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Hybrid panel of biomarkers can be useful in the diagnosis of pleural and peritoneal effusions

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ABSTRACT

Background: In clinical practice, pleural and peritoneal effusions are usual diagnosis. We evaluated the performance of a hybrid panel of biomarkers in the diagnosis of the main diseases affecting pleura and/or peritoneum.

Methods: Samples of pleural/ peritoneal fluid from 120 patients were evaluated for: CEA (carcinoembryonic antigen), VEGF-A (vascular endothelial growth factor A), PD-L1/B7-H1 (programmed death-ligand 1), NGAL (neutrophil gelatinase-associated lipocalin), TREM-1 (triggering receptor expressed in myeloid cells type-1) and IFNγ (gamma-interferon) by Luminex®; CALP (Calprotectin) by ELISA, and ADA (adenosine deaminase) by enzymatic deamination.

Results: For malignant effusion (ME) diagnosis, CEA and NGAL presented superior performance than VEGF-A, PD-L1 and CALP. A CEA-NGAL association showed good sensitivity (86.6%) and accuracy (79.2%). For non-tuberculous infectious effusion (NTBIE), NGAL presented the best performance with sensitivity (75.0%), specificity (62.0%) and accuracy (65.0%) higher than TREM-1 and CALP; however, when associated, although with good sensitivity, there was important decrease in specificity. For tuberculous pleural effusion (TPE), IFNy-ADA presented excellent sensitivity (100%), specificity (87.6%), NPV (100%) and accuracies (~90%).

Conclusions: CEA, NGAL, ADA and IFNy were useful in discriminating ME and TPE. However, for NTBIE diagnosis, the hybrid panel did not demonstrate advantages over the classic parameters.

1. Introduction

Pleural and peritoneal effusions are usual diagnosis in clinical practice. The quantification of biomarkers in cavity fluids may aid in the etiological diagnosis and in patients' management. Laboratory investigation of cavity effusions involves the evaluation of biochemical, immunological, microbiological, molecular and cellular parameters. However, laboratory tests do not always clarify the etiology of an effusion, stimulating the search for new biomarkers to be used with this purpose.

Cirrhosis and portal hypertension are the main causes of ascites (approximately 85% of cases), although infection and malignancy can occur with accumulation of peritoneal fluid [1,2]. Pleural effusion, in turn, may result from pressure imbalance in pleural space due to systemic diseases - such as cardiac transudate or, in the case of exudates, to reflect complications of infections, neoplasm, trauma, drug use, autoimmune diseases or idiopathic conditions [3].

This broad spectrum of etiologic causes of pleural effusion (PE) and peritoneal effusion (PerE) justifies studies with biomarkers quantified in samples obtained through thoracentesis and/or paracentesis, procedures considered minimally invasive and at a low risk for patients. The literature is varied regarding the performance of biomarkers for diagnosis in the different phases of disease's cycle [4]; however, to elect a biomarker with good sensitivity and specificity and that is effective and reproducible is not an easy task.

It is well studied the role of some biomarkers in the differential diagnosis of effusions [5,6]. In malignant effusions, we can cite as example, the carcinoembryonic antigen (CEA), a cell surface adhesion molecule that plays a role in the survival of tumor cells to apoptosis, tumor metastasis and angiogenesis, being a biomarker extensively studied in medical practice [7–10], and also the vascular endothelial growth factor A (VEGF-A), a permeability factor involved with fluid

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formation and tumor progression [11,12]. More recently, a rising biomarker - PD-L1 has been proposed for diagnosis of malignant pleural effusion, especially to those related with lung cancer [13,14].

Recent studies have shown the role of new biomarkers in the differential diagnosis of cavity effusions [5,15]. In this context, the neutrophil gelatinase-associated lipocalin (NGAL), used as a renal injury biomarker [16], has been proposed to differentiate infectious from malignant effusions [15,17–19], and in screening for bacterial peritonitis [20], as well as the Calprotectin (CALP), a protein routinely used for the diagnosis of inflammatory intestinal diseases [21,22]. The triggering receptor expressed in myeloid cells type 1 (TREM-1), a transmembrane receptor protein known to be a marker of sepsis, has also been proposed in the differential diagnosis of infectious effusions, especially to identify complicated parapneumonic effusions [23–25].

In day a day practice, the presumptive diagnosis of serous tuberculosis has been based on the quantification of adenosine deaminase (ADA), a ubiquitous enzyme involved in purine degradation [26,27], and on the gamma-interferon (IFNy), a pleiotropic cytokine produced by T and NK cells that interact with a specific receptor expressed on the cell surfaces; both biomarkers are considered good markers, mainly in countries with high and medium disease prevalence [28,29].

2. Materials and methods

2.1. Casuistic

Samples of pleural or peritoneal fluid from 120 patients submitted to thoracentesis or paracentesis for diagnostic investigation were included in this study. Each sample was representative of one patient. In addition to the routine exams, the samples were submitted to dosages of the biomarkers CEA, NGAL, VEGF-A, TREM-1, IFNγ, PD-L1, CALP, and ADA. The study was approved by the Research Ethics Committee and was conducted at the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo. Clinical and laboratory data were extracted from medical records and laboratory system database. The analyzed variables were age, sex and main etiological diagnosis according to the International Statistical Classification of Diseases and Related Health Problems (ICD), 10th Revision of Codes.

The samples were classified according to the site of the effusion, appearance, color (before and after centrifugation), cells counting and oncotic cytology. The study group included samples from patients with peritoneal and pleural effusions secondary to systemic diseases (transudates), malignancy, non-tuberculous infection and tuberculosis. Clinical history, laboratory and imaging tests and patient follow-up were used to confirm pleural and peritoneal transudates.

The diagnosis of malignant effusion (ME) was based on the presence of malignant cells in pleural/peritoneal fluid or tissue. The group of non-tuberculous infectious effusion (NTBIE) was composed by patients with PE associated with bacterial pneumonia (clinical history and positive blood or fluid culture), and patients with peritonitis (clinical history and ascites with polymorphonuclear leukocytes count ≥250/μL or positive fluid culture). The diagnosis of tuberculous pleural effusion (TPE) was considered when caseous granuloma was observed in the pleural biopsy and/or a positive culture for *Mycobacterium tuberculosis* was demonstrated in the PF or tissue.

Samples of PF were collected in EDTA tubes for quantification of biomarkers. After centrifugation for cell removal (900 *g* for 10 min), the supernatants were stored at −80 °C until analysis. Before analysis, the samples were re-centrifuged to avoid any possible matrix effect.

2.2. xMAP Luminex®

Samples of PE or PerE were prepared for analysis in a 96-well plate utilizing a custom Luminex Human Magnetic Assay (R&D System, Inc.) following the kit-specific protocols provided by R&D. CEA, VEGF-A, PD-L1, NGAL, TREM-1 and IFNγ were quantified using a Magpix analytical test instrument, which utilizes the xMAP technology in a multiple analyte profiling, and the xPONENT 4.2 software (Luminex Corp.). For data analysis was used Milliplex Analyst 5.1 software (Millipore Corp.). Biomarkers concentrations were expressed in pg/ml, and the detection limits were: CEA (56.4–13,700.0 pg/ml), VEFG-A (8.5–2070.0 pg/ml), PD-L1 (114.4–27,790.0 pg/ml), NGAL (139.6–33,920.0 pg/ml), TREM-1 (115.5–28,060.0 pg/ml) and IFNy (49.2–11,950.0 pg/ml).

2.3. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of Calprotectin were obtained by a 'sandwich' ELISA test with CALP human kit (Elabscience Biotechnology Inc.) following the kit-specific protocol provided by Elabscience, and analyzed on Victor X3 plate reader (PerkinElmer) in a 450 nm low-pass absorbance. The standard curve was plotted at 5-Plog and the results were calculated using the WorkOut 2.5 analysis software (PerkinElmer). Calprotectin concentrations were expressed in ng/ml, and the detection limit was 1.6–100 ng/ml.

2.4. Adenosine desaminase (ADA)

The assay for ADA is based on the enzymatic deamination of adenosine to inosine, defined as the amount of ADA which generates one mole of adenosine-to- inosine per minute at 37 °C. The assay used was the Quimiada Adenosina Deaminase kit (Ebram), and a concentration above 30.0 U/l is presumptive of tuberculosis.

2.5. Statistical analysis

Data are presented as median and 1st and 3rd interquartiles. Comparisons between groups were performed using one-way ANOVA, Kruskal–Wallis followed by Dunn's test for multiple comparisons since tumor markers data were skewed distributed by the Kolmogorov-Smirnov test. Receiver operator characteristic (ROC) analysis was performed to evaluate sensitivity (S), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and accuracy (ACU) of the markers. A biomarker cutoff was determined as the value that maximized the sum of specificity and sensitivity. The ACU was calculated from the true positive fraction (TPF) for a given false positive fraction (FPF). The SigmaStat program (Jandel Scientific, San Raphael, CA, USA) and Medcalc Statistical Software (Seoul, Korea) were used for statistical analysis; a p value $< .05$ was considered statistically significant.

3. Results

From the 120 included samples, 67 (55.8%) corresponded to ME (49 of PE and 18 of PerE); 28 (23.3%) to NTBIE (22 of PE and 06 of PerE); 15 (12.5%) to TPE, and 10 (8.3%) to transudates (TRANS). The study group was composed by 67 men (25–83 years) and 53 women (15–86 years), with no significant difference in gender ($p = .281$). Regarding to age, patients from TPE group were significantly younger than those from malignant group ($p = .037$).

The characteristics of the study group are demonstrated in Table 1. The median values (1st to 3rd interquartile range) of the biomarkers in the different disease groups are showed in Table 2. In ME, the concentrations of VEGF-A, TREM-1, IFNγ and ADA were significantly higher than in TRANS. CEA and NGAL were more expressed in ME when compared to NTBIE, TPE and TRANS. In the group NTBIE, CEA was higher when compared to TPE and TRANS, TREM-1 was higher than ME and TRANS and ADA was higher than TRANS. In TPE, TREM-1 was higher than TRANS and IFNγ and ADA was increased when compared to ME, NTBIE and TRANS.

Table 1
Characteristics of patients and of fluid samples.

Patients (n)	120
Age	
Mean ± SD	57.7 ± 16.6
Median	60.0
Male / Female (n)	67/53
Pleural / Peritoneal (n)	87/33
Aspect	
Before centrifugation	
Yellow/Ser-H / Hemorrhagic/Brownish/Colorless/ Lipemic	71/25/20/1/2/1
After centrifugation	
Color	
Before centrifugation	
Clear/Slightly cloudy/Cloudy	4/18/52
After centrifugation	
Clear/Slightly cloudy/Cloudy	116/2/2
Cell count (mm^3) ± SD	5420.8 ± 25937.0
Prevalence Neutrophils / Lymphocytes (n)	37/83

n: Number of cases; SD: Standard Deviation; Ser-H: Serum-hemorrhagic

3.1. Malignant effusion group

Sixty seven cases of malignant effusions were evaluated in the study. The primary sites of neoplasia were: lung ($n = 20$; 29.8%), gastrointestinal ($n = 15$; 22.3%), breast ($n = 9$; 13.4%), hematological ($n = 4$; 6.0%), thyroid ($n = 4$; 6.0%), uterus ($n = 3$, 4.5%), pancreas ($n = 3$, 4.5%), liver (n = 3, 4.5%), skeletal muscle ($n = 2$; 3.0%), ovary ($n = 2$; 3.0%), central nervous system ($n = 1$; 1.5%), and kidney ($n = 1$; 1.5%). The cutoffs obtained according to ROC curve for the diagnosis of ME were: CEA > 1.4 pg/ml, VEGF-A > 1.4 pg/ml, PD-L1 > 0.007 pg/ml, NGAL > 40.1 pg/ml and CALP > 30.0 ng/ml.

Tables 3 and 4 show the performance of individual and associated biomarkers in the diagnosis of ME. CEA alone presented 61.2% sensitivity, 84.9% specificity and 71.7% of accuracy; the combination CEA-NGAL improved sensitivity and accuracy (86.6% and 79.2%) with discrete loss of specificity (69.8%).

Table 3
Performance of each biomarker in the diagnosis of ME.

	VEGF-A	NGAL	PD-L1	CEA	CALP
S	52.2%	76.1%	52.2%	61.2%	74.0%
SP	73.6%	75.5%	58.5%	84.9%	56.7%
PPV	71.4%	79.7%	61.4%	83.7%	74.0%
NPV	54.9%	71.4%	49.2%	63.4%	56.7%
ACU	61.7%	75.8%	55.0%	71.7%	67.5%

ME: Malignant effusion; S: Sensitivity; SP: Specificity; PPV: Positive Predective Value; VPN: Negative Predective Value; ACU: Accuracy; VEGF-A: Vascular endothelial growth factor A; NGAL: Neutrophil gelatinase-associated lipocalin; CEA: Carcinoembryonic antigen; PD-L1: Programmed.
death-ligand 1; CALP: Calprotectin.

3.2. Non-tuberculous infectious effusion group

In the group of NTBIE diagnosis, TREM-1 (> 5.2 pg/ml), CALP (< 30.0 ng/ml), and NGAL (< 40.1 pg/ml) showed the best accuracy. The Tables 5 and 6 depict the performance of individual and associated biomarkers for the diagnosis of NTBIE. For the diagnosis of NTBIE, TREM-1 and CALP presented the higher sensitivity and specificity each one. However, when associated, although with good sensitivity, the specificity was very low.

3.3. Tuberculous pleural effusion group

In this group, were included only patients with pleural tuberculosis. A good accuracy was obtained for each biomarker, or when associated, with the cutoff of 1.8 pg/ml for IFNγ and of 29.6 U/l for ADA. The Table 7 shows the performance of individual and combined biomarkers in the diagnosis of tuberculous pleural effusion.

For the diagnosis of TPE, IFNγ alone showed high sensitivity, specificity and accuracy. The association IFNγ – ADA showed 100% sensitivity and 100% NPV, what is relevant in clinical practice, since a NPV of 100% exclude the diagnosis of pleural tuberculosis in a scenario of moderate and high disease prevalence. Fig. 1 illustrates the ROC curves according to effusions' etiology and the respective cutoff points.

Table 2
Biomarkers results in peritoneal and pleural fluid samples.

Biomarkers	TPE	NTBIE	ME	TRANS	*p*
	(*n* = 15)	(*n* = 28)	(*n* = 67)	(*n* = 10)	
CEA (pg/ml)	163.3 (61.1–207.8)	521.1 (269.8–2039.5)[b]	3185.0 (340.5–9077.5)[a]	96.8 (73.2–166.2)	< 0.001
VEGF-A (pg/ml)	782.6 (279.5–1155.2)	1231.0 (451.1–2708.5)	1539.0 (382.8–2853.0)[a]	290.6 (89.4–542.6)	0.018
NGAL (pg/ml)	37,603.0 (34,531.7–39,271.0)	29,578.0 (20,596.5–42,455.0)	45,660.0 (40,412.2–51,837.5)[a]	36,990.5 (27,843.0–44,933.0)	< 0.001
TREM-1 (pg/ml)	4548.0 (2673.7–8470.5)[c]	5735.0 (4068.0–9209.0)[b]	4548.0 (3026.0–5470.5)[a]	1145.6 (789.6–2030.0)	< 0.001
IFNγ (pg/ml)	668.2 (342.4–1244.2)[c]	56.5 (44.2–92.4)	51.1 (42.4–62.9)[a]	40.6 (34.9–51.1)	< 0.001
ADA (U/l)	38 (30.2–46.4)[c]	12.6 (1.2–26.5)[b]	6.5 (2.8–10.5)[a]	3.3 (0.2–6.3)[b]	< 0.001
PD-L1 (pg/ml)	6.6 (3.8–40.5)	5.4 (1.1–18.8)	11.34 (2.4–27.9)	3.7 (0.7–81.7)	NS
CALP (ng/ml)	28.2 (24.3–69.9)	24.3 (18.7–71.7)	43.7 (27.4–72.4)	_	NS

TPE: Tuberculosis; NTBIE: Non-Tuberculous Infectious Effusion; ME: Malignant Effusion; TRANS: Transudate; CEA: Carcinoembryonic antigen; VEGF-A: Vascular endothelial growth factor A; NGAL: Neutrophil gelatinase-associated lipocalin; TREM-1: Triggering receptor expressed in myeloid cells type 1; IFNγ: Gama-interferon; ADA: Adenosine Desaminase; PD-L1: Programmed death-ligand 1; CALP: Calprotectin
Data presented as median, with interquartile ranges (1–3); *p*: *p* value (*significance for $p < .005$).
[a] VEGF-A: ME > TRANS; NGAL: ME > NTBIE, TPE and TRANS; CEA: ME > NTBIE, TPE and TRANS; TREM-1: ME > TRANS; IFNγ: ME > TRANS; ADA: ME > TRANS;
[b] CEA: NTBIE > TPE and TRANS; TREM-1: NTBIE > ME, TRANS; ADA: NTBIE > TRANS;
[c] TREM-1: TPE > TRANS; IFNγ: TPE > ME, NTBIE and TRANS; ADA: TPE > ME, NTBIE and TRANS.

Table 4
Performance of associated biomarkers in the diagnosis of ME.

	NGAL-VEGF-A	PD-L1-VEGF-A	CEA-VEGF-A	VEGF-A CALP	NGAL-PD-L1	CEA-NGAL	NGAL-CALP	CEA-PD-L1	PD-L1-CALP	CEA-CALP	CEA-NGAL VEGF-A
S	82.1%	82.1%	79.1%	88.0%	88.1%	86.6%	92.0%	82.1%	90.0%	92.0%	89.6%
SP	60.4%	45.3%	69.8%	43.3%	49.1%	69.8%	46.7%	56.6%	33.3%	50.0%	58.5%
PPV	72.4%	65.5%	76.8%	72.1%	68.6%	78.4%	74.2%	70.5%	69.2%	75.4%	73.2%
NPV	72.7%	66.7%	72.5%	68.4%	76.5%	80.4%	77.8%	71.4%	66.7%	78.9%	81.6%
ACU	72.5%	65.8%	75.0%	71.3%	70.8%	79.2%	75.0%	70.8%	68.8%	76.3%	75.8%

ME: Malignant effusion; S: Sensitivity; SP: Specificity; PPV: Positive Predective Value; VPN: Negative Predective Value; ACU: Accuracy; VEGF-A: Vascular endothelial growth factor A; NGAL: Neutrophil gelatinase-associated lipocalin; CEA: Carcinoembryonic antigen; PD-L1: Programmed death-ligand 1; CALP: Calprotectin.

Table 5
Performance of each biomarker in the diagnosis of NTBIE.

	TREM-1	CALP	NGAL
S	64.3%	73.3%	75.0%
SP	70.7%	68.8%	62.0%
PPV	40.0%	35.5%	37.5%
NPV	86.7%	91.7%	89.1%
ACU	69.2%	69.6%	65.0%

NTBIE: Non tuberculous infectious effusion; S: Sensitivity; SP: Specificity; PPV: Positive Predective Value; VPN: Negative Predective Value; ACU: Accuracy; TREM-1: Triggering receptor expressed in myeloid cells type 1; CALP: Calprotectin; NGAL: Neutrophil gelatinase-associated lipocalin.

Table 6
Performance of associated biomarkers in the diagnosis of NTBIE.

	TREM-CALP	TREM-NGAL	CALP-NGAL	TREM-CALP-NGAL
S	93,3%	92,6%	93,3%	100,0%
SP	46,2%	44,6%	49,2%	32,3%
PPV	28,6%	32,9%	29,8%	25,4%
NPV	96,8%	95,3%	97,0%	100,0%
ACU	55,0%	55,5%	57,5%	45,0%

NTBIE: Non tuberculous infectious effusion; S: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; VPN: Negative Predictive Value; ACU: Accuracy; TREM-1: Triggering receptor expressed in myeloid cells type 1; CALP: Calprotectin; NGAL: Neutrophil gelatinase-associated lipocalin.

Table 7
Performance of individual and associated biomarkers in the diagnosis of TPE.

	IFNγ	ADA	IFNγ-ADA
S	93.3%	86.6%	100%
SP	93.3%	93.3%	87.6%
PPV	66.6%	65.0%	53.6%
NPV	99.0%	98.0%	100%
ACU	93.3%	92.5%	89.2%

TPE: Tuberculous pleural effusion; S: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; VPN: Negative Predictive Value; ACU: Accuracy; IFNγ: Gama-interferon; ADA: Adenosine Desaminase.

4. Discussion

In the present study, the performance of individual biomarkers for diagnosis of ME was variable. CEA and NGAL showed the best results each one, with higher sensitivity for NGAL e higher specificity for CEA. When associated, we obtained sensitivity, specificity and accuracy of 86.6%, 69.8% and 79.2%, respectively. In NTBIE, the diagnostic sensitivity and specificity of TREM-1 and CALP were good, with important loss of specificity when associated. For the diagnosis of TPE, the IFNγ presented the higher sensitivity, specificity and accuracy (93.3%). The association IFNγ-ADA showed 100% sensitivity. It is important to highlight the high NPV (100%) of both biomarkers, making it possible to exclude the diagnosis of TPE when ADA and IFNγ are below the proposed cutoff points.

Wang et al. [9] compared the potential diagnostic of 5 tumor biomarkers in the diagnosis of malignant pleural effusion: CEA, CA15-3, CA19-1, CA125 and alpha-fetoprotein. The authors report CEA as the best biomarker for mesothelioma and lymphoma/leukemia diagnosis, using a cutoff of 1,71 ng/ml. Chen et al. [10], in a recent study, evaluated the discriminatory capacity of CEA, CYFRA 21-1 and CTLA-4 for the diagnosis of MPE, obtaining an individual accuracy of 81.8%, 78.9% and 72.1%, respectively. In the present study, CEA and NGAL presented the best performance in the diagnosis of ME, but with a lesser accuracy than described by the cited authors.

NGAL is known worldwide as a renal injury biomarker [16]. This protein participates in the innate immune system and is released by neutrophils in infection and inflammation sites, with participation in the antibacterial strategy of iron depletion. [1,18–20]. Current studies have associated NGAL with cancer development and progression [17]. The oncogenic effect of NGAL may be related to the complex NGAL/MMP-9, that results in a protective action of MMP-9 from its auto-degradation and consequently in a higher gelatinolytic action of MMP-9 in extracellular matrix. Due to this function, the NGAL may promote cancer development and metastatic process [17,19]. Recent studies have associate high concentrations of NGAL with disease severity and poor survival in most cancer types [30]. To date, this is the first study to report the performance of this biomarker in cavity fluids for ME diagnosis, with a favorable performance for results above the estimated cutoff.

Wu et al. [18] and Gümüs et al. [15] used NGAL to differentiate complicated and uncomplicated parapneumonic effusions. In peritonitis of patients with non-malignant ascites of recent onset, this biomarker presented 96% sensitivity and 75% specificity to identify this condition; when combined with lactate dehydrogenase (DHL) showed 95% specificity and a good correlation with polymorph nuclear count (91%) [18]. In the present study, we observed that NGAL concentrations were significantly higher in ME than in NTBIE, TPE and TRANS, not being capable in differentiating NTBIE from TPE and TRANS.

PD-L1 is a ligand of the programmed death receptor 1, which is an immunoinhibitory receptor expressed by chronically stimulated CD4 and CD8 T cells it is which is expressed by non-lymphoid tissues and by activated antigen presenting cells (APC) [13]. PD-L1 overexpression is related to a wide variety of human tumors. It is a rising biomarker for MPE diagnosis, mainly related to lung cancer, which seems to be involved in the exhaustion and dysfunction of T cells, in patients with this type of tumor [13,14]. In this study, this biomarker did not present satisfactory performance for the diagnosis of malignant effusions, with an accuracy of only 43.3%. We highlight that only 20 cases of lung cancer were included in the evaluation. However, the values of PD-L1 were significantly higher in patients with lung cancer when compared to those with breast cancer (9 cases).

Recent meta-analysis showed that higher concentrations of VEGF are associated with MPE when compared to benign effusions [12]. This vascular permeability marker correlates with hypoxia-inducible transcription factors (HIFs), which play a central role in physiological adaptation to different oxygenation states. One of the most studied HIF

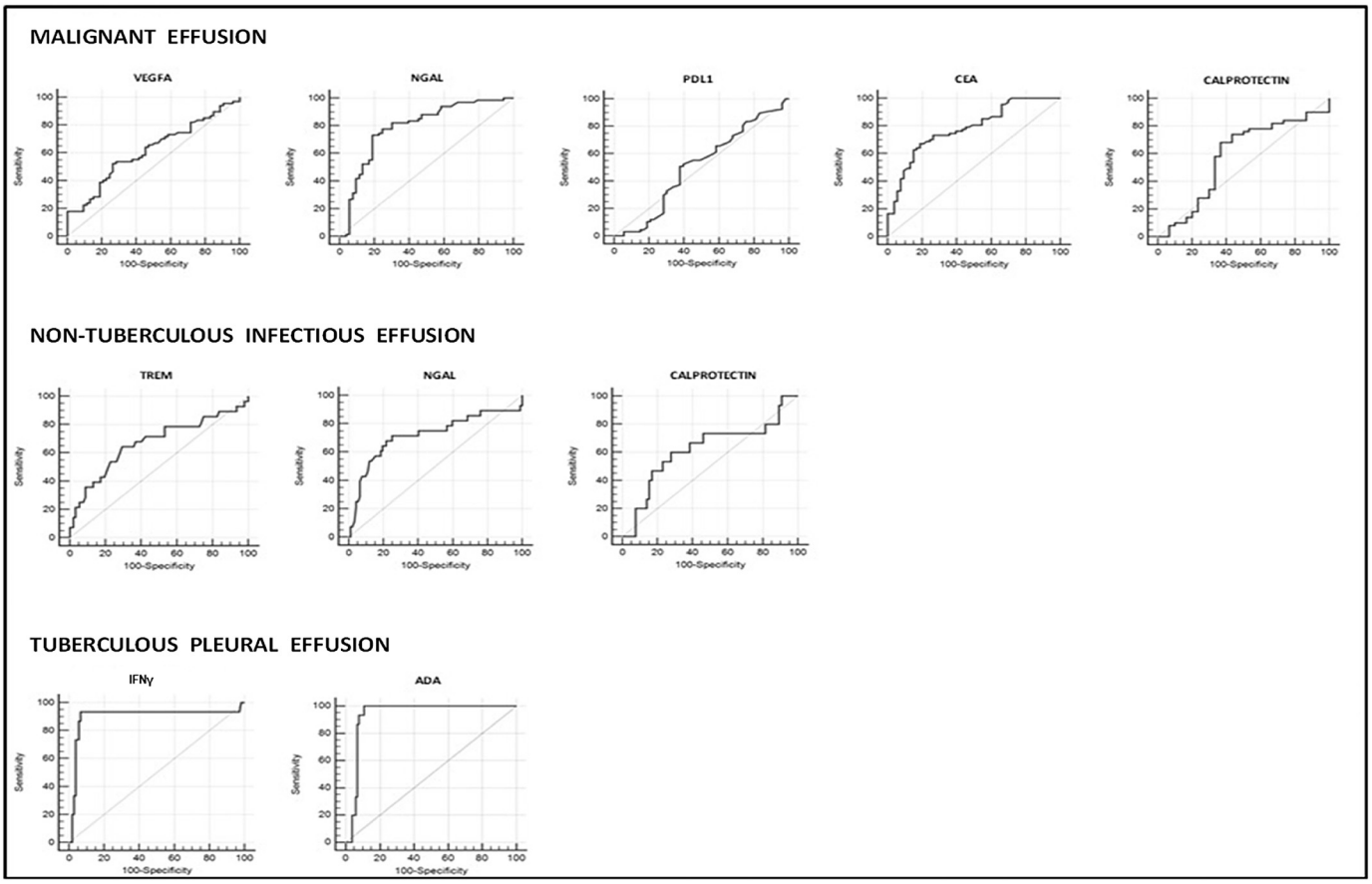

Fig. 1. Biomarkers receiver operating characteristic curves according to effusions' etiology.
VEGF-A: Vascular endothelial growth factor A; NGAL: Neutrophil gelatinase-associated lipocalin; PD-L1: Programmed death-ligand 1; CEA: Carcinoembryonic antigen; CALP: Calprotectin; TREM-1: Triggering receptor expressed in myeloid cells type 1; IFNγ: Gama-interferon; ADA: Adenosine desaminase.

target gene is the VEGF-A, which is expressed in stem cells and neoplastic cells, among others [11,12]. Although the cases included in the study presented advanced tumor staging, this biomarker did not show sufficient sensitivity to discriminate ME from effusions of infectious causes, discriminating only ME from transudates.

Calprotectin belongs to the S100 family of calcium binding proteins, implicated in the calcium-dependent regulation of cells differentiation, proliferation, motility and gene expression [22]. Although Sánchez-Otero et al. [21] reported a good accuracy for this marker in the diagnosis of ME, the performance of this biomarker in the present study was unsatisfactory for diagnosis, with an accuracy of 40.0%. Wu et al. [18] associated a super expression of CALP as promising in the distinction of uncomplicated parapneumonic pleural effusion. The small number of parapneumonic effusions in the present study may have influenced the unfavorable diagnosis performance of this biomarker.

TREM-1, a recognized biomarker associated with sepsis, has high sensitivity (from 71% to 94%) and specificity (from 74% to 93%) in differentiating between infectious (parapneumonic and empyema) and non-infectious pleural effusions [24]. The extracellular domain can be detected in cavity fluids such as soluble TREM-1, expressed on the surface of neutrophils, mature monocytes, macrophages and non-myeloid cells, such as epithelial and endothelial cells [23,25]. In agreement with the literature, we obtained, moderate sensitivity and high specificity with TREM-1 in the diagnosis of NTBIE, with discriminatory potential when compared to ME.

IFNγ and ADA are markers routinely used to diagnose pleural, peritoneal and pericardial tuberculosis with excellent diagnostic performance in countries with high and medium disease prevalence [6]. In tuberculosis, T helper 1 (Th1) cells play an essential role in the protection against *M. tuberculosis* producing IFNγ and tumor necrosis factor alpha (TNF-α) that synergistically activate microbicidal effector mechanisms in macrophages [28]. Individuals who have abnormalities of the Interleukin 12 (IL-12) or IFNγ receptors demonstrate increased susceptibility to diseases caused by mycobacteria [29]. ADA, a ubiquitous enzyme expressed by T lymphocytes and macrophages [15], presents increased activity in pleural tuberculosis [27]. In our study, and according to the literature, both biomarkers had a specificity of > 93% and, when associated, a sensitivity of 100% in the diagnosis of pleural tuberculosis, with a high discriminatory potential when compared to the other etiologies of effusions. In clinical practice, the high sensitivity and specificity of ADA permit its diagnostic use for patients with clinical suspicion of TPE, who presents a lymphocytic pleural exudate, anticipating the early introduction of specific treatment [27].

5. Conclusion

In medical practice, the evaluation of a hybrid panel of biomarkers in the traceability of the major diseases affecting the pleura and the peritoneum may be useful in the etiological discrimination of effusions. Although the cytological exam provides the diagnosis of ME in about 70% of cases, the association of biomarkers to cytology is particularly important for patients with clinical suspicion of ME and whose cytological results were doubtful or inconclusive. Not least important is the identification of complicated parapneumonic effusion, considering that most of these patients require more invasive therapeutic procedures, such as thoracic drainage. It is also important to highlight the importance of adenosine deaminase in the diagnosis of pleural

tuberculosis in developing countries, especially by its low cost and high diagnosis performance.

In conclusion, a hybrid panel composed by the biomarkers CEA, NGAL, IFNγ and ADA seems to be useful in discriminating between ME and TPE etiology, both lymphocytic effusions. For NTBIE, the panel used did not demonstrate diagnostic advantages over the classic literature parameters.

6. Study limitations

The study presented some limitations. First, the number of patients included is relatively small, considering to the composition of the three main diseases groups (ME, NTBIE and TPE). Also, in the ME group, the small number of cases in each primary site did not permit us to analyze the performance of the biomarker into a specific tumor group. Another point to be considered is that most of the biomarkers were quantified by xMAP Luminex® plataform, which is not routinely used for biomarkers quantification in Clinical Labs. So, the comparative analysis with literature data was prejudiced.

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