VALUE OF C-REACTIVE PROTEIN IN ETIOLOGIC DIAGNOSIS OF PLEURAL EFFUSION

Thesis

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ABSTRACT

Pleural effusion may represent a primary manifestation of many diseases, but most often they are observed as a secondary manifestation or complication of other diseases.

In most instances, the cause of effusion becomes apparent. However, in about 20% of cases, the effusion remain etiologically undiagnosed after the initial evaluation.

Recently many reports have been written on the biological use of the level of acute phase protein (APP) in numerous diseases, such as asthma, COPD, and pleural effusion. CRP levels have been shown to increase in a number of pulmonary diseases, notably bacterial infections, inflammation, neoplasia, pulmonary thrombo-embolism and some pleural effusion related to other conditions.

KEY WORDS,

VALUE OF C-REACTIVE PROTEIN

List of Abbreviations

A_1 AT	Alpha-1 antitrypsin
A_1 PI	Alpha-1 proteinase inhibitor
ADA	Adenosine deaminase
AIDS	Acquired immune deficiency
ANA	Anti-nucler antibody
APP	Acute phase protein
CAPD	Continuous ambulatory peritoneal dialysis
CBC	Complete blood count
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	Computerized tomography
DIC	Disseminated intravascular coagulopathy
DNA	Dexoyribonucleic acid
DVT	Deep venous thrombosis
ECG	Electric cardiography
ESR	Erythrocyte sedimentation rate
FDG	Fluro-deoxyglucose
GIT	Gastrointestinal tract
HDL	High density lipoprotein
h_s CRP	High sensitive C-reactive protein
IL	Interleukin
INF-γ	Interferon gamma

LDH	Lactate dehrydrogenase
LDL	Low density lipoprotein
MBL	Mannose binding lectin
MBP	Mannose binding protein
MPE	Malignant pleural effusion
MR	Magnetic resonance
PA	Postero-anterior
PAI-1	Plasminogen activator inhibitor type 1
PE	Pulmonary embolism
PET	Positive emission tomography
pH	Power of hydrogen ion
SAA	Serum amyloid protein
SAP	Serum amyloid P component
SLE	Systemic lupus erythromatosis
TB	Tuberculosis
TNF-α	Tumour necrosis factor alpha
$_{T}$PA	Tissue type plasminogen activator
VATS	Video assisted thoracic surgery
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoprotein

List of figures

Contents

introduction

Pleural fluid is a common problem in clinical practice. It can be caused by several mechanisms including increased permeability of pleural membrane, increased pulmonary capillary pressure, decreased negative intrapleural pressure, decreased oncotic pressure and obstruction of lymphatic flow ***(NaMaskall, 2003)***.

The first step in evaluation of patients with pleural effusion is to determine whether the effusion is a transudate or an exudates. This classification is currently based on Light's criteria ***(El-Sammak et al., 2003)***.

Malignancy is common and important cause of exudative pleural effusions. Because pleural fluid cytology and pleural biopsy specimens do not provide diagnosis in a high percentage of malignant effusion, several tumour markers have been studied. To overcome this limitation, it was hypothesized that measurement of C-reactive protein (CRP) may be useful in differentiating between transudates and exudates and between benign and malignant effusion ***(Kim et al., 2007)***.

C-reactive protein (CRP) is an acute phase protein widely used as a marker of inflammation and tissue injury. Its determination is simple, quick and inexpensive in pleural fluid, CRP levels have found higher in tuberculosis and parapneumonic effusions than in other causes of pleural effusions ***(Garvcia-Pachom et al., 2002)***.

Aim of the work

The aim of this work is to determine the validity of pleural fluid (CRP) concentration and pleural fluid/serum (CRP) ratio in etiologic diagnosis of pleural fluid.

Embryology and anatomy

By 3 weeks of gestational age, the pleural, pericardial, and peritoneal spaces begin to form the mesoderm, and, by 9 weeks, the pleural cavity has become separated from both the pericardial and peritoneal spaces ***(Gray and Skandalakis, 1985)***. Various cysts, diverticula, and defects can result from incomplete partitioning of the three mesodermal spaces. As the lung develops, the lung buds invaginate into the visceral pleura, and henceforth retain a pleural covering.

The pleural membranes are smooth, glistening coverings for the constantly moving lung. Overlying each pleural membrane is a single cell layer of mcsothelial cells. These cells can vary in shape from flat to cuboidal or columnar, perhaps based on the degree of stretching of the underlying submesothclial tissue. These cells, the most numerous cell type of the pleural space, may have a variety of functions important to pleural biology. Mesothelial cells can secrete the macromolecular components of the extracellular matrix and organize them into mature matrix, ***(Rannard et al., 1984)*** phagocytose particles, ***(Boylan et al., 1995)***, produce fibinolytic and procoagulant factors ***(Idell et al., 1992)*** and secrete neutrophil and monocyte chcmotactic factors ***(Antony et al., 1995)*** that may be important for inflammatory cell recruitment into the pleural spaces ***(Boylan et al., 1992)***. The mesothelial cells also produce cytokines such as transforming growth factor-β, epidermal growth factor, and platelet-derived growth factor, which arc important in pleural inflammation and fibrosis ***(Lee and Lane, 2003)***.

On the surface of the mcsothelial cells arc microvilli, which are irregularly distributed over the pleural surfaces. Although microvilli presumably exist to increase surface area for metabolic activity, the

function of these prominent features is unknown. Mesothelial cells produce hyaluronan but not mucin, express keratin microfilaments, stain negatively with epithelial-specific antibodies- (Ber-F,P4, B72.3, Leu.M1, and CHA), and stain positively for calretinin and mesothelin-all features that are important for histochemical and immunohistochemical identification of the cells in pleural effusions ***(Ordonez 2002)***.

The cells lie on a thin basement membrane overlying a varying region of connective tissue containing mostly collagen and elastin. Although the parietal pleural connective tissue layer is of a consistent thickness, the visceral connective tissue layer varies greatly. In a single individual, the visceral pleura varies from a thinner layer at the cranial region to a thicker layer at the caudal region ***(Albertine et al., 1982).*** Analysis of the constituents of the visceral pleura has shown that there is more collagen relative to elastin than is found in the lung parenchyma, a finding consistent with a mechanical role for the pleura ***(Oldmixon and Hoppin, 1956).*** This connective tissue layer contains blood vessels and lymphatics and joins with the connective tissue of the lung.

Blood Supply

The parietal pleura is supplied by intercostal arteries **(Fig.1)**. The visceral pleura in humans, like that in sheep, 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 pulmonary circulation. Both pleurae in humans 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.

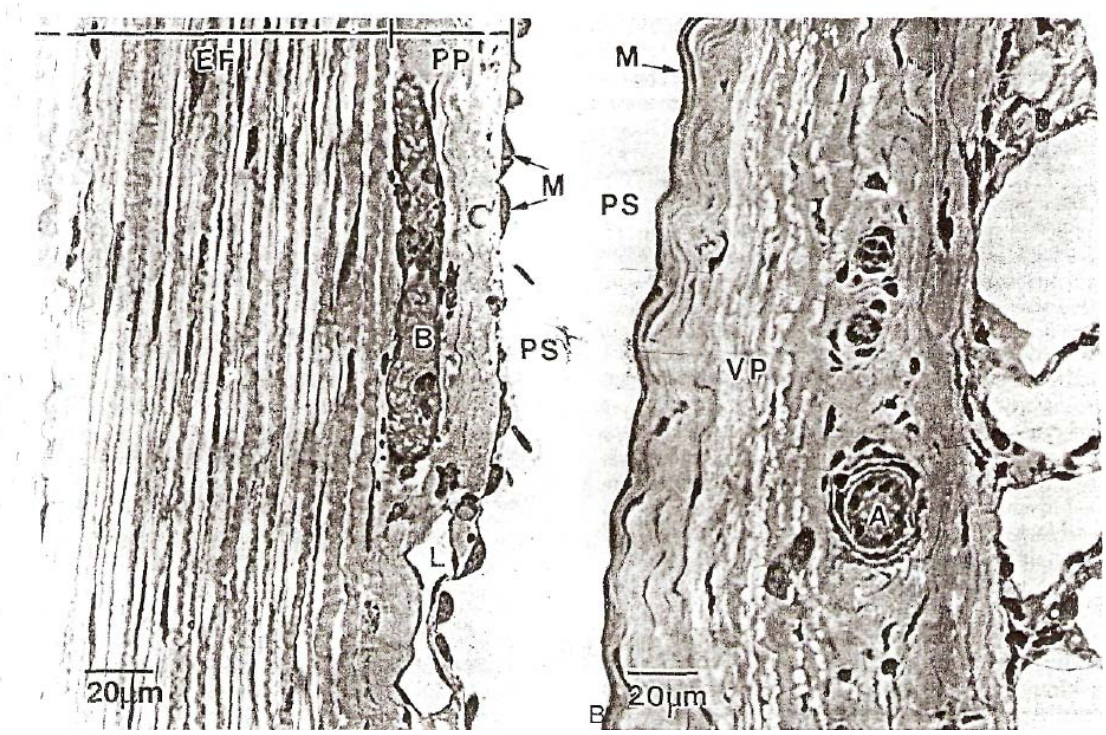

Fig. (1): Light micrographs showing the parietal and visceral pleurae of the sheep, an animal with a pleural anatomy similar to that1 of humans. Both membranes are covered by a single layer of mesothelial cells (M). A, The parietal pleura (PP) is the layer of loose connective tissue between the pleural space (PS) and the dense connective tissue of the endothoracic fascia (EF). Within the loose connective tissue are blood microvessels (B) from the intercostal arteries, and lymphatic lacunae (L) that open into the pleural space via stomata, B, The visceral pleural (VP) lies between the pleural space (PS) and the alveoli. The blood supply to the visceral pleura is via the bronchial arteries (A), which drain into pulmonary veins. The pleura contains dense bands of elastin and collagen ***(Chretien et al., 1985).***

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).*** Demonstrated in rabbits, sheep, and in humans, the parietal pleural lymphatics connect to the pleural space via stomas, holes of 8 to 10 μm in diameter that are formed by discontinuities in the mesothelial layer where mesothelium

joins to the underlying lymphatic endothelium ***(Liyy, 2003).*** The stromas can accommodate particles as large as erythrocytes. In various experimental studies, these lymphatic have been shown to be the major route of exit of liquid from the pleural space ***(Broaddus et al., 1988).*** From the stromas, liquid drains to lacunae-, spider-like submcsothelial 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 mesothclial cells, forming raised structures called Kampmeier's faci that may have an immune function ***(Pereira et al., 1994).***

Nerve Supply

Only the parietal pleura contains sensory nerve fibers, supplied by the intercostal and phrenic nerves. 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. The visceral pleura does not contain sensory nerve fibers; therefore, pain, whether from inflammation, tumor, or catheters advanced far out in the lung during bronchoscopy, indicates involvement of the adjacent parietal pleura.

HISTOLOGY OF THE PLEURA

The visceral pleura is composed of three layers:

The endopleura, which is composed of a continuous layer of mesothelial cells overlying a delicate network of irregularly arranged collagen and elastic fibers, is the most superficial ***(Peng et al., 1994).***

The mesothelial cells are active cells and are sensitive and responsive to various stimuli. In cell culture mesothelial cells have been shown to produce type I, type II and type IV collagen, elastin, fibronectin, and laminin, and express intermediate filaments typical of both epithelial cells, and fibroblasts ***(Light, 1995a)***. It also can express procoagulant activity due to a tissue factor that binds factor VII at the cell surface. It has also been demonstrated to produce transforming growth factor β1, platelet derived growth factor, epidermal growth factor and fibroblast growth factor ***(Lee and Lane, 2003).***

The mesothelial layer is very fragile. When irritated, they retract, but return continuity with adjacent cells by projections called cellular bridges. Mesothelial cells frequently are dislodged from the pleural surfaces and are thereby free in the fluid ***(Light, 1995a).***

The second layer (external elastic lamina, or the chief layer) is primarily responsible for pleural mechanical stability and consists of a thin layer of dense collagen elastic tissue, whereas the latter is arranged in a more or less haphazard manner in the plane of the pleural surface, the collagen fibers are grouped into, welldeveloped bundles whose orientation varies in different regions ***(Peng et al., 1994).***

The third layer (vascular or interstitial) of visceral pleura lies beneath the chief layer and consists of connective tissue containing lymphatic and blood vessels ***(Peng et al., 1994).***

The parietal pleura is composed of loose, irregular connective tissue covered by a single layer of mesothelial cells. Deep to the pleura, is the endo-thoracic fascia. This continuous band of dense irregular connective tissue composed mainly of collagen and elastin ***(Light, 1995a).***

Most of the vessels are located in the part farthest from the pleura, mainly the capillaries and the lymphatic lacunas. The lacunas are specialized initial lymphatics, shaped like flat cistern ***(Pistolisi et al., 1989).***

The Pleura: Form and Function

The pleura consists of two membranes, the visceral pleura covering the lung, and the parietal pleura covering the chest wall and diaphragm. These two pleural membranes meet at the hilar root of the lung. In the sheep, an animal with a pleural anatomy similar to humans, the surface area of the visceral pleura of one lung, including that invaginating into the lung fissures, is similar to that of the parietal pleura of one hemithorax, approximately 1000cm^2. The normal pleural space is approximately 18 to 20 μm in width, although it widens at its most dependent areas ***(Albertine et al., 1991)***. Although at one time controversial, ***Albertine et al. (1991)*** reported that pleural membranes do not touch each other, and that the pleural space is a real, not a potential, space **(Fig. 2)**.