Pleural Transport Physiology: Insights from Biological Marker Measurements in Transudates

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Abstract: *Aims*: The aim of this study was to evaluate the physicochemical properties of the pleural mesothelial barrier and of the biological markers that facilitate or eliminate the passage of molecules through the pleura.

Methods and Material: Pleural fluid samples from sixty-five patients with heart failure were analyzed. The biological markers studied were lactate dehydrogenase (LDH), adenosine deaminase (ADA), interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), carcinoembryonic antigen (CEA), copper/zinc superoxide dismutase (CuZnSOD), matrix metalloproteinase-2 (MMP-2), -3 (MMP-3), -7(MMP-7), -8 (MMP-8) and -9 (MMP-9). Based on the pleural fluid/serum ratio, these molecules were divided into three groups: a) the LDH-like group with a pleural fluid/serum ratio between 0,4 and 0,8 (LDH, CEA, CuZnSOD, ADA, CRP, MMP-8), b) molecules with a pleural fluid/serum ratio less than 0,4 (MMP-7 and MMP-9) and c) molecules with a pleural fluid/serum ratio equal or above 1 (TNF-α, IL-6, MMP-2 and MMP-3).

Results: No correlation between the molecular radius and the pleural fluid to serum ratio of the above biological markers was found.

Conclusions: The molecular size is not a major determinant for the passage of molecules through the mesothelial barrier. Several other factors may influence the transport of the above molecules to pleural cavity, such as their charge and shape.

Keywords: Biological markers, mesothelial barrier, pleural fluid/ serum ratio, transudates, lactate dehydrogenase, tumor necrosis factor.

INTRODUCTION

The first question to answer when a patient with an undiagnosed pleural effusion is evaluated is whether this effusion is a transudate or an exudate. The criteria established by Light[1] have become the "gold standard" for segregating transudative from exudative pleural effusions, especially because of their high sensitivity. This distinction determines not only the differential diagnosis and thus the following therapeutic intervention, but also the underlying pathophysiologic mechanisms which lead to the aggregation of fluid in the pleural cavity. Indeed, an exudative pleural effusion occurs when the permeability of the mesothelial-capillary barrier to albumin and other macromolecules is elevated. In contrast, the presence of a transudate indicates that the systemic or pulmonary pressures influencing the formation or reabsorption of pleural fluid are altered [2]. Because the barrier permeability characteristics are maintained to transudates, the transpleural transport of molecules is the same as in normal conditions. The aim of this study was to estimate the physicochemical properties of the pleural mesothelial barrier and of biological markers that

facilitate or eliminate their passage through the pleura. More specifically we hypothesized that molecular size, a physical property which may be represented by molecular radius, determines the passage of substances through the mesothelial barrier to the pleural cavity

SUBJECTS AND METHODS

We enrolled 65 patients with transudative pleural effusions who were admitted to the Respiratory Medicine Department of University Hospital of Larissa from September 2007 through February 2009. Local ethics committee approved the study protocol and all subjects provided written informed consent.

After the first successful thoracentesis of pleural fluid, samples were subjected to routine biochemical analysis including tests for glucose, total protein and LDH. In addition, samples were analyzed for total cell count and differential cell count. For biomarker's measurements, pleural fluid were immediately centrifuged at 1500g for 15 min at 4^0C to pellet the cellular elements and the supernatants were stored at -80^0C until the final procedure. Venous blood was also drawn for serum analysis and processed in the same way as the pleural fluid. ADA activity was measured by the colorimetric method of Giusti. CEA concentration levels were determined using an electrochemiluminescence immunoassay on the Roche

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Modular E 170 analyser (Roche Diagnostics, Mannheim, Germany). CRP measurements were performed by immunonephelometry with the Behring Nephelometer Analyzer II, using the N High Sensitivity kit (Dade Behring, Marburg, Germany), according to the manufacturer's instructions. IL-6, TNF-α, CuZnSOD and MMPs levels were measured with commercially available enzyme-immunosorbent assay kits (Biosource, Europe S.A., Bender MedSystems, Austria Europe and R&D Systems, Minneapolis MN USA), according to the manufacturer's protocols.

The patients were classified into exudate or transudate groups based on the clinical and laboratory findings of pleural effusions obtained by thoracentesis. Only patients with a transudative pleural effusion which was attributed to left heart failure were included in this study. Patients with traumatic thoracentesis were excluded from the study. Cut-off values for differentiating exudates from transudates were determined with the criteria of Light *et al.* as follows: pleural fluid to serum protein ratio>0.5, or pleural fluid to serum lactate dehydrogenase (LDH) ratio>0.6, or pleural fluid LDH greater than two-thirds of the upper limit of normal serum LDH level. Pleural effusion was defined as exudate when one or more of these findings were recognized [1].

In order to test our hypothesis, the pleural fluid to serum ratio of lactate dehydrogenase (LDH), adenosine deaminase (ADA), C-reactive protein (CRP), carcinoembryonic antigen (CEA), interleukin-6 (IL-6), tumor necrosis factor-a (TNF-α), copper/zinc superoxide dismutase (CuZnSOD) and matrix metalloproteinases (MMPs) [MMP-2, MMP-3, MMP-7, MMP-8, MMP-9] to patients with transudative pleural effusions. Based on the pleural fluid to serum ratio we can classify the biological markers to three subgroups: a) the LDH–like group with a pleural fluid to serum ratio between 0.4 and 0.8. Here belongs the majority of the molecules studied, i.e. LDH, CEA, CuZnSOD, ADA, CRP, MMP-8, b) molecules with a pleural fluid to serum ratio less than 0.4. MMP-7 and MMP-9 are included to this group c) molecules with a pleural fluid to serum concentration equal or above 1, to which TNF-α, IL-6 and MMP-2 and MMP-3 are included. The molecular radius of the biological markers was calculated according to the following empirical equation: $a = 0.483 \times (MW)^{0.385}$, where a is the molecular radius in Å and MW the molecular weight in Dalton [3]. This equation was calculated by Rippe and Stelin in order to evaluate the transperitoneal clearance of molecules with divergent MWs through pores of a wide range of radius.

Statistical analysis was performed using SPSS for Windows (SPSS, Chicago, USA). For mean values Student's t-test was performed. Spearman r test was used for correlation analysis. $P<0.05$ was regarded as significant.

RESULTS

Mean value of all twelve biological markers measured in pleural fluid and serum and their standard deviation are presented in Table **1**. Also the mean value of the pleural fluid/serum ratio, standard deviation and minimum and maximum value of the twelve biological markers measured to transudates are shown in Table **1**. The lowest ratio was observed for MMP-9 (0.09) whereas the highest for IL-6 (29.27).

No statistically significant correlation was found between the pleural fluid/serum ratio and the molecular radius of the molecules (correlation coefficient r = -0.339, p = 0.279). When we examined solely the biological markers with a molecular radius less than 30 Å (ADA, CuZnSOD, MMP-7, TNF-α and IL-6), the correlation coefficient between the molecular radius and the pleural fluid/serum ratio was -0.705 and it was not statistically significant (p value=0.117).

Table 1.   **Biological Markers Measured in Pleural Fluid, According to Pleural Fluid/Serum Ratio Value**

Molecule	MW[a]	Molecular Radius[b]	Pleural Fluid Mean (SD[c])	Serum Mean (SD[c])	Pleural Fluid/Serum Ratio		
					Mean (SD)	Minimum Value	Maximum Value
ADA	42	29.10	8.52 (3.26)	22.75 (8.37)	0.40 (0.17)	0.13	0.82
CRP	115	42.88	0.84 (0.76)	2.22 (1.61)	0.50 (0.63)	0.05	3.50
LDH	134	45.48	122 (62)	232 (88)	0.60 (0.48)	0.22	2.71
CEA	180	50.95	1.05 (0,69)	2.20 (1.56)	0.60 (0.51)	0.15	2.47
CuZnSOD	31	26.08	98.38 (50)	210 (139)	0.64 (0.37)	0.07	1.55
MMP-8	85	38.61	14.76 (5.2)	42.06 (12)	0.80 (2.87)	0.00	12.82
MMP-9	92	39.35	33.19 (15)	598 (226)	0.09 (0.26)	0.00	1.24
MMP-7	28	24.89	1.61 (2.29)	7.11 (2.78)	0.22 (0.24)	0.03	1.31
TNF-α	17	20.72	18.78 (11.78)	20.74 (14.21)	1.04 (0.57)	0.40	2.63
MMP-3	57	32.73	28 (16)	25.41 (19.77)	1.40 (0.86)	0.27	3.53
MMP-2	72	35.81	422.2 (122.2)	232.4 (68.21)	1.89 (0.46)	0.97	3.19
IL-6	20	21.99	43.82 (42.66)	29.27 (38.54)	29.27 (38.54)	5.09	208.60

a: molecular weight in kDa, b: molecular radius in Å, c: standard deviation. The mean value in pleural fluid and serum is given in ng/ml for MMPs, CuZnSOD, CEA, IU/L for ADA and LDH, mg/dl for CRP and pg/ml for IL-6 and TNF-α.

DISCUSSION

Based on the pleural fluid to serum ratio we classified the biological markers into three subgroups. The small ratio for MMP-7 and MMP-9 implies that these molecules can hardly transverse the mesothelial barrier from the blood circulation of the pleura to the pleura cavity. On the other hand, the local production of the MMP-2 and IL-6 by mesothelial cells and subsequent secretion to pleural cavity results to a high pleural fluid/serum ratio above unit [4]. For MMP-3 no study has been conducted regarding the expression of this enzyme by mesothelial cells. TNF-a is found to almost similar concentrations in blood circulation and pleural cavity (pleural fluid/serum concentration 1.04), and this reflects the convenience of this molecule to diffuse through the mesothelial barrier. It has been suggested that some of the above biological markers, either alone or combined, can contribute to the discrimination between the different kinds of exudative effusions [5, 6]. However, the factors that determine the transport of a molecule to pleural cavity, under conditions of normal or increased permeability, remain unknown.

Is Molecular Radius a Major Determinant for the Passage of Molecules Through the Pleura?

No correlation between molecular radius and pleural fluid/serum ratio of all twelve biological markers was found. At the peritoneal membrane of the abdominal cavity and at glomerular filter of the kidney it has been shown that small solutes (molecular radius < 30 Å) transport, through the peritoneal and glomerulal filter respectively, *via* diffusion. This means that osmotic pressure gradients determine the movement of small solutes. A different mechanism occurs for the transport of proteins and macromolecules (molecular radius ≥ 30 Å), as these solutes transport primarily *via* filtration, that is hydrostatic pressures lead the transport of macromolecules [7, 8]. Filtration does not depend on the MW and on the molecular radius of a solute whereas diffusion does. When we examined only the biological markers with a low molecular radius < 30 Å, which theoretically transport *via* diffusion, again no correlation was found. The above indicate that the mesothelial barrier is not primarily size-restrictive. Other factors, such as discussed below, may determine the passage of molecules to the pleural cavity.

Pathophysiological Significance

The properties that influence the sieving characteristics of the pleura can be divided in two main categories: i) the structure of mesothelial barrier, consisting of microvascular capillaries of the pleura and the mesothelial monolayer and ii) the physiochemical properties of the molecules. Pleural fluid is produced at parietal pleural level and represents a filtrate through capillary endothelium of the pleura and mesothelial cells [9]. For microvascular endothelium permeability, it has been suggested that small solutes (up to the size of albumin) diffuse through "small pores", represented by clefts in the intercellular junctions. In contrary, macromolecules use a population of "large pores", which could either be real openings between cells or be provided by an intracellular vesicular system [10]. No study on the microscopic structure of "small pores" located to mesothelium has been so far conducted. Macromolecule transfer to mesothelium mainly occurs through "large pores" [11]. The morphological counterpart for large pores is not known. Although cytoplasmic vesicles have been well described to mesothelial cells, these seem to account only for reabsorption of pleural fluid and not for its production. The microscopic structure of "small and large pores" should be an important factor to determine the feasibility of molecules to pass through the mesothelial barrier. Other factors that may influence the passage of molecules to pleural cavity involve the molecular radius, net charge, deformability and shape of molecules and their connection to plasma proteins, such as albumin.

CONFLICT OF INTEREST

None declared.

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