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DIAGNOSTIC UTILITY OF CHOLESTEROL IN DIFFERENTIATION OF PLEURAL EXUDATES FROM TRANSUDATES

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Summary. *Evaluation of the utility of measurement of pleural fluid cholesterol (CHOLpf and CHOLpf/s) in differentiation of exudates (Ex) from transudates (Tr), was the aim of our study.*
We analysed 200 patients with pleural effusions (PE) of known etiology, and the results were compared with the criteria of Light et al. According to their etiology 44 (22.2%) of PE were Tr and 156 (78.8%) were Ex. Receiver operating characteristics (ROC) curve, were used to determine the best cut-off values for CHOLpf and CHOLpf/s.
All analysed cut-off values of CHOLpf and of CHOLpf/s showed a significantly higher specificity (from 61.4% to 97.8%) in comparison to Light's criteria (56.8%) - ($p<0.01$). Using of 60 mg/dl cut-off value of CHOLpf and 0.40 of CHOLpf/s, we reached the highest specificity (79.5% and 97.8% respectively). The sensitivity and efficiency of all cut-off values of cholesterol were almost the same as in Light's criteria ($p>0.01$).
Light's criteria remain the best biochemical criteria for the classification of transudates and exudates. The high level of sensitivity, efficiency and significantly higher specificity in comparison to Light's criteria, makes CHOLpf with cut-off value of 60 mg/dl and CHOLpf/s with cut-off value of 0.40 to be used as alternative parameter in differentiation of transudates from exudates.

Key words: *Cholesterol, pleural effusions, transudates, exudates*

Introduction

If the pleural fluid (pf) is clinically and radiologically demonstrated, a clinician should consider performing a diagnostic thoracentesis. The first question to ask in assessing a patient with a PE, after diagnostic thoracentesis, is whether that effusion is a Tr or an Ex, which is without doubt of fundamental clinical significance (1-3). The criteria presented by Light et al. for an Ex are fully accepted even after more than 30 years of practical use. These are based on the determination of pleural/serum ratio of protein and lactate dehydrogenase (LDH) (4,5).

In recent years, other researchers have proposed a measurement of pleural fluid cholesterol (CHOLpf) as a biochemical parameter for the separation of Tr from Ex (6-8). Cholesterol can be produced by a mesothelial cell of pleural membrane during some inflamatory process on the pleura, so the CHOLpf increased in Ex PE, and in the cases of PE caused by pulmonary tuberculosis and reumatoid arthritis, its concentration can be extremely high (more than 1000 mg/dl) (9).

Hamm et al (10) showed that CHOLpf appears to be a more reliable parameter for the differentiation of Tr and Ex than protein and LDH. They found that protein and LDH levels, as well as their pleural fluid-to-serum ratios, resulted in erroneous classification of 11-15% of the effusions. Using pleural cholesterol with a cut-off value of 60 mg/dl to separate exudates from transudates, only 5% were incorrectly classified.

The present study was undertaken to evaluate the diagnostic utility of CHOLpf and pleural fluid to serum/cholesterol ratio (CHOLpf/s) in the separation of exudates from transudates.

Subjects and methods

Considering the etiology of effusion as the golden standard for the classification of pleural fluid, we studied 200 patients who had undergone a thoracentesis at the Department for Pleural Diseases, and who met the following conditions: 1) a confirmed diagnosis of a disease which, when causing a pleural effusion, is invariably associated with an exudate or with a transudate; 2) absence of other diseases capable of causing a pleural effusion or of modifying its nature; 3) measurement of pleural cholesterol concentration and indicators of Light et al in the same pleural effusion sample and blood for protein and LDH determination obtained within 8 h of the thoracentesis.

The diagnosis of the diseases causing the effusion was considered to be confirmed when the following conditions had been met (11,12):

congestive heart failure: presence of an enlarged heart with a clinical or echosonographic evidence of cardiac dysfuntion, and one or more of the following alterations: elevated venous pressure, oedema, tachycardia, or ventricular gallop;

liver cirrhosis: clinical and laboratory evidence of hepatic damage with portal hypertension or hypoalbuminemia;

renal failure: irreversibile damage to renal functions which lead to uremia;

pleural malignancy: cytologic or histologic demonstration of pleural involvement; *paramalignant pleural effusions*: A PE occuring in patients with known malignancy but without cytologic or histologic demonstration of malignancy, and for whom there was no obvious alternative diagnosis;

parapneumonic effusion: clinically and radiologically confirmed pneumonia with no direct or indirect evidence of bacterial invasion of the effusion;

empyema: pneumonia with one or more of the following indicators of bacterial invasion of the effusion: pus cells, bacteria in Gram's stein smear or culture, and pH under 7.0 or progressively decreasing to under 7.20;

tuberculosis: presence of tuberculous granulomas in pleural biopsy specimen or positive smear or culture of acid-fast bacilli.

PE associated with congestive heart failure, liver cirrhosis, renal failure and lymphomas were classified as Tr, and effusions associated with lung cancer, pneumonia, empyema and tuberculosis, were classified as Ex.

The first sample of pleural fluid obtained in each patient was considered for analysis. Cholesterol was measured by the color-enzymatic method GOT-PAP. Protein was measured by the biuret method and LDH by cinetic UV method with LD pyruvats as a substratum. The Synchron CX-5 Backman apparatus was used for all measurements.

For the laboratory classification of pleural fluid, *protein* and *LDH* were interpreted according to the criteria of *Light et al*, and a cut-off point of 60; 50; 45 md/dl and 0.30; 0.35; 0.40 was adopted for *CHOLpf* and *CHOLpf/s* respectively.

The operating characteristics of the measured indicators and their different combinations were assesed by their sensitivity, specificity and efficiency with relation to the etiology-based classification of pleural fluids. Receiver-operating characteristics (ROC) curve were used to determine the best cut-off values of cholesterol. The statistical significance was estimated by the non-parametric Mann-Whitney *U* test (13).

Results

With regard to the causal disease, of the 200 pleural effusions analysed 44 (22.2%) of pleural fluid samples were labeled as transudates and 156 (77.8%) as exudates (Table 1).

The classification based on the application of the criteria of Light et al and cholesterol (CHOLpf and CHOLpf/s) is compared with the etiologic classification, considered a golden rule. When Light's criteria are used, it may be observed that only 1 of 156 exudates was misclassified as a transudate, and 25 of 44 transudates were erroneously labeled as exudates. The one misclassified exudate corresponds to empyema, and of the erroneously classified transudates twenty were secondary to congestive heart failure and five to liver cirrhosis. When CHOLpf and CHOLpf/s were used, we noted that the number of misclassified exudates had become higher depending on the increase of the cut-off points level (ranging from 5 to 12 erroneously classified exudates). Contrarily, the increase of the cut-off points level of both CHOLpf and CHOLpf/s significantly decreased the number of erroneously labeled transudates as exudates (using a 0.40 cut-off point of CHOLpf/s only 1 transudate was misclassified as exudate in the case of pleural effusion secondary to congestive heart failure). Misclassified exudates correspond to parapneumonic effusions and empyema, and secondary to lung cancer. All misclassified transudates were significantly higher secondary to congestive heart failure, and in some cases secondary to liver cirrhosis (Table 2).

Table 1. Etiologic causes of pleural effusions

Biochemical type	Etiologic causes	number (n)	percentage (%)
Transudates (n=44; 22.2%)	Congestive heart failure	27	61.4
	Liver cirrhosis	7	15.9
	Lymphoma	6	13.6
	Renal failure	4	9.1
Exudates (n=156; 77.8%)	Lung cancer	65	41.7
	Tuberculosis	57	36.5
	Pneumonia	23	14.7
	Empyema	11	7.1
Total		200	100.0

Table 2. Accuracy of criteria of Light et al, and cholesterol (CHOLpf and CHOLpf/s) in the classification of 200 pleural effusions

Criteria / Parameters		Exudates (156)*	Transudates (44)*
Light et al			
	correctly classified	155	19
	misclassified	1	25
CHOLpf 45 mg/dl			
	correctly classified	151	27
	misclassified	5	17
CHOLpf 50 mg/dl			
	correctly clssified	149	33
	misclassified	7	11
CHOLpf 60 mg/dl			
	correctly classified	145	35
	misclassified	11	9
CHOLpf/s 0.30			
	correctly classified	150	37
	misclassified	6	7
CHOLpf/s 0.35			
	correctly classified	146	41
	misclassified	10	3
CHOLpf/s 0.40			
	correctly classified	144	43
	misclassified	12	1

* *Etiologic classification*

An analysis of the diagnostic accuracy of CHOLpf in separation of Tr from Ex, when the three diferent cut-off values of CHOLpf (45; 50; 60 mg/dl) were used, showed that the highest sensitivity was obtained with a 45 mg/dl cut-off value, the highest specificity with a 60 mg/dl cut-off value, and the highest efficiency has the 50 mg/dl cut-off value of CHOLpf. Despite the highest sensitivity (99.4%) and high efficiency (90%) of Light's criteria, there were no significant differences in comparison with the sensitivity and efficiency of all cut-off values of CHOLp ($p>0.01$). However, CHOLpf showed a significantly higher specificity in comparison with that of Light's criteria ($p<0.01$) (Table 3).

Table 3. Diagnostic accuracy of CHOLpf in separation of Tr from Ex (cut-off values: 60, 50,45 mg/dl)

Parameters/Criteria	Sensitivity (%)	Specificity (%)	Efficiency (%)
CHOLpf 45 mg/dl	97.4	61.4 *	90.0
CHOLpf 50 mg/dl	95.5	75.0 *	89.0
CHOLpf 60 mg/dl	92.9	79.5 */ **	91.0
Criteria of Light et al	99.4	56.8	90.0

* $p<0.01$ in comparison with specificity of criteria of Light et al.
** $p<0.05$ in comparison with specificity of 45 mg/dl cut-off values of CHOLpf

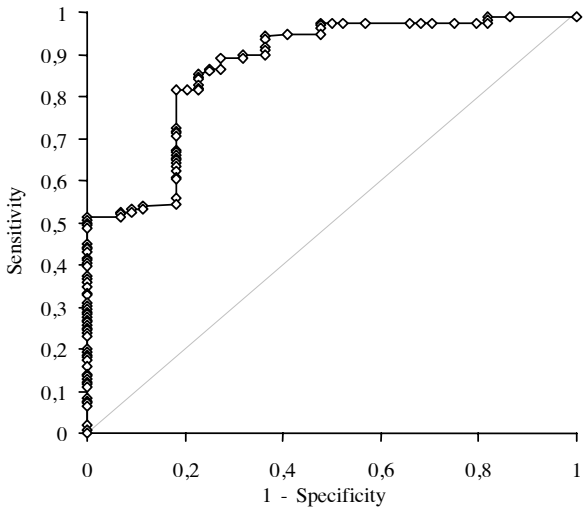
Using of the three different cut-off values of CHOLpf/s (0.30; 0.35 and 0.40), we noted that the highest sensitivity showed a 0.30 cut-off value and the highest specificity a 0.40 cut-off value of CHOLpf/s. Also, there is the same percentage of diagnostic efficiency of all cut-off values of CHOLp/s. Despite the highest sensitivity of Light's criteria (99.4%), there were no significant differences in comparison with the sensitivity of all cut-off values of CHOLp/s ($p>0.01$). However, CHOLpf showed a significantly higher specificity in comparison with the specificity of Light's criteria ($p<0.01$) - (Table 4).

Table 3. Diagnostic accuracy of CHOLpf /s in separation of Tr from Ex (cut-off values: 0.60; 0.35; 0.40)

Parameters/Criteria	Sensitivity (%)	Specificity (%)	Efficiency (%)
CHOLpf /s 0.30	96.1	84.0 *	93.5
CHOLpf/s 0.35	93.6	93.2 *	93.5
CHOLpf/s 0.40	92.3	97.8 */ **	93.5
Criteria of Light et al	99.4	56.8	90.0

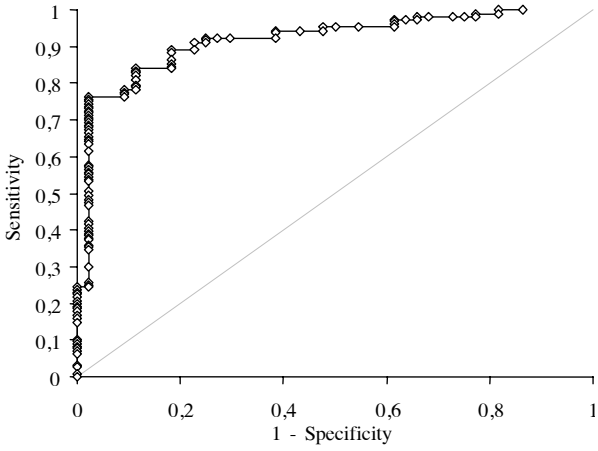
* $p<0.01$ in comparison with specificity of criteria of Light et al.
** $p<0.05$ in comparison with specificity of 0.30 cut-off values of CHOLpf/s

An estimate of ROC curve and AUC value, the most precise analysis of diagnostic accuracy, showed that both CHOLpf and CHOLpf/s had high AUC values (0,873 and 0,918 respectively), which gives them a diagnostic significance as biochemical parameters in the separation of pleural Ex from Tr (Figure 1 and 2).



Curve	Area	SE	p	95% CI of Area
CHOLpf	0. 873	0.0294	<0.0001	0.815 to 0.930

Fig. 1. ROC curve and AUC value of CHOLpf



Curve	Area	SE	p	95% CI of Area
CHOLpf/s	0. 918	0.0227	<0.0001	0.873 to 0.963

Fig. 2. ROC curve and AUC value of CHOLpf/s

Discussion

Similary to the data from the literature of recent years, the results obtained from our study have shown that the criteria of Light's et al, when used in the separation of pleural exudates from transudates, misclassified an important number of effusions (14-16).

Our results showed that the pleural fluid in a significantly high percentage of our patients with congestive heart failure were classified as exudates by Light's criteria. This misleading effect of Light's criteria is explained by the fact that the treatment of heart failure may change the chemistry of pleural fluid by withdrawing water and, thus, concentrating proteins. If this is so, the interpretation of protein ratio in heart failure would depend on the previous treatment, which is a variable that is difficult to standardize. This would mean that this indicator is not suitable in patients with heart

failure and, probaly, in those with liver cirrhosis in whom diuretics have been used (17).

Roth et al (18) showed that this limitation of the criteria of Light et al could be overcome by measuring the serum-effusion albumin gradient which, when over 1.2 mg/dl, indicated a transudate.

Similary to literature data (19-23), in comparison to Light's criteria, our results showed a high, almost same sensitivity and efficiency of all the analysed cut-off values of CHOLpf and CHOLpf/s. Also, there was a significantly higher specificity ($p<0.01$) of CHOLpf and CHOLpf/s in comparison to Light's criteria. When we used 60 mg/dl as a cut-off value of CHOLpf and 0.40 as a cut-off value of CHOL pf/s, the specificity obtained was the highest. In the correlation with our results in a large study of 232 patients (n = 48 for transudates), Gil Suay et al (6) demonstrated a sensitivity of 95,5% and a specificity of 91,6% for pleural cholesterol at a cut-off concentration of 54 md/dl, whereas the criteria presented by Light et al. achieved a sensitivity of 100%, but a disappointing specificity of only 64.5%. Another Spanish working group published the data obtained on 253 patients (n = 65 for transudates) and demontrated that with a treshold of 55 mg/dl for pleural cholesterol, the sensitivity was 91% at a specificity of even 100% (24). In accordance with the results of their colleagues from Valencia, the criteria presented by Light et al. achieved an acceptable sensitivity of 92.5%, but again a low specificity of 78.4%.

However, the factors infuencing pleural fluid cholesterol level have not been clearly delineated.

In their study, Marcelo A.C. Vaz et al (7) demonstrated that CHOL levels in pleural fluid are related to the serum cholesterol level and to the permeability of the pleura, as reflected by the ratio of the pleural fluid to the serum protein.

Since CHOL is not found free in the blood, it probably enters the pleural space linked to lipoproteins. The percentage of CHOL asociated with LDL (low-density lipoprotein) and HDL (high-density lipoprotein) in the pleural fluid was much lower than that associated with LDL and HDL in the serum. However, the observation that a much smaller fraction of CHOL in the pleural space is LDL cholesterol suggested that these lipoproteins are changed after thay enter the pleural space (HDL was modified to a lesser degree than the LDLs). Approximately 90% of the CHOL in the blood is associeted with LDL and HDL, which have an important role in cellular metabolism. But, the importance of the lipids in pleural physiology and in pleural diseases remains to be determined.

As mentioned above, the CHOLpf level was significantly related to the serum level. Moreover, the pleural fluid lipid level was always lower than the serum lipid level. This observation suggested that other factors, such as permeability of the pleural capillaries, might be influencing the pleural fluid level of each lipid. The ratio of the pleural fluid and serum level for all lipids was significantly lower than the ratio of pleural fluid to serum protein level. This indicates that the pleura is probably less permeable to the different lipids than it is to protein, because lipid molecules have a higher molecular weight.

So, the autors concluded that it is unlikely that pleural fluid cholesterol measurement would provide additional information on the ratio of the pleural fluid to serum protein because the pleural fluid cholesterol level can be accurately predicted from the serum cholesterol and the ratio of the pleural fluid to serum protein level.

On the basis of a significantly high sensitivity and specificity of both CHOLpf and CHOLpf/s, the practial use of its tests estimated throught ROC analysis, which is generally represented the relationships of sensitivity and specificity. The AUC, determined by ROC analysis, is the most accurate technique for comparing the discriminative properties of diagnostic tests (25). The AUC values obtained for cholesterol (0.873 for CHOLpf and 0.918 for CHOLpf/s) allow a practical (diagnostic) application of cholesterol for separation of transudates from exudates.

It conclusion, our study has shown that Light's criteria remain the best biochemical means by which pleural effusions can be clasified as transudates or exudates. The primary problem with Light' criteria is that they label some patients with transudative pleural effusions as having exudative pleural effusions. Because of high levels of sensitivity, specificity and efficiency too, pleural fluid cholesterol level with cut-off value of 60 mg/dl and pleural fluid to serum cholesterol ratio with cut-off value of 0.40, can be used as an alternative parameter for separation of transudates from exudates.

The accuracy in separation of transudates from exudates has a great significance, as it allows a proper choice of diagnostic procedures in diagnosing pleural effusions.

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DIJAGNOSTIČKI ZNAČAJ HOLESTEROLA PRI DIFERENTOVANJU PLEURALNIH EKSUDATA OD TRANSUDATA

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Kratak sadržaj: *Cilj ove studije bila je evaluacija značaja merenja koncentracije holesterola u pleuralnoj tečnosti (HOLp i HOLp/s)u cilju diferentovanja eksudata (E) od transudata (T).*
Analizirano je 200 pacijenata sa pleuralnim izlivom (PI) poznate etiologije a dobijeni rezultati komparirani su sa Light-ovim kriterijumima. Na osnovu etiološkog uzročnika diferentovano je 44 (22,2%) T i 156 (78,8%) E. Receiver-operating-characteristic (ROC) kriva korišćena je za odredjivanje najtačnijih graničnih vrednosti HOLp i HOLp/s. Sve granične vrednosti HOLp i HOLp/s pokazale su signifikantno veću specifičnost (od 61,4% do 97,8%) u poredjenju sa Light-ovim kriterijumima (56,8%)-(p<0,01). Granične vrednosti HOLp od 60 mg/dl i HOLp/s od 0,40 pokazale su najveću specifičnost (79,5% odnosno 97,8%). Senzitivnost i efikasnost svih graničnih vrednosti holesterola, gotovo su iste sa Light-ovim kriterijumima (p>0,01).
Light-ovi kriterijumi ostaju i dalje najbolji biohemijski kriterijumi za klasifikovanje transudata od eksudata. Visoka senzitivnost i efikasnost, kao i signifikantno veća specifičnost HOLp sa graničnom vrednosti od 60 mg/dl i HOLp/s sa graničnom vrednosti od 0,40 u odnosu na Ligh-ove kriterijume, omogućuje primenu holesterola kao alternativnog parametra pri diferentovanju eksudata od transudata.

Ključne reči: *Holesterol, pleuralni izliv, transudati, eksudati*