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ESTIMATION OF PLEURAL EFFUSION TYPE BY LEUKOCYTE FUNCTIONAL STATE

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ABSTRACT

PURPOSE was distinguishing between transudates and exudates on the basis of analysis neutrophils (PMN) activity by means of sensitive chemiluminescent methods**. METHODS** The chemiluminescent method was specially designed for assessment PMN activity in pleural fluids and blood. The procedure has in mind a small number of PMN in transudative effusions or turbidity in exudative effusions. On the basis of two approaches describing PMN with different functional state can be distinguished exudates from transudate. **RESULTS** The analysis based on the parameters of the luminol-amplified chemiluminescent (LCL) kinetics of stimulated PMNL was introduced chemiluminescent index - a maximum intensity of one PMN in pleural effusion to a maximum intensity of one PMN in the blood. At the chosen cut-off value the sensitivity and specificity was 87.5 % and 100 %, respectively. The analysis based on the model components of LCL generated by PMN showed the best sensitivity and specificity. In practice, the criterion used to separate completely separates the two types of effusions. **CONCLUSIONS** Described new chemiluminescent method will confirm with greater accuracy the differentiation of exudates from transudates in effusions of different aetiologies.

Key words: chemiluminescence, polymorphonuclear leukocyte, pleural effusion, transudate, exudate

INTRODUCTION

Distinguishing between transudates and exudates often presents a diagnostic dilemma because pleural effusion is not pathognomic. Frequently it is used Light's criteria (1), but the main problem is improper identifying some transudative effusions. Other workers have also demonstrated the utility of measuring the pleural fluid NT-pro-BNP for diagnosing pleural effusions due to congestive heart failure (2). Heffner and his co-workers have derived multilevel (3) and continuous (4) likelihood rations for the usual biochemical tests used to differentiate transudates and exudates. Post-test probabilities can be derived (4) when these likelihood ratios are used in conjunction with pretest probabilities using Bayes' theorem. Difficulties in using this approach occur because the pretest probabilities vary significantly from physician to physician. Nowadays continues finding of reliable and cheaper testes to discriminate transudates from exudates.

Pleural effusion has a different composition by comparison with blood (5) and then the pleural PMN toward the blood PMN probably will be in different functional state. Additionally different aetiologies of effusion follow to different PMN subpopulation and different functional properties. One of the properties that reflect different PMN state is activated oxygen species (AOS) production activity. The oxidative metabolites production can be evaluated by means of chemiluminescent methods and can be used to determine the transudative or exudative nature of pleural fluid.

PURPOSE was distinguishing between transudates and exudates on the basis of analysis PMN activity by means of sensitive chemiluminescent method. It was tested the LCL method specially designed for PMN in pleural fluids.

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METHODS

1. The chemiluminescent method was specially designed for assessment PMN activity in pleural fluids and blood. The procedure has in mind a small number of PMN in transudative effusions or turbidity in exudative effusions.

The tested samples contained luminol with final concentration10-5 mol/l, zymosan 0.3 mg/ml, diluted blood or pleural effusion and Krebs-Ringer phosphate medium (KRP). All components of the tested sample except blood (or pleural liquid) were preliminary thermostated in the luminometer's cuvettes at 37oC for 5 minutes. After addition of blood (or pleural liquid) the content of the cuvettes was mixed. LCL kinetics of three blood and three pleural samples was simultaneously recorded by a six-sample multiplexing luminometer operating in photon counting mode (6). As a result 2 groups of 3 LCL curves were acquired - for the investigated blood and pleural samples, respectively.

1.1 Analysis based on the parameters of the LCL kinetics of stimulated PMNL.

The obtained from each sample LCL kinetic was corrected for the background intensity levels of apparatus and cuvettes. Data were approximated using cubic spline.

For each kinetic curve several LCL parameters were calculated - Imax, T I max, T ½ I max and Twidth **(Figure 1).**

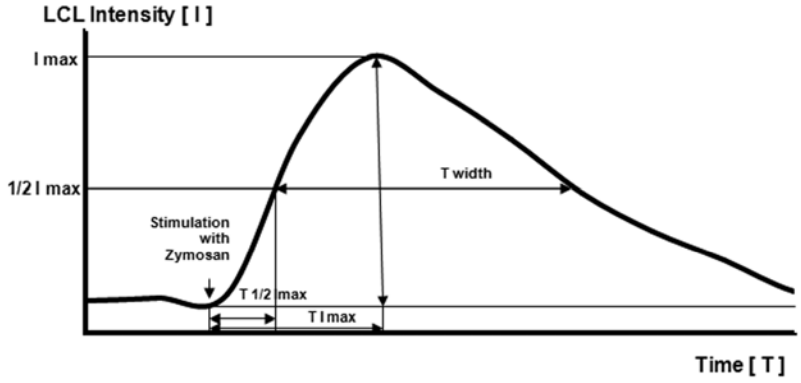

Figure 1. In CD -file name - Fig1_estimation of pleural effusion type by leukocyte functional

Having in mind the sample dilution and the number of PMN and erythrocytes, corrections of LCL kinetics in respect to PMNL and erythrocytes count were applied (7).

These parameters from LCL response from zymosan stimulated PMN in pleural liquid were compared with those of the response from PMN in blood for one and the same patient.

1.2 Analysis based on the model components of LCL generated by PMN

Because of the kinetics of LCL of stimulated PMN possesses a time-probabilistic nature, each obtained kinetic LCL curve was presented as a sum of three components - Poisson-type statistical distribution **(Figure 2).**

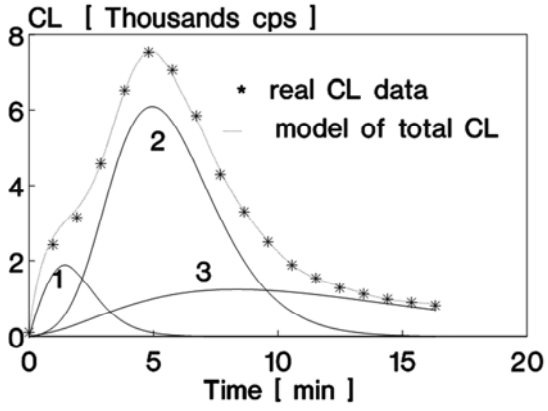

Figure 2. In CD -file name - fig2_estimation of pleural effusion type by leukocyte functional state.jpg

The values of the component parameters λi, mi, di and Ni (equation 1) were calculated using the iteration procedure to get the minimum sum of the squared differences between the real and the model LCL intensity (8).

$$I_{tot}(t) = \sum_{i=1}^{n} N_i . \lambda_i . m_i . \frac{(\lambda_i t)^{m_i}}{m_i !} e^{-\lambda_i t} \quad (1)$$

2. Determining type of pleural effusion according Light's criteria (1). Light's criteria state that a pleural effusion is an exudate if one or more of the following criteria are met: 1) The ratio of the pleural fluid to the serum protein exceeds 0.50; 2) the ratio of the pleural fluid lactic acid dehydrogenase (LDH) to the serum LDH exceeds 0.6 or 3) the absolute value of the pleural fluid exceeds two thirds the upper normal limit for serum (> 133 U/l).

RESULTS AND DISCUSSION

The LCL method specially designed for evaluation PMN activity in pleural fluids was used. It was tested for investigation the activity of PMNL in pleural effusion with different aetiology. On the basis of two approaches describing PMN with different functional state can be distinguished exudates from transudate. Furthermore preliminary results with the new methods showed better sensitivity and specificity compared with the standard criterion of Light (with not so good specificity).

The analysis based on the chemiluminescent parameters of LCL curves was introduced chemiluminescent index - a maximum intensity of one PMN in pleural effusion to a maximum intensity of one PMN in the blood (Imax pun / Imax bl) to differentiate between transudate of exudates. At the chosen cut-off value the sensitivity and specificity was 87.5 % and 100 %, respectively.

The analysis based on the model components of LCL generated by PMN showed the best sensitivity and specificity. In practice, the criterion used to separate completely separates the two types of effusions.

Table 1. *Brief title – Sensitivity and specificity of methods separating different types of effusion.*

Method	Sensitivity, %	Specificity, %
Light et al.	100	67
Chemiluminescent parameters of LCL curves	87,5	100
Model components of LCL generated by PMN	100	100

Tables 1 sum up the results of our research. In the table, take a gold standard expert assessment resulting from other clinical manifestations and clinical examinations. It is most likely to get better sensitivity and specificity using a chemiluminescent method. Apparently false positive exudates are less with the new test compared with the criteria of Light et all, which will increase the specificity of the method.

CONCLUSION

It is expected better sensitivity and specificity using a chemiluminescent method in separation of transudates and exudates, especially in comparison with the criteria of Light et all. Described new chemiluminescent method will confirm with greater accuracy the differentiation of exudates from transudates in effusions of different aetiologies.

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