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Original Article

Evaluation of a Newly Developed Non-Culture Test for Bacteriological Diagnosis of Ventilator-Associated Pneumonia in RMCH

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Abstract

Background: The diagnosis and treatment of pneumonia in patients who are receiving mechanical ventilation remain a difficult challenge**.**

Objective: To investigate the utility of soluble triggering receptor expressed on myeloid cells1(s TREM-1) levels in Endotracheal aspirate (ETA) samples as early biomarkers for the diagnosis of VAP compared with quantitative culture result of ETA.

Methods: A total of eighty patients with clinically suspected VAP cases were included in this study and were selected purposely. ETA was collected and level Wes TREM-1are measured. On the other hand, VAP was diagnosed by the quantitative culture of ETA.

Results: The concentration of s TREM-1 in ETA did not discriminate VAP positive from VAP negative patients when compared to quantitative cultures of ETA as the gold standard. A cut-off value of 68.72pg/ml for human s TREM1 in ETA resulted in a sensitivity of 60.81% and specificity of 62.5%.

Conclusion: It may be concluded that the proposed ELISA kit cannot be used as a rapid diagnostic test for the immediate diagnosis of VAP.

Keywords: Ventilator-associated pneumonia (VAP), infection, Non-culture Test

Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in the ICU and contributes disproportionately to both poor outcomes and the high cost of care in critically ill patients.[1]

Ventilator-associated pneumonia (VAP) is defined as pneumonia that arises more than forty-eight hours after initiation of mechanical ventilation by tracheostomy or endotracheal intubation. It has emerged as an important challenge in ICU as it contributes to approximately half of all cases of hospital-acquired pneumonia.[2]

VAP is estimated to occur in 9-27% of all mechanically ventilated patients with the highest risk being early in the course of hospitalization and mortality rates in patients with VAP range from 20-50% and may reach more than 70% when the infection is caused by multidrug-resistant and invasive pathogens [3,4]

VAP is clinically suspected usually on the basis of the presence of fever or hypothermia, leukocytosis or leucopenia, purulent tracheal secretion and the presence of a new or persistent radiographic infiltrate. But these clinical parameters individually have limited diagnostic value. In several studies, the clinical pulmonary infection score (CPIS) was used as a diagnostic tool for pneumonia and calculated on the basis of points assigned for 6 clinical criteria including body temperatures, leukocyte count, volume and appearance of endotracheal aspirate, oxygenation chest X-ray, and culture and Gram staining of endotracheal aspirate.[5-7] Pugin *et al.,* found that a CPIS of >6 was associated with a high likelihood of pneumonia with 93% sensitivity and 100% specificity.[5] To establish an infection, clinical parameters should coexist with the culture of lower respiratory tract secretion. [8]

Culture of the lower respiratory tract secretions obtained by bronchoscopically such as bronchoalveolar lavage (BAL) or protected specimen brush(PSB) are essential for deciding the antibiotic susceptibility of the etiological agent.[7] But bronchoscopy is an invasive procedure which

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cannot be performed in all patients suspected to have VAP. Bronchoscopy maylead to cardiac arrhythmias, hypoxemia or bronchospasm. So it is usually performed only in the later stages of VAP.[9] But any delay in the administration of appropriate antibiotic therapy is associated with higher morbidity and mortality. So there is a need for a non-invasive technique which can be performed in patients suspected to have VAP.[10]

Endotracheal suction is performed in ventilated patients as part of routine care and for tracheal toileting. Endotracheal aspiration does not require any special expert or instrument for its collection. It is easy to collect and less time-consuming and less.[11] Examination of endotracheal aspirate by gram staining allows rapid insight into the number and types of bacteria as well as the number of polymorphonuclear neutrophils that are suggestive of inflammation and infection. The presence of bacteria on Gram-stained smear correlates with the culture of approximate 10^5 bacteria per ml of aspirate.[12]

Diagnosis of VAP is both contentious and frustrating for the intensivist. Early detection of VAP and its causative micro-organisms is a key challenge for clinicians whose aim is to establish a rapid and adapted antibiotic therapy. Identification of the causative microorganism relies on quantitative or semi-quantitative cultures of BAL or ETA with microbiological data provided 24-48hoursafter the sampling. This study will evaluate the effectiveness of using soluble triggering receptor expressed on myeloid cells (s TREM)-1, expressed in response to bacterial infection, as a tool for the rapid diagnosis of VAP. The triggering receptor expressed on myeloid cells is a member of the immunoglobulin superfamily. Its expression on phagocytes is upregulated by exposure to bacteria. A soluble form of TREM-1(s TREM-1) is proposed as a new biomarker that had been tested for acute infections with different diagnostic and prognostic value. S TREM-1 can be found in different body fluids, such as serum, broncho-alveolar lavage fluid (BALF), endotracheal aspirate (ETA), and exhaled breath condensate (EBC) where it can be assayed by ELISA using commercial immunoassay kits. Some clinical studies have proved that s TREM-1did has the ability to identify patients with sepsis while others come to an opposite conclusion.

The real effect of s TREM-1 on the diagnosis of VAP is still unknown and has not been well evaluated yet.

With the above view this study will measure s TREM-1 levels in different body fluid samples from patients who are clinically suspected for VAP and at the same time microbiological test of the sample for diagnosis of VAP and correlation between them will be done

Materials and Methods

This is a cross-sectional type of analytical study. The study was conducted from January to December; 2018. Clinically suspected VAP patients admitted in the Intensive Care Unit Department of Rajshahi Medical College Hospital were selected purposively as cases in this study. Moreover, endotracheal aspirate was collected from patients under mechanical ventilation by endotracheal tube or tracheostomy tube for more than 48 hours and was also purposive in nature. Exclusion criteria were patient's attendant who refused to give consent and patient without infection. After all aseptic precaution, a narrow soft sterile disposable suction catheter was introduced through the endotracheal tube or tracheostomy tube. A 10 cc disposable syringe was used for aspiration and 3 to 5ml aspirate was collected and then injected into a sterile test tube. The tube was then brought to the laboratory for further processing. Rapid diagnosis of clinically suspected VAP cases was done by measuring the s TREM-1 level in body fluid (tracheal aspirate) which it can be assayed by ELISA using commercial immunoassay kits.

Results

A total of 80 patients' endotracheal aspirates were collected from clinically suspected VAP (Ventilator-associated pneumonia) cases according to CPIS (Clinical Pulmonary Infection Score) system and were tested for isolation. Endotracheal aspirates were also tested for ELISA.

Table 1: Culture positivity of collected samples. n=80

Culture positivity	Single isolated cases (%)	Multiple isolated cases (%)	Total (%)
Culture positive	72 (90%)	3 (3.75%)	75 (93.75%)
Culture negative	-	-	5 (6.25%)
Total			80 (100%)

Table 1 shows culture positivity of collected samples. Among 80 cases 75 (93.75%) cases were culture positive and 5 (6.25%) cases were culture negative.Single and multiple isolated cases were 72 (90%) and 3 (3.75%) respectively among the culture positive cases.

Clinically suspected 80 VAP patient's endotracheal aspirates were tested for ELISA. For negative control another 5 patient's (non-VAP case) endotracheal aspirates were also tested for ELISA.

Figure1 shows that, the concentration of human s TREM-1 in VAP cases and non-VAP cases did not show any significant difference. The average concentration of human s TREM-1in non-VAP cases were 68.72 pg/ml (OD-0.105).

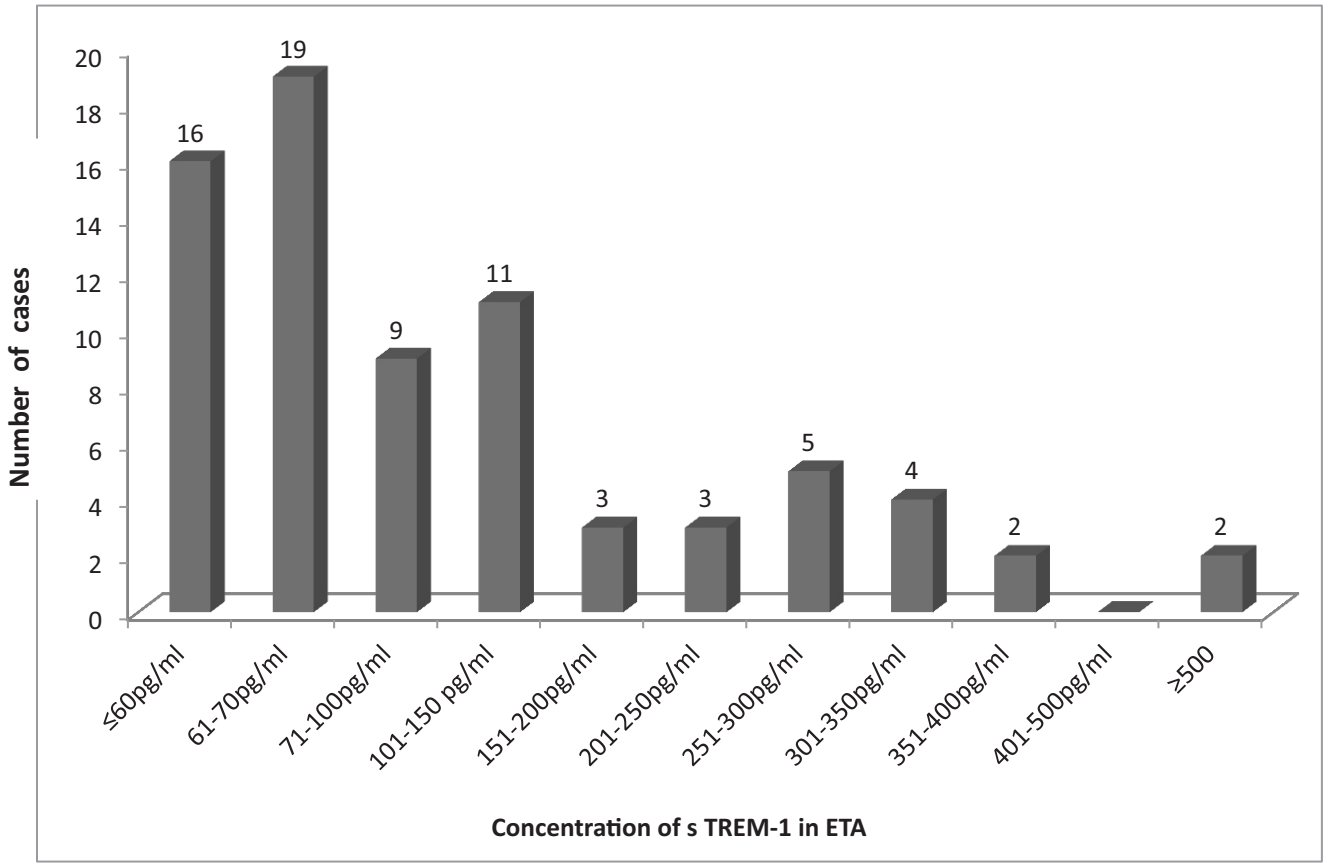

Figure- 1: Distribution of human s TREM-1 level in VAP cases. n=75

Table 2: Distribution of concentration of human s TREM-1 in VAP cases comparing with non-VAP cases.

Attributes	Above 68.72pg/ml	Average 68.72pg/m1	Below 68.72pg/ml	Total
VAP case	45 (60%)	(1.33%)	29 (38.67%)	75 (100%)
Non-VAP case	-	5	-	5 (100%)

A cut off value of 68.72 pg/ml for human s TREM-1 in ETA resulted in sensitivity of 60.81% and specificity of 62.5%.

Discussion

Ventilator associated pneumonia remains a major contributor to hospital-acquired infection in Asia. Early accurate diagnosis is fundamental in the management of patients with VAP.[1] Delayed diagnosis and subsequent delay in initiating appropriate therapy may be associated with worse outcomes in patients with VAP, on the other hand an incorrect diagnosis may lead to unnecessary treatment and subsequent complication related to therapy.[10]

In the present study soluble triggering receptor was measured which expressed on myeloid cell-1 in endotracheal aspirate by ELISA kit which is commercially available to see the diagnostic performance of s TREM-1.

It is evaluated that the concentration of s TREM-1 in Endotracheal aspirate (ETA) could not effectively categorize patients as VAP positive or VAP negative when using ETA culture samples as the comparison. This study found lower sensitivity 60.81% and specificity 62.5% and findings are consistent with some recent studies; a study Wang *et al.*, 2017 in china showed moderate sensitivity 85.5% and lower specificity 28.8%,[13] Palazzo *et al.*, 2012 in Washington also showed low sensitivity and specificity of s TREM-1 in ETA for VAP patient.[14] Dissimilarity was observed by Gibot*et al.*, 2004 in New England where they found high sensitivity 98% and specificity 90% but in that study they measures TREM-1 level in bronchoalveolar-lavage not ETA. Moreover that it could not differentiate between communitie acquired pneumonia from VAP.[15]

One of the goal of this study was to evaluate a rapid non-culture test that will be able to diagnose VAP effectively and will help the clinicians to start the treatment as early as possible so that ultimate reduction of the mortality rate of VAP patients can be obtained. It is decided that the proposed kit that measured the s TREM-1 in ETA needed a large scale evaluation to practice it as a rapid diagnostic procedure. Additionally, it would not be able to differentiate the micro-organism. It's expression on phagocytes is up regulated by exposure to any bacteria. As

a result, the microbiological diagnosis (culture) to know the exact causative organism and their drug sensitivity pattern to start accurate treatment of the VAP patient.

Conclusion

Ventilator associated pneumonia (VAP) is a significant cause of morbidity and mortality in critically unwell patients. In ELISA test, among five culture negative cases average concentration of human s TREM-1 was 68.72 pg/ml. But among 75 culture positive cases 45 cases showed higher concentration of human s TREM-1 in ETA (>68.72 pg/ml) and 29 cases showed lower concentration of human s TREM-1 (<68.72 pg/ml). The optimum cut-off value for s TREM-1 in ETA was 68.72 pg/ml, yielding sensitivity and specificity of 60.81% and 62.5%. Finally it may be concluded that the proposed ELISA kit need further evaluation to use it as a rapid diagnostic test for VAP.

Conflict of interest: No

References

1. Rea-Neto, A., Yousf, N.C.M., Tuche, F., Brunkhorst, F., Ranieri, V.M., Reinhart, K. and Sakr, Y. (April, 2008). Diagnosis of Ventilator associated Pneumonia : A systematic review off the literature. Available online: http:// ccforum.com/content/12/2/R56

02. Kalanuria, A.A., Zai, W. and Mirski, M. (2014). Ventilator associated Pneumonia in the ICU, BioMed Central, Critical Care; 18: 208. http:// Ceforum.com/ Content/18/2/208.

03. Selina, F.,Talha, K.A., Islam, A., Hasan, Z., Hyder, M. and S. Selvapandian. (2009). Organisms associated with ventilator associated pneumonia (VAP) in intensive care unit (ICU), Journal of the Bangladesh

04. Amanullah, S. and Mosenifar, Z. (Dec, 2015). Ventilator Associated Pneumonia Overview of Nosocomial Pneumonias. Online available https:// emedicine.medscape.com

05. Pugin, .J, Auckenthaler, R., Mili, N., Janssens, J.P., Lew, P.D. and Suter, P.M. (1991). Diagnosis of ventilator associated pneumonia by bacterilogical analysis of bronchoscopic lavage fluid. Am Rev RespirDis ; 143: 1121-1129.

06. Chastre J, Fagon JY. (2002).Ventilator associated pneumonia. AM J RespirCritCare Med; 165: 867-903.

07. Niederman, M.S. and Craven, D.E. (2005). Guidelines for the management of adults with hospital acquired ventilator associated pneumonia and health care associated pneumonia. AM J RespirCrit Care Med;171: 388-416.

08. Fartoukh, M., Maitre, B., Honone, S., Cerf, C., Zahan, J. and Brun-Buisson, C. (2003).Diagnosing Pneumonia during mechanical Ventilation: The clinical pulmonary infection score revisited.Am J RespirCrit Care Med; 168(2): 173-179

09. Joseph, N.M., Sistla, S., Dutta, T.K. and Badhe, A.S.(2010). Ventilator associated pneumonia in a tertiary care hospital in India: role of multidrug resistant pathogens. J infectdevCtries; 4(4): 218-225

10. Luna, C.M., Vujacich, P., Niederman, M.S., Vay, C., Gherardi, C., Matera, J. and Jolly, E.C. (1997). Impact of BAL data on the therapy and outcome of Ventilator associated Pneumonia. Chest; 676-685

11. EI-Ebiary, M., Torres, A., Gonzalez, J., Puig de la Bellacasa, J., Garcva, C. and Jiminez de Anta M.T. (1993). Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator associated pneumonia. Am Revrespire dis.; 148: 1552-1557.

12. Nagendra et al.(2001). Sampling variability in the microbiological evaluation of expectorated sputum and endotracheal aspirates.J ClinMicrobiol; 39(6): 2344-2342.

13. Wang, K., Chen, Y., Salido, M.M., Kohli, G.S., Kong, J.L. and Liang, H.J. (2017). The rapid in vivo evolution of Pseudomonas aeruginosa in ventilator associated pneumonia patients leads to attenuated virulence. Open Biol.7: 170029.Online available at https://dx.doi.org/10.6084/m9.figshare.c.385829

14. Palazzo, S.J., Simpson, T.A., Simmons, J.M. and Schnapp, L.M. (Dec, 2012). Soluble Triggering Receptor Expressed on Myeloid Cells-1 (s TREM-1) as a Diagnostic Marker of Ventilator associated Pneumonia. Respir Care; 57(12): 2052-2058. doi: 104187/respcare.01703.

15. Gibot, S., Cravoisy, A., Levy, B., Bene, M., Faure, G. and Bollaert P. (2004) Soluble Triggering Receptor Expressed on Myeloid Cells and the diagnosis of Pneumonia.N. Engl J Med; 350(5): 451-458