CD4/CD8 Ratio of Pleural Effusion Is A Prognostic Predictor for Non-Small Cell Lung Cancer Patients Under Immune Checkpoint Inhibitor Treatments

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Research Article

Keywords: ICIs, CD4/CD8, effusion, cancer

Posted Date: January 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-145181/v1

Abstract

Pleural effusion is a rare immune-related adverse event for lung cancer patients receiving immune checkpoint inhibitors (ICIs). We enrolled 281 lung cancer patients treated with ICIs and 17 were analyzed. We categorized the formation of pleural effusion into 3 patterns: type 1, rapid and massive; type 2, slow and indolent and type 3, with disease progression. CD4/CD8 ratio of 1.93 was selected as the cutoff threshold to predict survival. Most patients of types 1 and 2 effusions possessed pleural effusion with CD4/CD8 ratios > = 1.93. The median OS time in type 1, 2, and 3 patients were not reached, 24.8, and 2.6 months. The median PFS time in type 1, 2, and 3 patients were 35.5, 30.2, and 1.4 months. The median OS for the group with pleural effusion CD4/CD8 > = 1.93 and < 1.93 were not reached and 2.6 months. The median PFS of those with pleural effusion CD4/CD8 > = 1.93 and < 1.93 were 18.4 and 1.2 months. In conclusion, patients with type 1 and 2 effusion patterns had better survival than those with type 3. Type 1 might be interpreted as pseudoprogression of malignant pleural effusion. CD4/CD8 ratio > = 1.93 in pleural effusion is a good predicting factor for PFS.

Introduction

Immune checkpoint inhibitors (ICIs) have become promising agents against a variety of cancers. However, in some patients, concomitant immune-related adverse events (irAEs) develop. Among organs affected by immune checkpoint blockade, pleural involvement is rare. Under ICI treatment, pseudoprogression may develop, with a transient increase in the tumor size before regression[1]. Pseudoprogression in lung-cancer patients occurs not only in the solid part of the tumor, but also has been reported in malignant spread to pleural and pericardial space with the presentation of rapidly accumulating recurrent effusions[2]. The clinical course and outcomes of patients receiving ICIs followed by pleural effusion development are poorly known.

IrAEs involving different organs may result from various mechanisms[3]. For example, in myocarditis, the inflammatory infiltration of T cells is predominantly CD8[4], whereas in pericardial involvement, T cell infiltration is predominantly CD4[5]. Which types of lymphocytes are involved in pleural effusion under ICIs remained unknown.

In the present study, we aimed to categorize the clinical presentations of ICI-related pleural effusion and analyze the lymphocyte components in the pleural effusion in relation to the clinical outcomes of non-small cell lung cancer (NSCLC) patients receiving ICIs.

Methods

Study design

NSCLC patients were enrolled retrospectively at Taichung Veterans General Hospital from Oct 2015 to Dec 2019, during which ICI treatments were initiated. The last follow-up was on May 31, 2020. Eligible patients all had non-infectious pleural effusion after ICI use. The exclusion criteria were as follows: no cytology results of pleural effusion, mortality of unknown etiology, specimens for lymphocyte analysis not from pleural effusion, the duration from last dose of ICI to development of pleural effusion exceeded 12 months. Patients receiving ICIs and docetaxel were excluded, since docetaxel was known to cause pleural effusion[6]. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB No. CF16018A). Written informed consents for clinical data records, genetic and immunological testing were obtained from all patients. All methods were carried out in accordance with the relevant approved guidelines and regulations.

Definition of disease progression and development of pleural effusion

We categorized the pattern of pleural effusion formation of our patients into three types: (1) Type1: rapid production, without disease progression, within one month after ICIs use, (2) Type 2: slow production, without disease progression, one month after ICIs use, (3) Type 3: pleural effusion due to disease progression even with ICI treatments. Disease progression was defined as follows: (1) newly developed malignant pleural effusion which did not turn from positive to negative from serial cytology exams, (2) pleural effusion negative for malignancy but with disease progression at other locations.

We defined newly developed pleural effusion as follows: (1) no pleural effusion before ICI use, but effusion developed after treatments, (2) pleural effusion existed before ICI use and rate of effusion accelerated after treatments. The definition of acceleration was as follows: (1) a pigtail catheter was inserted for symptomatic relief of pleural effusion, or (2) the frequency of thoracentesis increased.

Pleural effusion analysis and lymphocyte subset measurement

We analyzed lymphocyte subsets in pleural effusion which was collected the first time patients had received thoracentesis after ICI use. Since no less than 150 ml of pleural effusion was required for analysis, the insufficient pleural effusions of some patients were not analyzed at the first time.

Mononuclear cells in the pleural effusion were collected through density gradient centrifugation with Ficoll-Paque. For cell type analyses based on surface molecules, cells were first stained with different fluorescence-labeled monoclonal antibodies and then analyzed with flow cytometry. Cells were gated based on forward scatter channel and side scatter channel to select lymphocytes. For T cell subset study, cells were stained with phycoerythrin (PE)-anti-CD3, PerCP-anti-CD4, and FITC-anti-CD8, and cells expressing CD3 were gated for CD4 and CD8 analyses. For B cell study, cells were stained with FITC-anti-CD3 and PE-anti-CD19, and the percentage of CD3-CD19 + cells was determined. Isotype-matched control monoclonal antibodies were obtained from BD PharMingen and BioLenged. Details of equipment and antibody are shown in online supplemental table 1.

Analyses of Cytokine productions in pleural effusion

Pleural effusion was first centrifuged to remove cells and debris. We then used the sandwich-enzyme-linked immunosorbent assay (ELISA) with the OptEIA kit (BD Pharmingen) to detect levels of IL-1, IL-2, IL-10, IL-12p70 and IFN-γ in the pleural effusion. We also used the DuoSet ELISA kit (R&D Systems Inc.,

Minneapolis, MN) to detect levels of IL-8 and IL-17. IL-6 and TNF-α levels were detected by ELISA (Invitrogen, Thermo Fisher Scientific, Waltham MA). Detection ranges with ELISA are shown in online supplemental table 2.

Identification of driver mutations and PD-L1 assay

Tumor specimens were procured for oncogenic mutation analyses as previously reported[7]. Five oncogenic drivers, including EGFR, KRAS, BRAF, HER2 and EML4-ALK, were tested. For patients with squamous cell carcinoma, oncogenic mutation analyses were not routinely performed.

Three commercial Programmed Death-ligand 1 (PD-L1) IHC assays, 22C3, SP142, and SP263, were performed for all patients when adequate specimens were available. The PD-L1 IHC 22C3 pharmDx was conducted on the DAKO Autostainer Link 48, while the Ventana PD-L1 SP142 and SP263 assays were conducted on the Ventana BenchMark platform.

Data records and response evaluation

Clinical data of individual patients included age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG PS), tumor stage, smoking status, and thyroid function. The age, ECOG PS, and tumor stage were evaluated while ICIs were initiated. The overall survival (OS) and progression free survival (PFS) were analyzed from the beginning of ICI treatment. TNM (tumor, node, and metastases) staging was performed according to the 8th edition of the American Joint Committee for Cancer (AJCC) staging system. We adopted here unidimensional measurements as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Statistical methods

Fisher's exact test and Mann-Whitney U test were used to compare inter-group differences for categorical and continuous variables as appropriate. Univariate and multivariate Cox proportional hazard regression models were used to estimate the hazard ratio. The OS and PFS were estimated using the Kaplan-Meier method, whereas the between-group differences were assessed using the stratified log-rank test. Two-tailed tests with p values < 0.05 were considered statistically significant.

All analyses were performed with the IBM SPSS Statistics package, version 23 (IBM Corporation, Armonk, NY).

Results

Patient characteristics

We included a total 281 advanced (stage IIIb/IV) NSCLC patients with ICIs initiated. Among these patients, 168 patients were treated with pembrolizumab, 43 with nivolumab, 47 with atezolizumab, and 23 with durvaluamb. Among them, 27 developed pleural effusion after ICI use, with 10 excluded. Among the remaining 17 patients, three were categorized as type 1, 5 as type 2, and 9 as type 3 (Fig. 1).

Their descriptive characteristics are summarized in Table 1. All the patients had reached advanced stages of lung cancer before ICI use. Adenocarcinoma was diagnosed in 13 patients, and three of them harboring *EGFR* mutations. Negative PD-L1 expression was found in 6 patients, low PD-L1 expression in 4 patients, and high PD-L1 expression in 5 patients. Of the 17 patients, 13 received lymphocyte subset analyses of their pleural effusions within one month after first thoracentesis.

Table 1
Demographic data and characteristics of different pleural effusion types

	All (N = 17)	Type 1 + Type 2(N = 8)	Type 3 (N = 9)	*p* Value[a)]
Age, medium (IQR)	60.1 (52.6 ~ 65.8)	63.0 (52.4 ~ 68.6)	58.9 (53.9 ~ 61.6)	0.423
Gender, N (%)				0.335
Male	10 (58.8)	6 (75)	4 (44.4)	
Female	7 (41.2)	2 (25)	5 (55.6)	
Smoking status, N (%)				0.347
Ever smoker	8 (47.1)	5 (62.5)	3 (33.3)	
Never smoker	9 (52.9)	3 (37.5)	6 (66.7)	
Stage, N (%)				1.000
IIIB ~ IIIC	2 (11.8)	1 (12.5)	1 (11.1)	
IVA ~ IVB	15 (88.2)	7 (87.5)	8 (88.9)	
Brain metastasis before ICI, N (%)				0.620
Yes	5 (29.4)	3 (37.5)	2 (22.2)	
No	12 (70.6)	5 (62.5)	7 (77.8)	
ECOG PS, N (%)				0.206
0 ~ 2	14 (82.4)	8 (100)	6 (66.7)	
3 ~ 4	3 (17.6)	0 (0)	3 (33.3)	
Pathology and driver mutation, N (%)				1.000
ADC without driver mutation	10 (58.8)	5 (62.5)	5 (55.6)	
ADC with *EGFR* mutation	3 (17.6)	1 (12.5)	2 (22.2)	
Non-ADC NSCLC	4 (23.5)	2 (25)	2 (22.2)	
PD-L1, N (%)				0.147
<1%	6 (35.3)	1 (12.5)	5 (55.6)	
1–49%	4 (23.5)	2 (25)	2 (22.2)	
>=50%	5 (29.4)	4 (50)	1 (11.1)	
N/A	2 (11.8)	1(12.5)	1(11.1)	
ICI type, N (%)				0.689
Pembrolizumab	10 (58.8)	4 (50)	6 (66.7)	
Nivolumab	1 (5.9)	1 (12.5)	0 (0)	
Atezolizumab	1 (5.9)	0 (0)	1 (11.1)	
Durvalumab	5 (29.4)	3 (37.5)	2 (22.2)	
Hypothyroidism after ICI use, N (%)				0.315
Yes	6 (35.3)	2 (25)	4 (44.4)	
No	9 (52.9)	6 (75)	3 (33.3)	
N/A	2 (11.8)		2 (22.2)	
Pericardial effusion requiring drainage after ICI, N (%)				0.576
Yes	3 (17.6)	2 (25)	1 (11.1)	
No	14 (82.4)	6 (75)	8 (88.9)	

ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; ADC, adenocarcinoma; NSCLC, non-small cell lung cancer; N/A, not applicable; PR, partial response; SD, stable disease; PD, disease progression

	All (N = 17)	Type 1 + Type 2(N = 8)	Type 3 (N = 9)	*p* Value[a)]
Interval from ICI to 1st thoracentesis, months, medium (IQR)	0.63 (0.30 ~ 4.87)	1.9 (0.3 ~ 6.9)	0.6 (0.3 ~ 1.9)	0.815
Interval from 1st thoracentesis to CD4/CD8 ratio, months, medium (IQR)	0 (0 ~ 0.97)	0.9 (0 ~ 2.7)	0 (0 ~ 0)	0.093
CD4/CD8 ratio, N (%)				0.036
>= 1.93	10 (58.8)	7 (87.5)	3 (33.3)	
< 1.93	7 (41.2)	1 (12.5)	6 (66.7)	
B cell ratio, N (%)				0.131
>= 6.09	5 (29.4)	4 (50)	1 (11.1)	
< 6.09	12 (70.6)	4 (50)	8 (88.9)	
a)Probability value by Mann-Whitney U test and Fisher's exact test.				
ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; ADC, adenocarcinoma; NSCLC, non-small cell lung cancer; N/A, not applicable; PR, partial response; SD, stable disease; PD, disease progression				

Different types of pleural effusion

Clinical data and outcomes of the enrolled patients are shown in Table 2. The disease course and treatment timeline for patients of types 1 and 2 are shown in Fig. 2. Type 1 patients within 2 weeks after ICI use developed pleural effusion with or without pericardial effusion (one patient also received pericardial drainage), and pigtail catheters were applied to two patients. All these patients presented with pleural effusion before ICI use and one of them was found to have malignant pleural effusion. Malignant cells were found in pleural effusion after the initial treatment, but were then absent in the following serial thoracentesis. Type 2 patients developed pleural effusion one month after ICI use. Malignant pleural effusion was not documented before or after ICI use. Among type 3 patients, malignant pleural effusion persisted in 6 patients while disease progression to other organs was found in three patients.

Table 2
Clinical data and outcomes of lung cancer patients developing pleural effusion after ICI use

No.	Age	Gender	Smoking	PS	Cell type/ *EGFR*	Stage	Brain mets	PD-L1	ICI	Cycle	PFS (m)	OS (m)	ICI to PE(m)	PE to CD4/CD8 (m)	Initial CD4/CD8	Initia B ce ratio
1	60.1	M	E	2	ADC	IVB	No	negative	n	92$	35.5	50.5#	0.2	1.7	1.89	0.3
2	79.0	F	N	2	ADC	IVA	No	high(+)	P	8	56.6*	56.6#	0.3	0.0	4.01	23.7
3	65.8	M	E	1	ADC	IVA	No	low(+)	D	4	7.8*	7.8#	0.3	0.1	7.22	12.6
4	47.6	F	N	1	IMA	IIIC	No	N/A	D	15	14.5*	14.5#	7.9	4.5	3.84	0.3
5	74.3	M	N	1	ADC@1	IVB	Yes	high(+)	P	14$	13.3*	13.3#	6.5	2.3	2.83	3.4
6	66.6	M	E	2	SqCC	IVB	Yes	high(+)	P	1	6.6*	6.6#	0.3	0.0	6.22	2.7
7	51.9	M	E	1	ADC	IVA	No	high(+)	D	26	14.0	24.8	3.5	5.9	2.10	7.3
8	52.6	M	E	1	ADC	IVB	Yes	low(+)	P	26$	30.2	31.3#	25.0	0.0	1.95	16.3
9	61.6	F	N	1	ADC	IVB	No	low(+)	P	1	0.5	0.6	0.2	0.0	1.07	2.7
10	56.4	M	E	3	ADC	IVB	No	negative	P	1	0.9	2.2	0.3	0.0	1.85	1.6
11	64.4	M	N	2	SqCC	IVB	No	negative	A	2	1.6	2.3	0.6	1.0	13.23	1.5
12	60.3	F	N	4	ADC@2	IVB	No	high(+)	P	5	3.2	4.7	0.2	0.0	4.78	4.9
13	45.7	F	N	1	ADC	IVA	No	negative	P	6	5.0	29.9#	4.9	0.0	1.36	0.8
14	47.6	M	N	1	ADC	IVB	Yes	negative	P	2	1.2	2.6	0.7	0.7	1.90	3.8
15	58.9	M	N	3	ADC@1	IVB	Yes	N/A	P	1	0.8	0.8	0.6	0.0	1.90	2.5
16	68.1	F	E	1	ADC	IVB	No	negative	D	6	1.4	14.0	1.9	0.0	1.90	4.5
17	53.9	F	E	1	SqCC	IIIB	No	low(+)	D	39$	18.4	20.9#	19.1	0.0	8.50	34.0

ICI, immune checkpoint inhibitor; M, male; F, female; E, ever smoker; N, never smoker; PS, Eastern Cooperative Oncology Group performance status; ADC, aden EGFR L858R mutation; SqCC, squamous cell carcinoma; @2, EGFR G719S mutation; Brain mets, brain metastasis before ICI use; n, nivolumab; P, pembrolizur month; *, no disease progression; #, survive; ICI to PE, time from ICI use to 1st thoracentesis; PE, pleural effusion; PE to CD4/CD8; interval from 1st thoracente before ICI, pleural effusion noted by image before ICI use; cytology (+), positive for malignant cell; cytology (-), negative for malignant cell; N/A, not applicable most times it showed negative for malignancy; PE once, the patient received thoracentesis for only one time.

Characteristics were compared between non-disease progression type (types 1 and 2) and disease progression type (type 3) (Table 1). In the non-disease progression group, 87.5%showed pleural effusion CD4/CD8 ratio > = 1.93, compared with 33.3% in the disease progression group (p = 0.036). The median OS in type 1 patients was not reached, whereas in type 2 was 24.8 months, and in type 3 was 2.6 months (Fig. 3B). The median PFS periods for patients with type 1, 2, and 3 effusion were 35.5, 30.2, and 1.4 months, respectively (Fig. 3D).

Pleural effusion with CD4/CD8 ratio > = 1.93 is a good predictor for survival

High pleural effusion CD4/CD8 ratios correlating with longer PFS and OS was more commonly found in patients of types 1 and 2. Cell surface expressions of CD4 and CD8 on T cells are shown in online supplemental Fig. 1. Patient no.1 and no.7 received serial pleural effusion CD4/CD8 ratios examination and the former had progressively elevated ratios (Fig. 2 and online supplemental Fig. 1 Patient no.1). Receiver operating curves analysis was applied to identify the optimal cutoff threshold (online supplemental Fig. 2). PFS of 3.7 months and OS of 12.0 months were used as cut-points according to median survivals in KEYNOTE-001 trial[8]. In predicting OS, two patients were excluded from analysis because the follow-up time failed to last > 12.0 months after ICI use. The cutoff threshold was set at 1.93 in predicting OS, PFS, and the type of pleural effusion.

In univariate and multivariate analyses for OS or PFS before ICI use, Cox-regression showed no significantly related risk factor, like age, gender, smoking history, PD-L1 status, and brain metastasis (Table 3A and 3B). ECOG PS was identified as significant predictors associated with OS in both univariate and multivariate analyses Similarly, ECOG PS and CD4/CD8 ratio were predictors of PFS. The median OS in CD4/CD8 > = 1.93 group was not reached, whereas the median OS in CD4/CD8 < 1.93 was 2.6 months (Fig. 3A). The median PFS of patients with CD4/CD8 > = 1.93 was 18.2 months, and with this ratio < 1.93 was 1.2 months (Fig. 3C).

Elevated pleural effusion B cell percentages in type 1 patients

Higher percentages of B cells were found in pleural effusions of patients without disease progression, especially in type 1 patients (Table 2). The optimal cutoff percentage of B cells was 6.09% in predicting OS, PFS, and pleural effusion types (online supplemental Fig. 3). In a previous study, Nieto et al. reported CD20 + B lymphocytes account for 5.81% of all leukocytes in malignant pleural effusion[9] so our finding of 6.09% is reasonable for defining “elevated” B cell ratio. Of the 17 patients, 5 had elevated B cell ratios in their initial pleural effusion analyses, and 4 of them were with type 1 and type 2 effusions.

Higher IL-8 levels in patients with pleural effusion CD4/CD8 ratio < 1.93

Expression levels of cytokines in pleural effusion were shown in online supplemental Fig. 4. IL-8 levels in pleural effusion of patients with CD4/CD8 ratios < 1.93 were higher than those with ratio > = 1.93 (online supplemental table 3A). Expression levels of cytokines were however similar across effusion types (online supplemental table 3B).

Table 3. Univariate and multivariate analyses of (A) Overall survival (OS) and (B) Progression free survival (PFS)

(A) Overall survival

	Univariate		Multivariate	
Variable	HR (95% CI)	*p* Value[a)]	HR (95% CI)	*p* Value[a)]
Age	1.00 (0.93-1.07)	0.866		
Male gender	1.38 (0.32-5.85)	0.666		
Never smoker	2.00 (0.48-8.43)	0.344		
ECOG PS				
0-2	1			
3-4	7.81 (1.52-40.29)	0.014	8.82 (1.48-52.66)	0.017
Brain mets before ICI	0.97 (0.19-4.89)	0.973		
PD-L1				
<1%	2.01 (0.36-11.11)	0.213		
1~49%	0.77 (0.07-8.50)	0.829		
>= 50%	1			
CD4/CD8 ratio				
<1.93	1			
>=1.93	0.31 (0.07-1.30)	0.108	0.285 (0.06-1.33)	0.111

(B) Progression free survival

	Univariate		Multivariate	
Variable	HR (95% CI)	*p* Value[a)]	HR (95% CI)	*p* Value[a)]
Age	0.95 (0.89-1.02)	0.158		
Male gender	1.07 (0.34-3.37)	0.915		
Never smoker	1.31 (0.41-4.12)	0.650		
ECOG PS				
0-2	1			
3-4	6.00 (1.30-27.72)	0.022	6.78 (1.29-35.49)	0.024
Brain mets before ICI	1.02 (0.27-3.86)	0.980		
PD-L1				
<1%	3.84 (0.77-19.33)	0.103		
1~49%	2.10 (0.34-12.91)	0.424		
>= 50%	1			
CD4/CD8 ratio				
<1.93	1			
>=1.93	0.27 (0.08-0.87)	0.028	0.25 (0.07-0.86)	0.027

a) Probability value by Cox regression model. ECOG PS, Eastern Cooperative Oncology Group performance status; mets, metastasis; ICI, immune checkpoint inhibitor; HR, hazard ratio; CI, confidence interval

Discussion

We have here found different presentations of lung cancer patients developing pleural effusion after receiving ICI. Three effusion developmental patterns were identified. Type 1 patients developed massive effusion within one month after initiating ICI treatment, usually within two weeks. The first time cytological examinations of thoracentesis after treatment revealed positive for malignancy in all these patients. Their development of effusion could be interpreted as "pseudoprogression", because the cytological examinations turned negative in the serial thoracentesis afterwards. Fulminant effusion development was resolved within two months after ICI use.

Most researchers reported survival benefits of pseudoprogression markedly better than that of typical progression[10–13]. In our study, type 1 patients had longer PFS and OS than those of type 3 and type 2 patients. Some studies reported that the malignant pleural effusion present before anti-PD-1 treatment is associated with shorter PFS and OS[14]. In our study, if pseudoprogression occurred as type 1 pleural effusion, long-term survival could be achieved. Therefore, ICI should still be considered in patients with malignant pleural effusion.

Kolla et al. reported similar cases in which pseudoprogression was suspected after nivolumab administration[2]. One patient developing massive pleural effusion had frequent thoracentesis for 8 weeks after nivolumab use. Cytological examination from pleural effusion was positive for malignancy. Nivolumab continued and there was a complete response. No more drainage was recorded after the first two months of therapy. That case shared a similar clinical presentation with our type 1 patients and may be categorized as "type 1" pleural effusion.

Type 2 pleural effusion developed one month after ICI treatment had begun. The time from treatment to first thoracentesis was as long as 25 months (case no.8). It is not surprising that the occurrence of irAEs was delayed, since Nigro et al. reported earlier that late-irAEs (after 12 months) are common (incidence 30.3%) in long responders to ICIs[15]. The cytology of pleural effusion was never documented positive before or after ICI use so this type was not categorized as pseudoprogression. Thoracentesis was usually infrequent. The exception was an atypical case no.7 who developed massive pleural effusion. Cytological results were variable, with alteration of positive or negative findings of malignancy, and subsequent infection of the effusion was also noted.

We identified those with CD4/CD8 ratios of pleural effusion > = 1.93 were well predicted for their survival. Though the initial CD4/CD8 ratio of patient no.1 (type 1) was only 1.89, serial pleural effusion CD4/CD8 ratios examined showed progressively elevated ratios, with the highest reaching 13.8. It is worth noting that the B cell ratio of pleural effusion was also elevated from 0.4–22.1% in serial analyses (Fig. 2). For such patients presented with the typical type 1 or type 2 pleural effusions with CD4/CD8 ratios < 1.93, serial follow up is recommended, because elevation of the ratio may indicate a good response to the ICI treatment. In type 1 patients, elevated B cell percentage is the feature distinguishing them from types 2 and 3 patients.

Three type 3 patients had initial pleural effusion CD4/CD8 ratios > = 1.93. Patient no.11 and 12 showed partial responses at the primary lesion, but development of new lesions was noted during follow up. No further treatment was given after disease progression due to poor performance status. This challenged the interpretation of OS. Patient no.17 developed pleural effusion 19 months after starting durvalumab medication, and CD4/CD8 and B cell ratios then increased, while cytological results were positive. Nevertheless, the PFS of this patient went up to 18.4 months. Infrequent thoracenteses was performed and there's no disease progression to the other organs beyond the pleura. Longer follow ups are desirable as the clinical presentation was different from other type 3 patients.

Infiltration of inflammatory cells with CD4 + predominant may contribute to elevated CD4/CD8 ratio in the pleural effusion. Scherpereel et al. evaluated T cell populations in patients with pleural effusion. Their blood CD4/CD8 ratios were 1.6. In healthy subjects, the ratio in pleural fluid is 0.59, compared with higher ratios of 3.8 in patients with pleural metastasis[16]. Aguiar et al. found CD4/CD8 ratios were similarly higher in malignant pleural effusion than in the peripheral blood (i.e., 3.6 vs 1.4)[17]. Nieto et al. reported that in patients after diagnosing malignant pleural effusion, their lymphocytes count in the pleural effusion is positively correlated with survival. CXCL10 helps attract lymphocytes in malignant effusion[9]. Accordingly, in patients with malignant pleural effusion, CD4/CD8 ratio of which is higher than the peripheral blood. This may be a defensive mechanism against cancer, and ICI likely reinforces the mechanism.

Regarding irAEs, cytokines or chemokines in response to ICIs have been studied. Khan et al. reported irAEs patients have initially low levels of CXCL9, 10, 11 and 19, but levels of CXCL 9, and 10 remarkably increase after treatment compared with those patients without irAEs[18]. Lim et al. found elevations of 11 cytokines in patients with severe irAEs, and even introduced a cytokine toxicity score[19]. IL-17 and IL-6 levels were reported as biomarkers in predicting irAEs[20,21]. In our study, we found IL-8 levels in patients with pleural effusion CD4/CD8 ratio < 1.93 were higher than those with ratio > = 1.93. IL-8, a chemokine produced by cancer cells, could play a role in cancer microenvironment. Higher IL-8 levels are correlated with poor prognosis[22]. Only one patient from the type 1 group has a higher level of IL-17. We also examined several other cytokines including IL-1, IL-2, IL-4, IL-6, IL-12p70, INF-γ, and TNF-α. However, the levels of these cytokines are either under detection limit or demonstrate no significant difference among the three types of patients.

There were several limitations of our study. First, its sample size was small, and was conducted retrospectively in a single medical center. Second, not all patients had their CD4/CD8 ratios determined at the initial thoracentesis. Also, their CD4/CD8 ratios were not determined before ICI treatment nor their ratio in the peripheral blood. Third, 11 of 17 patients received both chemotherapy and ICI, presenting a confounder on response evaluation. However, no patient was lost during follow up and all required clinical information was collected. We are the first to report two distinct types of pleural effusions after ICI use. These two types of patients both had relatively good prognosis. Our study is also the first to use the CD4/CD8 ratio in pleural effusion to predict patient survival after ICI use.

In conclusion, beside pleural effusion due to disease progression (type 3), two distinct effusion types were identified after ICI use: type 1, rapid (develop < 1 month) and massive and type 2, slow (develop > = 1 month) and relative indolent. Both types showed better overall and progression free survival than type 3. Type 1 could be interpreted as pseudoprogression of malignant pleural effusion. CD4/CD8 ratio > = 1.93 in pleural effusion after ICI use is a good predicting factor in PFS. In most patients of types 1 and 2, their CD4/CD8 ratios > = 1.93 in pleural effusion. In those patients presented with typical type 1 or type 2 pleural effusion but with CD4/CD8 ratios < 1.93, serial follow up is recommended because elevating ratio may indicate a good response to ICI.

Declarations

Acknowledgments

None

Author contributions

Po-Hsin Lee: Methodology, Methodology, Software, Formal analysis, Data Curation, Writing - Original Draft, Visualization

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Kun-Chieh Chen: Investigation, Resources

Yen-Hsiang Huang: Formal analysis, Resources

Jeng-Sen Tseng: Formal analysis, Resources, Writing - Original Draft

Kuo-Hsuan Hsu: Investigation, Resources

Yu-Chen Wu: Resources, Data Curation

Ko-Jiunn Liu: Conceptualization, Methodology, Validation, Resources, Data Curation, Writing - Review & Editing, Supervision

Gee-Chen Chang: Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision, Project administration

Additional Information (including a Competing Interests Statement)

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

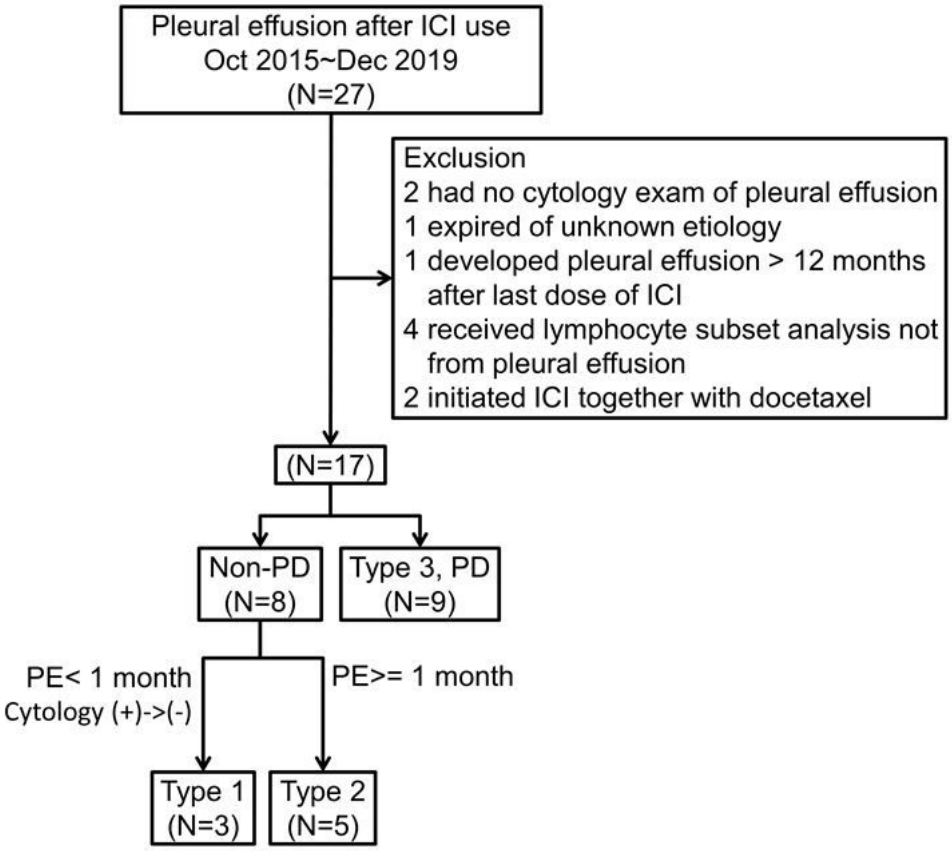

Figure 1

Algorithm for enrollment and follow-up of the study participants. Type 1: rapid growth of pleural effusion within one month after immune checkpoint inhibitor use. Malignant cells were found in pleural effusion after the initial treatment but were absent in the following serial thoracenteses. Type 2: slow growth of pleural effusion developed more than one month after immune checkpoint inhibitor use. Type 3: pleural effusion due to disease progression. ICI: immune checkpoint inhibitor, PD: disease progression, PE: pleural effusion

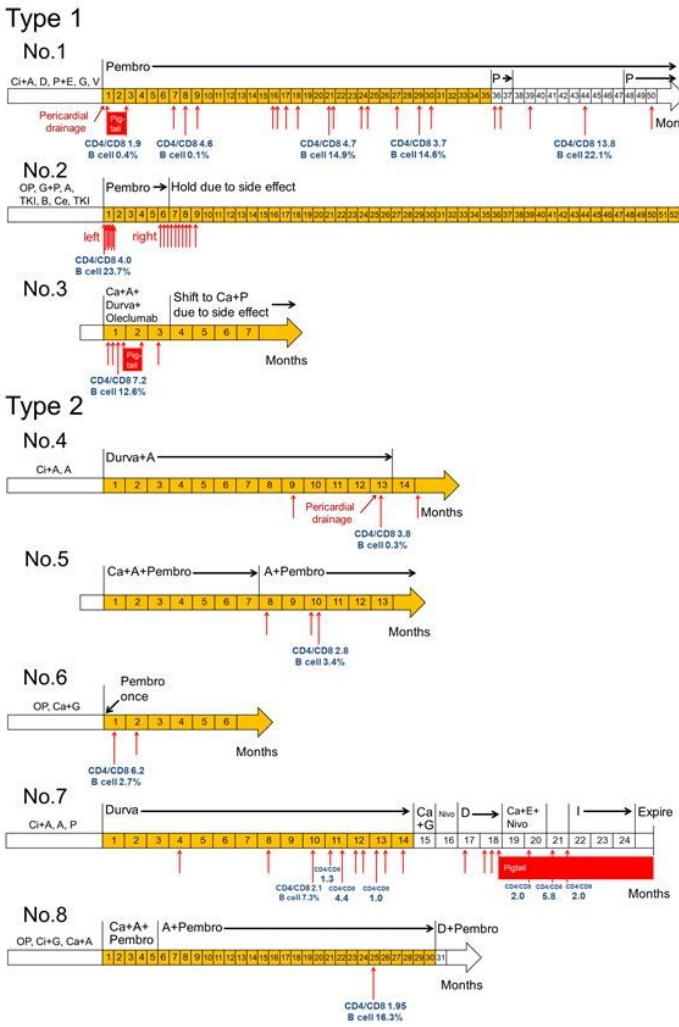

Figure 2

Disease course and treatment timeline for patients with type 1 and type 2 pleural effusions. Red Arrow: Pleural effusion requiring thoracentesis. Time line highlighted in yellow: progression free survival. Ci, cisplatin; A, pemetrexed (Alimta); D, docetaxel; P, paclitaxel; E, etoposide; G, gemcitabine; V, vinorelbine; Pembro, pembrolizumb; OP, surgical intervention; TKI, EGFR-tyrosine kinase inhibitor; B, bevacizumab; Ce, cetuximab; Ca, carboplatin; Durva, durvalumab; Nivo, nivolumab; I, irinotecan

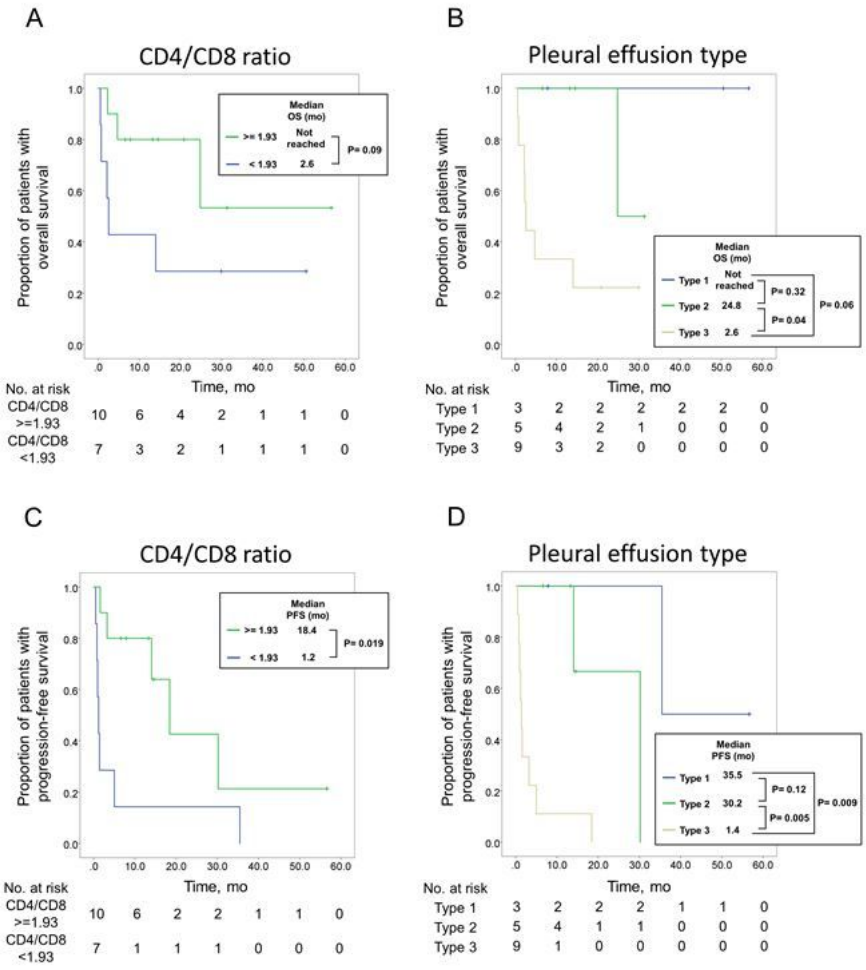

Figure 3

Overall survival (OS) and progression free survival (PFS) according to pleural effusion CD4/CD8 ratios and types. (A) OS and (C) PFS shown by Kaplan-meier methods according to their different CD4/CD8 ratios in pleural effusion. (B) OS and (D) PFS plotted by Kaplan-meier methods according to types of pleural effusion.

Supplementary Files

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- Supplementaryinformation14Jan2021.pdf