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BRIEF REPORT

Guy J. Oudhuis
Judith Beuving
Dennis Bergmans
Ellen E. Stobberingh
Guul ten Velde
Catharina F. Linssen
Annelies Verbon

Soluble Triggering Receptor Expressed on Myeloid cells-1 in bronchoalveolar lavage fluid is not predictive for ventilator-associated pneumonia

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G. J. Oudhuis · J. Beuving · E. E. Stobberingh · C. F. Linssen · A. Verbon (✉)
Department of Medical Microbiology, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, The Netherlands
e-mail: a.verbon@mumc.nl
Tel.: +31-43-3876644
Fax: +31-43-3876643

D. Bergmans
Department of Intensive Care, Maastricht University Medical Centre, Maastricht, The Netherlands

G. ten Velde
Department of Respiratory Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands

A. Verbon
Department of Internal Medicine, Division of General Internal Medicine, Section Infectious Diseases, Maastricht University Medical Centre, Maastricht, The Netherlands

Abstract *Purpose:* Soluble Triggering Receptor Expressed on Myeloid cells-1 (sTREM-1) has proven to be a good biomarker for sepsis. For the diagnosis ventilator-associated pneumonia (VAP), however, there have only been a few, relatively small, studies on the role of this receptor. The aim of the study was to evaluate the usefulness of sTREM-1 in bronchoalveolar lavage fluid (BALF) from Intensive Care Unit patients as rapid diagnostic test for VAP. *Methods:* The concentration of sTREM-1 in 240 BALF samples was measured using a quantitative sandwich enzyme immunoassay. Two researchers who were blind to the assay results determined whether a VAP was present or not. Clinical suspicion of a VAP was confirmed by the presence of $\geq$2% cells containing intracellular organisms and/or a quantitative culture result of $\geq 10^4$ colony forming units per millilitre BALF. *Results:* The mean concentration of sTREM-1 was significantly higher in the BALF of patients with confirmed VAP than in that of patients without confirmed VAP. However, the area under the receiver-operating characteristic curve was 0.58 (95% confidence interval 0.50–0.65, $P = 0.04$). *Conclusions:* The results imply that the sTREM-1 assay in BALF may not be discriminative for VAP.

Keywords Artificial respiration · Biological marker · Early diagnosis · Enzyme-linked immunosorbent assay · Intensive care · Respiratory tract infections

Introduction

The diagnosis of ventilator-associated pneumonia (VAP), a complication that often arises in patients receiving mechanical ventilation, is difficult. Clinical suspicion alone is not reliable [1] and may lead to an overestimation of VAP, resulting in unnecessary antibiotic therapy. Results from invasive procedures, such as quantitative culture of bronchoalveolar lavage fluid (BALF), have proven to be useful [2], but these are not available for 24–48 h. A potential candidate as a rapid diagnostic test for VAP is soluble Triggering Receptor Expressed on Myeloid cells-1 (sTREM-1). Testing for sTREM-1 in BALF has promising results with a sensitivity of 98% and a specificity of 90% [3].

TREM-1 is shed from membranes of activated phagocytes and is found in soluble form in body fluids. It is a glycoprotein, expressed as receptor on neutrophils and $CD14^{high}$ monocytes/macrophages [4]. The expression of TREM-1 is strongly up-regulated in

tissues infected with extracellular microorganisms [5]. In humans, sTREM-1 in the blood proved to be a good biomarker for sepsis [6]. For pneumonia, however, the value of sTREM-1 in BALF [3, 7–10] has received varying assessments. To evaluate the usefulness of sTREM-1 as rapid diagnostic test for VAP, we tested 240 BALF samples from intensive care unit (ICU) patients.

Materials and methods

Patients

This is a retrospective study that included patients admitted to the ICU of Maastricht University Medical Centre, who underwent BAL while receiving mechanical ventilation. In all patients, mechanical ventilation was initiated on the day of admission. Patients were screened daily for VAP using clinical criteria, and BAL was performed on the day of clinical suspicion. For each patient receiving more than one BAL, the BALs were included in the study when the time period between BALs was $\geq$2 weeks; when there was <2 weeks between BALs, only the first BAL was included.

Baseline criteria were recorded (Table 1). Time between ICU admission and BAL was also determined. Study protocols were approved by the institutional review board for human studies. Informed consent regarding the use of data for research purposes was obtained from patients or their legal representatives.

VAP diagnosis

Clinical suspicion of VAP was according to the definition of Bonten et al. [11], and VAP was considered to be acquired upon manifestation of the disease after $\geq$48 h of mechanical ventilation. Clinically suspected VAP was considered confirmed VAP when the microbiological results met the following criteria [11, 12]: presence of $\geq$2% cells containing intracellular organisms (ICO) and/or quantitative culture result of $\geq 10^4$ colony forming units (cfu) per millilitre of BALF.

Following BAL, antibiotic therapy was started empirically in patients who had not previously received treatment. When patients developed a VAP while receiving antibiotics, the therapy was adjusted. Antibiotic treatment before and during BAL does not influence the predictive value of the percentage ICO in BALF in diagnosing VAP [12]. Thus, due to the combination of quantitative BALF culture and presence of ICO, adjustment of the cfu/ml threshold was unnecessary to diagnose VAP correctly when antibiotics were being used before the BAL.

Two researchers (Guy J. Oudhuis and A. Verbon) retrospectively determined whether patients met the criteria of clinically suspected and confirmed VAP, independently from one another and blind to the sTREM-1 assay results. This yielded two study groups; a confirmed VAP group and a group in which VAP could not be confirmed. A consensus diagnosis was achieved in all cases.

Bronchoalveolar lavage

Bronchoscopy with directed BAL was performed as described previously [12]. Samples were processed within 1 h after the BAL. All aliquots were pooled, except for the first one. The BALF samples were Gram-stained, and quantitative culture was performed as described previously [13]. The urea concentration was determined by means of an enzymatic conductivity rate method using the SYNCHRON LX System (Beckman Coulter BV, Mijdrecht, The Netherlands) as previously described [14]. The remaining BALF was centrifuged at 250 g for 10 min. The supernatant was stored at –80°C in six different aliquots until further processing.

Soluble Triggering Receptor Expressed on Myeloid cells-1 assay

A quantitative sandwich enzyme immunoassay (Quantikine Human TREM-1 Immunoassay; R&D Systems, Minneapolis, MN), was used to detect sTREM-1 in BALF in accordance to the manufacturers' instructions, as described previously [7].

The measured concentration of sTREM-1 in BALF samples is not identical to that in the epithelial lining fluid (ELF), secondary to dilution of the BALF. To calculate the concentration sTREM-1 in ELF from the concentration of sTREM-1 in BALF, we applied a correction with a dilution factor using the ratio of urea concentration in the serum and BALF [15].

Statistical analysis

The Mann–Whitney *U* test was used for the numerical data; for sTREM-1 concentrations, we used Student's *t* test to compare logarithmic means. The Pearson χ^2 test was used for categorical data. The analyses were corrected, by clustered logistic regression, for the influence of multiple BALs performed in the same patient. To evaluate the diagnostic value of the sTREM-1 assay, we produced a receiver-operating characteristic (ROC) curve and measured the area under the curve. Statistical significance was defined as a P value of <0.05 in all cases. Analysis was performed with SPSS ver. 14.0 for Windows (SPSS, Chicago, IL).

Table 1 Characteristics of the study population

Characteristic	All patients[a] ($n = 207$)	Patients with confirmed VAP ($n = 90$)	Patients without confirmed VAP ($n = 117$)	*P* value (two groups compared)
Sex				
Male (%)	130 (62.8)	54 (60)	76 (65)	0.46
Female (%)	77 (37.2)	36 (40)	41 (35)	
Age, years, median (interquartile range)	62 (47–72)	65.50 (47–73.25)	61 (47.50–71)	0.43
Days in hospital				
Mean (± SEM)	68.0 (4.6)	75.7 (9.0)	62.1 (4.2)	0.76
Range	6–540	6–540	8–322	
Days in ICU				
Mean (± SEM)	39.8 (2.8)	45.1 (5.7)	35.8 (2.3)	0.77
Range	6–397	6–397	7–168	
Days in ICU before BAL[b]				
Mean (± SEM)	15.5 (0.9)	15.3 (1.5)	15.7 (1.2)	0.63
Range	2–83	3–83	2–73	
APACHE II score[c]				
Mean (± SEM)	23 (0.5)	23 (0.9)	23 (0.6)	0.83
Range	4–44	4–44	7–40	
Mortality in ICU (%)	72 (34.8)	33 (36.7)	39 (33.3)	0.62
Mortality in hospital (%)	83 (40.1)	41 (45.6)	42 (35.9)	0.16
History of COPD (%)	10 (4.8)	5 (5.6)	5 (4.3)	0.67
Medical specialty (%)				
Medical	71 (34.3)	24 (26.7)	47 (40.2)	0.26
Surgical	78 (37.7)	35 (38.9)	43 (36.8)	
Trauma	34 (16.4)	19 (21.1)	15 (12.8)	
Neurological	18 (8.7)	9 (10.0)	9 (7.7)	
Other[d]	6 (2.9)	3 (3.3)	3 (2.6)	
Reason for ICU admission (%)				
Respiratory insufficiency	25 (12.1)	10 (11.1)	15 (12.8)	0.21
Trauma	28 (13.5)	15 (16.7)	13 (11.1)	
Shock	52 (25.1)	19 (21.1)	33 (28.2)	
Cardiopulmonary failure	17 (8.2)	4 (4.4)	13 (11.1)	
Pneumonia	32 (15.5)	13 (14.4)	19 (16.2)	
Neurological disease	15 (7.2)	7 (7.8)	8 (6.8)	
Post-operative	38 (18.4)	22 (24.4)	16 (13.7)	

VAP, Ventilator-associated pneumonia; BAL, bronchoalveolar lavage; SEM, standard error of the mean; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; APACHE II, Acute Physiology and Chronic Health Evaluation II

[a] Seven patients underwent three BALs, 19 patients two BALs and 181 patients had one BAL. None of these patients had more than one positive BAL within a time period of 14 days

[b] All 240 BALs are included in this item

[c] 10% of the APACHE II scores could not be retrospectively calculated. The patients involved did not differ from the other patients in this study

[d] Including otorhinolaryngology, paediatrics, gynaecology and oral surgery

Results

Patients

Between January 2001 and October 2006, 361 BALs were performed, of which 59 were excluded from our retrospective analysis since they did not meet the quality criteria [12], and 62 were excluded because they were performed within 48 h after ICU admission. The remaining 240 BALs, performed in 207 patients were included in our study.

Seven patients underwent three BALs, 19 patients underwent two BALs and 181 patients had one BAL. The presence of VAP was confirmed in 97 cases (40.4%) and not confirmed in 143 cases (59.6%).

Confirmed VAP cases did not differ significantly from unconfirmed cases, and both groups did not differ significantly in terms of in-hospital mortality and ICU mortality (Table 1). The number of patients with chronic obstructive pulmonary disease (COPD) was low and did not differ between both study groups (Table 1).

Soluble Triggering Receptor Expressed on Myeloid cells-1 levels

The mean sTREM-1 concentration was significantly higher in the BALF of patients with confirmed VAP [1849 pg/ml, 95% confidence interval (CI) 1515–2256 pg/ml]

than in those without confirmed VAP (1424 pg/ml, 95% CI 1218–1664 pg/ml; $P = 0.04$; Fig. 1a).

Mean sTREM-1 levels did not differ between patients with COPD (1570 pg/ml, 95% CI 1081–2279 pg/ml) and those without (1617 pg/ml, 95% CI 1404–1863 pg/ml) ($P = 0.93$).

No significant difference in mean sTREM-1 level was observed between medical patients (1676 pg/ml, 95% CI 1352–2077 pg/ml) and surgical patients (2233 pg/ml, 95% CI 1769–2817 pg/ml) ($P = 0.08$), while the number of confirmed VAP cases did not differ between both groups ($P = 0.17$).

Diagnostic value of sTREM-1

A ROC curve analysis showed an area under the curve of 0.58 (95% CI 0.50–0.65; $P = 0.04$; Fig. 1b). Therefore, the sTREM-1 levels in our hands were not discriminative for VAP. Choosing a sensitivity of 95% resulted in a positive predictive value (PPV) of 41% and a negative predictive value (NPV) of 62% in our population. A specificity of 95% led to a PPV of 67% and a NPV of 62%.

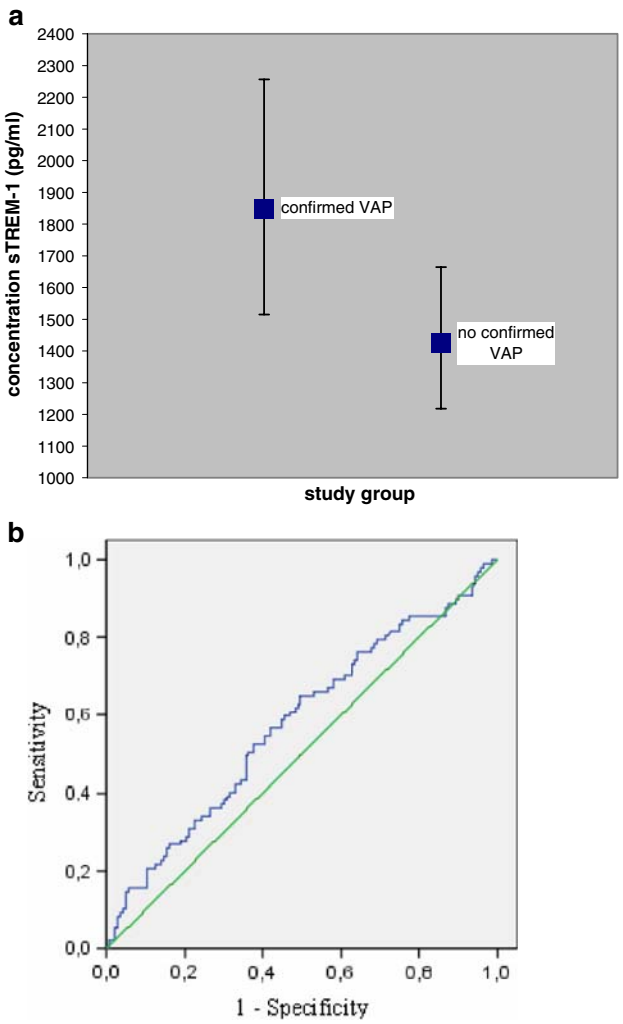

Fig. 1 **a** Mean soluble Triggering Receptor Expressed on Myeloid cells-1 (*sTREM-1*) levels in bronchoalveolar lavage fluid (BALF) samples of confirmed ($n = 97$) and non-confirmed ventilator-associated pneumonia (*VAP*) ($n = 143$) cases. *Vertical bars* 95% confidence intervals of the mean. **b** Receiver-operating characteristic (ROC) curve analysis of sTREM-1 in BALF samples of patients with and without confirmed VAP

Discussion

In this study, sTREM-1 levels were not discriminative for VAP. The differences between the results of our study and those of five other trials in terms of sTREM-1 and pneumonia are highlighted in Table 2. These may explain the differences in results. A correction for the dilution of BAL has been found to allow a better representation of the actual concentration of sTREM-1 in the alveoli [15]. However, this does not appear to explain the major differences in outcome, since using the concentration of sTREM-1 without correction for dilution did not result in a significantly better ROC analysis in our study (data not shown).

Samples from medical and surgical patients were included in this study. Our results show that the sTREM-1 levels tended to be higher in surgical patients, which is in accordance with the results of other studies in which surgery was reported to increase sTREM-1 expression levels in peripheral blood monocytes independent of infection [16]. We therefore conclude that in terms of diagnosing VAP in an every day ICU setting, the measurement of sTREM-1 levels in BALF does not seem to be discriminative for VAP.

The levels of sTREM-1 are not only increased by infecting micro-organisms [17]; cancer cells have also been shown to be able to directly up-regulate TREM-1 expression in patients' macrophages [18]. A number of inflammatory disorders, such as pancreatitis, are also known to increase levels of sTREM-1 [19]. In contrast, TREM-1 is not up-regulated in samples from patients with other inflammatory disorders, such as psoriasis, ulcerative colitis or vasculitis caused by immune complexes [5].

The strength of this study is that it was performed in a large, well-defined group of both medical and surgical ICU patients. One limitation may be that the analysis was performed retrospectively. Only patients that underwent BAL ≥48 h after being admitted to the ICU were included in the study. Therefore, these patients stayed longer in a ICU than the general ICU population. A second limitation may be that VAP is still difficult to diagnose. However, we used the widely accepted modified criteria of the Centers for Disease Control [11] to diagnose VAP and confirmed the diagnosis by both staining for ICO and culturing BALF samples [12].

Table 2 Characteristics of the six clinical studies regarding sTREM-1 and pneumonia

Study factors	Gibot [3]	Determann [7]	Horonenko [8]	Huh [9]	Anand [10]	Present study
Number of cases	148	28	23	80	105	240
Setting	Medical ICU	General ICU	Medical ICU	Medical ICU	Medical ICU	General ICU
Assay	Immunoblot	ELISA	ELISA	ELISA	ELISA	ELISA
Correction for dilution	No	No	Yes	No	No	Yes
Type of BAL	Mini-BAL	Non-directed	BAL, NS	Non-directed	Directed	Directed
Sensitivity/specificity	98/90	75/84	NS	86/90	42/76	65/48
Diagnosis	CAP/VAP	VAP	VAP	Bilateral lung infiltrates	VAP	VAP
Confirmation of VAP	$>10^3$ cfu/ml	$\geq 10^4$ cfu/ml	$\geq 10^3$ cfu/ml	$>10^4$ cfu/ml	$>10^3$ cfu/ml	$\geq 10^4$ cfu/ml and/or $\geq$2% ICO
Patients with confirmed VAP (%)	46 (31)	9 (32)	14 (61)	29 (36)	19 (18)	90 (43)
Mean sTREM-1 level (pg/ml) VAP patients[a]	34	894	403	521	172	227

VAP, Ventilator-associated pneumonia; BAL, bronchoalveolar lavage; NS, not specified; cfu, colony forming units; ICO, intracellular organisms; sTREM-1, soluble Triggering Receptor Expressed on Myeloid cells-1; ELISA, enzyme-linked immunosorbent assay; ICU, intensive care unit; CAP, community-acquired pneumonia

[a] Non-dilution corrected levels are presented

Conclusion

In conclusion, the results of this study imply that the sTREM-1 assay on BALF samples as a rapid diagnostic test for VAP may not be discriminative.

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References

1. Torres A, Ewig S (2004) Diagnosing ventilator-associated pneumonia. N Engl J Med 350:433–435
2. Torres A, El-Ebiary M (2000) Bronchoscopic BAL in the diagnosis of ventilator-associated pneumonia. Chest 117:198S–202S
3. Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE (2004) Soluble Triggering Receptor Expressed on Myeloid cells and the diagnosis of pneumonia. N Engl J Med 350:451–458
4. Bouchon A, Dietrich J, Colonna M (2000) Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 164:4991–4995
5. Bouchon A, Facchetti F, Weigand MA, Colonna M (2001) TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410:1103–1107
6. Gibot S, Kolopp-Sarda MN, Bene MC, Cravoisy A, Levy B, Faure GC, Bollaert PE (2004) Plasma level of a triggering receptor expressed on myeloid cells-1: its diagnostic accuracy in patients with suspected sepsis. Ann Intern Med 141:9–15
7. Determann RM, Millo JL, Gibot S, Korevaar JC, Vroom MB, van der Poll T, Garrard CS, Schultz MJ (2005) Serial changes in Soluble Triggering Receptor Expressed on Myeloid cells in the lung during development of ventilator-associated pneumonia. Intensive Care Med 31:1495–1500
8. Horonenko G, Hoyt JC, Robbins RA, Singarajah CU, Umar A, Pattengill J, Hayden JM (2007) Soluble triggering receptor expressed on myeloid cell-1 is increased in patients with ventilator-associated pneumonia: a preliminary report. Chest 132:58–63
9. Huh JW, Lim CM, Koh Y, Oh YM, Shim TS, Lee SD, Kim WS, Kim DS, Kim WD, Hong SB (2008) Diagnostic utility of the Soluble Triggering Receptor Expressed on Myeloid cells-1 in bronchoalveolar lavage fluid from patients with bilateral lung infiltrates. Crit Care 12:R6
10. Anand NJ, Zuick S, Klesney-Tait J, Kollef MH (2009) Diagnostic implications of Soluble Triggering Receptor Expressed on Myeloid cells-1 in BAL fluid of patients with pulmonary infiltrates in the ICU. Chest 135:641–647
11. Bonten MJ, Bergmans DC, Stobberingh EE, van der Geest S, De Leeuw PW, van Tiel FH, Gaillard CA (1997) Implementation of bronchoscopic techniques in the diagnosis of ventilator-associated pneumonia to reduce antibiotic use. Am J Respir Crit Care Med 156:1820–1824
12. Linssen CF, Jacobs JA, Schouten JS, van Mook WN, Ramsay G, Drent M (2008) Influence of antibiotic therapy on the cytological diagnosis of ventilator-associated pneumonia. Intensive Care Med 34:865–872
13. Jacobs JA, De Brauwer EI, Cornelissen EI, Drent M (2000) Accuracy and precision of quantitative calibrated loops in transfer of bronchoalveolar lavage fluid. J Clin Microbiol 38:2117–2121

14. Horak E, Sunderman FW Jr (1972) Measurements of serum urea nitrogen by conductivimetric urease assay. Ann Clin Lab Sci 2:425–431
15. Wiedermann FJ, Mayr AJ, Kaneider NC, Fuchs D, Mutz NJ, Schobersberger W (2004) Alveolar granulocyte colony-stimulating factor and alpha-chemokines in relation to serum levels, pulmonary neutrophilia, and severity of lung injury in ARDS. Chest 125:212–219
16. Gonzalez-Roldan N, Ferat-Osorio E, Aduna-Vicente R, Wong-Baeza I, Esquivel-Callejas N, Astudillo-de la Vega H, Sanchez-Fernandez P, Arriaga-Pizano L, Villasis-Keever MA, Lopez-Macias C, Isibasi A (2005) Expression of triggering receptor on myeloid cell 1 and histocompatibility complex molecules in sepsis and major abdominal surgery. World J Gastroenterol 11:7473–7479
17. Phua J, Koay ES, Zhang D, Tai LK, Boo XL, Lim KC, Lim TK (2006) Soluble Triggering Receptor Expressed on Myeloid cells-1 in acute respiratory infections. Eur Respir J 28:695–702
18. Ho CC, Liao WY, Wang CY, Lu YH, Huang HY, Chen HY, Chan WK, Chen HW, Yang PC (2008) TREM-1 expression in tumor-associated macrophages and clinical outcome in lung cancer. Am J Respir Crit Care Med 177:763–770
19. Yasuda T, Takeyama Y, Ueda T, Shinzeki M, Sawa H, Takahiro N, Kamei K, Ku Y, Kuroda Y, Ohyanagi H (2008) Increased levels of Soluble Triggering Receptor Expressed on Myeloid cells-1 in patients with acute pancreatitis. Crit Care Med 36:2048–2053