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Angiogenic molecule Tie-2 and VEGF in the pathogenesis of pleural effusions

Foteini Economidou[a], Katerina M. Antoniou[a], Nikolaos Tzanakis[b], Katerina Sfiridaki[c], Nikolaos M. Siafakas[a,*], Sofia E. Schiza[a]

[a]*Department of Thoracic Medicine, University Hospital, Medical School, University of Crete, Heraklion 71110 Crete, Greece*
[b]*Department of Epidemiology and Public Health, Medical School, University of Crete, Heraklion 71110 Crete, Greece*
[c]*Department of Hematology, Venizeleion General Hospital, Heraklion, Greece*

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Summary
Background: The role of angiogenesis in the pathogenesis of pleural effusion (PE) has not been determined. The expression of angiogenic factors may represent useful markers for the diagnosis and prediction of disease outcome. To measure the pleural fluid (PF) and serum levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and Tie receptor tyrosine kinase (Tie-2) in order to investigate their role in the pathogenesis of PEs.
Methods: Sixty-seven, 17 with transudative PEs due to heart failure and 50 with exudative PEs (malignant, 22; inflammatory, 15; undiagnosed, 13) were included in the study. PF and serum levels of the growth factors (VEGF, bFGF and Tie-2) were measured using enzyme-linked immunosorbent assays.
Results: PF and serum VEGF levels but not bFGF and Tie-2 levels were higher ($p<0.005$) in exudates than in transudates. PF VEGF levels were significantly higher in malignant than inflammatory and undiagnosed PEs ($p=0.03$). In addition, PF Tie-2 levels were not found different in malignant or in parapneumonic PEs.
Conclusion: Our results showed that VEGF is one of the main mediators in exudative PEs, but this effect is not mediated through the angiogenetic pathway Ang-1/Tie-2. However, the role of angiogenesis and its pathways in the pathogenesis of exudative PEs needs further exploration.

Abbreviations: Ang-1, angiopoietin 1; Ang-2, angiopoietin 2; bFGF, basic fibroplast growth factor; IQR, interquartile range, PE, pleural effusion; PF, pleural fluid; LDH, lactate dehydrogenase, Tie-2, tie receptor tyrosine kinase, VEGF, vascular endothelial growth factor.

*Corresponding author.
E-mail address: siafak@med.uoc.gr (N.M. Siafakas).

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Introduction

Pleural effusion (PE) is common finding in clinical practice. Increased vascular permeability and leakage play a principal role in the development of exudative PEs.[1] However, the role of angiogenesis in the pathogenesis of PE has not been determined. Angiogenesis is controlled by a balance of positive and negative regulators involved in multiple pathways that result in endothelial cell proliferation, differentiation and organization into a functional network of vascular channels.[2,3] These regulatory factors have a potent angiogenic effect, and their precise coordination is essential for vascular development. Angiogenic cytokines, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are candidates for the induction of PEs because they have been implicated in the induction of neovascularization, vascular permeability, and hemorrhage both in the inflammatory process and in tumor progression.[2–7] Thus, the expression of angiogenic factors may represent useful markers for diagnosis and prediction of disease outcome.

VEGF has been reported to play an important role in the development of certain types of effusion.[6,8–11] Several studies indicate that VEGF is consistently higher in exudative than in transudative PEs.[12–14] Effusions associated with malignancies seem to have higher levels of VEGF than benign effusions.[8,9,13,15,17–19] It has been suggested that increased VEGF levels in the malignant PEs increase vascular permeability and contribute to fluid accumulation.[9,16] Despite the statistically significant differences in pleural fluid (PF) VEGF levels between malignant and non-malignant effusions, substantial overlap exists, suggesting that VEGF levels are unlikely to be useful diagnostically as a single marker.[20]

Recently, another endothelial cell-specific receptor tyrosine kinase (Tie-2) and its ligand family, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), have been shown to mediate different functions of angiogenesis.[20–23] Identified on the basis of homology screening, Ang-2 acts as an alternative ligand for Tie-2[20,21] and binds to Tie-2 with similar affinity, but competitively antagonizes Ang-1 effects with blockage of Tie-2 phosphorylation and activation. Functionally, transgenic mice over-expressing Ang-2 show similar defects as the Ang-1 or Tie-2 deficient mice, suggesting that Ang-2 acts as a natural antagonist to Ang-1/Tie-2 action.[20–23] In line with this notion, it has been recently reported that Ang-2 levels but not Ang-1 levels are elevated in exudative PEs suggesting that Ang-2 along with VEFG participate in pleural inflammation and the pathogenesis of exudative PEs.[17] On the contrary, it was suggested that Ang-2 may have a direct role in stimulating Tie-2 receptor signaling and inducing in vitro angiogenesis.[24] These findings indicate that the physiological role of Ang-2 is more complex than previously recognized: acting alternately to promote or blunt Tie-2 receptor signaling in endothelial cells, depending on local conditions.[24]

To the best of our knowledge, the role of Tie-2 in the pathogenesis of PEs has not been investigated. The aim of the present study was to determine the levels of VEGF, bFGF, and Tie-2 in PF and corresponding serum samples of patients with PEs, in order to further evaluate the signaling pathway of VEGF through the angiopoietins' receptor, Tie-2. Our results indicate that VEGF does not act through the Tie-2 receptor pathway in exudative PEs.

Methods and materials

Between January 1, 2004 and August 30, 2005, we prospectively studied 67 consecutive patients with PEs. The study was approved by the Ethics Committee of our hospital, and before the thoracentesis all patients signed an informed consent.

PEs were categorized as exudates or transudates according to Light's criteria.[25–27] A PE was attributed to heart failure when it was transudative, the patient had symptoms and signs of left ventricular failure, a heart ultrasound study revealed systolic or diastolic dysfunction of the left ventricle, and the PE responded to the appropriate therapy. A malignant PE was diagnosed if the PF cytology or pleural biopsy findings were positive for malignant cells, (i.e., proven malignant PE), or if the patient had a persistent PE with a known malignancy and alternative diagnoses were excluded (i.e., probable malignant PE). A parapneumonic PE was defined as one associated with bacterial pneumonia, including empyema. A PE was categorized as tuberculous if Mycobacteria tuberculosis were found in PF, sputum, bronchial lavage fluid, or pleural biopsy specimen (positive smear or culture) [i.e., proven tuberculous PE] or if pleural biopsy revealed granuloma and other granulomatous diseases were excluded (i.e., probable tuberculous PE). A specific diagnosis could not be made even after open pleural biopsy, and they were included in the ''undiagnosed'' group.

The PF was aspirated and blood was drawn immediately after the thoracentesis, and the specimens were collected in plain sterile tubes. PF and blood samples were centrifuged at 1000*g* at 4 °C for 10 min. PF supernatants and serum samples were stored at −80 °C. The following characteristics were recorded: PF and serum total protein levels; lactate dehydrogenase (LDH) concentration (upper limit for serum LDH, 480 IU/L); glucose levels; PF pH; PF nucleated cell counts; and differential cell counts.

The levels of VEGF, bFGF Tie-2 in PE and serum were measured by enzyme-linked immunosorbent assay using a duoset methodology (R&D Systems; Minneapolis, MN). Briefly, after standard procedures, the cell-free samples were pipetted into the wells of the microtitre plates, specific horseradish peroxidase-linked polyclonal antibodies were added and immunoreactive levels of VEGF, bFGF and Tie-2 were determined. Values below the detection limit were assumed as zero.

Statistical analysis

Values were reported as the median (interquartile range [IQR]) for non-normally and as mean (SD) for normally distributed variables. Mann–Whitney, Kruskal–Wallis, and Wilcoxon ranked sum tests were used to assess the difference between different groups, as appropriate. The Spearman test was used to assess the correlation between variables. Values below the detection limit were assumed to be zero for statistical analysis. For statistical analysis, a statistical software package SPSS, version 11.0; SPSS Inc., Chicago, IL was used.