International Journal of Advances in Medicine
Kalaisezhian N et al. Int J Adv Med. 2018 Aug;5(4):1041-1046
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: http://dx.doi.org/10.18203/2349-3933.ijam20183144

Original Research Article

Validity of leukocyte esterase reagent (LER) strip in diagnosing infectious pleural effusion

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Received: 31 May 2018
Accepted: 28 June 2018

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ABSTRACT

Background: Pleural fluid sampling and analysis are essential to confirm an infection. In a resource-limited health care setting or if biochemistries of the aspirated fluid are not available on an emergency basis, urine reagent strips applied to pleural fluid may expedite diagnostic information. Present study was aimed to evaluate the effectiveness of leukocyte esterase reagent (LER) strip in diagnosing infectious pleural effusion and to correlate the reaction of LER strip with various stages of infectious effusions.
Methods: A prospective longitudinal study was conducted in the medical ward of Government Mohan Kumaramangalam Medical College Hospital for a period of one year. Pleural effusion patients with associated immuno-compromised conditions like diabetes mellitus, patients on steroids, HIV reactive patients, chronic obstructive lung disease were included for the study. A totalof 84 patients were included for the study. Exploratory thoracocentesis was done with an 8 mm needle and pleural fluid was obtained and sent for cell count and biochemical testing. The pleural fluid was also tested using the leukocyte esterase reagent strip. The results were recorded as 0, 1+, 2+, 3+ based on the density of violet colour.
Results: The leukocyte esterase reagent strip test showed totally negative results in all patients with transudative pleural effusion and also in patients with tuberculous pleural effusion, whereas among patients with infectious pleural effusion other than tuberculosis LER strip test showed negative to only 15% of the patients and for remaining it ranged from 1+ to 3+. The validity of leukocyte esterase strip test was tested in comparison with the gold standard culture test. The accuracy of LER strip test was found to be 88.6%, and the sensitivity and specificity was 90.2% and 66.2% respectively.
Conclusions: Reagent strips may speed up the bedside diagnosis of infectious effusions. However, where access to standard biochemical pleural fluid analysis is not available this dipstick tests would add value to the management of the patients.

Keywords: Infectious effusion, Leukocyte esterase reagent strip, Pleural effusion, Validity

INTRODUCTION

Globally, pleural cavity infection occurrence rate has been constantly increasing for each age group with an unknown cause.[1-3] For example, from 1996 to 2008, admission rate has been increased two times for the patients suffering from empyema in America (3.04-5.98/100,000).[1] These changes are possibly related to the enhancement of clinical diagnosis awareness and the increasing number of available examination methods,

allowing physicians to better identify pleural cavity infection. Furthermore, this may also be related to the increasing age of the elderly year by year.

Pleural cavity infection is often secondary to pulmonary infection. Pleural effusion occurs in 15-44% of admitted patients suffering from pneumonia, in which 40% of patients are complicated with parapneumonic effusion or abscess.[4,5] For pneumonia treatment, exceeding non-steroidal anti-inflammatory drugs are applied at the early stage, which could easily cause pleural effusion.[6-8] The empyema in 50% of patients was deprived from pneumonic pleural effusion. The occurrence rate of thoracic cavity infection for males was two times than that for females. Furthermore, the incidence rate of diabetes, long-term excessive drinking, drug taking and rheumatoid arthritis for these patients were higher than that for the normal population. In addition, 2/3 of patients with chronic lung disease or immunodeficiency disease are complicated with parapneumonic effusion or empyema; and anaerobic pleural cavity infection occurred in patients with poor oral hygiene and those who accidentally inhaled the infection.[9] Other patients were secondary to operative wound and iatrogenic injury, while 1/3 patients failed to be influenced by high-risk factors. Moreover, the fatality rate of hospital-acquired pleural infection was higher than that of community-acquired pleural infection.[10,11]

Multiple proinflammatory factors would stimulate neutrophils for migration and fibrocytes for chemotaxis.[12] Furthermore, the endothelial permeability of blood vessels would be further improved. The bacteria enter the pleural cavity, and bacteria and bacterial degradation products can be detected in the effusion. Due to the phagocytosis of the bacterial metabolism and neutrophils, lactic acid would increase, pleural effusion pH and glucose would decrease, and lactic dehydrogenase would be elevated.

The terms pleural infection and parapneumonic effusions are used interchangeably, although one-fourth of pleural infection cases occur without a concurrent bacterial pneumonia. The typical patient with pleural bacterial infection presents with symptoms of pneumonia (i.e., fever, chest pain, dyspnea, cough) along with leukocytosis, raised serum C-reactive protein (CRP) levels, and a chest radiograph showing the effusion and radiological lung infiltrates. However, patients may have a more indolent presentation and several conditions (eg, tuberculosis, connective tissue diseases, pulmonary embolism, pancreatic diseases, or malignancy) can all mimic pleural bacterial infection.

Pleural fluid sampling and analysis are essential to confirm an infection.[13] In a resource-limited health care setting or if biochemistries of the aspirated fluid are not available on an emergency basis, urine reagent strips applied to pleural fluid may expedite diagnostic information. One study tested commercially available reagent strips for leukocyte esterase in the pleural fluid of 42 patients with bacterial infections, 15 with tuberculosis, and 71 with noninfectious causes.[14] A positive test yielded 42% sensitivity, 100% specificity, and an LR positive of 75 for the identification of bacterial infections in the pleural space.

As of today, most of the studies done to assess the efficacy of leukocyte esterase reagent (LER) strip for diagnosing infective etiology was mainly done on patients with spontaneous bacterial peritonitis only very few studies was conducted on pleural fluid. So, the present study was conducted to assess the efficacy of LER is diagnosing the infective cause among the patients with pleural effusion.

Present study was aimed to evaluate the effectiveness of leukocyte esterase reagent (LER) strip in diagnosing infectious pleural effusion and to correlate the reaction of LER strip with various stages of infectious effusions.

METHODS

A prospective longitudinal study was conducted in the medical ward of Government Mohan Kumaramangalam Medical College Hospital for a period of one year. The study was started after getting the clearance from the institutional ethical committee. Patients presented with pleural effusion based on clinical and radiological examination were included for the study. Pleural effusion patients with associated immuno-compromised conditions like diabetes mellitus, patients on steroids, HIV reactive patients, chronic obstructive lung disease were also included for the study. Patients who had recurrent pleural effusion and patients who had taken antibiotics in the recent past (within one month) were excluded from the study. Based on the above-mentioned inclusion and exclusion criteria the total subjects included in the study was 84. A written informed consent was obtained from all the patients involved in the study.

A detailed socio-demographic history along with clinical history was obtained from all the patients. A complete general physical examination was conducted on all patients. Pleural effusion was diagnosed clinically in these patients on the basis of reduced tactile/vocal fremitus, dull note on percussion and by absence of breath sounds on auscultation. Chest x-ray was obtained and the diagnosis was confirmed.

Exploratory thoracocentesis was done with an 8 mm needle and pleural fluid was obtained and sent for the following analysis. Pleural fluid was sent for cell count. Biochemical testing for pH, lactate dehydrogenase, protein and glucose was done. Microbiological studies for identification of pathogens in smear - Gramstain, AFB and cultures for bacteria, mycobacteria and fungi. Cytological studies for leukocyte, lymphocyte count and malignant cells were also done.

The cause of effusion was made out on basis of clinical findings and pleural fluid analysis results. It was interpreted on the basis of Lights criteria18.

The exudate was differentiated from a transudate when any one of the following was present:

- The ratio of pleural fluid to serum protein was greater than 0.5.
- The ratio of pleural fluid to serum LDH was greater than 0.6.
- Pleural fluid LDH was more than two thirds of upper limits of normal serum LDH.

If the patient had an exudative pleural effusion, then infectious etiology was made based on the pleural fluid description, glucose level, differential cell count, microbiological studies and cytology. The non tuberculous parapneumonic effusion was further classified into simple parapneumonic, complicated parapneumonic effusion and empyema.

The pleural fluid was tested using the leukocyte esterase reagent strip (UROCOLOR10 SD, Biostandard Diagnostics Pvt Ltd). The basis of this test is the ability of esterase enzyme which is present in polymorphonuclear leukocytes of the pleural fluid, to split the heterocyclic carboxylates and formation of a pyrrole. This in turn reacts with diazonium salt and produces a violet colour in the reagent strip. A drop of non-centrifuged pleural fluid that was collected in heparinised tubes was applied against the leukocyte label of the strip. Precisely after 2 minutes, the colour change in the strip was visually read against that provided in the container. The results were recorded as 0, 1+, 2+, 3+ based on the density of violet colour. A result of 0 was considered negative, less than 2 was considered less significant and greater than and equal to 2 were considered significant, strongly positive.

All data were entered and analysed using SPSS version 21. Mean and standard deviation was derived for all the parametric variables. Statistical inference was derived for two categorical variables by using Chi-square test. The validity of the leukocyte esterase strip was assessed by deriving sensitivity and specificity.

RESULTS

Table 1 shows the age and sex wise distribution of the study subjects. It is seen from the table that majority of the subjects were in the age group between 30 and 50 years with a mean age of 45.9 years. Males are comparatively higher in number than the females. The male: female ratio was 1.7: 1, but the distribution of age group between males and females was found to be almost similar. All our patients presented with symptoms such as cough, dyspnoea and chest pain and the diagnosis of pleural effusion was made by clinical examination such as percussion and auscultation and the final diagnosis was confirmed by doing a chest X-ray and the thoracocentesis. The pleural fluid was sent for complete biochemical analysis and for culture for identification of the organism. With the help of biochemical report we further classified the patients as exudative and transudative pleural effusion.

Table 1: Age and gender wise distribution of the study subjects.

Age group	Male	Female	Total	P value
20-30	2 (3.7%)	1 (3.2%)	3 (3.5%)	0.816
31-40	6 (11.3%)	4 (12.9%)	10 (11.9%)	
41-50	28 (52.8%)	17 (54.8%)	45 (53.5%)	
51-60	10 (18.8%)	7 (22.5%)	17 (20.2%)	
>60	7 (13.2%)	2 (6.4%)	9 (10.7%)	
Total	53 (100%)	31 (100%)	84 (100%)	
Mean±SD	46.8±7.1	44.5±6.8	45.9±6.5	

In present study subjects we found majority (72.5%) of the patients had exudative type of pleural effusion which is mainly of infective etiology and among them 20% had tuberculous etiology and the remaining had other infective organisms. Only 27.3% of the subjects had transudative type of pleural effusion (Table 2).

Table 2: Various type of pleural effusion detected in the study subjects.

Type of infectious agent	Frequency	Percentage
Streptococcus	14	31.8
Staphylococcus	12	27.2
Pseudomonas	8	18.1
Hemophilus influenza	4	9
E. coli	3	6.8
Klebsiella	3	6.8
Total	44	100

Table 3: Various types of infective agent identified among patients with infectious pleural effusion.

Type of pleural effusion	Frequency	Percentage
Non-infectious pleural effusion (transduate)	23	27.3
Infectious pleural effusion	44	52.3
Tuberculous pleural effusion	17	20.2
Total	84	100

All the infective exudative pleural effusion patients pleural fluid was sent for culture for identifying the organism in which majority (59%) of the patients had

gram positive cocci which is either streptococcus or staphylococcus followed by pseudomonas (18%). Gram negative bacilli such as E. coli and Klebsiella were seen in 14% of the patients (Table 3).

The biochemical report of the pleural fluid analysis shows that mean pleural fluid leukocyte count was maximum in infectious pleural effusion followed by tuberculous pleural effusion and it was very minimum among patients with transudative pleural effusion which is a non-infective etiology and a similar type of results was also seen with mean pleural fluid neutrophil count. The neutrophil count was high in patients with infectious pleural effusion and among them it was maximum in empyema type of pleural effusion patients. The leukocyte esterase reagent strip test showed totally negative results in all patients with transudative pleural effusion and also in patients with tuberculous pleural effusion, whereas among patients with infectious pleural effusion other than tuberculosis LER strip test showed negative to only 15% of the patients and for remaining it ranged from 1+ to 3+ and in that majority had 2+ results followed by 3+ and 1+. The validity of leukocyte esterase strip test was tested in comparison with the gold standard culture test. The accuracy of LER strip test was found to be 88.6%, and the sensitivity and specificity was 90.2% and 66.2% respectively. In our study the sensitivity and the positive predictive value (97.3%) was found to be comparatively higher than the specificity and the negative predictive value (33.3%). Since the detection of true positives was high the LER strip test can effectively be used as a screening test in the detection of infectious pleural effusion.

Table 4: Results of the leukocyte reagent strip and the pleural fluid cell count among the study subjects.

Etiology	Pleural fluid leukocyte count (μL^{-1})	Pleural fluid neutrophil count (μL^{-1})	Leukocyte reagent strip			
			0	1+	2+	3+
Non-infectious pleural effusion (transduate) (n=23)	600 (230-1300)	130 (45-426)	23 (100%)	0	0	0
Infectious pleural effusion (n=44)	7250 (1126-11287)	6840 (1013-10980)	7 (15.9%)	9 (20.4%)	16 (36.3%)	12 (27.2%)
Tuberculous pleural effusion (n=17)	2300 (1100- 3800)	168 (101-387)	17 (100%)	0	0	0

Table 5: Validity of LER test in comparison with the gold standard test (culture test).

Variable	Culture positive	Culture negative	Total
LER positive	37	1	38
LER negative	4	2	6
Total	41	3	44
Sensitivity		90.2%	
Specificity		66.7%	
Positive predictive value		97.3%	
Negative predictive value		33.3%	
Accuracy		88.6%	

DISCUSSION

The dipstick leukocyte esterase test is intended to detect leukocytes in urine, but it has also been applied to other biological specimens for the rapid diagnosis of infection. The test uses the ability of the esterase enzyme present in the polymorphonuclear leukocytes of the sample to split heterocyclic carboxylates and form a pyrrole. The latter reacts with a diazonium salt producing a violet colour in the reagent strip.[15] In present study subjects out of 84 patients with pleural effusion 44 had infectious exudative type of pleural effusion and among that 44 patients LER strip test was found to be positive for 37 patients and in that 2+ results was seen in 16 patients and 3+ results was seen in 12 patients. Among the 37 patients simple parapneumonic effusion was seen in 10 patients, 21 patients had complicated parapneumonic effusion and empyema was seen in 6 patients. This strip test was useful in the rapid diagnosis of infection in pleural effusion. Since the causes of pleural effusions were many, the importance of ruling out an infectious etiology is essential to decide upon the use of antibiotics. If the cause of effusion had been secondary to cardiac, liver or renal failure, use of diuretics would be recommended and unnecessary use of antibiotics would be prevented. This test could be used as a bed side test and would be useful in deciding upon the treatment till the laboratory results were available.

A study done by Azoulay et al in the year 2000 among the patients admitted in ICU used two parameters in the strip one was protein and the other was LER for differentiating the infective and non-infective etiology and another study in 2010 conducted by Porcel etal on 128 patients with pleural effusion used only LER strip test in classifying exudative and transudative type of pleural effusion.[16,14]

The present study shows the accuracy of LER strip test was found to be 88.6%, and the sensitivity and specificity was 90.2% and 66.2% respectively and the positive and negative predictive value was 97.3% and 33.3%. As of today, only very few studies had been conducted to study the validity of reagent strips to identify a pleural effusion as an exudate or as a manifestation of infection. A study done by Castellote et al in assessing the validity of LER strip test had found the sensitivity as 91% and specificity of 80% in diagnosing exudative pleural effusion and the study done by Azoulay et al and Porcel et al had found the sensitivity to be 42% and the specificity as 100%.[16-18]

The validity of LER strip test was done by many authors on patients with spontaneous bacterial peritonitis. Butani et al used leukocyte esterase reagent strip/nitrite strip test to diagnose SBP in 136 specimens by using grade 2 as a cut-off scale, and found the sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strip/nitrite strip test as 83%, 99%, 91%, and 98% respectively.[19,20] In study done by Vanbiervliet et al, nine of 72 patients included were diagnosed with SBP, another leukocyte esterase reagent strip/nitrite reagent strip test was positive in all cases with 100% sensitivity and specificity.[20] Sapey et al found sensitivity, specificity, PPV, NPV of leukocyte esterase reagent strip as 64.7%, 99.6%, 91.7%, and 97.4% respectively.[21] Kim et al revealed 50% sensitivity, 100% specificity, 100% PPV and 87% NPV of the leukocyte esterase reagent strip/nitrite reagent strip test in his study.[22] Thevenot et al found 89% sensitivity, 100% specificity, 100% PPV and 99% NPV.[23]

In this era, HIV has been highly prevalent infection worldwide. The immuno compromised nature of the disease made the patients susceptible to pneumonia of various infectious etiologies including tuberculous infection.[24,25] Both have been associated with effusions and the management being different for both the etiologies. The use of LER strip in such cases could be of valuable help in differentiating a non tuberculous from a tuberculous infection, as in our study none of the patients with tuberculous pleural effusion had shown positive for LER strip test.

CONCLUSION

Reagent strips may speed up the bedside diagnosis of infectious effusions. As the value of a diagnostic test lies in its ability to distinguish between otherwise commonly confused disorders, it is thought that the main application for this use of dipsticks is bacterial-mycobacterial effusion discrimination, particularly in the resource limited healthcare setting. However, where access to standard biochemical pleural fluid analysis is not available this dipstick tests would add value to the management of the patients. Further studies in a large number of patients are needed to determine the value of this rapid, easy-to-use, and inexpensive tool for identification of pleural fluids that do not require more expensive tests, notably the impact of its use on patient outcome.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kalaisezhian N, Irsath S, Shankar R. Validity of leukocyte esterase reagent (LER) strip in diagnosing infectious pleural effusion. Int J Adv Med 2018;5:1041-6.