




Higher Concordance of PD-L1 Expression Between Biopsies and Effusions in Epithelioid than in Nonepithelioid Pleural Mesothelioma

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BACKGROUND: Malignant mesothelioma (MM) is a therapy-resistant tumor, often causing an effusion. Drugs targeting the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway have shown promising results, but assessment of PD-L1 expression to select patients for therapy has mainly been performed on histologic tissue samples. In a previous study, we showed that MM effusions are suitable for PD-L1 assessment with results comparable to those reported in histologic studies, but no studies have compared PD-L1 expression in histologic and cytologic samples. **METHODS:** PD-L1 expression was determined immunohistochemically (clone 28-8) in 61 paired samples of effusions and biopsies from patients with pleural MM, obtained at the time of diagnosis. Only cases with >100 tumor cells were included. Membranous staining in tumor cells was considered positive at $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$ cutoff levels. **RESULTS:** Of 61 histologic samples, PD-L1 expression was found in 28 and 7 samples at $\geq 1\%$ and $>50\%$ cutoffs, respectively; the corresponding figures for cytology were 21 and 5, respectively. The overall percentage agreement between histology and cytology was 69% and 84%, with a kappa (κ) of 0.36 and 0.08 at $\geq 1\%$ and $>50\%$ cutoffs, respectively. The concordance between cytology and histology tended to be higher for epithelioid MM versus nonepithelioid MM at a $\geq 1\%$ cutoff. PD-L1 positivity in biopsies, but not in effusions, correlated with the histologic subtype at a $\geq 1\%$ cutoff. **CONCLUSIONS:** A moderate concordance of PD-L1 expression between biopsies and effusions from pleural MM, especially for the epithelioid subtype, indicates biological differences between the 2 types of specimens. Cytology and histology may be complementary. *Cancer Cytopathol* 2021;129:468-478. © 2021 The Authors. *Cancer Cytopathology* published by Wiley Periodicals LLC on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

KEY WORDS: cell block; clone 28-8; cytology; histology; immunohistochemistry.

INTRODUCTION

Malignant mesothelioma (MM) is a rare but highly aggressive tumor that develops in the mesothelial cells lining the serous cavities of the body, most commonly in the pleura.¹ MM is divided into 3 main histologic subtypes,

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epithelioid, sarcomatoid, and biphasic, containing both histologic components.² The main etiology is long-term, predominantly occupational exposure to asbestos^{1,3}; however, despite the ban on asbestos in several countries, the incidence of MM remains high worldwide.⁴

The prognosis of MM remains poor, and recent therapy modalities have improved survival only marginally⁵; thus, the development of novel therapeutic approaches is urgent, and there is a clinical need for better prognostic and predictive biomarkers.⁶

Programmed cell death 1 (PD-1) and its ligand, programmed cell death ligand 1 (PD-L1), play an important role in the regulation of T cell activity, and PD-1 acts as a coinhibitory receptor to prevent immune activation.^{7,8} PD-L1 can be upregulated in tumor cells and has been demonstrated in several malignancies, providing a mechanism for the malignant cell to escape the immune system,⁹ by avoiding T cell cytotoxicity and facilitate survival.¹⁰

Several drugs targeting PD-1 or PD-L1 are being developed or are currently in clinical use.¹¹ Recently, immunotherapy for MM has garnered wide interest, and several trials of PD-1/PD-L1 blockade have shown promising results.^{12,13}

Cytology is less traumatic to the patient, and an effusion of the pleural cavity usually causes symptoms, hence an evacuation of the fluid is performed both for therapeutic and diagnostic purposes. An effusion specimen is often the first material to be received in the laboratory, and cytology is often the first method used in the diagnosis of pleural MM.¹⁴ Effusion cytology may provide an earlier diagnosis, increasing the chances for better therapy effect and longer survival times.¹⁵ In cases of malignancy, the effusions are often abundant and offer enough material for various ancillary analyses.

According to previous recommendations, the diagnosis of MM requires a histologic sample, but in the case of epithelioid and biphasic MM, the diagnosis can be established in effusion cytology, although the sensitivity may be lower.¹⁶

Immunohistochemical (IHC) confirmation of the expression of PD-L1 in the tumor cells is a common prerequisite for the initiation of therapy,¹⁷ and histologic material is generally used for the IHC assessment of PD-L1. Several studies have compared PD-L1 reactivity in histologic and cytologic material from non-small cell lung cancer (NSCLC); according to a recent review, the concordance is described as good, but the studies that have met the inclusion criteria are few.¹⁸ The cytologic material

included in these studies comprised several different types of specimens, and few studies have focused on effusions.

PD-L1 expression has also been identified in histologic MM material, but the studies are comparatively few.¹⁹ Only a few studies have been performed on cytologic material, and few effusion specimens have been included.^{20,21}

In a previous study, performed on 74 cases, we showed that IHC assessment of PD-L1 in MM effusions is feasible, and we found that the level of positivity was similar to the outcomes of previous studies performed on histologic material.²²

The present study included 26 effusions from our previous study, fulfilling the inclusion criteria, and an additional 35 cases, a total of 61 cases. Based on the extended material, we made a paired comparison with the corresponding histologic specimens. To the best of our knowledge, no study comparing PD-L1 reactivity in histologic and cytologic material from MM has been performed previously.

MATERIALS AND METHODS

A total of 61 paired, formalin-fixed, paraffin-embedded (FFPE) pleural biopsies and cell pellets from pleural effusions from patients with pleural MM, collected from January 1999 through April 2020, were retrieved from the archives of Skåne University Hospital and Halland Hospital. For 26 of the cases the cytologic specimens were included in a previous study.²² The specimens had been diagnosed at the Department of Genetics and Pathology, Skåne University Hospital in Malmö/Lund, Sweden (43 cases), and the Department of Pathology and Cytology, the Halland Hospital in Halmstad, Sweden (18 cases).

All samples had been collected before each patient had received oncological treatment, and the biopsy had been performed at the same time as the collection of the effusion or within 12 weeks afterward. Samples containing fewer than 100 malignant cells were excluded. One cell block and 1 biopsy from each case were stained with PD-L1.

There were no differences in the sampling or preparation methods. The histologic diagnoses were based on hematoxylin and eosin (H&E)-stained slides, supported by IHC assessment. The cytologic diagnoses were based on conventional wet-fixed H&E- and air-dried May-Grünwald-Giemsa-stained smears at both hospitals, as well as cell blocks at Halland Hospital, and were confirmed by immunocytochemistry. The diagnostic immunostains were performed on cell block slides at Halland

Hospital and, in the majority of cases, cytospin slides at Skåne University Hospitals.

The antibodies used varied slightly over time and between hospitals, but the basic immunopanel for histologic specimens consisted of CEA, EMA, BerEp4, CK5/6 or CK5, and calretinin. The basic immunopanel for cytologic specimens used at Skåne University Hospital consisted of CEA, EMA, and BerEp4,²³ with addition of CK5 and desmin in some of the later cases. If a complete immunopanel had not been used, the staining was supplemented with the missing antibodies. If needed, appropriate antibodies were added to exclude possible differential diagnoses. For effusions containing mesothelial cells without obvious malignant characteristics, desmin staining or, in more recent cases from Skåne University Hospital, BAP1, was performed to strengthen the diagnosis of malignancy.

Patient characteristics, including age, sex, and sampling dates, and the original diagnostic reports, including antibody reactivity and histologic subtype, were retrieved from the databases of the Department of Genetics and Pathology at Skåne University Hospital in Malmö/Lund and the Department of Pathology and Cytology at the Halland Hospital in Halmstad and from the patients' charts. All cases were treated as MM in the clinical setting, and computed tomography and positron emission tomography scans were typically performed. All histologic samples were reassessed to confirm the histologic subtype according to the World Health Organization classification of pleural tumors² by at least 1 experienced cytopathologist (H.B. [all cases] and T.S. [Halland Hospital cases]) together with a certified cytotechnologist (M.S.I.M [all cases]).

From all biopsies and cell blocks, an H&E-stained slide was made and examined by M.S.I.M., in unclear cases together with an experienced cytopathologist (T.S., H.B., A.D.) to confirm the presence of malignant cells.

The study was approved by the Regional Ethical Board Southern Health Care Region, Lund University (no. 2006/399 with addition 2017/708) and was conducted in adherence to the Declaration of Helsinki. Informed consent was obtained from all patients included.

IHC Assay

PD-L1 antibody

PD-L1 IHC 28-8 pharmDx is a detection kit for PD-L1 protein with a qualitative IHC assay using monoclonal

immunoglobulin G rabbit anti-PD-L1, clone 28-8 (Dako North America, Carpinteria, California). The antibody is intended to detect the PD-L1 protein in FFPE tissue blocks using EnVision FLEX visualization system on Autostainer Link 48, AS48430 (Dako North America). The staining procedure has been described previously in detail.²²

In addition to the manufacturer's controls, a positive and a negative control tissue block including specimens from the tonsil (+ in histiocytes of germinal centers, ++ in crypt epithelium), placenta (+++), and either the small intestine or appendix (–) was produced and used as an in-house control.

Immunostaining of specimens with anti-PD-L1

Identical cut sections from controls, biopsies, and cell blocks were stained with the PD-L1 antibody. The samples were cut at 4- μ m thickness, no longer than 2 days before the PD-L1 staining procedure. The PD-L1 sections were placed on charged slides and incubated in an oven for 1 hour at 60°C and subsequently immunohistochemically stained using the automated staining system on Autostainer Link 48, AS48430 (Dako North America) according to the manufacturer's protocol.

Target retrieval solution (Dako North America) buffer was diluted 50 times with distilled water and preheated to 65°C using a Digital Decloaking Chamber, PT10030 (Dako Colorado, Fort Collins, Colorado). Heat-induced antigen retrieval was performed in the PT Link Pre-Treatment Module, PT10030 (Dako Colorado) with target retrieval solution buffer for 20 minutes at 97°C at low pH. After 20 minutes, the buffer was cooled down to 65°C, and the slides were taken out and subsequently washed for 5 minutes in wash buffer. A wash buffer stock solution was diluted 20 times with distilled water before usage. The slides and reagent bottles were loaded in the Autostainer Link 48, AS48430 (Dako North America). Before the automated immunostaining process, wash buffer was added to each slide to prevent drying of the cut sections. Immunostainings were performed in batches. To prevent drying, avoiding the nonspecific binding of antibodies, automatic rehydration with distilled water was performed.

Each block was cut at least twice, producing 2 identical slides of each specimen: one for staining with the PD-L1 antibody, the other for an additional negative

antibody control for every sample slide, stained with negative control reagent, a buffer that contains immunoglobulin G antibodies that lack specificity for PD-L1 and works as an isotype control. Finally, the slides were covered with coverslips using permanent mounting media. All steps were performed according to the manufacturer's instructions.

Evaluation of immunohistochemical staining with anti-PD-L1

PD-L1 reactivity was assessed light microscopically, using a BX45 microscope (Olympus, Tokyo, Japan). The PD-L1 staining in malignant cells was evaluated by the criteria recommended in an assessment manual from Dako (Agilent/pharmDx, Santa Clara, California).²⁴

The positivity of PD-L1 staining was defined as the percentage of well-preserved malignant cells exhibiting positive complete surrounding or linear partial membranous staining. All membranous reactivity was considered positive, regardless of intensity. Cytoplasmic reactivity in malignant cells was ignored, and any PD-L1 reactivity in nonmalignant cells, necrotic areas, and immune cells (eg, lymphocytes or macrophages) was disregarded and was not included in the assessment. The same evaluation criteria were applied to histologic and cytologic specimens.

All well-preserved malignant cells on the entire slide were evaluated, and the percentage of malignant cells showing membranous staining at any intensity in the whole specimen was assessed as negative if viable malignant cells with reactivity were <1% or positive at the different cutoff levels $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$.

The scoring was performed blindly, independently, and without side-by-side comparison, first by a certified cytotechnologist (M.S.I.M.) and then by 1 or 2 experienced cytopathologists (T.S., K.D. [cytologic samples], A.D.). The evaluation was supported by correlation with a slide stained with H&E and additional immunostains. The percentages of well-preserved malignant cells expressing PD-L1 were semiquantified. Cases with discordant PD-L1 reactivity were reassessed (by H.B. or A.D. together with M.S.I.M.) to achieve consensus without knowledge of the previously reported result by manual counting of malignant cells and PD-L1-positive cells.

Statistical Analysis of Data and Outcome

Patient characteristics, including age, sex, sampling date, histologic subtype, and the description of cytologic

specimens—including reactivity to diagnostic antibodies—were retrieved from the databases of both hospitals and from the patients' charts.

The association of PD-L1 expression with patient characteristics was evaluated using the Mann-Whitney *U* test for age and Fisher's exact test for sex and histologic subtypes.

The prevalence of PD-L1 positivity for histology and cytology at the $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$ cutoffs was analyzed. The agreement between histology and cytology was statistically analyzed using Cohen's kappa (κ) agreement statistics at the $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$ cutoff levels (with bootstrapped 95% CI).²⁵ To calculate κ between histology and cytology for PD-L1 positivity at the $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$ cutoff values, the overall percentage agreement (OPA), positive percentage agreement (PPA), negative percentage agreement (NPA) taking histology as the nonreference standard, and McNemar's test were calculated.

In the terminology of Altman,²⁵ $OPA = \frac{a+d}{N}$, $PPA = \frac{a}{a+c}$, and $NPA = \frac{d}{d+b}$, and the strength of agreement for κ is considered poor (<0.2), fair (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80), or very good (0.81-1.00).

All *P* values were determined using 2-sided tests, and $P > .05$ was not considered statistically significant. CIs using a modified Wald method with a Wilson score of 95% were calculated using GraphPad QuickCalcs (<https://www.graphpad.com/quickcalcs/>). All other data analyses and summary graphs were produced using IBM's SPSS Statistics for Windows, version 26.0 (Armonk, New York).

RESULTS

Relationship Between PD-L1 Expression in Histologic and Cytologic Specimens and Patient Characteristics

A total of 61 patients with the diagnosis of pleural MM and pairs of histologic and cytologic specimens were analyzed for PD-L1 expression.

Expression of PD-L1 in both histologic and cytologic specimens related to patients' age, sex, and histologic subtypes of MM, at 2 cutoff levels ($\geq 1\%$ and $>50\%$), are shown in Table 1. The PD-L1 positivity did not differ with respect to age or sex, neither in histologic nor cytologic specimens. However, a significant difference for

TABLE 1. Expression of PD-L1 Correlated With Patient Characteristics and Histologic Subtypes of Malignant Mesothelioma

Characteristics	Histology ^a										Cytology					
	1% Cutoff Level					50% Cutoff Level					1% Cutoff Level		50% Cutoff Level			
	All Patients	Negatives (<1%)	Positives (≥1%)	P	Negatives (≤50%)	Positives (>50%)	Negatives (<1%)	Positives (≥1%)	P	Negatives (≤50%)	Positives (>50%)	Negatives (<1%)	Positives (≥1%)	P	Negatives (≤50%)	Positives (>50%)
Age at diagnosis, y, median (range)	72 (50-90)	72 (50-90)	73 (51-86)	.529	72 (50-90)	73 (60-86)	72 (50-90)	73 (51-81)	.879	72 (50-90)	73 (60-81)	72 (50-90)	73 (51-81)	.879	72 (50-90)	73 (60-81)
Sex, n (%)																
Men	52/61 (85.2)	26/52 (50.0)	26/52 (50.0)	.160	46/52 (88.5)	6/52 (11.5)	33/52 (63.5)	19/52 (36.5)	.479	48/52 (92.3)	4/52 (7.7)	48/52 (92.3)	4/52 (7.7)	.563	48/52 (92.3)	4/52 (7.7)
Women	9/61 (14.8)	7/9 (77.8)	2/9 (22.2)	.049 ^b	8/9 (88.9)	1/9 (11.1)	7/9 (77.8)	2/9 (22.2)	.736 ^b	8/9 (88.9)	1/9 (11.1)	8/9 (88.9)	1/9 (11.1)	1.00 ^b	8/9 (88.9)	1/9 (11.1)
Histology, n (%)																
Epithelioid	49/61 (80.3)	30/49 (61.2)	19/49 (38.8)		45/49 (91.8)	4/49 (8.2)	33/49 (67.3)	16/49 (32.7)		45/49 (91.8)	4/49 (8.2)	45/49 (91.8)	4/49 (8.2)		45/49 (91.8)	4/49 (8.2)
Nonepithelioid	12/61 (19.7)	3/12 (25.0)	9/12 (75.0)		9/12 (75.0)	3/12 (25.0)	7/12 (58.3)	5/12 (41.7)		11/12 (91.7)	1/12 (8.3)	11/12 (91.7)	1/12 (8.3)		11/12 (91.7)	1/12 (8.3)
Biphasic	10/61 (16.4)	3/10 (30.0)	7/10 (70.0)		7/10 (70.0)	3/10 (30.0)	7/10 (70.0)	3/10 (30.0)		10/10 (100)	0/10 (0.0)	10/10 (100)	0/10 (0.0)		10/10 (100)	0/10 (0.0)
Sarcomatoid	2/61 (3.3)	0/2 (0.0)	2/2 (100)		2/2 (100)	0/2 (0.0)	0/2 (0.0)	2/2 (100)		1/2 (50.0)	1/2 (50.0)	1/2 (50.0)	1/2 (50.0)		1/2 (50.0)	1/2 (50.0)
Total, n (%)	61 (100)	33/61 (54.1)	28/61 (45.9)		54/61 (88.5)	7/61 (11.5)	40/61 (65.6)	21/61 (34.4)		56/61 (91.8)	5/61 (8.2)	56/61 (91.8)	5/61 (8.2)		56/61 (91.8)	5/61 (8.2)

Abbreviation: PD-L1, programmed cell death ligand 1.

^aFor biphasic mesothelioma, the positivity of the histologic component with the highest score was chosen.

^bComparison between PD-L1 reactivity in epithelioid and nonepithelioid subtypes.

TABLE 2. Prevalence of PD-L1 Positivity in Histologic and Cytologic Specimens for Different Cutoff Levels

PD-L1 Cutoff Levels	Positivity Prevalence, % (95% CI)	
	Histology ^a	Cytology
≥1%	46 (34-58)	34 (24-47)
>5%	34 (24-47)	20 (11-31)
>10%	26 (17-39)	15 (8-26)
>50%	11 (5-22)	8 (3-18)

Abbreviation: PD-L1, programmed cell death ligand 1.

^aFor biphasic mesothelioma, the positivity of the histologic component with the highest score was chosen.

PD-L1 expression in the histologic specimens at the ≥1% cutoff level was found between epithelioid and nonepithelioid MM ($P = .049$).

Status of PD-L1 Expression in Histologic and Cytologic Specimens

Differences in PD-L1 expression between histologic and cytologic specimens were found, either as discrepancies regarding positivity in the sample types, or in different percentages of positive cells when both sample types were positive. Detailed data for all cases are shown in Supporting Table 1.

The prevalence of PD-L1 reactivity in histologic and cytologic specimens for the different cutoff levels is reported in Table 2. The prevalence of positivity was marginally lower in cytologic samples, but considering the width of the CIs, the relevance of the difference is questionable. Images from samples illustrating different cell components and proportions of positive cells are shown in Figures 1 and 2.

The agreement in paired cases between histologic and cytologic specimens for PD-L1 expression at the ≥1%, >5%, >10%, and >50% cutoff values is shown in Table 3.

Correlation of PD-L1 Expression between Paired Histologic and Cytologic Specimens

The concordance was calculated for both positives and negatives (OPA) and for positives (PPA) and negatives (NPA) separately. OPA ranged between 62% and 84%. PPA decreased with increasing cutoff values, whereas NPA improved (Table 4).

Cohen's κ at the ≥1% cutoff level was 0.36 (CI, 0.13-0.59) which statistically is considered fair to moderate. The κ decreased at higher cutoff thresholds (Table 4). McNemar's test was not statistically significant at any cutoff level (Table 4).

A moderate concordance between histologic and cytologic specimens at the ≥1% cutoff level was found for epithelioid ($\kappa = 0.43$), but not for nonepithelioid MM ($\kappa = 0.08$). The κ values decreased for higher cutoff levels. Detailed data including κ , OPA, PPA, NPA, and McNemar probabilities are shown in Table 5.

DISCUSSION

The PD-1/PD-L1 pathway plays a crucial role in tumor immune escape. PD-L1 expression has been demonstrated in several types of malignant tumors, and IHC determination of PD-L1 is performed to select patients for treatment with immune checkpoint inhibitors.

Most studies of PD-L1 expression have been performed on histologic samples, mainly on NSCLC tissue samples.²⁶ Several studies comparing PD-L1 reactivity between histologic and cytologic material have also been performed for NSCLC.¹⁸ Various types of cytologic samples have been included, and only a few studies have reported the results of paired comparisons between pleural effusions and histology. In studies on NSCLC, the concordance between cytologic and histologic samples has been reported in different ways, as agreement (positive, negative overall, as κ or even correlation coefficient), thus it is difficult to compare the results of different studies. In a systematic review based on 141 studies, OPA was 88.3% between histologic and cytologic specimens at the ≥1% cutoff level in 9 studies, fulfilling the inclusion criteria.¹⁸

In studies performed on histologic MM specimens, positivity ranged from 11% to 72% at the ≥1% cutoff level.¹⁹ Studies performed on MM effusions are scarce. In a previous study comprising 74 MM effusions, 61 of which fulfilled the inclusion criteria, we found PD-L1 positivity in 38% and 10% of the cases at the ≥1% and >50% cutoff levels, respectively.²² Aside from our own study, we found only 2 studies, including in total 8 MM.^{20,21} A study performed on various types of cytologic material included 3 pleural effusions, 1 of which was MM.²⁰ Another study including 7 MM effusions with matching biopsies was reported by Khanna et al.²¹

Among our histologic samples, we found PD-L1 positivity in 28/61 (41%) cases at ≥1% cutoff level and in 7/61 (11%) at >50% cutoff, respectively (Table 1). The positivity range reported in previous studies performed on histologic MM samples is wide.¹⁹ A recent study that used the same antibody clone (28-8 pharmDx) and is

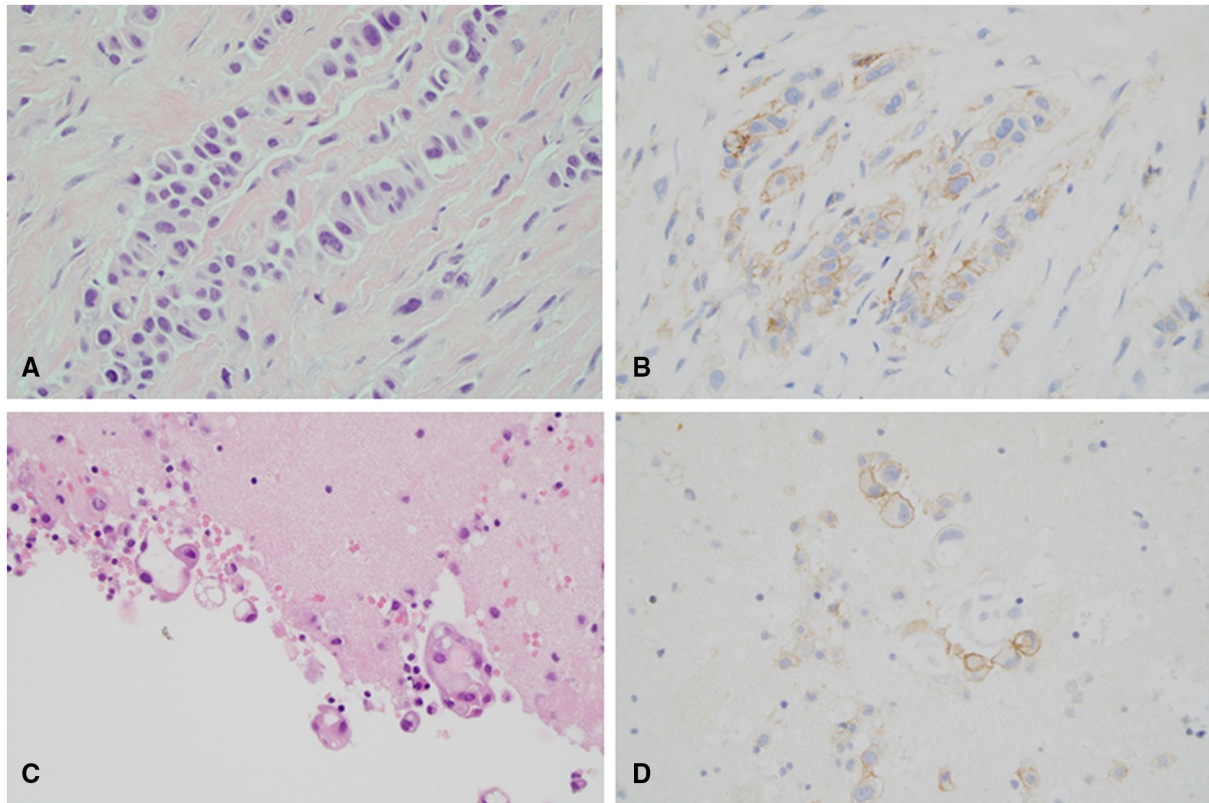


Figure 1. PD-L1 reactivity in paired histologic and cytologic specimens from an epithelioid mesothelioma (original magnification $\times 400$). (A) Histologic specimen, H&E staining. (B) Histologic specimen, PD-L1 immunostaining (>50% PD-L1-positive malignant cells). (C) Cytologic specimen, H&E staining. (D) Cytologic specimen, PD-L1 immunostaining (>50% PD-L1-positive malignant cells).

most comparable to ours reported a positivity rate of 23% (25/112) in all pleural effusions and positivity rates of 16% (9/56), 30% (14/47), and 22% (2/9) in epithelioid, biphasic, and sarcomatoid MM, respectively.²⁷

In our study, the prevalence of PD-L1-positive samples in epithelioid and nonepithelioid MM differed for the histologic specimens at the $\geq 1\%$ cutoff level, the epithelioid MM being positive in 19 of 49 (39%) cases, the nonepithelioid in 9 of 12 (75%) cases (Table 1). This is in accordance with previous studies that have demonstrated higher positivity rates in sarcomatoid MM.^{28,29} The positivity rates in the cytologic samples (34% [21/61] at the $\geq 1\%$ cutoff and 8% [5/61] at the $>50\%$ cutoff) did not differ from the results in our previous study.

We report concordance between histologic and cytologic specimens as OPA, PPA, and NPA; as Cohen's κ ; and as McNemar's probability.

In the absence of studies comparing effusions and biopsies from MM, we compared our results with those reported in similar NSCLC studies. A review of studies

comparing histologic and various types of cytologic specimens reveals a high degree of concordance, but only 3 of the included studies were performed on effusions. One of the studies, in which antibody clone 22C3 was used on 30 effusions and 40 bronchial wash samples, revealed very high agreement for the total material ($\sim 90\%$), reported as OPA, PPA, and NPA at both the $\geq 1\%$ and $\geq 50\%$ cutoff levels.³⁰ Our results were similar for the $\geq 1\%$ cutoff level but were lower for the $\geq 50\%$ cutoff level. Another study reported fair to moderate concordance ($\kappa = 0.39$) in 15 effusions and the corresponding histologic samples,³¹ which is in agreement with our results. As shown in Table 2, the prevalence of positivity was marginally lower in cytologic samples compared with histologic samples. The κ values at the different cutoff levels indicate that for highly positive samples, cytology and histology disagree and may be complementary. McNemar's test yielded no evidence of systematic discrepancies between histologic and cytologic samples (Table 4). The agreement decreased at higher cutoff levels, which may be explained by the

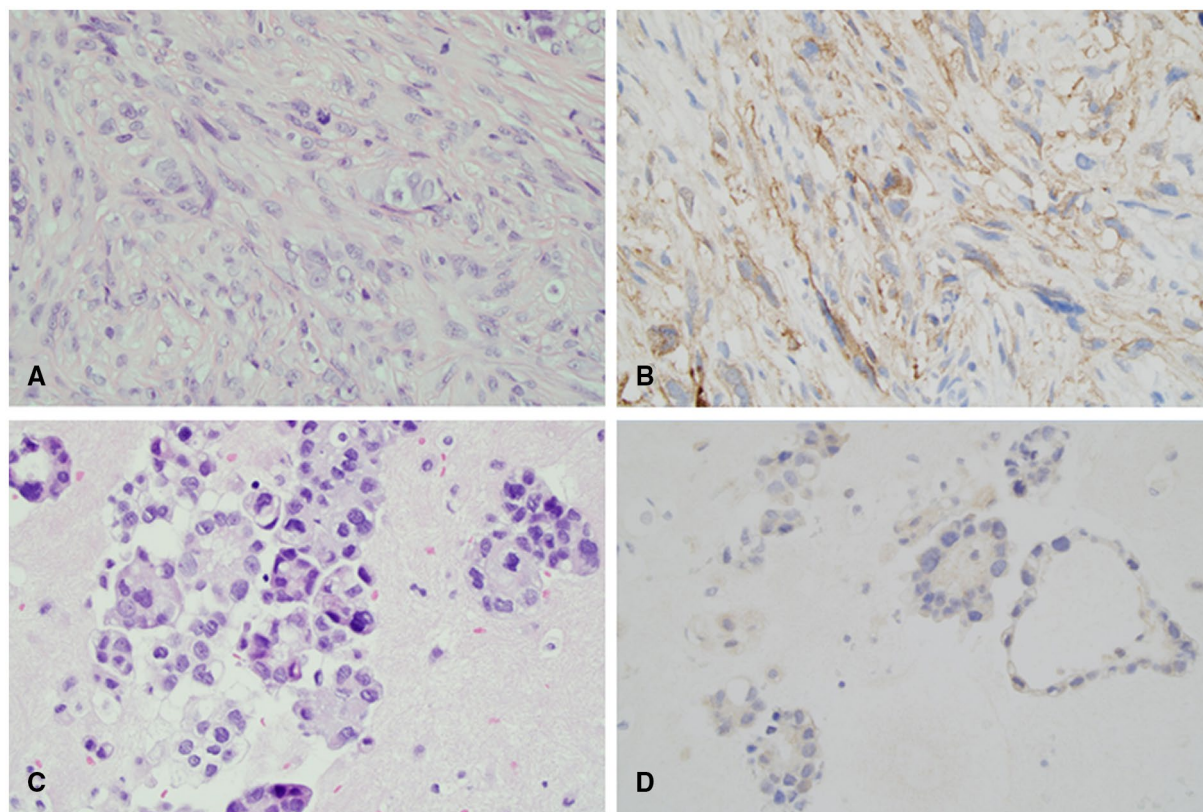


Figure 2. PD-L1 reactivity in paired histologic and cytologic specimens from a biphasic mesothelioma. The histologic specimen is shown with the sarcomatoid component. The epithelial component was PD-L1-negative (original magnification $\times 400$). (A) Histologic specimen, H&E staining. (B) Histologic specimen, PD-L1 immunostaining (>50% PD-L1 positive malignant cells). (C) Cytologic specimen, H&E staining. (D) Cytologic specimen, PD-L1 immunostaining (negative).

TABLE 3. Overview of Agreement Between Histologic and Cytologic Specimens for PD-L1 Expression at $\geq 1\%$, $>5\%$, $>10\%$, and $>50\%$ Cutoff Levels

Histology ^a	Cytology	
	Positive	Negative
$\geq 1\%$ Cutoff level		
Positive	15	13
Negative	6	27
$>5\%$ Cutoff level		
Positive	5	16
Negative	7	33
$>10\%$ Cutoff level		
Positive	4	12
Negative	5	40
$>50\%$ Cutoff level		
Positive	1	6
Negative	4	50

Abbreviation: PD-L1, programmed cell death ligand 1.

^aFor biphasic mesothelioma, the positivity of the histologic component with the highest score was chosen.

comparatively small size of this study and the small number of positive cases at higher cutoff levels, increasing the effect of random variation. It indicates, however, that the

selected cutoff level and the material used have an influence on the outcome.

Discrepancies between PD-L1 expression in histologic and cytologic material in different studies may be explained by methodological and biological factors, such as different cutoff values and evaluation criteria used, antibody clone, preparation method, interobserver variability, the use of different kinds of samples, and intratumor heterogeneity.

The design of our study minimizes discrepant results due to methodological differences. We used the same preparation method for both types of material, we used only 1 antibody clone, and we strictly applied the criteria for positivity recommended by the manufacturer.²⁴ We studied just 1 type of tumor at the same tumor stage and included only cases with a maximum of a 12-week interval between the sampling dates of effusions and biopsies to avoid the influence of biological changes during cancer progression. All material was sampled at the time of the diagnosis—that is, before

TABLE 4. Agreement of PD-L1 Reactivity in Histologic and Cytologic Specimens at Different Cutoff Levels

PD-L1	Cell Positivity (95% CI)			
	≥1% Cutoff Level	>5% Cutoff Level	>10% Cutoff Level	>50% Cutoff Level
Cohen's κ coefficient	0.36 (0.13-0.59)	0.07 (-0.17-0.31)	0.16 (-0.10-0.43)	0.08 (-0.23-0.38)
OPA	69 (56-79)	62 (50-73)	72 (60-82)	84 (72-91)
PPA	54 (36-70)	24 (10-45)	25 (10-50)	14 (1-53)
NPA	82 (65-92)	83 (68-92)	89 (76-96)	93 (82-98)
McNemar's test	.17	.09	.14	.75

Abbreviations: NPA, negative percentage agreement; OPA, overall percentage agreement; PD-L1, programmed cell death ligand 1; PPA, positive percentage agreement.

Cohen's κ coefficient is presented as range -1-1. OPA, PPA, and NPA values are presented as percent with histology as the nonreference standard. McNemar analyses are presented as *P* values. For biphasic mesothelioma, the positivity of the histologic component with the highest score was chosen.

the patients had received oncological treatment. Some studies indicate that chemotherapy may affect PD-L1 expression in cancer cells, which could skew the apparent outcome.³²

The agreement between histologic and cytologic samples at the ≥1% cutoff level differed between epithelioid and nonepithelioid MM. For epithelioid MM, the concordance was moderate ($\kappa = 0.43$), whereas there was no concordance for nonepithelioid MM ($\kappa = 0.08$) (Table 5). The κ values decreased considerably for higher cutoff thresholds. Different proportions of histologic subtypes of MM in studies comparing histologic samples with effusions affect the results, as sarcomatoid MM seldom sheds tumor cells into the effusion. A plausible explanation for the different concordances for epithelioid and nonepithelioid MM in our study is that the more frequently positive sarcomatoid tumor component is not found in the cytologic material. Hence, the malignant cells in effusions from epithelioid MM may better represent the whole tumor. A recent study showed that overall survival and proportion of long-term survival were significantly better for patients with MM diagnosed by cytology than by histology, indicating that malignant cells in effusions represent a different population.¹⁵

The low proportion of sarcomatoid MM (2/61) and biphasic MM (10/61) in our study is explained by the fact that our basic inclusion criterion was an effusion containing diagnostic malignant cells.

Immunological characteristics may differ between primary tumors, metastatic tumors, circulating tumor cells, and effusions, and there might be heterogeneity within the same tumor manifestation.³³

From a biological point of view, microenvironment of cells in effusions is different from cells fixed in solid tissue. Malignant cells in effusions occur in a liquid

TABLE 5. Agreement of PD-L1 Reactivity in Epithelioid and Nonepithelioid Mesothelioma and Corresponding Cytology Specimen

PD-L1	Cell Positivity (95% CI)	
	Epithelioid	Nonepithelioid
≥1% Cutoff level		
Cohen's κ coefficient	0.43 (0.16-0.69)	0.08 (-0.36-0.51)
OPA	73 (60-84)	50 (25-75)
PPA	58 (36-77)	44 (19-73)
NPA	83 (66-93)	67 (20-94)
McNemar's test	.58	.22
>5% Cutoff level		
Cohen's κ coefficient	0.01 (-0.27-0.28)	0.00 (-0.40-0.40)
OPA	67 (53-79)	42 (19-68)
PPA	17 (4-46)	33 (12-65)
NPA	84 (68-93)	67 (20-94)
McNemar's test	.45	.13
>10% Cutoff level		
Cohen's κ coefficient	0.14 (-0.18-0.46)	0.08 (-0.36-0.51)
OPA	78 (64-87)	50 (25-75)
PPA	22 (5-56)	29 (8-65)
NPA	90 (76-97)	80 (36-98)
McNemar's test	.55	.22
>50% Cutoff level		
Cohen's κ coefficient	0.18 (-0.24-0.60)	-0.14 (-0.37-0.08)
OPA	88 (75-95)	67 (39-86)
PPA	25 (3-71)	0 (0-62)
NPA	93 (81-98)	89 (54-100)
McNemar's test	1.00	.63

Abbreviations: NPA, negative percentage agreement; OPA, overall percentage agreement; PD-L1, programmed cell death ligand 1; PPA, positive percentage agreement.

Cohen's κ coefficient is presented as range -1-1. OPA, PPA, and NPA values are presented as percent with histology as the nonreference standard. McNemar analyses are presented as *P* values. For biphasic mesothelioma, the positivity of the histologic component with the highest score was chosen.

environment, whereas the tumor cells in biopsies grow in solid stroma. Furthermore, malignant effusions often contain a mixture of malignant cells, benign mesothelial cells, and various types of inflammatory cells and soluble substances that may interact with the malignant cells, causing phenotype changes.^{34,35} We found that the biopsies were strongly predominated by tumor cells, whereas

the effusions contained various proportions of lymphocytes, macrophages, and tumor cells. An interaction between tumor cells and the immune cells in effusions may affect the expression of PD-L1, though this was beyond the scope of the present study.

Heterogeneity in malignant tumors may cause variability due to sampling errors in both histology and cytology, especially when dealing with small samples.³⁶ Two studies demonstrated that the differences in scoring of histologic and cytologic specimens seem to be explained in part by sampling from heterogeneous tumors in histologic material, and insufficient material available in the cytologic specimens.^{20,31} The analysis of malignant effusions may also be hampered by low overall cellularity, and the tumor cells may be few compared with inflammatory background cells. On the other hand, small histologic samples may be damaged or may not contain tumor cells, whereas the cells in effusions are usually well preserved and contain malignant cells that may have exfoliated from the whole tumor area. Hence, an effusion may be more representative than a small biopsy.

The rationale behind the determination of PD-L1 activity in tumor tissue is the assumption that PD-L1 positivity would predict the response to treatment with PD-L1 inhibitors. Whereas several studies have indeed shown a correlation between therapy response and PD-L1 expression, good response has also been recorded in some patients with low levels of PD-L1 expression in their MM tumors,³⁷ and there is no consensus as to whether tumor PD-L1 expression predicts outcomes.³⁸

In many of our cases, there were large differences in the fraction of positive cells in biopsies and effusions. The variability of histology could well be an effect of the narrowly localized sampling, which may not be representative of the whole tumor. An effect of local sampling does not exist to the same degree in effusions, which are well mixed and thus represent to some degree all of the exposed tumor mass. The “age” of the effusion could be a confounding factor if different cell types are shed at different rates. However, the most likely cause of the differences is biologic in terms of the characteristics of the lesions and their shedding propensity. If and in which way this is predictive of therapy response can only be answered by studies in which histologic and cytologic samples that have been collected before therapy are available.

In conclusion, the present study demonstrates a moderate concordance at the $\geq 1\%$ cutoff value for

PD-L1 expression in paired samples of MM effusions and corresponding biopsies. The concordance was higher for epithelioid than for nonepithelioid MM. Discordant results may be explained by inherent differences between the sampled cell populations. Whether basing therapy decisions on PD-L1 positivity in effusion cytology samples would be an improvement compared with histology—or whether the methods would be complementary—can only be determined by investigating paired samples together with therapy response.

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AUTHOR CONTRIBUTIONS

Mohammed S. I. Mansour: Full access to all study data; responsibility for integrity of data and accuracy of data analysis; study concept and design; acquisition, analysis, and interpretation of data; assessment of immunostaining; writing—original draft; writing—review and editing; statistical analysis; acquisition of funding. **Tomas Seidal:** Analysis and interpretation of data; assessment of immunostaining; writing—original draft; writing—review and editing; critical revision of manuscript for important intellectual content; administrative and material support. **Ulrich Mager:** Analysis and interpretation of data; writing—original draft; writing—review and editing; critical revision of manuscript for important intellectual content; administrative and material support. **Katalin Dobra:** Analysis and interpretation of data; assessment of immunostaining; critical revision of manuscript for important intellectual content; acquisition of funding; administrative and material support. **Hans Brunnström:** Full access to all study data; responsibility for integrity of data and accuracy of data analysis; study concept and design; analysis and interpretation of data; assessment of immunostaining; writing—review and editing; critical revision of manuscript for important intellectual content; acquisition of funding; technical and material support. **Annika Dejmek:** Full access to all study data; responsibility for integrity of data and accuracy of data analysis; study concept and design; analysis and interpretation of data; assessment of immunostaining; writing—original draft; writing—review and editing; critical revision of manuscript for important intellectual content; statistical analysis; acquisition of funding; administrative, technical, and material support; study supervision.

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