and PD-L1 (22C3) status in TNBC.

Key Words: Triple-negative breast cancer, tumor-infiltrating lymphocytes, PD-L1, immunohistochemistry, pathology

INTRODUCTION

Tumor-infiltrating lymphocytes (TILs) are one of the components of the tumor microenvironment. In breast cancer, higher TIL level is associated with higher pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC) as well as better prognosis in patients with triple-negative breast cancer (TNBC).[1-4] Programmed cell death ligand-1 (PD-L1) expressed in the tumor cells interacts with T cells and inhibits the host anti-tumor immune response.[5] Immune checkpoint inhibitors (ICIs) are antibodies targeting the PD-L1, and the level of PD-L1 expression correlates with the treatment response.[6,7] In breast cancer, ICIs including atezolizumab and pembrolizumab were approved for use in the treatment of metastatic TNBC.[5,7] Not only for advanced TNBC but also the current standard treatment of early-stage TNBC is NAC combined with ICIs, with pCR being the primary endpoint.[8-10] In the neoadjuvant setting, evaluation of TIL level and PD-L1 expression can only be performed on core needle biopsy (CNB). In addition, TIL and PD-L1 expressions are intratumorally heterogeneous.[11,12] Thus, whether the TIL level and PD-L1 status in CNBs could repre-

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sent the status of the whole tumor is important for establishing further treatment plans for TNBC patients. Since comparative studies of the PD-L1 status between CNBs and resected specimens are limited,[13-15] we aimed to evaluate the concordance of the TIL level and PD-L1 status in paired CNB samples and resected specimens of TNBC patients.

MATERIALS AND METHODS

This retrospective study was approved by the Institutional Review Board of Gangnam Severance Hospital (no. 3-2021-0452), and the requirement for an informed consent was waived.

Patient selection and clinicopathologic evaluation

A total of 49 patients diagnosed with TNBC by CNB, who underwent upfront surgery in Gangnam Severance Hospital between January 2018 and March 2021, were selected for the study. The clinical and pathological data were obtained from electronic medical records. The following data were obtained: patient's age at initial diagnosis, size of the invasive tumor, number of cores containing tumor tissues obtained by CNB, TIL levels in the CNBs and resected specimens, and PD-L1 status (SP142 and 22C3 clones).

All hematoxylin and eosin-stained slides and immunohistochemistry (IHC) slides of PD-L1 (SP142) and PD-L1 (22C3) of CNBs and matched resected specimens were retrospectively reviewed by two pathologists (Hyungwook Choi and Yoon Jin Cha).

Evaluation of TILs

The stromal TIL levels were scored according to the guidelines for TIL assessment.[16] Briefly, the intratumoral stromal area occupied by the mononuclear inflammatory cells (MICs), including lymphocytes and plasma cells in the total intratumoral stromal area, was calculated as a percentage. The tumor area, defined by the boundaries of invasive tumor cells, was evaluated. Meanwhile, those of polymorphonuclear leukocytes, granulocytes, dendritic cells, and macrophages were not scored. The areas outside the tumor border, around the intraductal component, and normal lobules were also excluded. Within the tumor border, TILs in the tumor zones with extensive fibrosis, crush artifacts, necrosis, and regressive hyalinization were excluded. A full assessment of the average TILs in the tumor area was performed on a representative section of the whole tumor; the average score was reported as a percentage.

In the CNBs, all cores containing invasive tumor cells were evaluated. Since the true tumor borders may be unclear in CNBs, imaginary tumor borders were defined as the area containing the invasive carcinoma in each core. As in the resected specimens, a full assessment of the average TIL levels was performed in the CNBs within the imaginary tumor borders. The TIL scores were subclassified as low (<30%) and high (≥30%).[17] Cases harboring consistently low- or high TIL in both CNB and resected specimen were defined as low-TIL and high-TIL groups, respectively.

IHC and clinical classification of TNBC

All immunohistochemical markers were assessed using a light microscope. A cut-off value of ≥1% positively stained nuclei was used to define estrogen receptor (ER, clone 6F11; dilution 1:200; Leica Biosystems, Wetzlar, Germany) and progesterone receptor (PR, clone 16; dilution 1:500; Leica Biosystems) positivity.[18] Human epidermal growth factor receptor 2 (HER2) (clone 4B5; dilution 1:5; Ventana Medical System, Oro Valley, AZ, USA) staining was analyzed according to the American Society of Clinical Oncology/College of American Pathologists guidelines using the following categories: 0=no immunostaining; 1+=weak incomplete membranous staining in <10% of tumor cells; 2+= complete membranous staining, either uniform or weak, in ≥10% of tumor cells; 3+=uniform intense membranous staining in ≥10% of tumor cells.[19] HER2 IHC was considered positive when strong (3+) membranous staining was observed, whereas 0 and 1+ were considered negative. Samples showing 2+ HER2 expression were further evaluated for HER2 amplification by silver in situ hybridization. Based on the abovementioned interpretation, only samples of ER-negative, PR-negative, and HER2-negative breast cancers were defined as TNBC and included in this study.

PD-L1 (clone SP142; dilution prediluted; Ventana Medical System) and PD-L1 (clone 22C3; dilution 1:50; Dako, Carpinteria, CA, USA) IHC were performed. The result of PD-L1 IHC was reported as the immune cell (IC) percentage (for SP142 clone) or combined proportional score (CPS) (for 22C3 clone) according to the interpretation guidelines and scoring algorithm provided by the manufacturer.[10,20] The IC scoring of PD-L1 (SP142) expression was performed based on the proportion of tumor area covered with any discernible PD-L1 staining of any intensity in ICs, including lymphocytes, macrophages, granulocytes, and dendritic cells. The tumor area was defined by the area occupied by tumor cells as well as their associated intratumoral and contiguous peritumoral stroma. The area with necrosis or foreign material was excluded from the tumor area. Only tissues containing at least 50 viable tumor cells with associated stroma were considered adequate and included in this study. The PD-L1-stained IC associated with ductal carcinoma in situ or lobular carcinoma in situ, PD-L1-stained giant cells associated with biopsy, and speckling granular cytoplasmic staining pattern were excluded from the scoring. In the biopsy tissue, the final IC score was calculated based only on the biopsy cores containing invasive tumor cells. The IC score in the resected specimen was calculated as described above. PD-L1 (SP142) positivity was defined as an "IC score ≥1%."

The CPS of PD-L1 (22C3) expression was calculated as the number of PD-L1 staining cells, including tumor cells, lymphocytes, and macrophages, divided by the total number of all via-

ble invasive tumor cells, and then multiplied by 100. Only invasive viable tumor cells with any perceptible and convincing partial or complete linear membrane staining at any intensity were considered as positive PD-L1 staining. Only MICs, that is, lymphocytes and macrophages, which showed membrane and/or cytoplasmic staining at any intensity within the invasive tumor nests and/or adjacent supporting stroma were considered as positive PD-L1 staining. The adjacent MICs were present within the same ×200 field as the invasive tumor and directly associated with tumor response. The CPS was determined at ×200 magnification. Areas with edge artifact, crushing artifact, or necrosis were excluded from scoring. Only tissue specimens containing at least 100 viable tumor cells were considered adequate and were included in this study. In the biopsy tissue, the final CPS was only calculated in the biopsy cores containing invasive tumor cells. The CPS in the resected specimen was calculated as described above. PD-L1 (22C3) positivity was defined as a "CPS ≥10."

Statistical analysis

Data were analyzed using SPSS (v.26.0; IBM Corp., Armonk, NY, USA). A p value<0.05 was considered statistically significant. The TIL scores of CNBs and resected specimens were compared using paired t-tests and Pearson's r correlation. To further evaluate the concordance between the TIL scores in CNBs and resected specimens, the intraclass correlation coefficients (ICCs) and their 95% confidence intervals (CIs) were calculated. ICCs of <0.5, 0.5–0.75, 0.75–0.9, and >0.9 indicated poor, moderate, good, and excellent reliability, respectively.[21] For agreement analysis of PD-L1 (SP142) and PD-L1 (22C3), Cohen's Kappa coefficient was calculated after setting the "positive" or "negative" values as categorical data, according to criteria described above. Kappa values of <0.00, 0.00–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 indicated poor, slight, fair, moderate, substantial, and almost perfect agreement, respectively.[22]

RESULTS

Patients' baseline characteristics

The patients' baseline clinicopathologic characteristics are shown in Table 1. The median core number of CNB was 4 (range, 1–38). Invasive ductal carcinoma was the most common histologic subtype (n=40, 85.1%), and the mean invasive tumor size was 1.9 cm. All patients included in the analysis had a single tumor mass.

Comparison of TIL levels in CNBs and resected specimens

Correlation analysis showed very strong positive correlation of the TIL scores between CNBs and resected specimens (r=0.865, p<0.001) (Figs. 1 and 2). The ICC value was 0.847 (95% CI 0.733–0.913, p<0.001), showing good reliability. Overall, the average TIL score was approximately 5.51% higher in the resected specimens compared with that in the CNBs (24.59% in

Table 1. Baseline Characteristics of the Patients

	Value
Age, yr	57.1±14.7
Biopsy core numbers*	4 (1–38)
Histologic subtype	
Invasive ductal carcinoma	40 (85.1)
Mixed metaplastic carcinoma and IDC	2 (4.3)
Metaplastic carcinoma	1 (2.1)
Carcinoma with apocrine differentiation	1 (2.1)
Adenoid cystic carcinoma	1 (2.1)
Medullary carcinoma	1 (2.1)
Invasive lobular carcinoma	1 (2.1)
Size of invasive carcinoma, cm	1.9 (0.4–9.0)

IDC, invasive ductal carcinoma.
Data are presented as mean±standard deviation, median (range), or n (%).
*Cores containing invasive tumor.

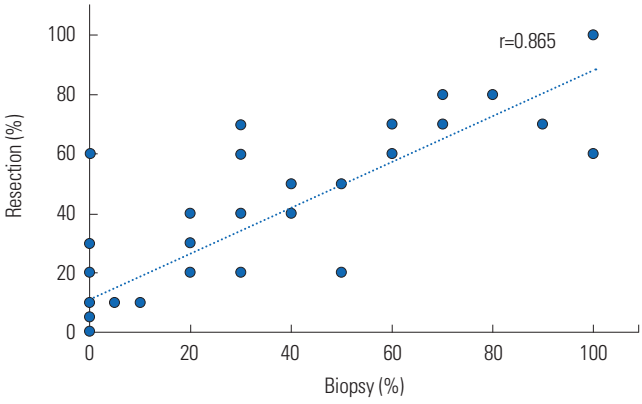

Fig. 1. Tumor-infiltrating lymphocyte (TIL) scores between core needle biopsies (CNBs) and resected specimens. Very strong positive correlation was observed, with r=0.865 (p<0.001). TIL scores of CNBs and resected specimens are shown on the x-axis and y-axis, respectively.

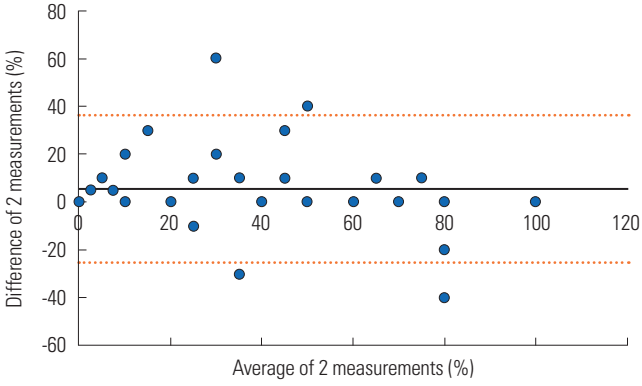

Fig. 2. Bland–Altman plots of tumor-infiltrating lymphocyte (TIL) levels in core needle biopsies (CNBs) and resected specimens. The difference in TIL levels between the CNBs and resected specimens versus the corresponding mean for each of the 49 pairs. Solid line represents the average difference (5.51%), and dotted line shows the 95% confidence interval around this estimate.

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CNBs and 30.10% in resected specimen, p=0.018). With a cut-off value of 30%, the agreement of TIL level was considered substantial (κ=0.747, p<0.001) (Table 2). A total of 6 (12.2%) tumors had discordant TIL scores with the two-tiered sub-classification. The ICC values according to the number of cores are shown in Table 3. When the core number was more than four, the ICC increased to >0.9 (p<0.001).

Concordance of PD-L1 status in CNBs and resected specimens

The positive rates of both PD-L1 (SP142) and PD-L1 (22C3) were higher in resected specimen with discordant PD-L1 status in 12 cases (24.5%) on SP142 assay and 14 cases (31.1%) on 22C3 assay. The overall agreement of PD-L1 positivity in two assays was moderate in SP142 (κ=0.503, p<0.001) and fair in 22C3 (κ=0.380, p=0.010) (Table 4).

The kappa values according to the number of CNB cores are shown in Table 5. In PD-L1 (SP142) assay, agreement was consistently moderate even if the core number exceeded four. However, the agreement of PD-L1 (22C3) status significantly improved from fair (κ=0.363, p=0.031) to moderate (κ=0.750, p=0.028) with more than four tumor cores included in CNB.

When the agreement of PD-L1 status was analyzed separately in the high-TIL group and the low-TIL group, the agreements were significantly substantial in the high-TIL group (κ=0.757, p=0.002) (Table 6) with PD-L1 (SP142). Fig. 3 presents representative images of discordant cases where PD-L1 expression changed from initially negative in CNB samples to positive in the resected specimens.

CPS was also analyzed as continuous value. The overall ICC of CPS value of 22C3 assay between CNB and resected specimens was 0.357 (p=0.008), indicated poor reliability. Subset analysis based on the tumor core numbers (≤4 vs. ≥5) revealed poor reliability with ≤4 tumor core number (ICC=0.315, p=0.022), which was markedly improved with ≥5 tumor core number (ICC=0.890, p=0.010) (Table 5). Meanwhile, subset analysis based on the TIL levels of CNB (≤30% vs. >30%) exhibited insignificant result in reliability (low-TIL in CNB, ICC=0.118, p=0.272; high-TIL in CNB, ICC=0.360, p=0.067) (Table 6).

In order to understand the features of cases with discordant PD-L1 results, we conducted a comparison of several factors including the TILs in the CNB and the resected specimens, the number of tumor cores in CNB, and the tumor size. The cases with concordant PD-L1 results revealed higher TIL in both

Table 2. Distribution and Agreement of TIL Scores Based on the Two-Tiered Subclassification

CNB	Resected			Kappa	p value
	Low (<30%)	High (≥30%)	Total		
Low (<30%)	26 (low-TIL group)	4	30 (61.2)		
High (≥30%)	2	17 (high-TIL group)	19 (38.8)	0.747	<0.001
Total	28 (57.1)	21 (42.9)	49 (100.0)		

CNB, core needle biopsy; TIL, tumor-infiltrating lymphocyte.
Data are presented as n or n (%).

Table 3. Reliability of TIL Level Based on the Core Number of CNBs

Core number of CNB	ICC	95% CI	p value
1–3 (n=12)	0.791	0.421–0.935	0.001
4 (n=25)	0.875	0.692–0.947	<0.001
≥5 (n=10)	0.911	0.636–0.978	<0.001

CNB, core needle biopsy; TIL, tumor-infiltrating lymphocyte; ICC, intraclass correlation coefficient; CI, confident interval.

Table 4. Distribution and Agreement of PD-L1 Status

CNB	Resected				
	Positive	Negative	Total	Kappa	p value
PD-L1 (SP142)				0.503	<0.001
Positive*	22	4	26 (53.1)		
Negative†	8	15	23 (46.9)		
Total	30 (61.2)	19 (38.8)	49 (100)		
PD-L1 (22C3)				0.380	0.010
Positive‡	17	5	22 (48.9)		
Negative§	9	14	23 (51.1)		
Total	26 (57.8)	19 (42.2)	45 (100)		

CNB, core needle biopsy; PD-L1, programmed cell death ligand 1.
Data are presented as n or n (%).
*Immune cell (IC) score ≥1%; †IC <1%; ‡combined proportional score (CPS) ≥10; §CPS<10.

Table 5. Agreement of PD-L1 Status according to the Core Number of CNBs

PD-L1 (SP142)			PD-L1 (22C3)			ICC	p value
Core number	Kappa	p value	Core number	Kappa	p value		
≤4 (n=37)	0.493	0.002	≤4 (n=37)	0.363	0.031	0.315	0.022
≥5 (n=10)	0.600	0.058	≥5 (n=8)	0.750	0.028	0.890	0.010

CNB, core needle biopsy; PD-L1, programmed cell death ligand 1; ICC, intraclass correlation coefficient.

Table 6. Agreement of PD-L1 Status in High- and Low-TIL Groups

TIL group	PD-L1 (SP142)		PD-L1 (22C3)		ICC	p value
	Kappa	p value	Kappa	p value		
High-TIL	0.757	0.002	0.474	0.057	0.360	0.067
Low-TIL	0.373	0.054	0.333	0.098	0.118	0.272

PD-L1, programmed cell death ligand 1; TIL, tumor-infiltrating lymphocyte ; ICC, intraclass correlation coefficient.

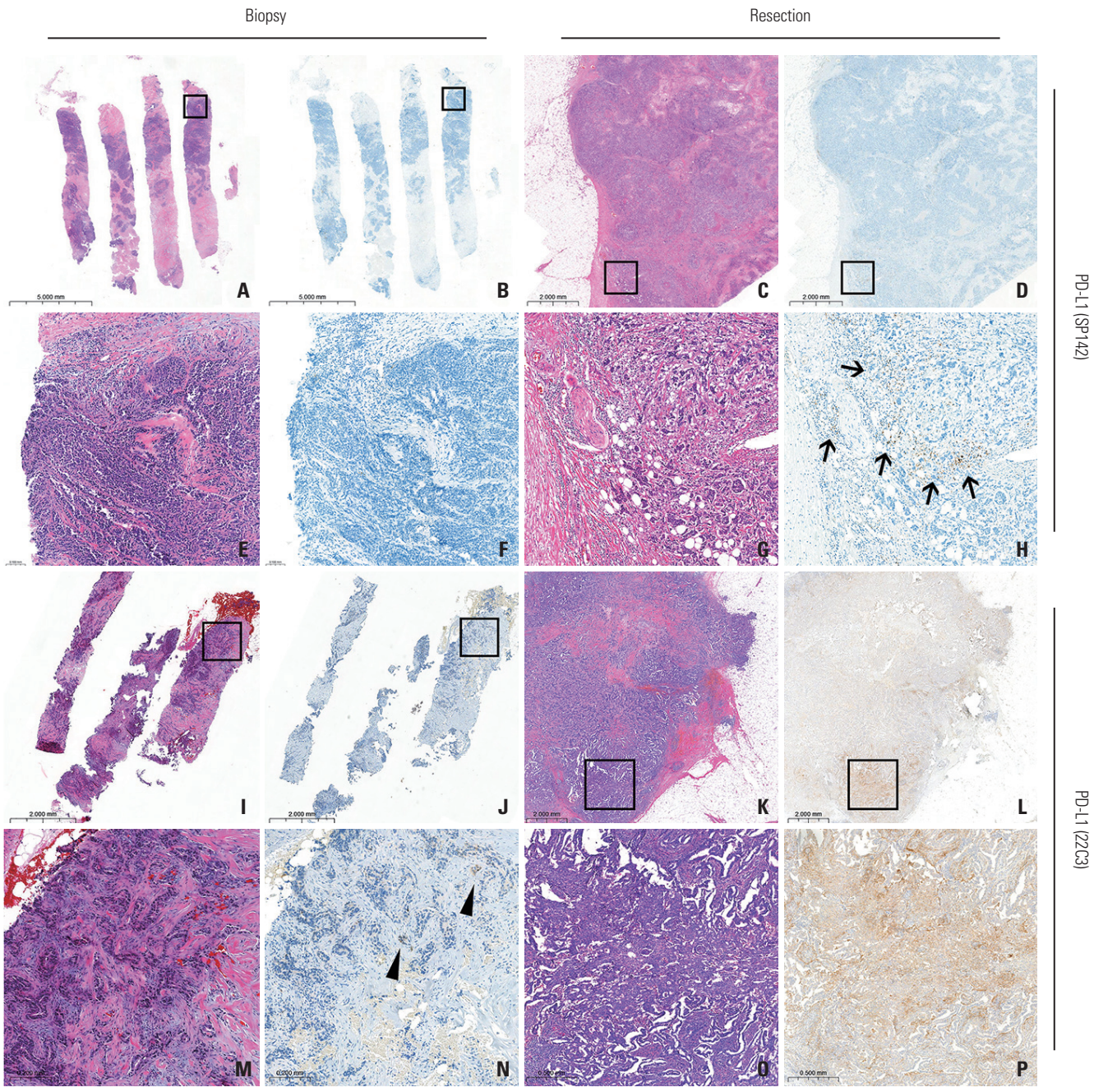

Fig. 3. Representative programmed cell death ligand 1 (PD-L1) discordant cases: core needle biopsies (CNBs) and resected specimens. Representative images illustrating the discordant PD-L1 expression between CNB and matched resected specimens. In the CNB samples (A and I), negative PD-L1 results were observed (B: SP142 assay, J: 22C3 assay). The images in the first and third rows (A-D and I-L) were magnified in the subsequent rows (E-H and M-P). The corresponding resected specimens (C and K) demonstrated positive PD-L1 expression (D: SP142 assay, L: 22C3 assay). Notably, higher tumor-infiltrating lymphocytes (TILs) were observed in the resected specimen (G), displaying dot-like PD-L1 (SP142) expression (H, arrows). The combined proportional score (CPS) in the CNB sample (J) was 5, with low TILs and only focal PD-L1 (22C3) expression in tumor cells (N, arrowheads). In contrast, the resected specimen (K) exhibited relatively higher PD-L1 (22C3) expression in both TILs and tumor cells (P), resulting in a CPS of 20, which is considered positive.

CNB and the resected specimens, a greater number of tumor cores in CNB, and larger tumor size. However, these differences were not statistically significant. Supplementary Table 1 (only online) provides further details on these comparisons.

DISCUSSION

Herein, we evaluated the agreement of the TIL scores and the status of two PD-L1 antibodies between CNBs and matched resected specimens of TNBC.

The TIL scores of CNBs were significantly representative of

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those of resected specimens, when analyzing both continuous and categorical variables (ICC=0.847, $p<0.001$; κ=0.747; $p<0.001$), and the agreement was almost perfect (κ=0.911; $p<0.001$) when ≥5 tumor cores were included in CNBs. This result was consistent with our previous study examining the agreement of TIL level between CNBs and matched resected specimens of breast cancer, which showed ICC >0.9 when ≥5 tumor cores were included in the CNBs.[13] A number of studies have shown good correlation between TIL levels in CNB and surgical samples.[13,23,24] Mani, et al.[25] suggested that a single core may represent the entire tumor. However, a single core might be insufficient to represent the whole tumor, especially for TNBC, as it had the most discordant TIL levels as reported in a previous study.[13] However, in line with the previous study,[13] we confirmed that CNB containing ≥5 tumor cores would likely represent the whole tumor and five cores appeared to be sufficient.

Akin to the TIL levels, the PD-L1 status of CNBs samples was at least moderately reliable overall. Interestingly, an increase in core numbers did not improve the kappa value of PD-L1 (SP142) status, whereas ≥5 cores showed substantial agreement in PD-L1 (22C3) status. This difference between the two antibodies could be attributed to the spatial heterogeneity of TIL and PD-L1 expression as well as the difference in the scoring method for each antibody. The expression of PD-L1 (SP142) is only measured based on the PD-L1-positive IC occupancy, which is heterogeneously distributed. Owing to the additional PD-L1 heterogeneity, improvement of the kappa value to more than moderate agreement might be difficult. By contrast, the CPS of PD-L1 (22C3) was determined based on the tumor cell staining as well as IC staining, which increases the chances of positive result compared with PD-L1 (SP142), especially when the tumor has high level of PD-L1 positive tumor cells despite the low TIL levels.

Although the PD-L1 status of CNBs was representative of the resected specimens, PD-L1 status showed weaker consistency than that of TIL scores. This may be explained as follows. First, the weaker consistency observed for PD-L1 (SP142) may have been derived from the low cutoff value for positivity (IC ≥1%).[20] Although the clinical significance is most pronounced with an IC 1% cutoff,[26] a substantial number of cases exhibit equivocal positivity, which becomes even more challenging in resected specimens with subjective visual interpretation. A previous study conducted on non-small cell lung cancer demonstrated an increase in positive cases using artificial intelligence-powered analysis to aid in the interpretation of equivocal IHC cases.[27] Significant higher agreement of high-TIL group implies that the high TIL level also increases the possibility of PD-L1 expression. Second, for PD-L1 (22C3), an analytical difference exists between the calculation of the CPS of PD-L1 (22C3) and IC score of PD-L1 (SP142). When interpreting PD-L1 (SP142) staining, IC score is calculated from the proportion of area occupied by PD-L1-expressing ICs in the total tumor area. Meanwhile, CPS requires the number of ICs expressing PD-L1, the number of tumor cells expressing PD-L1, and the total number of tumor cells. If a tumor has high cellularity with PD-L1-expressing tumor cells and sparse stroma, that case might have highly concordant CPS with PD-L1 (22C3) assay. Conversely, if a tumor has densely packed tumor cells that outnumber the PD-L1-expressing ICs, the CPS could be negative (less than 10) while the IC score is more likely to be over 1%.

The kappa value of PD-L1 (22C3) was relatively low and insignificant in both high-TIL group and low-TIL group. As PD-L1 (22C3) expression is evaluated using tumor cell and IC staining, TIL level alone appeared to have no significant impact on CPS, considering the fact that the high-TIL group showed significantly substantial agreement with PD-L1 (SP142) whose scoring relied on the IC. Rather, the increment of core number markedly increases the kappa value as well as ICC in the PD-L1 (22C3) assay.

In present study, there was a discrepancy in the PD-L1 expression, both with SP142 and 22C3 antibodies. Most of discordant cases exhibited a positive conversion of PD-L1 results in resected specimens compared to the CNB samples. This finding could be explained by the heterogeneity of TIL and PD-L1 expression within the tumor. The cases with discordant PD-L1 results exhibited lower levels of TIL in both the CNB and the resected specimens. Additionally, these cases had fewer tumor cores in CNB and smaller tumor sizes compared to cases with concordant PD-L1 results. It is important to note that a higher TIL and a greater number of tumor cores in CNB might significantly improve the concordance between CNB and resected specimens in a larger sample size. However, the larger tumor sizes could contribute to increased heterogeneity in TIL and PD-L1, which may be compromised by the number of tumor cores in CNB. Further study with a larger number of cases would enhance the reliability and confidence of the results.

A previous study compared the PD-L1 (SP142) status in CNB and resected specimen demonstrated lower discrepancy rate compared to the present study.[15] Unlike the previous study, we retrospectively examined the pre-existing slides, where IHC was performed on a representative section of each resected specimen. Typically, if a positive PD-L1 result is obtained from the preoperative CNB tissue, additional IHC in the resected specimen is not commonly performed, as any PD-L1 positivity, irrespective of tissue type, is considered to be PD-L1 positive. However, in the study conducted by Dobritoiu, et al.,[15] IHC was carried out on all available sections of the resected specimens. Considering the heterogeneous distribution of PD-L1 expression and TILs within the tumor, this approach might have resulted in an increased positive rate of PD-L1 in the resected specimens, as well as a reduction in the discrepancy rate.

This study had several limitations that need to be addressed. First, it had a small sample size, which could affect the analytic power. As the current standard treatment of early-stage TNBC is NAC,[28] collecting treatment-naïve surgical samples and matched CNBs of TNBC patients is difficult. Our previous study

only included 32 patients with TNBCs among total of 220 patients with breast cancer.[13] As the availability of treatment-naive TNBC surgical samples declines due to the NAC, a multicenter study or a large-scale retrospective study is required to gather sufficient data for meaningful analysis. Second, despite the good reliability of TIL scores and PD-L1 status in the CNBs in TNBC, further detailed analysis of the tumor immune microenvironment is required. Some tumors have high TIL scores but lack PD-L1 expression. This might be attributed to the different subtype and spatial distribution of TILs,[29,30] and should be unraveled by additional studies. Lastly, the TNBC included in this study was a clinical subtype of breast cancer that lacked ER, PR, and HER2 expression on IHC. However, TNBC is composed of molecularly heterogenous subtypes,[31] and this might have affected the different TIL scores and PD-L1 status. Hence, further precise studies are needed to elucidate the immune characteristics of TNBC.

In conclusion, the PD-L1 status of both SP142 and 22C3 assays evaluated in CNBs could represent the status of the entire tumor in TNBC. In addition, more than four CNB cores can improve the agreement of PD-L1 status as well as the TIL levels of the entire tumor.

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AUTHOR CONTRIBUTIONS

Conceptualization: Yoon Jin Cha. Data curation: Hyungwook Choi and Yoon Jin Cha. Formal analysis: Hyungwook Choi and Yoon Jin Cha. Funding acquisition: Yoon Jin Cha. Investigation: all authors. Methodology: all authors. Project administration: Yoon Jin Cha. Resources: all authors. Supervision: Yoon Jin Cha. Validation: Hyungwook Choi and Yoon Jin Cha. Visualization: Hyungwook Choi and Yoon Jin Cha. Writing—original draft: Hyungwok Choi and Yoon Jin Cha. Writing—review and editing: all authors. Approval of final manuscript: all authors.

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