



## Data Article

# Experimental supporting data on the influence of platelet-derived factors of malignant pleural effusions on T cell effector functions and their relevance in predicting prognosis of lung adenocarcinoma patients with pleural metastasis



Maria Mulet<sup>a,#</sup>, Carlos Zamora<sup>a,#</sup>, José M. Porcel<sup>b</sup>, Juan C. Nieto<sup>a</sup>, Virginia Pajares<sup>c</sup>, Ana M. Muñoz-Fernandez<sup>c</sup>, Nuria Calvo<sup>d</sup>, Aureli Esquerda<sup>e</sup>, Silvia Vidal<sup>a,\*</sup>

<sup>a</sup> Department Immunology, Institut Recerca Hospital de La Santa Creu i Sant Pau, Barcelona, Spain

<sup>b</sup> Pleural Medicine Unit, Department of Internal Medicine, Hospital Universitari Arnau de Vilanova, IRBLleida, University of Lleida, Lleida, Spain

<sup>c</sup> Department Pneumology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

<sup>d</sup> Department Oncology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

<sup>e</sup> Department of Laboratory Medicine, Hospital Universitari Arnau de Vilanova, IRBLleida, University of Lleida, Lleida, Spain

## ARTICLE INFO

## Article history:

Received 3 August 2020

Revised 24 August 2020

Accepted 28 August 2020

Available online 3 September 2020

## Keywords:

Lung cancer

Malignant pleural effusion

Platelet-derived factors

Overall survival

## ABSTRACT

The data described in this article are supplementary to our primary article "Platelet factor 4 regulates T cell effector functions in malignant pleural effusions". Malignant pleural effusion (MPE) is a common complication of advanced lung adenocarcinoma (LAC) associated with a poor life expectancy [1]. Several challenges need to be addressed to identify non-invasive molecular biomarkers that help to predict the prognosis of LAC patients with MPE [2]. In the primary publication, we proposed that platelet-derived factors, especially platelet factor 4 (PF4), can negatively regulate T lympho-

DOI of original article: [10.1016/j.canlet.2020.06.014](https://doi.org/10.1016/j.canlet.2020.06.014)

\* Corresponding author.

E-mail address: [svidal@santpau.cat](mailto:svidal@santpau.cat) (S. Vidal).

# These authors contribute equally.

<https://doi.org/10.1016/j.dib.2020.106266>

2352-3409/© 2020 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

cyte activation and granzyme B expression in pleural metastasis and its levels were associated with a worse prognosis. Here, we provide data on the influence of other platelet-derived factors, including transforming growth factor  $\beta$  (TGF- $\beta$ ), vascular endothelial factor (VEGF), and P-selectin on T lymphocyte response in MPE and their relevance as prognostic factors in lung cancer patients with pleural metastasis. Pleural fluids from 35 lung adenocarcinoma (LAC) and 20 heart failure (HF) patients were collected by thoracentesis and its platelet-derived factors' content was measured by specific enzyme-linked immunosorbent assay (ELISAs). Correlations between pleural levels of platelet-derived factors and T cell functions were analyzed by Pearson coefficients. Kaplan-Meier curves were used to estimate the effect of pleural concentrations of platelet-derived factors on overall survival of LAC patients with pleural metastasis. These analyses showed that the concentration of platelet-derived factors was not associated with T cell proliferation and cytotoxicity. Furthermore, their levels do not predict the survival of LAC with MPE.

© 2020 Published by Elsevier Inc.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

---

## Specifications Table

Subject	Cancer Research
Specific subject area	Malignant pleural effusion, lung cancer, platelet-derived factors
Type of data	Graphs and figures
How data were acquired	Concentrations of soluble factors of pleural fluids were determined by specific ELISA kits in LabSystems Multiskan microplate readers. The proliferation and cytotoxic capacity of T cells in the presence pleural fluids was evaluated by flow cytometry in a MACSQuant <sup>R</sup> Analyzer 10.
Data format	Statistical analysis was performed using GraphPad Prism 7 software.
Parameters for data collection	Analyzed
Description of data collection	Pleural fluids were collected from 35 advanced lung adenocarcinoma patients (stage IV) and from 20 heart failure patients as a control group. Molecular composition of pleural fluids and clinical parameters of patients were analyzed. Overall survival was measured from the date of MPE diagnosis to the date of the last-follow-up or death.
Data source location	Pleural fluid samples were collected by thoracentesis. Pleural concentrations of PF4, TGF- $\beta$ , VEGF and P-selectin were determined by specific ELISAs. An <i>ex vivo</i> model with conditioned culture was proposed to study the effect of pleural fluid on T lymphocyte functions. Data of cultures was analyzed by flow cytometry.
Data accessibility	Pleural fluid samples from both lung adenocarcinoma and heart failure patients were collected from patients who attended to the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) and the Hospital Universitari Arnau de Vilanova (Lleida, Spain). Blood samples were also obtained from healthy volunteers who attended to blood bank of the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain).
Related research article	Data is provided in the article. Clinical characteristics of patients are provided in Table 1 of the related research article. Author's name: Maria Mulet, Carlos Zamora, José M. Porcel, Juan C. Nieto, Virginia Pajares, Ana M. Muñoz-Fernandez, Nuria Calvo, Aureli Esquerda, and Silvia Vidal. Title: Platelet factor 4 regulates T cell effector functions in malignant pleural effusions, Cancer Letters. Journal: Cancer Letters. Status: In press.

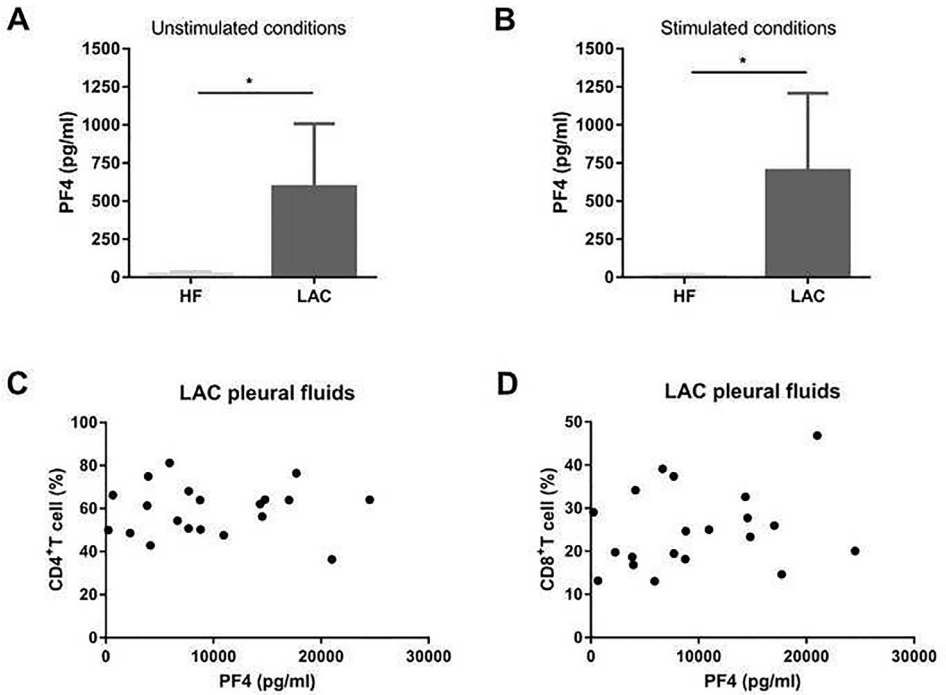
## 1. Data Description

### 1.1. PF4 production by pleural fluid cells

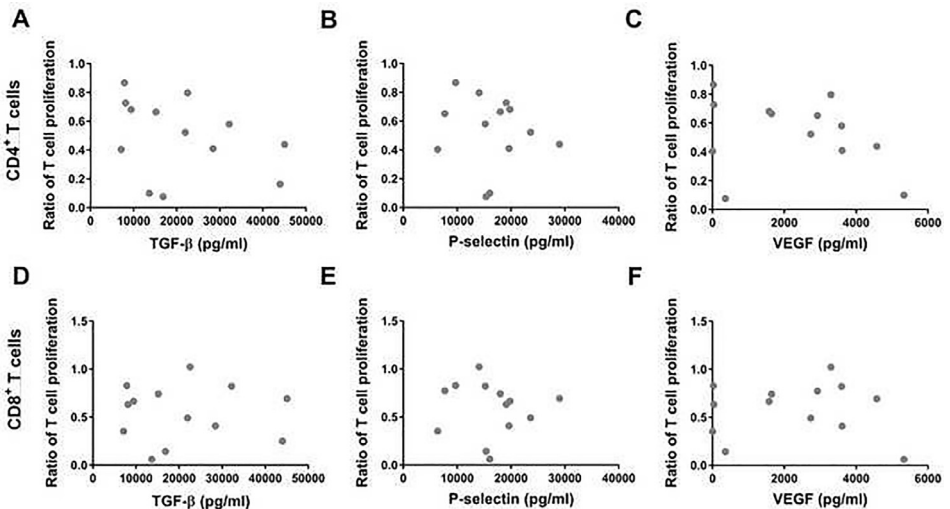
We quantified PF4 production by pleural cells isolated from LAC or HF pleural fluids under spontaneous conditions and after T cell stimulation. In both cases, our *in vitro* experiments revealed that pleural cells isolated from LAC pleural fluids were able to release higher amounts of PF4 than those isolated from HF-related effusions (Fig. 1A-B). Regardless of the culture conditions, PF4 production by pleural cells did not reach the high concentrations of PF4 previously measured in pleural fluids (HF:  $2411 \pm 1490$  pg/ml; LAC:  $9884 \pm 6708$  pg/ml) [4]. The levels of PF4 in pleural fluids were not correlated with the number of CD4<sup>+</sup> or CD8<sup>+</sup> T cells in LAC pleural fluids (Fig. 1CD).

### 1.2. Association between T lymphocyte response impairment and platelet-derived factors in pleural fluids

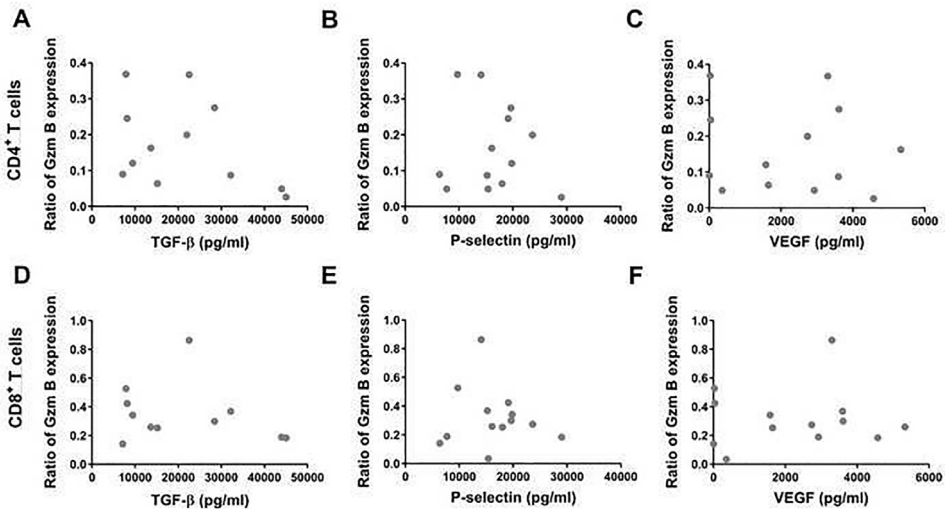
We previously observed that PF4 pleural levels were negatively associated with T cell functions (proliferation and granzyme B expression) in MPE [4]. Here, we provide data about the association of other platelet-derived factors, also increased in MPE (TGF- $\beta$ , P-selectin and VEGF), with the observed T cell impairment. None of soluble factors measured were correlated with



**Fig. 1.** Origin of platelet factor 4 in pleural fluids and its association with T lymphocyte composition. Comparison between PF4 production by pleural fluid cells from HF (n=4) and LAC (n=5) pleural fluids under (A) spontaneous conditions or (B) after T cell stimulation. Association between PF4 pleural levels and the frequencies of (C) CD4<sup>+</sup> and (D) CD8<sup>+</sup> T lymphocytes. Levels of PF4 in pleural fluids and *in vitro* cultures were determined by ELISA. Mann-Whitney test and Spearman coefficient were used for statistical analyses. \*p < 0.05. HF, heart failure patients; LAC, lung adenocarcinoma patients.



**Fig. 2.** Association between levels of platelet-derived factors in pleural fluids and T lymphocyte proliferation. Correlation of (A) TGF-β, (B) P-selectin and (C) VEGF with CD4<sup>+</sup> T cell proliferation. Correlation of (D) TGF-β, (E) P-selectin and (F) VEGF with CD8<sup>+</sup> T cell proliferation. Statistical analysis was performed using Pearson coefficient.



**Fig. 3.** Association between levels of platelet-derived factors in pleural fluids and T lymphocyte granzyme B expression. Correlation of (A) TGF- $\beta$ , (B) P-selectin and (C) VEGF with CD4<sup>+</sup> T cell granzyme B expression. Correlation of (D) TGF- $\beta$ , (E) P-selectin and (F) VEGF with CD8<sup>+</sup> T cell granzyme B expression. Statistical analysis was performed using Pearson coefficient.

neither CD4<sup>+</sup> nor CD8<sup>+</sup> T cell proliferation (Fig. 2) and cytotoxicity (measured by granzyme B expression) (Fig. 3).

### 1.3. The association between pleural levels of platelet-derived factors and the overall survival of LAC patients

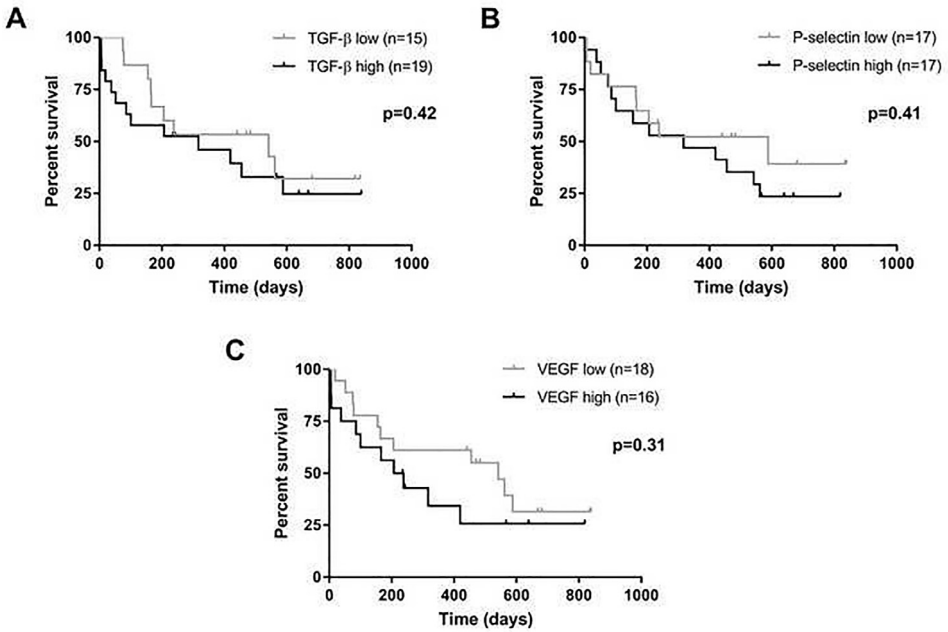
In order to determine the implication of platelet-derived factors from pleural fluids on predicting the prognosis of LAC patients with MPE, we distributed patients in two groups according to their pleural concentrations. The cut-off values for each platelet factor were calculated by the mean levels of HF pleural fluids + 2 SD. With these cutoff values, we distributed LAC patients in two groups, high and low levels.

Using Kaplan-Meier curves, we found that pleural levels of TGF- $\beta$ , P-selectin and VEGF did not seem to influence the overall survival rates as there were no statistical differences between patients with high or low levels (Fig. 4).

## 2. Experimental Design, Materials and Methods

### 2.1. Pleural fluid samples

Pleural fluid samples were collected from 35 LAC and 20 HF patients from the Hospital de la Santa Creu i Sant Pau (Barcelona) and Hospital Universitari Arnau de Vilanova (Lleida). Heparin (10 U/ml; Hospira, Lake Forest, IL, USA) was added to fresh pleural fluids before filtering them with a 40  $\mu$ m sterile filter. After spinning down the cellular pellet, leukocyte composition (T cells, macrophages, and neutrophils) was determined by flow cytometry as it is explained in detail in the primary article [4]. Cell-free pleural fluids were stored at -80°C to further determine concentrations of platelet-derived factors. Clinical and molecular profiles of patients are presented in the related research article (Table 1 and Fig. 3, respectively) [4].



**Fig. 4.** Survival of LAC patients according to pleural levels of platelet-derived factors measured at the time of MPE diagnosis. Kaplan-Meier survival curves comparing LAC patients with high or low concentrations of (A) TGF- $\beta$ , (B) P-selectin and (C) VEGF. Estimated survival rates were compared using the log-rank test. Pvalues are provided in the graphs.

## 2.2. Quantification of platelet-derived factors in pleural fluids

Pleural concentrations of TGF- $\beta$  (Mabtech, Sweden), VEGF (Peprotech, Rocky Hill, NJ, USA), P-selectin (R&D Systems, MN, USA) and PF4 (Peprotech) were measured by specific ELISAs.

## 2.3. In vitro culture of pleural fluids cells

Cells from pleural fluids were collected by centrifugation (574 x g, 5 min) and cultured in complete medium (RPMI 1640 (HyClone, Logan, Utah, USA) supplemented with 10% fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel), 2 mM glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin (BioWhittaker, Verviers, Belgium)) for 72 h at 37°C in 5% CO<sub>2</sub>. On the other hand, pleural fluid cells were also stimulated with T cell activation/expansion kit (cocktail of anti-CD3, -CD28 and anti-CD2) according to manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Supernatants of cultures were kept at -20°C to further determine PF4 production by ELISA.

## 2.4. In vitro culture of PBMCs in the presence of pleural fluids

Peripheral mononuclear cells (PBMCs) were isolated from healthy volunteers' blood by Ficoll-density gradient (Lymphoprep, AXIS-SHIELD PoCAs, Oslo, Norway) as previously described [3] and adjusted to 10<sup>7</sup> cell/ml in RPMI medium. Then, cells were stained with 10  $\mu$ M of carboxyfluorescein succinimidyl ester (CFSE; Sigma, St. Louis, Missouri) to further determine T cell

proliferation. After washing, cells were adjusted to  $5 \cdot 10^6$  cell/ml in RPMI medium and cultured in 96-well plates in the presence of 50% of pleural fluids from LAC or HF patients for 72 h at 37°C in 5% CO<sub>2</sub>. Some cells were also cultured in the presence of 50% FCS in order to have a control of activation and normalize results. T cell stimulation was performed with T cell activation/expansion kit which contains a cocktail of anti-CD3, anti-CD2 and anti-CD28 antibodies according to manufacturer's instructions (Miltenyi Biotec).

### 2.5. Cellular staining and flow cytometry analysis

After 72 h of culture in the presence of pleural fluids, PBMCs were harvested and surface stained with anti-CD4-PECy7 (BioLegend, San Diego, CA, USA) and antiCD8-PErCP (BD Bioscience, San Jose, USA) and intracellular stained with anti-granzyme B-PE (eBioscience, San Diego, CA, USA).

For data analysis, doublets were excluded and cells were gated according to their morphology by forward- versus side-scatter (FSC-SSC) dotplot. The cellular viability was assessed by flow cytometry using the LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (Thermo Fisher Scientific, Oregon, USA). Proliferation cycles of T cells were analyzed in the B1 channel (FITC). Percentages of positive cells and mean fluorescence intensity (MFI) were calculated using the MACSQuantify™ software (Miltenyi Biotec).

### 2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA). Comparison between PF4 production by LAC and HF pleural cells was determined by the Mann-Whitney test. Correlations between the pleural concentration of platelet-derived factors and T lymphocyte functions were analyzed by Pearson test (for PF4, P-selectin and TGF- $\beta$ ) and Spearman test (for VEGF) according to their respective data distribution. Survival analyses were assessed by Kaplan–Meier curves and Log-rank statistics. Differences were significant at  $p \leq 0.05$ .

### Ethics Statement

Written informed consent was obtained from all patients and healthy volunteers. Samples were anonymized and ethical approval of the study was granted by the Institutional Ethics Committee of the Hospital de la Santa Creu i Sant Pau.

### Declaration of Competing Interest

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.

### Acknowledgments

This research was funded through a collaboration with Merck KGaA, Darmstadt, Germany. Silvia Vidal was supported by Fondo de Investigaciones Sanitarias (FIS) and she is a participant in the Program for Stabilization of Investigators of the Direcció d'Estrategia i Coordinació del Departament de Salut, Generalitat de Catalunya. This work has been performed within Ph.D. Immunology Program of Universitat Autònoma de Barcelona (UAB).

## References

- [1] I. Psallidas, I. Kalomenidis, J.M. Porcel, B.W. Robinson, G.T. Stathopoulos, Malignant pleural effusion: From bench to bedside, *Eur. Respir. Rev* 25 (2016) 189–198 <https://doi.org/10.1183/16000617.0019-2016>.
- [2] A.O. Clive, B.C. Kahan, C.E. Hooper, R. Bhatnagar, A.J. Morley, N. ZahanEvans, O.J. Bintcliffe, R.C. Boshuizen, E.T.H. Fysh, C.L. Tobin, A.R.L. Medford, J.E. Harvey, M.M. Van Den Heuvel, Y.C.G. Lee, N.A. Maskell, Predicting survival in malignant pleural effusion: Development and validation of the LENT prognostic score, *Thorax* 69 (2014) 1098–1104 <https://doi.org/10.1136/thoraxjnl-2014-205285>.
- [3] C. Zamora, E. Cantó, J.C. Nieto, M.A. Ortiz, C. Diaz-Torné, C. Diaz-Lopez, J.M. Llobet, C. Juarez, S. Vidal, Functional consequences of platelet binding to T lymphocytes in inflammation, *J. Leukoc. Biol.* 198 (2013) 3099–3108 <https://doi.org/10.1189/jlb.0213074>.
- [4] M. Mulet, C. Zamora, J.M. Porcel, J.C. Nieto, V. Pajares, A.M. MuñozFernandez, N. Calvo, A. Esquerda, S. Vidal, Platelet factor 4 regulates T cell effector functions in malignant pleural effusions, *Cancer Lett* (2020) In press.