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The Role of Secretory Immunoglobulin A, Neutrophils and Pathogens in the Early Onset-Ventilator Acquired Pneumonia, Based on Analysis of Bronchoalveolar Lavage Specimen

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Abstract: Background and Aim: Early-onset VAP cause high morbidity/mortality, longer/higher cost of treatment. Secretory-IgA and neutrophils play important role in early-onset VAP. Exploration the role of these components in local immunity of respiratory system, has been done toward concentration of s-IgA, percentage of neutrophil and kind of pathogens of BAL specimen in mechanical ventilator - patients > 48 hours.
Methods: Cohort study of s-IgA levels, percentage of neutrophil and pathogensof BAL specimen, in mechanical ventilator-patients.Sampling was carried out, on 1st and 3rd dayof using mechanical ventilator. On the 3rd day, subjects were divided into 2 groups: VAP(-) group:28 persons;and VAP(+)group: 33 persons.VAP (+), if scores of CPIS (Clinical Pulmonary Infection Score)>6.
Results: In early-onset VAP patients, the level of s-IgA has increased significantly andcan reduce theneutrophils. The kind of pathogens play important role in stimulatingor destroying s-IgA, and contribute significantly to the VAP incidence, whereas percentage of neutrophil increased in all of pathogens infections. InVAP(-)MRSAinfection group, s-IgA level decreased belowthe baseline and percentage of neutrophil increased non-significantly and was controlled.In VAP(+) group,percentage ofneutrophil increased significantly and play important role in the incidence and deterioration of VAP.In this study, the four highestpathogensin mechanical ventilator - patientsare A. baumannii (42,31%), MRSA (21,79%), K. pneumonia (12,82%) and P. aeruginosa (8,97%).
Conclusion: S-IgA and neutrophilscooperate each otherin local immunity mechanism,and s-IgA can suppress excessive reaction of neutrophils.S-IgAgive different responses to pathogens pattern and can destroyed by certain pathogen, while neutrophils do not give different response to different pathogens and give contribution in worsening the incidence of VAP.The most kind of pathogensin mechanical ventilator-patientsare A. baumanii, MRSA, K. pneumonia andP. Aeruginosa.
Keywords: Early-onset VAP, CPIS score, S-IgA, neutrophils and pathogens inLRT.

Introduction

Ventilator Acquired Pneumonia (VAP) is pneumonia that obtained in patients using mechanical ventilators, after >48 hours.[1,2,3,4] This relates to increased high morbidity and mortality, costs, longer hospitalization.[5] The incidence of approximately 5-52 cases per 1000 ICU treatment patient.[4,5]Especially for Asian countries, Chawla, 2008, has a survey of the epidemiology, diagnosis and management of HAP and VAP in 10 Asian countries reached 3.5 to 46/1000 patients per day with a mortality of 33-50%, can even reach 70%.[6,7]Observation of the long treatment of pneumonia patients in some countries such as the United States, reported that longer treatment increased on average 4-13 days, the number of cases 250,000-300,000 cases a year, and costs $ 5000-20000 / case and even can be increased to $ 40,000 / in patient VAP.[8,9,10]

The incidence of VAP represents an increase of 70% in pneumonia caused by pathogens Multi Drug Resistance (MDR), VAP-causing pathogens, approximately 87% are gram-negative pathogens, especially *Acinetobacter baumannii* (39%), Pseudomonas aeruginosa (31%) and Klebsiellaspp (20%). In addition, also the contamination of pathogens by means of mechanical ventilators such as endotracheal tube, ventilator pipe, sucker sputum, water in a hospital or air conditioner. Aspiration of gastric fluid has the potential to cause colonization of pathogenic gram (-).[11,12,13]

Infections that occur in VAP, is linked to the immune system found in the respiratory system. Secretory Immunogblobulin A (s-IgA), a component of humoral immunity are very fundamental in the respiratory system, which will bind pathogens at mucosal surfaces and the amount is 65-80% more than in serum (systemic).[14,15] The interaction between the s-IgA with innate immunity in mucosal secretions, can protect mucosal surfaces from infection.[16,17] Research on s-IgA trakeabronkial activity in vitro, suggesting that the activity of s-IgA increases when there is a pathogen, and vary in quantity and quality.[18] S-IgA protects the mucosa of pathogens, as it can react with molecular adhesion of potential pathogens, so will prevent the adherent and colonization of pathogens in the host cell.[19,20] In addition, s-IgA serves as opsonin, and together with neutrophils, monocytes and macrophages have the same receptor, in order to enhance the effect of complement bacteriolitic, which will neutralize toxins and viruses, thus preventing the harmful component contact with tissues and cells in the respiratory system.[21,22] The function of neutrophils from two different sides, namely as a component of the body's defenses along macrophages and s-IgA destroying germs, but on the other hand, excessive increase in neutrophils can damage lung tissue and worsen lung function.[23,24]

Samples s-IgA and neutrophil lower respiratory tract, as well as the culture of pathogens, can be obtained by bronchoscopy procedure rinses bronchoalveolar (BAL).[26,27,28] Sampling through BAL chosen, because BAL had a sensitivity of 97% and specificity of 100% compared to the tracheobronchial and sputum samples.[29,30] Assessment of VAP, is done by using the Clinical Pulmonary Infection Score. CPIS Scores is a number to assess the clinical condition of the patient, and the strong presence of VAP allegations, determined by using 5 variables, namely: the shape and amount of sputum, extensive consolidation on chest X-ray, temperature, leukocyte count, which increases oxygen demand, CPIS score> 6, expressed as VAP (+).[31,32] Score CPIS, has a sensitivity of 93% and specificity of 100%, when cultured pathogens were extracted using BAL fluid.[33,34,35]This background, to do research and find out who will observe and ensure the role of s-IgA, neutrophils and the presence of infection with various types of pathogens taken from the respiratory tract distal to do BAL procedures.

Methods

This isacohort study, a prospective observational analytic with s-IgA, neutrophils and culture lower respiratory tract pathogens from fluid BAL as independent variables and VAP as dependent variable. Sample size was determine and by using cohort formula and number of sample was 28 subjects VAP(-) and 33 subjects VAP(+), cases were found by consecutive sampling. This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia.

Subjects

Case were VAP subjects with a mechanical ventilators were recruited from at ICU Adam Malik Hospital in Medan city, North Sumatera,Indonesia, from August 2013 to July 2014. The inclusion criteria the cases inpatients at ICU using a mechanical ventilator, aged ≥ 18 - <61 years, scores CPIS ≤6, no contra indications bronchoscopy procedures and the family agreed to follow the study by signing the consent form.

The exclusion criteria in cases anatomical abnormalities or lower respiratory tract trauma, pneumonia, pulmonary tuberculosis, HIV disease, malignancy, diabetes mellitus, hemodynamic unstable patients and died before <48 hours (drop out).

Bronchoscopy techniques and BAL

Fibreoptic bronchoscopy (FOB) using a large channel bronchoscope was performed on all patients was inserted through endotracheal tubes viaa sterile connector to maintain ventilation during procedure. The FOB was inserted to the chosen bronchial subsegment left subsegment lingula or right middle lobe or the location of sampling was selected on the basis of a chest radiograph. Bronchoalveolar Lavage Balloon Catheter was introduced into the suction channel of the FOB into the selected subsegment and the balloon was then inflated with 1.5–2 ml of air to occlude the subsegmental bronchial lumen. The irrigation lumen of distal plug was then ejected by flushing 2 mL of sterile saline.[26,29,30] Fluid BAL was performed with ten times in a row of 20 mL sterile saline. Finally the fluid BAL was immediately delivered to the microbiology laboratory for quantitative bacterial culture, neutrophil and s-IgA analysis process.

Examination

Secretory-IgA fluid BAL sample was sent to a laboratory for analysis is introduced into theice bucket standard delivery research laboratory specimens, immediately, centrifuged2500g10min, the supernatant was taken, enter into thesamplecup3@0.3cc. Supernatant (give identity, name, date, and type of examination) immediately freeze and store in the refrigerator-70^{o}C, fluid samples can last up to6months. Culture, and sensitivity test performed on microbiology and neutrophils analysis in laboratory pathology clinic Adam Malik Hospital.

Reagent Kit for the examination of s-IgA human is used Elisa, a product Immundiagnostik AG, Stubenwald-Allee8a, D64625Bensheim, Cat: K8870, Lot: K8870-120817, ED: 31-05-2014. Standard rangeis22.2 to 600,000ng/ml, the limit ofdetection: 13.4ng/ml, dilution factor: 1000x. Kitis used exclusively for research(for research use only, not for use indiagnostic or therapeutic procedures). Principles Examination using the technique of quantitative sandwich enzyme immuno-assay to measure secretory IgA. In the first incubation step, sIgA in the sample will be bound by polikonal antibody (rabbit anti-human IgA), which is found on the surface of microtiter wells. To eliminate the substance is not bound, washing steps performed. In the second incubation phase, conjugate solution was added which specifically identify secretory IgA binding. After washing steps back, to remove particles that are not bound, substrate solution is added and incubated back. Acidic stop solution was added to stop the reaction. Changes color from blue to yellow occurs. The intensity of the yellow color of the measured concentrations of secretory IgA describe.

Statistical Analysis

Univariate analysis used to determine the sample s-IgA and neutrophil, which consists of average, minimum and maximum values, median, standard deviation. Bivariate Analysis to determine the relationship between two variables. Before performing these tests, the data tested first with the alkaline Kolmogorov-Smirnov test to determine the normality of the data. Then the paired t-test, Mann-Whitney test, Spearman's correlation test and multivariate analysis were performed to determine the relationship of the independent variables with the incidence of VAP by testing at the same variables that have statistical significance in the bivariate analysis, through logistic regression analysis. $p< 0.05$ was considered as a significant.

Result

The characteristics of all subjects, VAP(-) group and VAP(+) group are summarized in table 1. Age, sex, inpatient days and early diagnose using a mechanical ventilator in ICU.

Table 1.characteristic subjects

Characteristic	**All subjects n = 61**	***VAP*(-) n = 28**	***VAP*(+) n = 33**
Age (years)	39.92±14.04	38.89±13.66	40.79±14.52
Sex (%) - Male - female	 41(67.2) 20(32.8)	 20(80.0) 8(20.0)	 21(63.6) 12(36.4)
Inpatient (days)	9.34±7.38	6.82±4.65	11.48±8.58
early diagnose: -head injury -stroke -post op -enchepalopaty -pneumonia	 37 10 8 6 0	 22 3 2 1 0	 15 7 6 5 0
total	61	28	33

In Table 2. After the Mann-Whitney test the scores CPIS on the third day between VAP(-) and VAP(+), *p* value of 0.0001(<0.05), there was significant difference. After the independent t-test between the levels of s-IgA third day on VAP(-) and VAP(+), *p* value of 0.309(>0.05), there was no significant difference average levels of s-IgA third day between the two groups of subjects. While After the independent t-test between neutrophil third day on VAP(-) and VAP(+), *p* value of 0.0001(<0.05), that there are differences in average neutrophil third day curry between the two groups of subjects.

Table 2.The results ofthe examinationCPIS scores(pts), s-IgA levels(ng/ml) and Neutrophils(%) inallsubjects, VAP(-) andVAP(+) onday1and day3

Subjects	**Day 1**	**Day3**	***p***	**hypothesis Test**
CPIS score (pts)				
Allsubjects (n =61)	2.25±1.47	5.61±2.77	0.0001	Wilcoxon test
VAP(-) (n = 28)	1.82±1.31	2.86±1.24	0.0001	Pairedt-test
VAP(+) (n = 33)	2.61±1.52	7.94±0.90	0.0001	Wilcoxon test
S-IgA (ng/ml)				
Allsubjects (n =61)	60752.87±31579.27	88225.14±70664.70	0.008	Wilcoxon test
VAP(-) (n = 28)	64437.29±43225.43	78144.03±35637.32	0.019	Wilcoxon test
VAP(+) (n = 33)	57626.70±16474.05	96778.82±90149.08	0.048	Wilcoxon test
Neutrophils(%)				
Allsubjects (n =61)	62.55±14.10	75.61±17.10	0.0001	Pairedt-test
VAP(-) (n = 28)	61.04±16.28	66.57±16.05	0.111	Pairedt-test
VAP(+) (n = 33)	63.84±12.08	83.28±14.08	0.0001	Pairedt-test

Table 3.Typeof lower respiratory tractpathogensinPatientsUsingMechanicalVentilator >48hours

No.	Pathogenstype	n	%
1	Nopathogens	8	8.97
2	*A. baumanii*	32	42.31
3	*MRSA*	17	21.79
4	*K. pneumoniae*	10	12.82
5	*P. aeroginosa*	7	8.97
6	*Burkholderiacepacia*	2	2.56
7	*E. coli*	1	1.28
8	*Stapylococcusaerius*	1	1.28
total		78	100.00

Based on Table 3, it is known that the sequence of the most four kinds of pathogensin Patients Using Mechanical Ventilator >48hours are *A.baumanii*42.31%, *MRSA* 21.79%, *K. pneumoniae* 12.82%, and *P. aeroginosa*8.97%.

Table 4.The relationships betweenS-IgA andNeutrophils inPatientsUsingMechanicalVentilator >48 hours

Relationships between variables	All subject			*VAP*(+)			*VAP*(-)		
	n	***r***	***p***	***n***	***r***	***p***	***n***	***r***	***p***
S-IgA with Neutrofil	61	-0.299	0.019	33	-0.461	0.007	28	-0.008	0.967

Pearson correlation test

It is seen that in all subjects, the variables-IgA has relationship with neutrophils variable, the number of correlation of-0.299. This correlation value indicates that if the s-IgA decreased, the neutrophils will be increase and the contrast. In the VAP(+), it appears that the variable, s-IgA has relationship with neutrophils variables, the number of correlation of-0.461. This correlation value indicates that if the s-IgA decreased, the neutrophils will be increase. While the VAP(-) there was no correlation inverse between s-IgA and neutrophils (p =0.967), because both variables together in maintaining lower respiratory systimimmunity are summarized in table 4.

Table 5. Results of Kruskal-Wallis test Influence Variable Types of Pathogens to other variables on day3.

Independent Variables	Dependent Variables	p
types ofpathogens	Skor CPIS (VAP)	0.010
	S-IgA	0.049
	Neutrofil	0.227

From the results of the Kruskal-Wallis testis known that only the average variable VAP ands-IgA were differ significantly based on the types of pathogens of the lower respiratory tract. While thetypes of pathogens no significantly to the neutrophils, the types of pathogens do not affect the value of neutrophils, overall value of neutrophils increased, without being influenced by the type of pathogens in detail.

Table 6.Results ofRegression Logistic AnalysisAllVariablesIndependent for VariablesVAP

Variable	*p.*	B	Exp(B) OR	CI 95%
S-IgA	0.073	1.574	4.828	[0.862;27.037]
Neutrofil	0.001	-4.079	0.017	[0.003;0.181]
Patogen	0.045	2.429	11.347	[1.057;121.863]
Constanta	0.022	-5.552	0.004	

The variables-Ig A no effect on the incidence of VAP(p=0.073), but the T-test, unpaireds-Ig A increased significantly in both groupsofVAP(-) and especially in VAP(+),on the day third. In this case thes-IgA are influenced by the type of pathogen, where the value ofs-IgA are varies. While two other variables, based on test results have an impact on the incidence of VAP.

Discussion

This study uses an internal control where the control data taken shortly before exposure to risk factors or before any effects of infection caused by using a mechanical ventilator. Basic data is taken from the patient's geographic data, CPIS scores and fluid samples from the lower respiratory tract with BAL procedure for examination of s-IgA, neutrophils and cultures of pathogens. Furthermore, subjects were observed, recorded, and underwent measurement CPIS scores by researchers, at the appointed time. Observation of the patientis continued until the patient is moved to ward or died. Subjects exposed to the risk factor of a mechanical ventilator, some became VAP(+), while the others are VAP(-).

VAP diagnosis method in this research is to use a score CPIS, a tool to measure the clinical condition of the patient, which is judged by fever, chest X-ray, leukocyte count, oxygen demand, changes of color and volume the sputum. The range of values of 0-12, when the value >6, then declared there VAP(+).[31,32] Several studies have shown that repeated chest X-ray examination of the diagnostic accuracy of more than 68% are generally accompanied by a picture of consolidation nonhomogeneous (air bronchogram).[33] Torres, 2009, stated that the diagnosis of VAP include signs of new or progressive infiltrate on chest radiograph, fever, leukocytosis or leukopeni and purulent discharge. Chest X-ray picture with two of the three criteria mentioned symptoms provide sensitivity 69% and specificity of 75%.[36]Junshan, 2011, a meta-analysis study in several hospitals, 13 research designed with the same method, stating the score CPIS simple and easy to do, score CPIS very helpful for diagnosis, reasonably accurate, can be used to evaluate the success of the treatment, the rate sensitivity of 93% and specificity of 100%.[33]Harde, 2013, scores measuring CPIS to head trauma and neurological subjects who use mechanical ventilation >48 hours, when the score CPIS more than 6 points then performed with a number of mini-BAL 29 subjects, most pathogens are A. baumannii and Enterobacter and summarized that very well score CPIS are used for evaluation of alleged cohorts VAP, reanabel and can be used to decide when given antibiotics and reduction of antibiotics.[32]

Secretory-IgA from the lower respiratory tract subjects of patients using mechanical ventilator. Increased level s-IgA occurred in both groups, but showed no significant difference. This can occur because the levels of s-IgA significantly influenced by varies the patterns of pathogens. In grouping pathogens, s-IgA is highest in patients with non-MRSA, whereas the s-IgA is lowest in the group of pathogens MRSA. Not yet known exactly this phenomenon, whether there is a relationship between these variables, researchers found in previous research literature. Diebel 2009 examines the evolution of bronchial albumin, IgA and IgG levels of sputum in patients with mechanical ventilation in ICU are associated with nosocomial pneumonia, the results showed albumin and IgG showed no significant difference from the group into pneumonia and not pneumonia. But the s-IgA increased during the patient on a ventilator, but the production of s-IgA decreased in the majority of patients who develop into VAP.[18] Daniele 1990, in vitro finding of gram-negative pathogens in nosocomial pneumonia patients had IgA protease activity that causes lysis s-IgA. Destruction of s-IgA by pathogens also affect the function of IgA anti-inflammatory. Gram-negative pathogen Pseudomonas aeruginosa and *Acinetobacter baumannii* can damage cellular effector function of s-IgA, that developed into pneumonia and severity of the disease increases as the uncontrolled inflammatory response.[15]Diebel 2006 observed levels of s-IgA, can be influenced by confounding types of pathogens. Where pathogens can form IgA proteases, which destroyed the s-IgA, but the specific pathogen can stimulate the production of s-IgA increased. Each pathogen causes of its own contribution to destroy the ability of local immunity.[19]

The variable s-IgA all subjects had relationship with variable neutrophils, this correlation value indicates that if the s-IgA decreased the neutrophils will increases, and in contrast. In this study, s-IgA increased after the patient using a mechanical ventilator and increasing after the VAP(+), but this condition is also influenced by the types of pathogen, so that s-IgA in some subjects, especially with MRSA pathogens decreased. S-IgA correlated inversely with neutrophils, it is known that excessive increase in neutrophils can damage lung tissue, worsen lung function and destroy the s-IgA.[24]

Neutrophils in both groups then obtained significant difference, increased significantly in case of VAP(+). The neutrophils as alveol pulmonary immune defense, and also act as a pro-inflammatory in large,

easy-going migration into the alveolar cavity, as the only PMN that can enter into the alveoli and so the response to pathogen infection.[23,25] That neutrophils did not have a significant relationship with the type of pathogen, but descriptively average neutrophil increase in MRSA pathogens, the cause that s-IgA decreased in subjects with the same pathogen. This phenomenon should be done more research. So in this study, showed that the significant increase in the percentage of neutrophils in the incidence of VAP(+), and plays an important role for the worsening.

Incidence of VAP.

Results pattern of pathogens, the four highest in patients using mechanical ventilator are *A. baumannii, MRSA, K. pneumoniae*and *P. aeroginosa*. Variable pathogens has an influence on the incidence of VAP(+), *A. baumannii* the highest number of pathogen isolation, statistically significant role in the incidence of VAP. Joseph, 2010, research colonization of pathogens in patients using mechanical ventilators, beginning the installation of a mechanical ventilator (pre-VAP), showed that there had been colonized by pathogens such as *A. baumannii, P. aeroginosa and MRSA*. He advocated if MDR bacteria colonization has occurred pre-VAP, then extended Antibiotic therapy.[37] While Mai 2007 in central Taiwan Hospital South, finding pathogens MDR *A. baumannii* most, but statistically not increase morbidity and mortality.[12,38]Zaccard 2009 did 399 times BAL, concluded that BAL bilaterally perform better than the unilateral, to get multiple pathogens.[31] While doctors Asian Group, pathogens, approximately 87% were gram-negative pathogens, and the majority were *Acinetobacter baumannii* 39%, *Pseudomonas aeruginosa* 31% and *Klebsiella pneumoniae* 20%.[7]Guler, 2012, pathogens reproduce observed in early-onset VAP, CPIS score (7-9), finding the most consecutive pathogens: *P. aeruginosa, A. baumannii* and *MSSA*.[39] The colonization of number of phatogens are the most frequently encountered and often comes from the oropharynx are pushed when intubation in early mechanical ventilator, which later evolved into the early VAP after 48-96 hours. In addition, VAP is endemic often occurs due to contamination of diagnostic tools or respiratory therapy device as a bronchoscope, a nebulizer, water or air.

Conclusion

In this study which is conducted in subjects inpatient at ICU Adam Malik Hospital, Medan city, Indonesia, summarized:

1. Levels of s-IgA in patients using mechanical ventilator and the incidence of early-onset VAP, experienced a significant improvement changes. But pathogens play an important role in stimulating or destroying the s-IgA. In the MRSA pathogen, s-IgA decreased from baseline.
2. The percentage of neutrophils increased not significantly and controlled in VAP(-), however neutrophils play an important role in the incidence of VAP(+) and deterioration.
3. Type of pathogens in this study are the four highest in patients using mechanical ventilator is *A. baumannii, MRSA, K. pneumoniae* and *P. aeroginosa*, contribute significantly to the incidence of VAP.

Suggestion, do more research to improve the levels of s-IgA lower respiratory tract, such as nutrition and vaccination to improve lung health and the percentage of neutrophils in this study can be used for further research neutrophil development suppressive therapy on the incidence of VAP deterioration.

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