Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Role of GSDMD and VEGF in differentiating between malignant and non-malignant pleural effusions

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Abstract

Background It is crucial to differentiate between benign and malignant pleural effusions while making a diagnosis. The purpose of this research was to investigate the diagnostic significance of GSDMD and VEGF in discriminating between different kinds of pleural effusion and their correlation with both progression-free and overall survivals in the malignant type.

Methods Ninety-one pleural fluid samples, which were classified as transudates or exudates (tuberculous, para-infectious, or malignant) by pleural fluid classifications, were tested for GSDMD using sandwich ELIZA kits, and 41 of the exudative samples were randomly selected for VEGF testing. Both markers' diagnostic accuracy was assessed.

Results The lowest level of GSDMD was associated with the transudate group (mean and SD of 2.35 ± 0.44 ng/mL) and the highest in the malignant effusion group (mean and SD of 4.38 ± 1.67 ng/mL). The specificity and sensitivity of GSDMD in the diagnosis of exudative PE were 97% and 98%, respectively ($p = 0.001$) with the cutoff point = 2.89). Regarding VEGF, its level was 222.3 ± 53.4 pg/ml for all studied samples where MPE ($n = 21$) was 261.2 ± 48.2 pg/ ml (mean ± SD), TBPE ($n = 7$) was 185.4 ± 6.96 pg/ml (mean ± SD), and PIPE ($n = 13$) was 179.3 ± 13.9 pg/ml (mean ± SD). The diagnostic accuracy of VEGF for the detection of MPE was 90% with a sensitivity of 100% and specificity of 80% and the cutoff point was 191.5 pg/ml. There were highly significant inverse correlations between progression-free survival and both GSDMD ($r = -0.531$, $p = 0.009$) and VEGF ($r = -0.582$, $p = 0.006$) in MPE.

Conclusion Pleural effusion GSDMD can be an effective marker for differentiating the different kinds of PE, and VEGF levels can be a useful adjuvant marker in screening out MPE as a possible diagnosis, leading to the proper selection of patients who may benefit from more invasive procedures.

Keywords GSDMD, VEGF, Pleural effusion

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1 Background

Pleural effusion (PE) is connected with a variety of benign and malignant illnesses and determining its cause could be difficult. One of the most difficult aspects of its diagnosis is separating exudates from transudates [1]. A further critical concern is whether the effusion is benign or malignant. Although the cytological evaluation of PE samples is easy and has good specificity, it has a low sensitivity [2]. Clarifying the etiology of malignant PE in a patient with negative cytology necessitates more complex diagnostic methods. As a result, distinguishing between non-malignant and malignant PE for some patients may be challenging and confined by the risks associated with invasive methods [3].

The evaluation of inflammatory markers and growth factors is a newer and underutilized diagnostic technique for PE.

Gasdermin D (GSDMD) is an agent that causes pyroptosis, a type of programmed cell death caused via inflammatory caspases that fragment GSDMD and activate and secrete interleukin-18 (IL-18) and IL-1. [4] A significant variety of research has been done on the structure and functionality of GSDMD and its isoforms [5]. The GSDMD concentration is considerably elevated in pulmonary tuberculosis, pneumonia, and non-small cell lung cancer. [6–8] However, limited studies have demonstrated its relevance in distinguishing between malignant and non-malignant PE.

VEGF (vascular endothelial growth factor) is a key regulator of vasculogenesis and capillary permeability as it promotes the development of capillaries and has unique chemotactic and mitogenic actions on the vascular endothelium [9]. As a result, VEGF may be a significant marker in the formation of malignant PE and, as a result, can help in distinguishing between it and the benign type.

The objective of this study was to investigate the efficacy of GSDMD and VEGF in discriminating PE of different etiologies and their correlation with both progression-free and overall survivals in the malignant type.

2 Methods

2.1 Study population

This prospective study involved 91 consecutive patients with newly diagnosed PE. The study was conducted from June 2021 to December 2022. The study was authorized by the Menoufia University Hospital's Ethics Committee (**IRB: 10/2022EONC21**). The sample was classified as transudate or exudate based on the definition of pleural fluid causes. GSDMD was detected in all pleural fluid (PF) samples ($n=91$), while VEGF was detected in 41 samples selected randomly from exudative samples.

The following are some of the causes of pleural effusion: Para-infectious pleural effusions (PIPE) were defined when associated with pneumonia, positive bacterial culture, or lung abscesses provided that no TB bacilli were in the pleural fluid. When malignant cells were found in the pleural space via cytological analysis or tissue biopsy, malignant PE (MPE) was diagnosed. Tuberculous PE (TBPE) was identified depending on the existence of TB bacilli (acid & alcohol fast) in pleural fluid or biopsies tissue (and/or the presence of caseating granuloma).

Transudate PE (TPE) was verified using Light's criteria: the PF/serum protein ratio is < 0.5, the PF lactate dehydrogenase (LDH) is < 2/3 the normal maximum limit and the PF /serum LDH ratio is < 0.6 [10]. When there was a conflict between the Light's criteria and the clinical aspect, the albumin gradient between the serum and PE of more than 1.2 g/dl identified the TPE [11].

Patients presented with combined cancer lung and TB pneumonia, malignancy associated with transudate pleural effusion, thoracotomy tube, undiagnosed pleural effusion, patients under the age of 18, or patients who declined to participate in this research were all omitted.

2.2 Collection of samples

Each patient provided informed and written consent. At the time of the first tapping, a thoracocentesis was conducted, and the PF and serum samples were taken.

The pleural fluid (PF) was collected in two different types of tubes one containing EDTA (for cytological examination) and one plain (for examination of LDH, total protein, adenosine deaminase (ADA), Albumin, total cell count, TB bacilli, culture for bacteria, GSDMD, and VEGF-A). Levels of total protein, albumin, and LDH were investigated in peripheral venous blood Samples collected in the plain ones.

All samples (except for GSDMD and VEGF) were immediately analyzed using established automated procedures, and Light's criteria were used to discriminate transudate from exudate effusion samples. If the analysis does not yield a diagnosis, a pleural biopsy may be needed to reach the final diagnosis.

Centrifugation of PF samples was done directly at 3000 rpm for 10 min to measure GSDMD and VEGF. The pleural fluid supernatant was collected and stored at − 20 °C for future investigation.

2.3 Gasdermin D assay

A double-antibody sandwich enzyme-linked immunosorbent test (ELISA) to detect the presence of Human GSDMD in all PF samples (by SunRed Biological Technology Co., Ltd, Shanghai). In the Standard wells, 50 µl of each standard was added. 40 µl of each sample was added

to the test wells. Both standard and test wells received 50 µl of prepared streptavidin-HRP solution before 10 µl of GSDMD-antibody were added to each well of the microtiter ELISA plate. At 37° C, the plates were incubated for an hour. After that, the plates were washed five times with wash buffer and each well received 50 µl of chromogen solution A and 50 µl of chromogen solution B, which caused the liquid to turn blue. The wells were then incubated at 37 °C for 10 min without exposure to light. To each well, 50 µl of stop solution was added (the blue color changes into yellow immediately). A microplate reader was used to determine the absorbance of each colored solution at a wavelength of 450 nm. According to the supplier, the sensitivity of this test was 0.089 ng/ml, with the range of the assay being 0.1—15 ng/ml.

2.4 Vascular endothelial growth factor A assay

A double-antibody sandwich ELISA (SunRed Biological Technology Co., Ltd, Shanghai) was used in the kits to determine the level of Human VEGF in randomized 41 exudative pleural effusion samples. It was done as performed with the GSDMD assay. According to the supplier, the sensitivity of this test was 2.677 pg/ml and the assay range was 3–900 pg/ml.

For GSDMD and VEGF: The standard curve linear regression equation was developed based on the concentration of the standards and the associated optic density values, and the optic density values of the sample were then used in the regression equation to compute the concentration of the corresponding sample.

2.5 Response to treatment and survival data

All of the participants with MPE had received systemic anticancer therapy either chemotherapy and\or targeted therapy. Follow-up was done for all MPE patients starting from the date of the first diagnosis.

The treatment response of participants was evaluated using the updated RECIST guidelines (version 1.1) [12] as the following: Complete response: necessitates the clearance of all targeted lesions and any pathologic lymph nodes on the short axis to less than 10 mm. The partial response: a reduction of at least 30% in the total targeted lesion diameters from the baseline sum. Progressive disease: A 20% increase in the overall diameters of targeted lesions. Additionally, the total needs to have a minimum absolute rise of 5 mm. Stable disease: Neither adequate shrinking to meet for the partial response nor sufficient rise to meet for progressive disease. It is important to note that the presence of one or even more extra lesions is also termed progression.

Progression-free survival was estimated from the date of the diagnosis to the date of the disease progression (biochemical relapse, pathological progression, or radiological progression). While the duration from the diagnosis to the latest follow-up or death (due to cancer), whichever came first, was used for the estimation of overall survival. When accessing a patient's records, if the patient was still living, the case was censored at that time.

2.6 Statistical analysis of the data

By using the Statistical Package for Social Science version 22, (SPSS, Inc, Chicago, Illinois, USA), qualitative data were presented as numbers and percentages, whereas quantitative data were reported as mean and standard deviation. The Chi-square test was employed to investigate the relationship between 2 qualitative variables. Furthermore, the Mann–Whitney test was used for the comparison of 2 groups that were not normally distributed and had quantitative factors. The ANOVA test was used for the comparison between 3 or more normally distributed groups with quantitative variables, while for the comparison between 3 or more groups that were not regularly distributed and had quantitative factors we used the Kruskal–Wallis test. The diagnostic accuracy of both markers in the differential diagnosis of PE was determined using ROC curve analysis. The log-rank test and Kaplan–Meier graphs were used for the comparison between medians of survival times between high and low marker expressions. A statistically significant p value of 0.05 or less was used.

3 Results

This study involved 91 patients (54 Males, and 37 Females) admitted to Menoufia University Hospital's Chest Department with newly diagnosed pleural effusion. According to PF analysis, the patients were divided into the transudate PE (TPE) group ($n=36$ with an age of 47.8 ± 10.7 years, mean $\pm$ SD) and exudative PE (EPE) group which redivided into 3 subgroups: MPE ($n=23$ with an age of 48.3 ± 13.6 years), TBPE ($n=8$ with an age of 37.8 ± 12.0 years, mean $\pm$ SD), and PIPE ($n=24$ with an age of 42.2 ± 11.5 years, mean $\pm$ SD). The differences in demographic and laboratory investigations between groups are shown in Table 1.

The pleural fluid GSDMD level for exudative PE was 3.73 ± 1.23 ng/ml (mean $\pm$ SD), where the MPE group was 4.38 ± 1.67 ng/ml (mean $\pm$ SD), TBPE was 3.44 ± 0.38 ng/ml (mean $\pm$ SD) and PIPE was 3.20 ± 0.30 ng/ml (mean $\pm$ SD) while for TPE, it was 2.35 ± 0.44 ng/ml (mean $\pm$ SD) as presented in Table 2.

Regarding VEGF, its level was 222.3 ± 53.4 pg/ml for all studied samples where MPE ($n=21$) was 261.2 ± 48.2 pg/ml (mean $\pm$ SD), TBPE ($n=7$) was 185.4 ± 6.96 pg/ml (mean $\pm$ SD), and PIPE ($n=13$) was 179.3 ± 13.9 pg/ml (mean $\pm$ SD). Except for TBPE and PIPE, there were

Table 1 Demographic data and laboratory investigations among the studied patients ($N=91$)

Studied variables	Malignant pleural effusion group (MPE) ($N=23$)	Tuberculous pleural effusion (TBPE) ($N=8$)	Para-infectious PIPE ($N=24$)	transudative pleural effusion group (TPE) ($N=36$)
Age (years)	48.3 ± 13.6	37.8 ± 12.0	42.2 ± 11.5	47.8 ± 10.7
Mean ± SD	47.0	39.0	40.5	44.0
Median	28.0 – 71.0	19.0 – 58.0	23.0 – 68.0	33.0 – 69.0
Range				
Sex	15(65.2)	5(62.5)	16(66.7)	18(50.0)
Male	8(34.8)	3(37.5)	8(33.3)	18(50.0)
Female				
Pleural Effusion total protein gm/dl	6.62 ± 1.21	5.63 ± 0.90	5.48 ± 1.27	2.03 ± 0.73
Mean ± SD	6.40	5.56	5.18	2.10
Median	4.20 – 8.97	4.28 – 6.96	3.48 – 8.20	0.40 – 3.38
Range				
Pleural Effusion LDH (IU/L)	652.0 ± 192.1	503.3 ± 144.9	516.2 ± 248.8	98.8 ± 28.2
Mean ± SD	573.3	469.1	420.7	95.0
Median	357.8– 988.0	302.0 – 768.0	282.5 – 1100	59.7 – 187.0
Range				
ADA (IU/L)	36.6 ± 9.94	76.3 ± 24.6	21.2 ± 7.52	7.72 ± 1.46
Mean ± SD	37.5	72.0	21.0	8.00
Median	11.0 – 55.0	49.0 – 128.0	4.90 – 35.0	4.30 – 10.0
Range				
Total leucocytic cells/mm3	9(39.1)	4(50.0)	11(45.8)	36(100.0)
< 1000	14(60.9)	4(50.0)	13(54.2)	0(0.00)
> 1000				
Differential cell count	2(8.70)	0(0.00)	0(0.00)	1(2.80)
eosinophilic	21(91.3)	8(100)	4(16.7)	30(83.3)
lymphocytic	0(0.00)	0(0.00)	0(0.00)	1(2.80)
neutrophil	0(0.00)	0(0.00)	0(0.00)	4(11.1)
polymorphs				
Type of malignancy in MPE	7(30.4)	-	-	-
Bronchogenic carcinoma	5(21.7)			
Breast cancer	3(13.1)			
Mesothelioma	4(17.4)			
Lymphoma	3(13.1)			
Hepatocellular carcinoma	1(4.3)			
Ovarian carcinoma				

LDH lactate dehydrogenase, *ADA* Adenosine deaminase, *MPE* Malignant pleural effusion

statistically significant variations in GSDMD and VEGF levels among all groups as presented in Table 2.

The correlation between GSDMD and laboratory investigations in the EPE group is demonstrated in Table 3 where there were significant positive correlations between GSDMD and total protein, LDH, ADA, and VEGF levels in the PF. Furthermore, in the exudative pleural effusion group, there were substantial positive correlations between VEGF-A and pleural fluid levels of total proteins, LDH, and ADA, but only ADA reached a significant level ($p=0.031$).

The results in Table 4 indicate the GSDMD marker's diagnostic accuracy in EPE diagnosis together with the diagnostic accuracy of the VEGF marker in the diagnosis of MPE, and Fig. 1 shows the ROC curve for their sensitivity and specificity, where the AUC was 0.994 with a cutoff point of 2.89 ng/ml for differentiating between transudate and exudative PE, and the sensitivity and specificity were 98% and 97%, respectively, for GSDMD, while for VEGF, the AUC was 1.00 with a cutoff point of 191.5 pg/ml for detection oF MPE, and the sensitivity and specificity were 100% and 80%, respectively.

Furthermore**,** the diagnostic accuracy of GSDMD in distinguishing between TPE and different kinds of EPE and in distinguishing different types of EPE is presented in Table 5, and the ROC curves for their sensitivity and specificity are illustrated in Figs. 2 and 3.

The response to first-line treatment was assessed in MPE patients, most of the cases achieved partial response to therapy (52.2%), six cases had stable disease (26.1%), three cases (13%) with complete response to therapy, and two cases (8.7%) had their disease progressed. Eighteen cases (78.3%) progressed after first-line therapy and five cases (21.7%) didn't. At the end of our study, four cases

Table 2 GSDMD and VEGF levels among the studied patients ($N=91$)

Studied variables	Exudative pleural effusion (EPE) group (N=55)			Transudative PE (TPE) (N=36)	Test of sig	P value
GSDMD (ng/ml) Mean±SD Median Range	3.73±1.23 3.30 2.63 – 8.21			2.35±0.44 2.43 1.24 – 2.90	U 7.93	0.001*
	MPE (N=23)	TBPE (N=8)	PIPE (N=24)		-	
	4.38±1.67 3.51 2.92 – 8.21	3.44±0.38 3.30 3.11 – 4.24	3.20±0.30 3.14 2.63 – 4.04		K 68.4	Post hoc P_1:0.014* P_2:0.001* P_3:0.520 P_4:0.001* P_5:0.003* P_6:0.001*
VEGF (pg/ml) Mean±SD Median Range	N=41 222.3±53.4 197.6 154.0 - 362.0			-	-	-
	MPE (N=21)	TBPE (N=7)	PIPE (N=13)	-	-	-
	N=21 261.2±48.2 256.4 198.0 - 362.0	N=7 185.4±6.96 183.7 176.0 - 194.0	N=13 179.3±13.9 183.8 154.0 - 197.0	--	F 25.1	Post hoc P_1:0.001* P_2:0.001* P_3:0.722

F ANOVA test, *K* Kruskal–Wallis test, *U* Mann–Whitney test, *MPE* Malignant pleural effusion, *TBPE* Tuberculous pleural effusion, *PIPE* Para-infectious pleural effusion

* Significant, P_1: Comparison between MPE and TBPE, P_2: Comparison between MPE and PIPE, P_3: Comparison between TBPE and PIPE, P_4: Comparison between MPE and TPE, P_5: Comparison between TBPE and TPE, P_6: Comparison between PIPE and TPE

Table 3 Correlation between GSDMD and VEGF with laboratory investigations of exudative pleural effusion group

Studied variables	GSDMD		VEGF	
	r	*P* value	*r*	*P* value
Effusion total protein	0.463	0.001*	0.125	0.435
Effusion LDH	0.317	0.019*	0.296	0.061
ADA	0.531	0.001*	0.336	**0.031***
VEGF	0.488	0.001*	–	

LDH Lactate dehydrogenase, *ADA* Adenosine deaminase, *Significant

died (17.4%) and nineteen cases (82.6%) were still alive (Table 6).

The median progression-free survival was 12 months (11.4 ± 2.85 mean ± SD), while the median overall survival was 6 months (7.13 ± 3.93 mean ± SD) as presented in Table 7. Furthermore, there are highly significant inverse correlations between progression-free survival and both GSDMD (r -0.531, p 0.009) and VEGF (r -0.582, p 0.006) but regarding the overall survival the correlation was not statistically significant.

The median level of GSDMD and VEGF was taken as a cutoff point to classify the low and high expression

Table 4 Diagnostic accuracy of GSDMD (in the detection of exudative pleural effusion and VEGF (in the detection of malignant pleural effusion)

Studied variable	AUC	*P* value	Cutoff point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
GSDMD	0.994	0.001*	2.89	98%	97%	98%	97%	98%
VEGF	1.00	0.001*	≥ 191.5	100%	80%	84%	100%	90%

AUC Area under the curve, ***PPV*** Positive predictive value, ***NPV*** Negative predictive value, *Significant

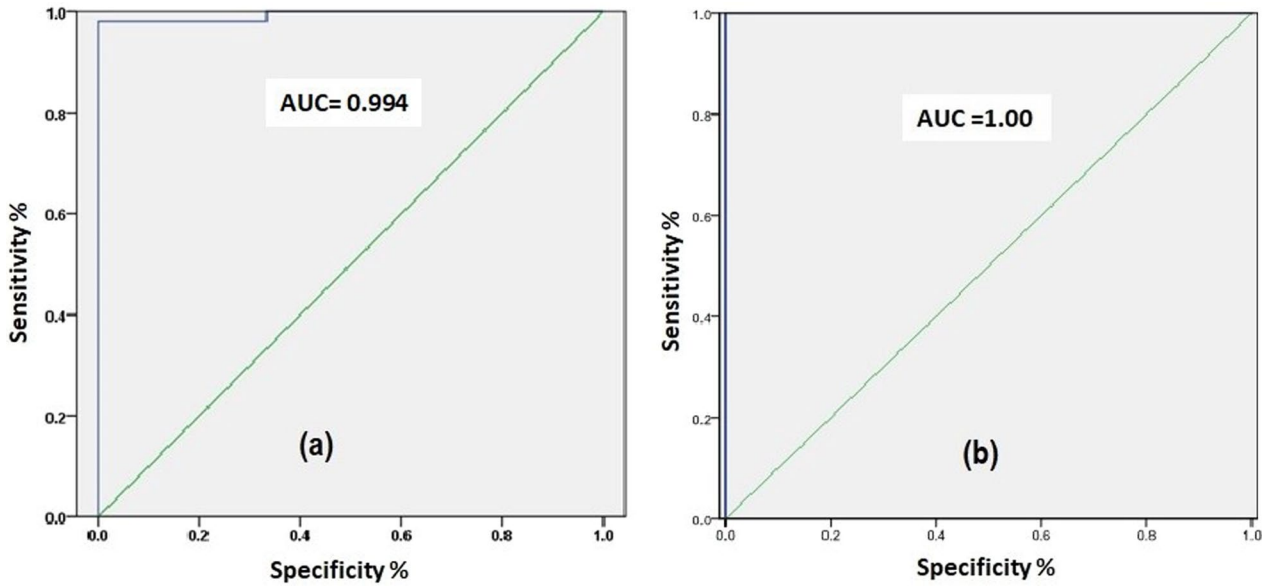

Fig. 1 Roc curves for sensitivity and specificity of **a** GSDMD for detection of exudative Pleural effusion and **b** VEGF for detection of malignant pleural effusion

Table 5 Diagnostic accuracy of GSDMD in the differential diagnosis of pleural effusion

GSDMD	TPE versus TBPE	TPE versus MPE	TPE versus PIPE	MPE versus TBPE	MPE versus PIPE	TBPE versus PIPE
AUC	1.00	0.971	0.985	0.646	0.803	0.714
P value	0.001*	0.001*	0.001*	0.249	0.001*	0.074
Cutoff point	≥ 2.80	≥ 2.94	≥ 2.89	≥ 3.25	≥ 3.26	≥ 3.11
Sensitivity (%)	100%	96%	91%	83%	87%	75%
Specificity (%)	92%	95%	94%	57%	75%	71%
PPV (%)	73%	92%	93%	86%	81%	46%
NPV (%)	100%	97%	91%	50%	77%	89%
Accuracy (%)	93%	95%	93%	77%	81%	72%

TPE Transudate pleural effusion, *TBPE* Tuberculous pleural effusion, *MPE* Malignant pleural effusion, *PIPE* Para-infectious pleural effusion, *AUC* Area under the curve, *PPV* Positive predictive value, *NPV* Negative predictive value, *Significant

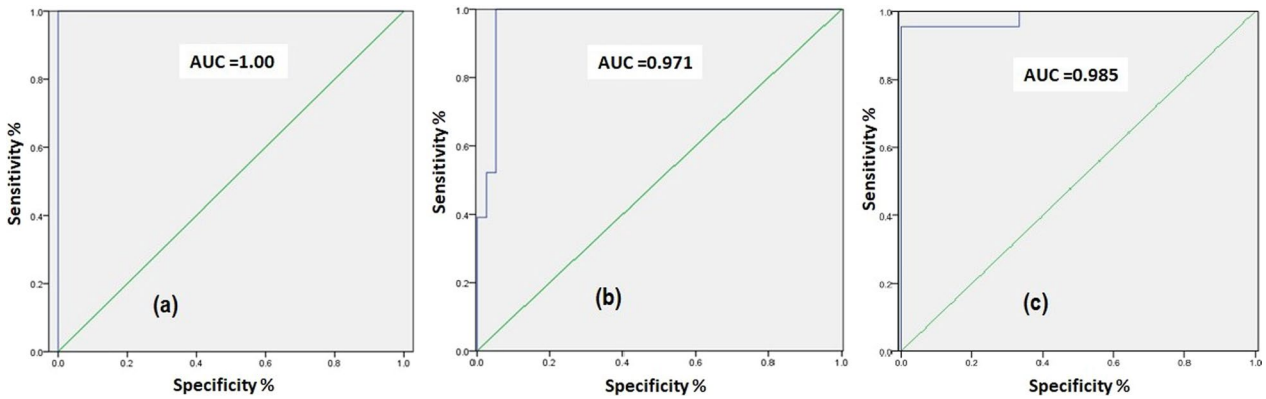

Fig. 2 Roc curves for sensitivity and specificity of GSDMD for detection of **a** TBPE, **b** MPE, and **c** PIPE

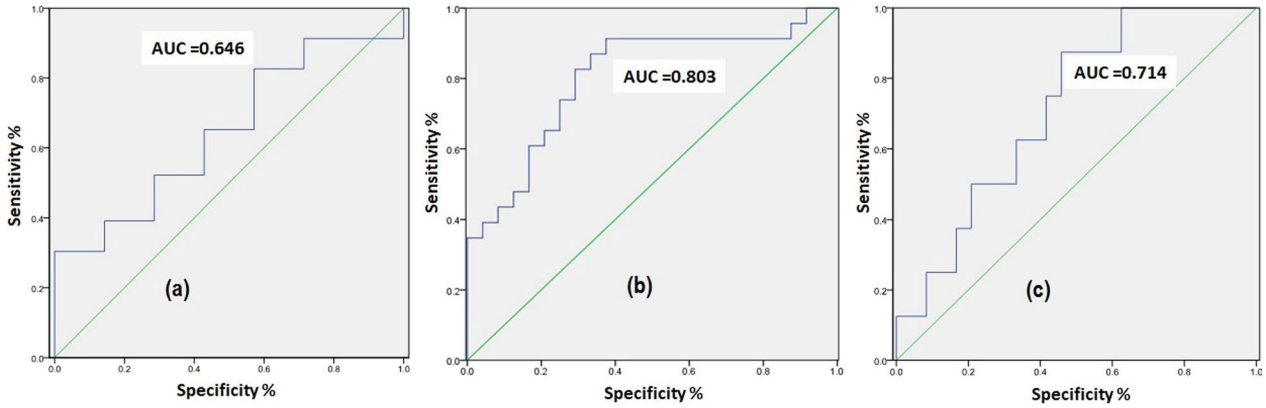

Fig. 3 Roc curves for sensitivity and specificity of GSDMD for differentiating between **a** MPE from TBPE, **b** MPE FROM PIPE, and **c** TBPE from PIPE

Table 6 Relation between GSDMD and VEGF with the response, progression, and outcome among malignant pleural effusion group

Studied variables	Total no No. (%)	GSDMD Mean ± SD	Test of sig. *P* value	VEGF Mean ± SD	Test of sig. *P* value
Response					
Complete response	3(13.0)	3.45 ± 0.20	K	235.9 ± 33.1	K
Progressive disease	2(8.70)	4.97 ± 1.27	1.79	195.7 ± 49.5	2.89
Partial response	12(52.2)	4.45 ± 1.83	0.617	258.8 ± 56.5	0.409
Stable disease	6(26.1)	4.50 ± 1.97		267.7 ± 36.6	
Progression			U		U
Progressed	18(78.3)	4.66 ± 1.79	1.41	274.9 ± 46.1	2.47
Not progressed	5(21.7)	3.37 ± 0.14	0.157	217.3 ± 22.3	0.013*
Outcome			U		U
Alive	19(82.6)	4.39 ± 1.79	1.13	257.8 ± 50.8	0.896
Dead	4(17.4)	4.34 ± 1.05	0.256	275.5 ± 37.1	0.370

U: Mann–Whitney test *K*: Kruskal–Wallis test, * significant

Table 7 Correlation between GSMD and VEGF with Progression-free survival and Overall survival among malignant pleural effusion group

Studied variables	Mean ± SD Median (months)	GSDMD		VEGF	
		r	*P* value	*r*	*P* value
Progression-free survival	11.4 ± 2.85 12.0	− 0.531	**0.009***	− 0.582	**0.006***
Overall survival	7.13 ± 3.93 6.00	− 0.390	0.066	− 0.304	0.181

r Spearman's correlation * Significant

(GSDMD ≤ 3.51 versus ˃ 3.51) and (VEGF ≤ 256.4 versus ˃256.4). The results in Table 8 and Fig. 4 indicate significantly shorter median progression-free survival in the patients with high both GSDMD and VEGF expression ($P = 0.023$ and $P = 0.003$ respectively). Also, the median of overall survival in the patients with high both GSDMD and VEGF expression was shorter but the difference was statistically not significant ($P = 0.108$ and $P = 0.359$ respectively).

4 Discussion

The pyroptosis is a type of programmed cell death. The GSDMD is released in significant amounts following cell rupture [4]. These might be the causes of GSDMD detection in PE.

Table 8 Relation between GSMD and VEGF with progression-free survival and overall survival among malignant pleural effusion group

Studied variables	GSDMD						Log-rank *P* value
	≤ 3.51			> 3.51			
	Median	Std. Error	95% CI Lower – upper	Median	Std. Error	95% CI Lower – upper	
Progression-free survival	8.00	3.15	1.82 – 14.1	4.00	0.57	2.86 – 14.1	5.15 0.023*
Overall survival	14.0	0.351	13.9 – 15.3	13.0	1.37	10.9 – 16.3	2.58 0.108
Studied variables	**GSDMD**						**Log-rank *P* value**
	≤ 256.4			> 256.4			
	Median	Std. Error	95% CI Lower – upper	Median	Std. Error	95% CI Lower – upper	
Progression-free survival	13.0	5.02	3.15 – 22.8	4.00	0.63	2.76 – 5.24	9.06 0.003*
Overall survival	13.0	0.583	12.9 – 15.2	10.0	1.56	10.6 – 16.8	0.843 0.359

St. Error Standard error, *CI* Confidence interval *Significant

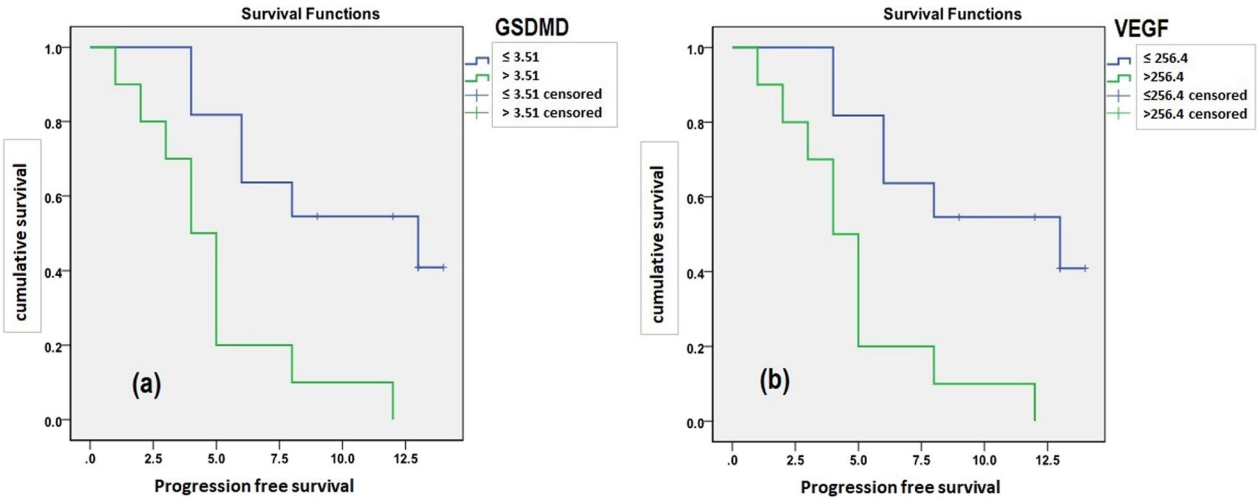

Fig. 4 Relation between **a** GSDMD and **b** VEGF with progression-free survival among malignant pleural effusion group

The alveolar macrophages, airway epithelium, and type II alveolar cells along with mesothelial, inflammatory, and tumor cells participate in VEGF concentration in PE. [13–15]

The purpose of this study was to evaluate the diagnostic value of GSDMD in distinguishing transudate PE from various types of exudate PE, and the diagnostic value of VEGF in distinguishing between malignant and non-malignant exudative pleural effusions, as well as the correlation between GSDMD and VEGF with progression-free survival and overall survival in malignant pleural effusion.

The GSDMD level in PF had statistically significant variations among all groups (except TBPE and PIPE, $P=0.520$) in this study, with the lowest level in the transudate group (mean and SD of 2.35 ± 0.44 ng/mL) and the highest in the malignant effusion group (mean and SD of 4.38 ± 1.67 ng/mL). GSDMD level in PE was found to be correlated positively with PF protein (r 0.463, p 0.001), LDH (r 0.317, p 0.019), and ADA (r 0.531, p 0.001), suggesting that the GSDMD could be a useful marker for differentiating different types of PE.

The sensitivity and specificity of GSDMD in the diagnosis of PE were 98% and 97%, respectively ($p=0.001$) and the ROC curve demonstrated that the GSDMD not only could be an effective marker to differentiate between the transudate and exudative PE (AUC 0.994 & the cutoff point = 2.89), but also GSDMD exhibited a strong diagnostic capability for separating TPE from TBPE (AUC = 1.0, the cutoff value = 2.80 ng/mL, sensitivity = 100%,

and specificity = 92%), MPE (AUC = 0.971, the cutoff value = 2.94 ng/mL, sensitivity = 96%, and specificity = 95%), and PIPE (AUC = 0.985, the cutoff value = 2.89 ng/mL, sensitivity = 91%, and specificity = 94%). GSDMD also performed satisfactorily in differentiating MPE from PIPE (AUC = 0.803, cutoff value = 3.26 ng/ml).

Our results were in line with those of Li et al., [16] who solely looked at the GSDMD as a new marker in differentiating between the types of PE. They observed a statistically significant link between GSDMD, LDH, and ADA after evaluating all patients with pleural exudates, and the GSDMD levels in PE were significantly connected with the level of ADA and LDH ($p = 0.0001$). GSDMD's diagnostic accuracy was 94% for specificity and 96% for sensitivity. Their ROC curve's AUC was > 0.973 to differentiate between transudate PE and other groups. Their greatest AUC was 0.990 for TB diagnosis, while the value of 18.40 ng/ml was the cutoff point. Furthermore, 9.35 ng/ml was the identical cutoff point for both parapneumonic and malignant PE. Although they found significance in the levels of GSDMD in various PE types, as we did, they found no significance between MPE and parapneumonic PE (in our study was between TBE and PIPE).

Recent research indicates that pyroptosis can be activated by a variety of pathogenic triggers, including infectious disorders, neurological diseases, and malignancy. Surprisingly, there was a strong positive association between the amount of GSDMD together with the number of nucleated cells, suggesting that the GSDMD is released by such local pleural nucleated cells. [17–20]

In terms of VEGF levels in PF, there was a significant variation (with $p = 0.001$) between various exudative PE (except TBPE and PIPE, $P = 0.722$) with the greatest level in the MPE (261.2 ± 48 pg/mL, mean ± SD). The sensitivity and specificity of VEGF for the identification of MPE were 100% and 80%, respectively (p 0.001), and the AUC was 1.0, with a cutoff value of 191.5 pg/ml.

Multiple studies agree with our findings that the greatest VEGF concentrations were identified in malignant pleural effusions, although the sensitivity, specificity, and cutoff value differ between studies in ROC analysis.

In Fiorelli et al.'s study, the median level of pleural VEGF in patients with MPE was 753 [458–1200 pg/ml], which was substantially higher than the value identified in benign PE ($P = 0.005$). Furthermore, there were no significant variations between malignant PE due to cancer lung and other malignant causes. The sensitivity of VEGF was 63%, specificity was 83%, and the cutoff threshold was > 652 pg/ml. [3]

Furthermore, Momi et al. investigated the sensitivity and specificity of VEGF concentrations in MPE, which were 100 and 84%, respectively with a cutoff value of 2000 pg/ml [21].

Sack et al. observed that malignancies had the greatest VEGF concentrations in pleural effusions, which allows it to be distinguished from all other causes; however, there was no difference between lung and secondary cancers. Apart from congestive heart failure and TB PE, all diagnostic other categories could be distinguished with sufficient confidence. Although substantial differences were found, ROC analysis revealed that VEGF did not have sufficient diagnostic power when employed as a single parameter [22]

Additionally, Duysinx et al. observed 69% sensitivity and 54% specificity with a cutoff value of 382 pg/ml [23], whereas Shu et al. [24] demonstrated 47% sensitivity and 96% specificity with a cutoff was 959 pg/ml.

Kaya et al., on the other hand, found no significant difference between MPE and TBPE, despite that MPE had higher median and mean VEGF levels, and its levels in subtypes of cancer lung and different malignant effusions were not substantially different [25].

The gold standard for the diagnosis of MPE is cytological and/or histopathological evidence of pleural neoplasia, whereas VEGF may only imply the diagnosis. But, because thoracoscopy is an invasive procedure with some limitations in its accessibility, the measurement of the VEGF in PF might assist in identifying patients who would benefit from invasive procedures.

Without a doubt, regular VEGF assessment in all pleural effusions is likely not a cost-effective method and couldn't be always advised. But, it might be beneficial in people with evidence of cancer either clinically or radiologically and may signal the necessity for a diagnostic biopsy [26, 27]. In contrast, given the rareness of a carcinogenic etiology for transudate, the patients with TPE should be excluded.

In the current study, there were inverse correlations between VEGF and both progression-free survival and overall survival; however, it was only statistically significant with progression-free survival ($r = -0.582$, $p = 0.006$). These findings corroborated the findings of Cheng et al. who observed that pleural effusion with high levels of VEGF was correlated with shorter overall survival and disease-free survival [28]. Higher VEGF expression is linked to a poor prognosis and shorter progression-free survival and overall survival. VEGFs' contributions to angiogenesis and cancer development in the location of cancer progression might account for our findings in addition to their potential to promote vascular permeability. There is concrete proof that malignant cells generate VEGF, which enhances tumorigenesis and the progression of cancer and plays a significant role in malignant cell migration from the vasculature into the pleural cavity which may explain our results [3, 9, 29].

Thickett et al., on the other hand, didn't observe any significant correlation between malignant survival and pleural VEGF levels and observed that when empyema was eliminated using standard clinical criteria, only individuals with malignancies used to have a VEGF level above 1000 pg/ml. [30]

Gao, et al., found that overexpression of GSDMD reveals a poor prognostic value for adenocarcinoma of the lung similar to our results that found significantly longer median progression-free survival with low GSDMD expression which could be explained by depletion of the GSDMD activates PARP and caspase 3 cleavages and promotes tumor cell death through the intrinsic apoptotic pathway. Furthermore, co-expression results indicate the relation of GSDMD with EGFR/Akt signaling. The median of overall survival in low GSDMD expression was longer than that in high GSDMD expression but the difference was not significant as might longer follow-up period of our patients is needed for a better assessment of this difference in overall survival.[7]

One of the current study's limitations is the limited number of participants together with not all the samples of exudative PE examined for VEGF. Future studies for studying the GSDMD in the pleural fluid are advised with its relation to the treatment in various types of PE.

5 Conclusion

Pleural effusion GSDMD can be an effective marker for differentiating the different kinds of PE, and VEGF levels can be a useful adjuvant marker in screening out MPE as a possible diagnosis, leading to the accurate identification of individuals who may advantage from more invasive treatments. Higher expression of GSDMD and VEGF might indicate longer median progression-free survival in MPE patients.

Abbreviations

ADA	Adenosine deaminase
ELISA	Enzyme-linked immunosorbent assay
EPE	Exudative pleural effusion
GSDMD	Gasdermin D
LDH	Lactate dehydrogenase
MPE	Malignant pleural effusion
PE	Pleural effusion
PIPE	Para-infectious pleural effusion
TBPE	Tuberculous pleural effusion
TPE	Transudate pleural effusion
VEGF.	Vascular endothelial growth factor

Acknowledgements

Not applicable.

Author contributions

M.E. performed study design. A.E, M.E, R.H, and H.E performed data collection. S.A, E.A., M.E, and A.E. carried out data analysis. S.A, E.A., M.E, and A.E. performed interpretation of results. A.E, R.H, and H.E. performed initial draft. All authors made final review of the manuscript content. All authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

The data generated and/or evaluated during the present work are obtainable upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The study was authorized by the Menoufia University Hospital's Ethics Committee (**IRB: 10/2022EONC21**), and written informed consent from the participants was acquired following the Menoufia University Hospital's local ethical committee and the principles of the Helsinki Declaration.

Consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 26 December 2022 Accepted: 20 February 2023
Published online: 06 March 2023

References

1. Korczynski P et al (2009) Diagnostic utility of pleural fluid and serum markers in differentiation between malignant and non-malignant pleural effusions. Eur J Med Res 14(Suppl 4):128–133
2. Loddenkemper R (1998) Thoracoscopy–state of the art. Eur Respir J 11(1):213–221
3. Fiorelli A et al (2011) Vascular endothelial growth factor in pleural fluid for differential diagnosis of benign and malignant origin and its clinical applications. Interact Cardiovasc Thorac Surg 12(3):420–424
4. Jorgensen I, Miao EA (2015) Pyroptotic cell death defends against intracellular pathogens. Immunol Rev 265(1):130–142
5. Pandeya A et al (2019) Gasdermin D (GSDMD) as a new target for the treatment of infection. Medchemcomm 10(5):660–667
6. Cheng KT et al (2017) Caspase-11–mediated endothelial pyroptosis underlies endotoxemia-induced lung injury. J Clin Investig 127(11):4124–4135
7. Gao J et al (2018) Downregulation of GSDMD attenuates tumor proliferation via the intrinsic mitochondrial apoptotic pathway and inhibition of EGFR/Akt signaling and predicts a good prognosis in non-small cell lung cancer. Oncol Rep 40(4):1971–1984
8. Gong Z et al (2019) Regulation of host cell pyroptosis and cytokines production by Mycobacterium tuberculosis effector PPE60 requires LUBAC mediated NF-κB signaling. Cell Immunol 335:41–50
9. Zebrowski BK et al (1999) Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. Clin Cancer Res 5(11):3364–3368
10. Light RW et al (1972) Pleural effusions: the diagnostic separation of transudates and exudates. Ann Intern Med 77(4):507–513
11. Roth BJ, O'Meara TF, Cragun WH (1990) The serum-effusion albumin gradient in the evaluation of pleural effusions. Chest 98(3):546–549
12. Eisenhauer EA et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45(2):228–247
13. Grove CS, Lee YC (2002) Vascular endothelial growth factor: the key mediator in pleural effusion formation. Curr Opin Pulm Med 8(4):294–301
14. Nishigaki Y et al (2003) Increased vascular endothelial growth factor in acute eosinophilic pneumonia. Eur Respir J 21(5):774–778

15. Maitre B et al (2001) Vascular endothelial growth factor synthesis in the acute phase of experimental and clinical lung injury. Eur Respir J 18(1):100–106
16. Li P et al (2021) Pleural fluid GSDMD is a novel biomarker for the early differential diagnosis of pleural effusion. Front Microbiol 12:620322
17. Beckwith KS et al (2020) Plasma membrane damage causes NLRP3 activation and pyroptosis during mycobacterium tuberculosis infection. Nat Commun 11(1):2270
18. Derangère V et al (2014) Liver X receptor β activation induces pyroptosis of human and murine colon cancer cells. Cell Death Differ 21(12):1914–1924
19. Jiang Z et al (2017) miRNA-214 inhibits cellular proliferation and migration in glioma cells targeting caspase 1 involved in pyroptosis. Oncol Res 25(6):1009–1019
20. Shi J et al (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526(7575):660–665
21. Momi H et al (2002) Vascular endothelial growth factor and proinflammatory cytokines in pleural effusions. Respir Med 96(10):817–822
22. Sack U et al (2005) Vascular endothelial growth factor in pleural effusions of different origin. Eur Respir J 25(4):600–604
23. Duysinx BC et al (2008) Diagnostic value of interleukine-6, transforming growth factor-beta 1 and vascular endothelial growth factor in malignant pleural effusions. Respir Med 102(12):1708–1714
24. Shu J et al (2007) Clinical utility of vascular endothelial growth factor in diagnosing malignant pleural effusions. Acta Oncol 46(7):1004–1011
25. Kaya A et al (2005) Vascular endothelial growth factor in benign and malignant pleural effusions. Arch Bronconeumol 41(7):376–379
26. Elia S et al (2008) Tumor type M2-pyruvate-kinase levels in pleural fluid versus plasma in cancer patients: a further tool to define the need for invasive procedures. Eur J Cardiothorac Surg 33(4):723–737
27. Ferrer J et al (2005) Predictors of pleural malignancy in patients with pleural effusion undergoing thoracoscopy. Chest 127(3):1017–1022
28. Cheng D, Kong H, Li Y (2013) Prognostic values of VEGF and IL-8 in malignant pleural effusion in patients with lung cancer. Biomarkers 18(5):386–390
29. Chen Y, Mathy NW, Lu H (2018) The role of VEGF in the diagnosis and treatment of malignant pleural effusion in patients with non-small cell lung cancer (Review). Mol Med Rep 17(6):8019–8030
30. Thickett D, Armstrong L, Millar A (1999) Vascular endothelial growth factor (VEGF) in inflammatory and malignant pleural effusions. Thorax 54(8):707–710

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