Relationship Between Pleural Fluid and Serum Cholesterol Levels*

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Introduction: Since the criteria of Light and colleagues for differentiating transudates and exudates were described, other tests, including the pleural fluid (PF) cholesterol test, have been proposed for the same purpose. However, the factors influencing PF cholesterol levels have not been clearly delineated.

Purpose: To analyze the relationships among total cholesterol (CHOL), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TRIG) in serum (S) and PF.

Methods: PF and S from 99 patients (transudates, 13 patients; exudates, 86 patients) were analyzed for CHOL, HDL, LDL, TRIG, apolipoprotein AI, apolipoprotein B, and protein. The relationship between the PF and S level for each of these measurements was analyzed with linear regression and multiple regression using the ratio of PF to S protein for that measurement as a second independent variable.

Results: This study demonstrated that CHOL levels in PF are related to S cholesterol levels and to the permeability of the pleura \( (r = 0.88; p < 0.001) \). However, the percentage of CHOL associated with LDL and HDL (56%) in the PF was much lower than that associated with LDL and HDL in S (93%), suggesting that lipoproteins are modified once they enter the pleural space. The PF TRIG was not closely related to its S level or to the PF/S protein ratio \( (r = 0.49) \).

Conclusion: PF cholesterol levels can be closely predicted from the S cholesterol levels and the permeability of the pleura, as reflected by the ratio of PF protein to S protein. Therefore, the CHOL ratio should not provide additional information to that provided by the protein ratio when trying to differentiate transudates from exudates. PF lipoproteins (LDL and HDL) undergo metabolic alterations once they enter the pleural space. PF TRIG levels are not closely related to S levels or to the permeability of the pleura.

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Key words: cholesterol; chylothorax; lipid; pleural effusion; pleural fluid

Abbreviations: ApoA = apolipoprotein AI; ApoB = apolipoprotein B; CHOL = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PF = pleural fluid; PROT = total protein; S = serum; TRIG = triglyceride; VLDL = very low-density lipoprotein

The criteria of Light et al.\(^1\) have been used for the differentiation of transudative and exudative pleural effusions for the past 3 decades. These criteria use the ratio of pleural fluid (PF) to serum (S) protein and lactic acid dehydrogenase, and the absolute level of the lactic acid dehydrogenase in the PF to make this differentiation. Since those criteria originally were proposed, other tests for the separation of transudates and exudates have been proposed. One proposed test is the level of total cholesterol (CHOL) in the PF.\(^2\)–\(^4\)

In order to assess properly the role of PF cholesterol measurements in separating transudates from exudates, a better understanding of the relationship between the CHOL level in S and that in PF would be useful. CHOL is not found free in blood but, rather, is linked to lipoproteins.\(^5\) The preponderance of S cholesterol (90%) is carried in the low-density lipoproteins (LDLs) (52% of LDL weight is due to CHOL) and high-density lipoproteins (HDLs) [19% of HDL weight is due to CHOL].\(^3\) It is
supposed that PF cholesterol also is related to LDLs and HDLs. Previous studies analyzing the relationship between S and PF LDLs have presented conflicting results. Rerabek\(^6\) reported that in patients with parapneumonic effusions, the PF LDL level ranged from 11 to 32% of the S LDL level. In contrast, Pfalzer and coworkers\(^7\) reported that transudative PFs had low levels of LDL cholesterol (mean, 16% of simultaneous S values); while exudative PF had high levels of LDL cholesterol (mean, 68% of simultaneous S values).

The purpose of the present study was to analyze the relationships among CHOL, LDL cholesterol, and HDL cholesterol in the S and PF. It was hypothesized that the PF cholesterol would be related to the S cholesterol and that the PF cholesterol also would tend to be higher if the permeability of the pleura (as reflected by the ratio of PF protein to S protein) was higher.

### MATERIALS AND METHODS

PF from 99 patients, including 13 transudates and 86 exudates, was analyzed. The separation of transudates and exudates was made using the criteria of Light et al\(^8\) and the clinical picture. All patients gave informed consent for participation in the study, which was approved by the Ethics Committee of the Heart Institute (InCor) at the University of São Paulo Medical School. At the time that the thoracentesis was performed, a PF sample and an S sample were collected. For each patient, the levels of CHOL, HDL cholesterol, LDL cholesterol, apolipoprotein AI (ApoA), apolipoprotein B (ApoB), triglyceride (TRIG), and total protein (PROT) were measured both in S and in the PF. A different aliquot of the PF or the S was used for each measurement.

The CHOL level was measured using an enzymatic, colorimetric method (Cobas Integra system; Roche Diagnostics; Indianapolis, IN). With this method, all cholesterol is freed from the lipoproteins and is subsequently oxidized. When the oxidized CHOL is formed, H\(_2\)O\(_2\) is produced, which in turn reacts with the reagent 4-chlorophenol and 4-aminoantipyrine to form a red compound. The increase in absorbance at 520 nm is proportional to the cholesterol level.

The HDL level was measured by the enzymatic, colorimetric method without sample pretreatment (Cobas Integra system; Roche Diagnostics). In this measurement, the first step is to add polyanion solutions that link with LDLs, very low-density lipoproteins (VLDLs), and chylomicrons, but not with HDLs. These lipoprotein-polyanion complexes are resistant to the actions of detergents and enzymes. The second step is the addition of a detergent that liberates CHOL from the HDL but not from the other lipoproteins. The freed cholesterol is then measured by the same methods as CHOL. The sensitivity of this technique is 4.1 \times 10^{-3} \text{mg/dL}. The test range is within 0 and 155 mg/dL.\(^9\)

The LDL cholesterol was measured with an automated clinical chemistry analyzer (Boehringer Mannheim Systems; Mannheim, Germany). With this method, a specific sugar (eg, α-cyclodextrin sulfate and dextran sulfate) is added to the solution that renders the VLDLs and the chylomicrons resistant to degradation by the enzymes. Then a reagent that contains LDL-specific enzymes (ie, enzymes that have a small reactivity with HDL) is added to break down the LDL, and subsequently the CHOL is measured. This oxidation forms H\(_2\)O\(_2\), which reacts with the reagent, forming a purple solution. The increase in absorbance at 585 nm is proportional to the CHOL level. The lowest detectable level is 3 mg/dL of CHOL. The test range is between 3 and 550 mg/dL.

ApoA and ApoB were measured using a nephelometer (Behringwerke AG; Frankfurt, Germany), which measured the difference in the scattered light coming through the samples after pretreatment with specific antibodies against ApoA and ApoB. When antibody against ApoA or ApoB is added to a sample, it reacts with the specific apolipoprotein forming immune complexes, producing turbidity which is proportional to the apolipoprotein concentration. Different samples are used for the measurement of ApoA and ApoB. The lowest detectable level by this technique is from 20 mg/dL for ApoA and 27 mg/dL for ApoB. In the present study, a value equal to three fourths of the lowest detectable value was assigned when there was no detectable apolipoprotein in the sample.

TRIG was measured using an enzymatic, colorimetric method (Cobas Integra system; Roche Diagnostics). In this method, TRIG is hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol then is phosphorylated and undergoes oxidation that liberates H\(_2\)O\(_2\), which reacts with 4-chlorophenol and 4-aminonitripyrine to form a red compound. The increase in absorbance at 520 nm is proportional to the TRIG level.

### Statistical Analysis

Results were expressed as the mean ± SEM. The relationship between the PF and S levels of the various lipids measured was analyzed using linear regression, with the PF level as the dependent variable. To determine whether the permeability of the pleura, as reflected by the ratio of PF to protein, influenced the PF level of the lipids, multiple regression analysis was used, with the pleural lipid level used as the dependent variable and the S lipid level and the ratio of PF level to protein level used as independent variables. The data were analyzed via computer software (SigmaStat, version 2.03, and Sigma Plot, version 4.0; Jandel Scientific; San Rafael, CA). p < 0.05 was considered to be statistically significant.

### RESULTS

We studied a total of 99 patients. Thirteen of the patients had transudative pleural effusions with the following etiologies: congestive heart failure, 9 patients; cirrhosis with ascites, 2 patients; and renal failure, 2 patients. Eighty-six of the patients had exudative pleural effusions with the following etiologies: malignancy, 40 patients; tuberculosis, 28 patients; parapneumonic effusions, 6 patients; pancreatitis, 2 patients; superior vena cava syndrome, 1 patient; pulmonary embolism, 1 patient; postcoronary artery bypass graft surgery, 1 patient; and undetermined exudates, 7 patients. Patients with chylothoraces were excluded from the study.

The mean PF level for all the lipid measurements was lower than the mean S level (Table 1). The level of each lipid in the PF was significantly correlated with the level of the respective lipid in the S. However, there was not a close relationship between the level of a lipid in the PF and the level of the same...
lipid in the S, since none of the correlation coefficients exceeded 0.60. This indicates that < 35% of the variance in the PF lipid levels could be explained by the relationship to the S lipid levels.

Since there was not a close correlation between the PF and S lipid levels, an attempt was made to take into consideration the permeability of the pleural capillaries. The ratio of the PF protein level to the S protein level was used as a measure of this permeability. When the relationship between the lipid ratios and the protein ratios was analyzed, it was found that the mean lipid ratio was lower than the mean protein ratio for each of the lipids (Fig 1). This suggests that the movement of lipid from the S into the pleural space is more restrained than is the movement of protein from the S into the pleural space, or that the lipids are metabolized once they are in the pleural space.

A reasonable hypothesis is that the PF level of a lipid is influenced by the permeability of the capillaries as well as by the S level of that lipid. To evaluate this possibility, multiple regression analysis was performed with the PF lipid level as the dependent variable and the S lipid level and the PF/S protein ratio as the independent variables (Table 2). In general, the addition of a measure of permeability (ie, the PF/S protein ratio) led to a significant reduction in the variance. When the patients were considered as a group, the reduction in variance was significant at the p < 0.001 level with both the S lipid level and PF/S protein ratio for each of the six different lipid measurements. The only exception was the analysis for the LDL, where the reduction in variance due to the S lipid level reached a significance level of only 0.069.

When the S cholesterol and the PF/S protein ratio were used to predict the PF cholesterol level (Fig 2), there was a good correlation (r = 0.88; standard error of the estimate, 15.0 mg/dL). This observation suggests that the level of cholesterol in the pleural space is primarily dependent on the passive movement of cholesterol from the S into the PF.

The major portion of cholesterol in the S is associated with LDL. In our population, the mean S LDL cholesterol level was 125 mg/dL, while the mean total S cholesterol level was 171 mg/dL, indicating that 73% of the S cholesterol was LDL. If cholesterol moved passively into the PF and re-

### Table 1—Results of Linear Regression Analysis of the Relationship Between the Levels of Lipids in the PF and Their Corresponding S Levels

<table>
<thead>
<tr>
<th>Measurement Type</th>
<th>Measurements, No.</th>
<th>S, mg/dL</th>
<th>PF, mg/dL</th>
<th>r Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>99</td>
<td>171 ± 3.8</td>
<td>73 ± 3.2</td>
<td>0.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>97</td>
<td>125 ± 3.9</td>
<td>27 ± 1.9</td>
<td>0.39</td>
<td>0.003</td>
</tr>
<tr>
<td>ApoB</td>
<td>92</td>
<td>121 ± 3.3</td>
<td>43 ± 1.9</td>
<td>0.43</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>98</td>
<td>38 ± 1.5</td>
<td>13 ± 0.7</td>
<td>0.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoA</td>
<td>93</td>
<td>105 ± 4.1</td>
<td>37 ± 1.9</td>
<td>0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TRIG</td>
<td>99</td>
<td>128 ± 6.2</td>
<td>33 ± 1.8</td>
<td>0.26</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM, unless otherwise indicated.
†p < 0.001 compared with S.

**Figure 1.** Comparison of the PROT PF/S ratio and the lipid PF/S ratio in all patients (mean ± SEM).
mained unchanged once in the PF, it would be expected that the ratio of the PF LDL to S LDLS and the ratio of the PF cholesterol to S cholesterol would be tightly correlated. But our data reveal that the ratio of the PF LDL to the S LDL (0.22) is significantly (p < 0.001) lower than the ratio of the PF to S cholesterol (0.43) (Fig 1). Since ApoA is the protein associated with LDL, one would anticipate that the mean ApoA PF/S ratio would be similar to the LDL PF/S ratio. However, the ApoA PF/S ratio (0.37) was significantly (p < 0.001) higher than the LDL PF/S ratio (Fig 1). These findings suggest that once the LDL enters the pleural space, it is modified such that less CHOL is associated with each molecule of ApoA. Another piece of evidence supporting a change in the lipoproteins once they enter the pleural space is the observation that 93% of the cholesterol in the S is associated with either LDL or HDL, while only 56% of the cholesterol in the PF is associated with LDL and HDL.

Figure 2. Comparison of the observed PF cholesterol level and the cholesterol level predicted from the level of S cholesterol and the PF/S protein ratio.

<table>
<thead>
<tr>
<th>Measurement Type</th>
<th>Measurements, No.</th>
<th>r Value</th>
<th>S Lipid Level</th>
<th>PF/S PROT Ratio</th>
<th>Overall p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>99</td>
<td>0.88</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>97</td>
<td>0.72</td>
<td>0.069</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>91</td>
<td>0.70</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>98</td>
<td>0.70</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ApoA</td>
<td>92</td>
<td>0.73</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TRIG</td>
<td>99</td>
<td>0.49</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
In contrast, when the S TRIG and the ratio of the PF to S protein were used to predict the PF TRIG level (Fig 3), the correlation was much poorer ($r = 0.49$).

**DISCUSSION**

The present study demonstrates that cholesterol levels in PF are related to the S cholesterol levels and the permeability of the pleura, as reflected by the ratio of the PF to the S protein. However, the percentage of CHOL that was associated with LDL and HDL in the PF was much lower than that associated with the LDL and HDL in the S. These findings suggest that the lipoproteins were modified once they entered the pleural space. The PF TRIG level was not closely related to the S TRIG level or to the permeability of the pleura.

In the S, lipids are not free but, rather, are linked to lipoproteins. Lipoproteins are spherical molecules composed of apolipoproteins, CHOL, TRIGs, and phospholipids. The apolipoprotein subtype and the proportion of CHOL, TRIGs, and phospholipids are different for each type of lipoprotein. The known lipoproteins are chylomicron, VLDL, LDL, HDL, and intermediate-density lipoprotein. Approximately 90% of the CHOL in the blood is associated with LDL and HDL. LDL is synthesized in the vessels, while HDL is synthesized in both the liver and the vessels. Once the lipoproteins are in blood, they can be captured by specific receptors that are present on the cell membranes. The density of the receptors is greater when cells need cholesterol than when they do not. The lipoproteins also are removed by the liver and are secreted through the bile into the intestine. Cells use the constituents of the lipoproteins to help construct their membranes and to produce hormones, and for intracellular regenerating processes. In summary, these lipids have an important role in cellular metabolism. But, the importance of the lipids in pleural physiology and in pleural disease remains to be determined.

It was hypothesized that the lipid level in the PF would be dependent on the lipid level in the S. Since the PF cholesterol levels are less than the S cholesterol levels, it was also hypothesized that capillaries in the pleura would restrain the movement of larger molecules more than they would restrain the move-
ment of smaller molecules. Indeed, the PF cholesterol level was significantly (p < 0.001) related to the S level. However, the correlation coefficient was only 0.41, which indicates that < 20% of the variance in the PF cholesterol level could be explained by its dependence on the S cholesterol levels. Similar relationships were found for the other lipids. Moreover, the PF lipid level was always lower than the S lipid level. This observation suggested that other factors, such as the permeability of the pleural capillaries, might be influencing the PF levels of each lipid.

Since the level of lipid in the PF was not closely correlated with the S level, it was hypothesized that the level of lipid in the PF also would be related to the permeability of the pleura. The ratio of the PF to the S protein level is thought to be a good measure of the permeability of the pleural capillaries since protein is probably not metabolized in the pleural space and protein is not thought to be concentrated in the pleural space. The ratio of the PF to the S level for all lipids was significantly lower than the ratio of the PF to the S protein level (Fig 1). This indicates that the pleura is probably less permeable to the different lipids than it is to protein. This is not surprising since the lipid molecules have a higher molecular weight. Another possibility is that the lipids could be metabolized in the pleural space, as suggested by Pfalzer et al.

When regression analysis was used with the PF cholesterol level as the dependent variable, the addition of the PF/S protein ratio as an independent variable resulted in a significant reduction in the variance and an increase in the correlation coefficient from 0.41 to 0.89. This relatively high correlation coefficient suggests that the PF cholesterol level is dependent almost completely on the S cholesterol level and the permeability of the pleura.

When the same analysis was used to analyze the relationship between the levels of LDL cholesterol, HDL cholesterol, ApoA, and TRIG in S and PF, the addition of the PF/S protein ratio resulted in higher correlation coefficients (Table 2), but none of the correlation coefficients were nearly as high as that for CHOL alone but was similar to that for LDL cholesterol (0.72). This is compatible to the suggestion of Pfalzer et al that the HDL was modified in PF. However, the percentage of the cholesterol in the S (22%) and in the PF (17%) that was associated with HDL was similar. This suggests that HDLs were modified to a lesser degree than the LDLs after they entered the pleural space.

The PF TRIG level was not closely related to either its S level or the PF/S protein ratio. This result has three possible explanations. First, the pleural permeability might be less for TRIG than it is for the other lipids. This possibility is supported by the observation that the ratio of the PF to S lipid level is lower for TRIGs than it is for any other lipid. A possible explanation for this observation is that TRIG is carried mainly by chylomicrons with a diameter of 80 to 1,200 nm and by VLDL with a diameter of 30 to 80 nm. In contrast, the diameter of an LDL is 18 to 25 nm and the diameter of an HDL is 5 to 12 nm. Second, the TRIGs in the pleural space could be modified by the inflammatory process, through the delivery of free radicals, or by the interaction between TRIGs and molecules that contain iron in their structure. This interaction could transform the TRIGs into fatty acids, which would not be detected by our methodology.

Third, the level of TRIGs in the S fluctuates much more than that of other lipids, and, therefore, at a single time point, assuming that there is a slow equilibration between the S and the PF, there would not be a close relationship between PF and S TRIG levels. Our patients were not necessarily fasting when the S and PF samples were obtained. Obviously, all of these factors could be operative to a greater or lesser extent. The present study was not designed to identify which of the above factors was predominantly responsible for the above observations.

There are several clinical implications from the present study. First, since the PF cholesterol level can be predicted from the S cholesterol level and
from the PF/S protein ratio, measurements of PF cholesterol probably add little to measurements of PF protein in the differentiation of pleural transudates and exudates. Second, since there is such a weak relationship between the S and PF TRIG levels, one need not take into consideration the S TRIG levels when assessing whether or not a patient has a chylothorax.

In conclusion, the present study demonstrates that the level of cholesterol in the PF can be closely predicted from the S cholesterol level and from the PF/S protein ratio. A much lower fraction of the cholesterol in the PF than in the S is associated with LDL. This suggests that the LDLs undergo metabolic alterations once they enter the pleural space. The PF TRIG levels are not closely related to either the S TRIG level or to the ratio of or the permeability of the pleura to protein.

REFERENCES