CORRELATION BETWEEN LEVEL OF ANGIOPOIETIN-2 (PLEURAL FLUID AND SERUM) AND AETIOGENESIS OF PLEURAL EFFUSION

Thesis

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بسم الله الرحمن الرحيم

"وقل اعملوا فسيري الله عملكم ورسوله والمؤمنون وستردون إلي عالم الغيب والشهادة فينبئكم بما كنتم تعملون"

صدق الله العظيم

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Abbreviations

ABIN-2: A20-binding inhibitor of NF-κB 2

ADA : Adenosine de aminase.

AFB : Acid fast bacilli.

ANA : Antinuclear antibody.

Ang-1: Angiopoietin-1.

Ang-2: Angiopoietin-2.

Ang-3: Angiopoietin-3.

Ang-4: Angiopoietin-4.

ANOVA: Analysis of variances.

BM : Bone marrow.

CBC: Complete blood count.

CHF: Congestive heart failure.

CIE: Countercurrent immunoelectrophoresis.

CRP: C-reactive protein.

CSF: Cerebrospinal fluid.

CT: Computerized tomography.

Dok : Downstream of tyrosine kinase.

ECs: Endothelial cells.

EGFR : Epidermal growth factor receptor.

ELISA : Enzyme-linked immunosorbent assay.

EPCs: Endothelial progenitor cells**.**

ERK : Extra cellular signal-regulated kinases.

FISH: Fluorescent in situ hybridization.

FOXO1: Forkhead box protein O1.

HUVECs: Human umbilical vein endothelial cells.

IL: Interleukin.

IL-1 α : Interleukin-1 alpha.

IL-1 β : Interleukin-1 beta.

IL-1RA: Interleukin-1 receptor antagonist.

IL-2R: Interleukin-2 receptor.

INF γ: Interferon gamma.

KDa: Kilo Dalton.

LDH: Lactate dehydrogenase.

MCP-1: Monocyte chemotactic peptide-1.

MDD: Minimum detectable dose.

MMP-9: Matrix metalloproteinase-9.

MODS: Multiple organ dysfunction syndrome.

NF-κB: Nuclear factor kB**.**

NK: Natural killer cells.

NT-BNP: N-terminal brain natriuretic peptide.

PAF: Platelets activating factor.

PAI-1: Plasminogen activator inhibitor-1

PCR : Polymerase chain reaction.

PEs : Pleural effusion.

PF : Pleural fluid.

PH : Power of hydrogen ion.

PH2O: Water pressure.

PI3K : Phosphoinositide-3 kinase.

PKB: Protein kinase B.

PN2: Nitrogen pressure.

PO2: Oxygen pressure.

PPD : Purified protein derivative.

SAPK/JNK: Stress-activated protein kinase/c-Jun NH_2-terminal kinase.

SLE: Systemic lupus erythromatosis.

SPSS: Statistical package for scientific studies.

TGF- β : Transforming growth factor beta.

Th: T-helper cell.

TIE: Tyrosine kinase with immunoglobulin and epidermal growth factor domains.

TNF : Tumor necrosis factor .

TNF α: Tumor necrosis factor alpha.

Tpa : Tissue plasminogen activator.

VATS : Video-assisted thoracoscopic surgery.

VEGF: Vascular endothelial growth factor.

WPBs : Weibel-Palade bodies.

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Abstract

Key Words : (Pleural effusion ,exudates, transudates ,angiopioetin2)

Pleural effusion is a common clinical problem which differentiated into transudates and exudates according to Light's criteria, based on the protein and lactate dehydrogenase levels in pleural fluid and serum.

The angiopoietins are protein growth factors that promote angiogenesis.There are now four identified angiopoietins: Ang1, Ang2, Ang3 and Ang4.

Ang-2 levels are elevated in exudative PEs, and they correlate with levels of PF VEGF levels and markers of pleural inflammation.

INTRODUCTION

Pleural effusion is the abnormal accumulation of fluid in the pleural space. The first step in the evaluation of a pleural effusion is a detailed history and physical examination; the importance of the history and physical examination arises from the fact that a significant percentage of pleural effusions have no definitive diagnostic features on pleural fluid analysis or pleural biopsy. Diagnosis of the cause of pleural effusions is based on the clinical setting and exclusion of other alternative causes. The next step is sampling of the pleural fluid and categorization as a transudate or exudate ***(Martin, 2008).***

Transudative pleural effusions result from systemic diseases that do not directly involve the pleura but instead produce an imbalance of' Starling's forces, resulting in movement of fluid into the pleural space. The diagnostic focus for transudates call for recognition of the systemic disease. Such systemic diseases include congestive heart failure, cirrhosis with ascites, and the nephrotic syndrome. Treatment of transudative effusions should focus on treatment of the underlying disease. Exudative pleural effusions result from local or systemic diseases that directly injure the pleural surface. The diagnostic locus for exudative effusions is to recognize the responsible intrapleural disease. Exudative pleural effusions have any one or more of the following characteristics:

(1) pleural fluid protein–to–serum protein greater than 0.50.
(2) pleural fluid LDH–to–serum LDH greater than 0.60.
(3) pleural fluid LDH greater than two thirds of the upper normal limit for serum.

Transudative pleural effusions meet none of the above three criteria. In addition to the measurement of pleural fluid protein and LDH to differentiate transudate from exudate, other tests that can be helpful include: white blood cell count and differential, glucose, amylase, cytological examination and cultures for aerobic and anaerobic bacteria, mycobacteria and fungi ***(Martin, 2008).***

Inflammation and associated vascular hyper permeability resulting in plasma leakage are fundamental to the development of exudative, protein-rich pleural effusions, increased permeability of the pleural microvasculature is generally attributed to factors that are released in inflammatory and malignant pleural diseases ***(Lee et al., 2003)***.

Although the exact pathogenetic mechanisms of exudative Pleural effusions are unclear. Vascular endothelial growth factor (VEGF) has been shown to play an important role in the formation of exudative Pleural effusions ***(Grove and Lee, 2002).***

Besides its pro angiogenic properties, VEGF is a pro inflammatory agent ***(Kim I et al., 2001).***

VEGF is a potent inducer of vascular hyper permeability ***(Robberts and Palade, 1995).***

VEGF levels are higher in pleural exudates than in transudate ***(Hamed et al., 2004),(Sack et al., 2005).***

More importantly, VEGF blockage significantly reduces vascular permeability and pleural fluid accumulation in a murine model of malignant Pleural effusion ***(Yano et al., 2000).***

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 ***(Tsigkos et al., 2003).***

In addition, in vivo studies ***(Thurston et al., 2000)*** and in vitro studies ***(Pizurki et al., 2003)***, have demonstrated that Ang-1 has anti-inflammatory and anti permeability 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. The angiopoietin family of growth factors includes four members, all of which bind to the endothelial receptor tyrosine kinase Tie2.

Two of the angiopoietins , Ang-l and Ang-4, activate the Tie2 receptor, whereas Ang-2 and Ang-3 inhibit Ang-l induced Tie2 phosphorylation ***(George, 2003).***

In addition, Ang-1 inhibits vascular permeability caused by VEGF and inflammatory agents. The effect of Angiopoietin-2 on inflammation and vascular permeability has not been examined as thoroughly. However, the observation that Angiopoietin-2 antagonizes the effects of Ang-l in endothelial cells ***(Maisonpierre et al., 1997).***

Scharpfenecker et al. (2005) suggested that Angiopoietin 2 promotes vascular permeability and destabilizes the endothelial cell monolayer integrity leading to the detachment of endothelial cells in vitro**.**

Aim of work

The aim of this study is to determine the diagnostic relevance of Angiopoietin-2 in the pleural fluid and serum of patients with pleural effusions of different etiology.

Chapter 1

THE PLEURA

INTRODUCTION :

The pleura is the serous membrane that covers the lung parenchyma, the mediastinum, the diaphragm, and the rib cage. This structure is divided into the visceral pleura and the parietal pleura. The visceral pleura covers the lung parenchyma, not only at its points of contact with the chest wall, diaphragm, and mediastinum but also in the inter-lobar fissures. The parietal pleura lines the inside of the thoracic cavities. In accordance with the intrathoracic surfaces that it lines, it is subdivided into the costal, mediastinal, and diaphragmatic parietal pleura. The visceral and the parietal pleura meet at the lung root. At the pulmonary hilus, the mediastinal pleura is swept laterally onto the root of the lung. Posterior to the lung root, the pleura is carried downward as a thin double fold called the pulmonary ligament ***(Light, 2007a).***

A film of fluid (pleural fluid) is normally present between the parietal and the visceral pleura. This thin layer of fluid acts as a lubricant and allows the visceral pleura covering the lung to slide along the parietal pleura lining the thoracic cavity during respiratory movements. The space, or potential space, between the two layers of pleura is designated as the pleural space. The mediastinum completely separates the right pleural space from the left in humans ***(Light, 2007a).***

Blood Supply

The parietal pleura is supplied by intercostal arteries ***(Albertine et al., 1984).***

The visceral pleura is exclusively supplied by the bronchial circulation, which drains into pulmonary veins ***(Albertine et al., 1982).***

The drainage route via pulmonary veins may have contributed to earlier confusion about whether the visceral pleural blood supply was from a systemic (bronchial) or a pulmonary circulation. Both pleurae, therefore, have a systemic circulation, although the visceral pleural bronchial circulation may have a slightly lower perfusion pressure than the parietal pleural intercostal circulation because of its drainage into a lower-pressure venous system ***(Broaddus and Light, 2010).***

Lymphatics

If one injects carbon particles into the pleural space as a visible marker of lymphatic drainage pathways, one later finds that the black carbon has been taken up into lymphatics on the parietal side, not the visceral side. The visceral pleura has extensive lymphatics, but they do not connect to the pleural space ***(Albertine et al., 1982).***

The parietal pleural lymphatics connect to the pleural space via stomata, holes of 8 to 10 ým in diameter that are formed by discontinuities in the mesothelial layer where mesothelium joins to the underlying lymphatic endothelium ***(Y-Y Li and J-C Li, 2003).***

The stomata can accommodate particles as large as erythrocytes. In various experimental studies, these lymphatics have been shown to be the major route of exit of liquid from the pleural space ***(Broaddus et al., 1988).***

From the stomata, liquid drains to lacunae, spider-like submesothelial collecting lymphatics, which then drain to infracostal lymphatics, to parasternal and periaortic nodes, to the thoracic duct, and into the systemic venous system. Lymphoid cells have been described lying within aggregates underneath morphologically different mesothelial cells, forming raised structures called Kamp Meier's foci that may have an immune function, as shown for the peritoneal space ***(Jantz and Antony , 2008).***

Nerve Supply

The parietal pleura contains sensory nerve fibers, supplied by the intercostal and phrenic nerves, and has long been thought to be the major site of pain sensation in the pleura. The costal and peripheral diaphragmatic regions are innervated by the intercostal nerves, and pain from these regions is referred to the adjacent chest wall. The central diaphragmatic region is innervated by the phrenic nerve, and pain from this region is referred to the ipsilateral shoulder ***(Broaddus and Light, 2010).***

The visceral pleura has more recently been shown to have sensory nerve fibers that may participate in pain or other sensations such as dyspnea ***(Pintelon et al, 2007).***

In addition, pleural adhesions may contain pain fibers and contribute to post-thoracotomy or post pleurodesis pain ***(Montes et al., 2006).***

PHYSIOLOGY OF THE PLEURAL SPACE

Normal Pleural Liquid and Protein Turnover

Since the 1980s, a consensus has developed that normal pleural liquid arises from the systemic pleural vessels in both pleura, flows across the leaky pleural membranes into the pleural space, and exits the pleural space via the parietal pleural lymphatics ***(Broaddus, 2008).***