Angiopoietin-2 Levels Are Elevated in Exudative Pleural Effusions*

Ioannis Kalomenidis, MD; Androniki Kollintza, MSc; Ioanna Sigala, MD; Andreas Papapetropoulos, PhD; Spyros Papiris, MD, FCCP; Richard W. Light, MD, FCCP; and Charis Roussos, MD, PhD, FCCP

Objective: **To examine the pleural fluid (PF) and serum levels of angiopoietin (Ang)-1, Ang-2, and vascular endothelial growth factor (VEGF) in patients with pleural effusions (PEs).**

Methods: **One hundred fifteen patients, 16 with transudative PEs due to heart failure and 99 with exudative PEs (malignant, 40; parapneumonic, 24; tuberculous, 13; miscellaneous etiologies, 22) were included in the study. PF and serum levels of the growth factors were measured using enzyme-linked immunosorbent assay.**

Results: **PF Ang-2 and VEGF levels but not Ang-1 levels were higher ($p < 0.001$) in exudates than in transudates. PF Ang-2 levels were higher in tuberculous PEs than in PEs of any other etiology and were lower in heart failure PEs than in PEs of any other etiology. The highest PF VEGF levels were observed in patients with malignant and parapneumonic PEs. The lowest PF VEGF levels were observed in patients with transudates. In PEs, Ang-2 levels correlate with VEGF levels ($p < 0.001$), RBC count ($p = 0.002$), nucleated cell count ($p < 0.001$), total protein levels ($p < 0.001$), and lactate dehydrogenase levels ($p < 0.001$). PF Ang-1 levels were lower than serum Ang-1 levels both in patients with exudates ($p < 0.001$) and in those with transudates ($p = 0.001$). PF Ang-2 levels were higher than serum Ang-2 levels both in patients with exudates ($p < 0.001$) and in those with transudates ($p = 0.045$). PF VEGF levels were higher than serum VEGF levels in patients with malignant PEs ($p < 0.001$) and parapneumonic PEs ($p = 0.003$), but lower than serum VEGF levels in heart failure PEs ($p < 0.001$). In patients with tuberculous PEs and exudative PEs of miscellaneous etiology, PF and serum VEGF levels did not differ significantly.**

Conclusion: **Ang-2 levels but not Ang-1 levels are elevated in exudative PEs, and they correlate with levels of VEGF and markers of pleural inflammation. It is thus possible that Ang-2 along with VEGF participate in pleural inflammation and the pathogenesis of exudative PEs.**

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Key words: angiopoietins; exudates; pleural effusions; vascular endothelial growth factor; vascular hyperpermeability

Abbreviations: Ang = angiopoietin; IQR = interquartile range; LDH = lactate dehydrogenase; PE = pleural effusion; PF = pleural fluid; VEGF = vascular endothelial growth factor

Inflammation and associated vascular hyperpermeability resulting in plasma leakage are fundamental to the development of exudative, protein-rich pleural effusions (PEs).[1] Increased permeability of the pleural microvasculature is generally attributed to factors that are released in inflammatory and malignant pleural diseases,[1] although the exact

*From the Marianthi Simou Laboratory (Drs. Kalomenidis, Sigala, Papiris, and Roussos, and Mrs. Kollintza), Department of Critical Care & Pulmonary Services, Athens Medical School, "Evangelismos" Hospital, Athens, Greece; the Department of Molecular Pharmacology (Dr. Papapetropoulos), School of Pharmacy, University of Patras, Patras, Greece; and the Department of Allergy, Pulmonary and Critical Care Medicine (Dr. Light), Vanderbilt University, Nashville, TN.

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Correspondence to: Ioannis Kalomenidis, MD, Department of Critical Care and Pulmonary Services, Athens Medical School, "Evangelismos" Hospital, 45–47 Ipsilandou St, 10675 Athens, Greece; e-mail:jkalomenidis@hotmail.com

pathogenetic mechanisms of exudative PEs are unclear. Vascular endothelial growth factor (VEGF) has been shown to play an important role in the formation of exudative PEs.[2] Besides its proangiogenic properties, VEGF is a proinflammatory agent[3,4] and a potent inducer of vascular hyperpermeability.[5] VEGF levels are higher in pleural exudates than in transudates.[6–9] More importantly, VEGF blockage significantly reduces vascular permeability and pleural fluid (PF) accumulation in a murine model of malignant PE.[10,11]

Angiopoietin (Ang)-1 and Ang-2 are receptor tyrosine kinase ligands that act in conjunction with VEGF in promoting angiogenesis occurring under both physiologic and disease conditions.[12] In addition, *in vivo* studies[13,14] and *in vitro* studies[15–17] have demonstrated that Ang-1 has antiinflammatory and antipermeability properties; it blocks the expression of adhesion molecules on the endothelial cell surface, leukocyte adherence on endothelial cells and transmigration into tissues, and interleukin-8 production by endothelial cells. In addition, Ang-1 inhibits vascular permeability caused by VEGF and inflammatory agents. The effect of Ang-2 on inflammation and vascular permeability has not been examined as thoroughly. However, the observation that Ang-2 antagonizes the effects of Ang-1 in endothelial cells[18] suggests that Ang-2 promotes vascular permeability. In line with this notion, Ang-2 destabilizes the endothelial cell monolayer integrity leading to the detachment of endothelial cells *in vitro*.[19] More evidence supporting a hyperpermeability and proinflammatory function of Ang-2 comes from a recent *in vivo* study[20] in which Ang-2 was found to induce edema formation and to exert a weak stimulatory effect on leukocyte migration when injected into a mouse paw.

The role of Ang in the pathogenesis of PEs has not been examined. The aim of the present study was to determine the levels of VEGF, Ang-1, and Ang-2 in PF and corresponding serum samples of patients with PEs. We hypothesized the following: (1) Ang-2 and VEGF levels, but not Ang-1 levels, are higher in exudative PEs than in transudative PEs; and that (2) Ang-2 and VEGF levels are higher in PF than in the corresponding serum samples in patients with pleural exudates but not in those with pleural transudates.

Materials and Methods

The study was approved by the Ethics Committee of our hospital, and every patient signed an informed consent form. Patients were prospectively recruited between March 2003 and December 2004. PEs were categorized as exudates or transudates according to the criteria of Light.[21] 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 (*ie*, *proven malignant PE*), or if the patient had a persistent PE and a known malignancy and alternative diagnoses were excluded (*ie*, *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) [*ie*, *proven tuberculous PE*] or if pleural biopsy revealed granuloma and other granulomatous diseases were excluded (*ie*, *probable tuberculous PE*). A lymphocyte-predominant PE with no other explanation and with favorable response to antituberculous treatment was also considered to be of tuberculous origin. Other diagnoses were established based on clinical and laboratory data.

The PF was aspirated and blood was drawn immediately after the thoracentesis, and the specimens were collected in plain tubes. PF and blood samples were centrifuged at 1,000*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 RBC counts; PF nucleated cell counts; and differential cell counts. The levels of Ang-2 and VEGF in PE and serum were measured by enzyme-linked immunosorbent assay using a duoset methodology (R&D Systems; Minneapolis, MN). Ang-1 protein levels were measured by a sandwich noncompetitive enzyme-linked immunosorbent assay consisting of a primary mouse antihuman Ang-1 antibody and a secondary biotinylated goat antihuman Ang-1 antibody (both from R&D Systems). Streptavidin-HRP (R&D Systems) was used to amplify the antibody-antigen reaction, and the color was developed using a TMB-H_2O_2 kit (Pierce; Rockford, IL). Standard curves were generated using recombinant human Ang-1 protein (R&D Systems) at concentrations of 0 to 50 ng/mL. The minimum detectable dose of the assays for Ang-1, Ang-2, and VEGF were 200, 65, and 15 pg/mL, respectively.

Statistical Analysis

Values were reported as the median (interquartile range [IQR]) since they were found not to be normally distributed. 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.

Results

Patient Characteristics

One hundred fifteen patients, 77 men and 38 women were included in the study. The median age was 68 years (IQR, 19 to 90 years). Sixteen patients had transudative PEs secondary to heart failure, and 99 patients had exudative PEs of different origin (Table 1): malignant PEs, 40 (proven, 32; probable, 8); parapneumonic PEs, 24 (empyemas, 4); tuberculous PEs, 13 (proven, 9; probable, 4); and