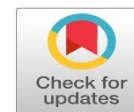*Research Article*

Can nCD64 be used as a marker for VAP diagnosis in mechanically ventilated traumatic brain injury patients and monitoring antimicrobial therapy?

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Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most severe complications in traumatic brain injury (TBI) patients and is considered a risk factor for poor prognosis. The Aim of the work: The primary goal of this study was to assess the role of CD64 in the diagnosis of VAP in TBI in the intensive care unit (ICU) and monitoring antimicrobial treatment, the secondary goal was to detect the prevalence of commonly causing organisms of VAP. **Methods:** This study was conducted on 40 isolated traumatic brain injury patients with the Glasgow Coma Scale (GCS ≤ 8) admitted to ICU. Each patient was subjected to three times sets of samples; at the time of admission, after 3 days of admission, and after 7 days of admission. Routine investigations such as CBC, CRP, urine analysis, and routine blood culture plus standard bacterial culture for endotracheal aspirate (ETA). nCD64 % was evaluated by flowcytometry. **Results:** nCD64 % was significantly higher at the second time sample set when compared with the first and third sample time sets. ETA culture gave positive results in all patients at the first time sample set while, 90% and 85.8% in the second and third time sets respectively. **Conclusion:** nCD64 alone is not a dependable tool for diagnosis and monitoring of antibiotic response in mechanically ventilated patients. Clinical and radiological data collectively with ETA culture results are still the best for diagnosis of VAP in hospitalized patients

Keywords: Ventilator-associated pneumonia; nCD64; traumatic brain injury; endotracheal aspirate

Introduction

Traumatic brain injury (TBI) is defined as brain damage caused usually by an external force. It is the main reason of death and disability in traumatized patients [1]. Patients who have undergone TBI suffer from severe affection of their impaired consciousness level and cellular immunity [2], and may be need tracheal intubation for ventilator support which may increase the incidence of ventilator-associated pneumonia (VAP) [3].

VAP is a pattern of nosocomial pneumonia developed 48 hours or more after receiving mechanical ventilation. Many studies concluded that VAP can

lead to increased mortality and morbidity among their patient, prolonged hospital stayincrease and medical costs [4].

In VAP, ETA samples exploring high bacterial load are considered confirmatory of microbial etiology according to the user guidelines from the Infectious Diseases Society of America [5]

CD64 is expressed on the surface of antigen presenting cells as monocytes, macrophages and dendritic cells but only a small amount is expressed on the surface of neutrophils (nCD64) [6]. Many studies found that CD64 is one of the most useful markers for diagnosis of infection or sepsis close to CRP and procalcitonin [7]. Expression of nCD64 rapidly increases at the onset of bacterial infection, while decline was observed within 48 hours then return to normal baseline levels after about 7 days [8]. So, it could be useful at time of admission for diagnosis of infection and monitoring objective done with serial evaluation [9].

For treatment of pneumonia most of antibiotic protocols depend upon the empirical therapy. However, distribution of causative bacteria and incidence of antibiotic resistance which vary between countries, so, obligatory development of an appropriate antibiotic protocols based on epidemiological data for each place [10].

The current protocol for treatment states using antimicrobials for 7–8 days in VAP patients without any respiratory comorbidities such as pulmonary empyema, lung abscess, cavitation or necrotizing pneumonia to avoid the developing of multidrug resistant strains [1].

The aim of this study was to assess the expression of nCD64 in traumatic brain injury patients with GCS ≤ 8 who mechanically ventilated at time of admission in ICU and verified its relationship with antimicrobial treatment response. Also, to detect the prevalence of causative organisms of VAP in ICU.

Subjects and methods:

This prospective cross-sectional study included 40 patients with isolated traumatic brain injury with GCS ≤ 8 who mechanically ventilated at time of admission to Intensive Care Unit (ICU), Minia Health Insurance Hospital during the period from December 2018 to July 2019.

The required sample size had been estimated to be 40, through sample size estimation for comparison of ratios based on the significance level of 0.05, power of 0.80, and a VAP prevalence of 15% as estimated in previous studies using ClinCalc Sample Size Calculator.

After obtaining written informed consent from their relatives, all patients were subjected to history taking from their relatives with emphasis on history of chronic diseases (chest disease, heart disease, liver diseases, and kidney disease) and history of antibiotics taken or medical center admission at the last 2 months before the study and all patients were subjected to

clinical examination for symptoms and signs of pneumonia.

Each patient was subjected to routine and special investigations for three times set of samples: the first set was done at time of admission (as a basal sample) and the second was done after three days of admission (fulfilling the criteria for diagnosis of VAP) and on empirical antibiotics (Ceftriaxone and clarithromycin) according to ICU protocol for all patients. While, the third set of sampling was done after 7 days of starting of specific antibiotic therapy according to the culture antibiotic sensitivity tests (AST) (7th day of admission) done for only 14 patients, as 26 patients discontinued due to death. Ten apparently healthy individuals (with normal TLC and CRP) were used as a negative control for nCD64 percentage.

Blood sampling protocol: Under complete aseptic conditions, suitable venous blood samples were withdrawn from each patient for the first and second sample sets for routine blood culture using (BACTEC, Becton Dickinson Diagnostic Instrument Systems, Sparks, Md), TLC with differential and nCD64 using flowcytometry (BD FACSCanto™ II, USA) [11]. At the third time set of sample, nCD64 by flowcytometry was evaluated plus other routine investigations.

EndoTracheal Aspirate (ETA): Before collection of endotracheal aspirates, the inspiratory fraction of oxygen (FiO2) was increased to 100% for one minute and, during the procedures, arterial pressure, cardiac frequency, oxygen saturation via pulse oximetry and hemorrhages were observed. Under complete aseptic technique, Intensive care specialist performs tracheal aspiration through passing a new suction catheter into the endotracheal tube as a part of rapid post intubation care. The tip of the suction tube collected into a sterile screw-caped container with the cap tightly secured and sent to the microbiology unit lab for; ETA semi-quantitative culture and sensitivity as ETA specimens were processed using 1-microliter calibrated loops on routine culture media. Colonies for each strain were counted for bacterial concentrations (CFU/mL). Threshold of $> 10^5$ CFU/ml is suggestive of infection rather than colonization [5]. Identification and antimicrobial sensitivity testing were done using (VITEK-2, bioMérieux - France).

Evaluation of nCD64: EDTA venous blood was used within 24 hours of collection. For each sample, two tubes labeled; one for monoclonal antibodies labeled fluorescein iso-thiocynate (FITC) mouse anti-human CD64monoclonal antibody (BD-Bioscience). The other tube was used for negative isotopic control (FITC) Mouse IgG1 k isotype control. monoclonal antibodies were added to respective tubes, lysing

solution were added for each tube, washing by phosphate buffer saline (PBS) solution were added to each tube. By flowcytometry software, cell surface expression of nCD64 was evaluated at wavelength 468nm laser excitation and the emitted fluorescence was monitored with a detector optimized to collect peak emission at 504-541nm. Neutrophils phenotyping was done by gating according to forward scatter (size) and side scatter (granularity) strategy. Results were expressed as percentage of cells positive for CD64. The positivity of CD64 expression was determined to exceed 20% as cutoff and the negative was considered to be lower than this cutoff. [11].

Statistical analysis:

Using SPSS program (Statistical Package for Social Sciences) software version 25. Descriptive statistics were done for parametric quantitative data by mean ± standard deviation and for non-parametric quantitative data by median and interquartile range (IQR). Distribution of the data was done by Shapiro Wilk test. Analyses were done for non-parametric quantitative data using Mann Whitney test between each two groups and Wilcoxon Signed rank test non-parametric quantitative data between each two times. Analyses were done for qualitative data using Fisher's exact test (more than 20% of cells have expected < 5).

Correlations between different variables were done using Spearman's rho correlation coefficient and Pearson's correlation coefficient. ROC (Receiver Operating characteristic) curve analysis was done to determine area under the curve (AUC), optimal cutoff point, sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) and accuracy for prediction of cases. The $p<0.05$ was considered significant.

Results

This study included 40 patients, with mean age (71.5±10.2). The majority of patients (80%) were males, and 60% of patients died during the first week after admission and only 14 patients (40%) completed the study (Table 1). CD64 was significantly decreased in the third sample when compared with the second one with a p-value (0.01). There was no significant difference in CD64 in the first sample when compared with the second one with a p-value (0.23), and there was no significant difference in CD64 of the first sample when compared with the third one with a p-value (0.729), as shown in table (2).

Percentage of CD64 at the optimal cut-off point of >3.4 was detectable. It was found that CD64 has a sensitivity of (100%). While the specificity of CD64 was (50%). The positive predictive value was (80%) and the negative predictive value was (100%), as shown in table (3).

In figure (1a), ETA (endotracheal aspirate) culture results for the 40 patients at the first set of samples shows that the most prevalent microorganisms were Klebsiella+acinetobacter and the least was Enterobacter aerogenes. In the second set of samples, 10% revealed no growth, 20% Klebsiella and 20% Streptococcus viridans. (figure 1b). ETA culture results for 14 patients at the third set of samples revealed no growth in 28.6% and Acinetobacter baumanii in 28.6% (figure 1c). There was no significant difference in the frequency of positive ETA of the first sample when compared with either the second sample or the third one, with p value (0.13) (0.157) respectively, also there was no significant difference of ETA of the second sample when compared with the third one, with p value (0.157) as shown in table (4).

There was significantly higher TLC level during the first sample compared to the second sample with of P value (0.002). Staff cells and CRP was significantly higher in the first sample compared to the second and the third sample. However, there was no significant difference when comparing staff of the second sample with the third one. Segmented neutrophil was significantly higher at the third sample when compared with the first one with P-value (0.005) (Table 5). The study revealed a significant negative correlation between nCD64 and TLC in 2nd sample and 3rd sample with p-value (0.002, 0.032 respectively), while there was no significant correlation between nCD64 and TLC in 1st sample (p-value: 0.145). There was no significant correlation between nCD64 and CRP in all samples. In addition, there was no significant correlation between nCD64 and ETA culture results in 2nd sample and 3rd sample with p-value (0.239, 0.356 respectively), as shown in table (6). The prevalence of multi-drug resistant organisms (MDR) was increasing during a hospital stay. They were 60% of isolated organisms at first ETA culture samples, 65% of 2nd ETA culture samples and 71.4%, of 3rd ETA culture samples as shown in table (7).

Table (1): Demographic data for studied patients

Parameter	
Age (years) range Mean± SD	51-82 years 71.5±10.2
Sex Male Female	32(80%) 8(20%)
Fate Died Complete the study 7 days duration	26(60%) 14(40%)

Table (2): nCD64 % in all patients' samples and control group

CD 64%	1st sample N=40	2nd sample N=40	3rd sample N=14	Control N=20	P value		
					1 vs C	2 vs C	3 vs C
Median IQR	8.7 (5-12.4)	9.7 (7.3-13.2)	9.7 (5.5-10.3)	3.8 (2.8-6.2)	<0.001*	<0.001*	<0.001*
P value 1st vs 2nd sample = 0.23; 1st vs 3rd sample = 0.72; 2nd vs 3rd sample = 0.01							

Table (3): ROC curve results for nCD64

	CD 64
Optimal Cutoff point	>3.4
Area under the curve (AUC)	0.832
95% CI	0.652-0.943
P value	<0.001*
Sensitivity	100
Specificity	50
Positive predictive value	80
Negative predictive value	100
Accuracy	83.3

Table (4): ETA culture results in the three culture sets

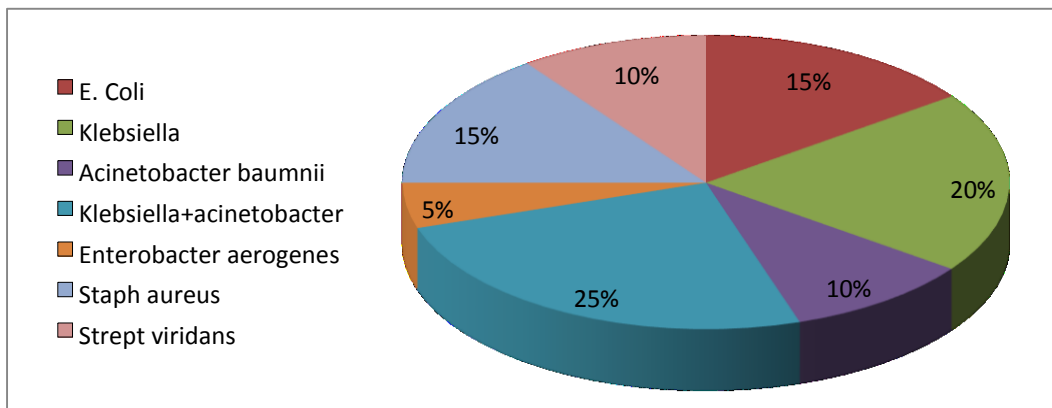| ETA culture	1st sample N=40	2nd sample N=40	3rd sample N=14	P value		
				1 vs 2	1 vs 3	2 vs 3
-Ve *+Ve*	0(0%) 40(100%)	4(10%) 36(90%)	2(14.3%) 12(85.7%)	0.13	0.157	0.157

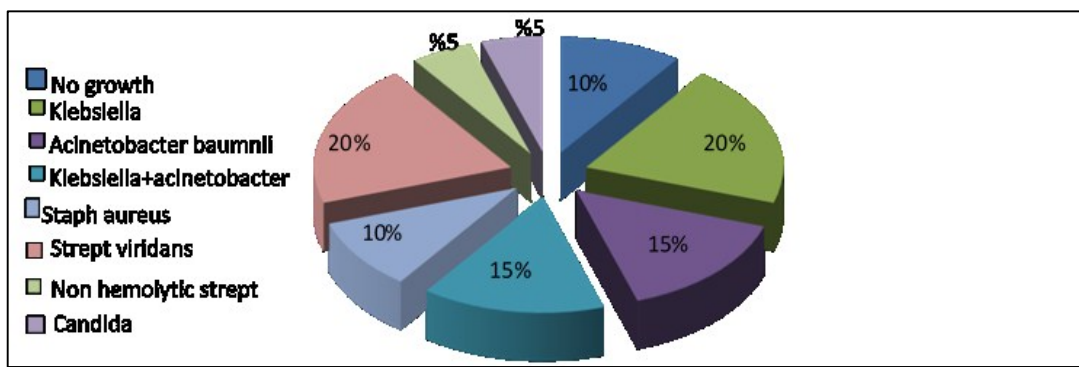**Figure (1a): ETA culture results after the 1st set of samples**

Figure (1b): ETA culture results after the 2nd set of samples
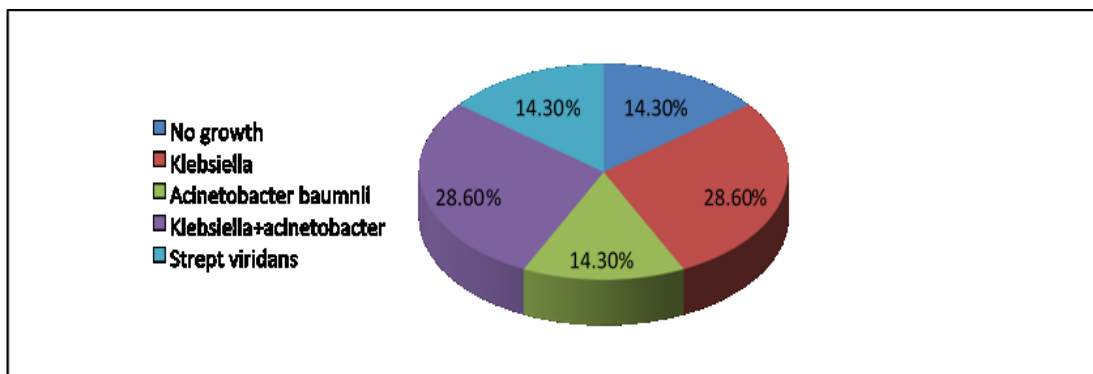

Figure (1c): ETA culture results after the 3rd set of samples

Figure (2) shows the different groups of antibiotics used to the most common gram-positive organisms isolated by culture. Figure (3) shows the different groups of antibiotics used to the most common gram-negative organisms isolated by culture.

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Figure (2): Antibiotic sensitivity testing for Gram-positive bacteria from ETA culture at the three sample sets

Figure (3): Antibiotic sensitivity testing for Gram-negative bacteria from ETA culture at the three sample sets

Table (5): TLC and differential leucocytic count, CRP and CD64 in the three sample sets

Parameters	1st sample Median (IQR) N=40	2nd sample Median (IQR) N=40	3rd Sample Median (IQR) N=14	P value		
				1 vs 2	1 vs 3	2 vs 3
TLC (cell/mm^3)	14.6 (9.4-17.8)	11.3 (6.5-14.6)	12.9 (4.5-16.4)	*0.002**	*0.139*	*0.180*
Staff (cell/mm^3)	5 (3-7)	3 (2-4)	3 (2-4)	*0.001**	*0.015**	*0.557*
Segmented (cell/mm^3)	79.5 (69.3-83.8)	76 (66.3-84.5)	85 (63-90)	*0.271*	*0.005**	*0.241*
CRP (mg/dl)	17 (12-39)	10.5 (4.5-22.5)	6 (4-22)	*0.002**	*0.009**	*0.307*
nCD64%	8.7 (5-12.4)	9.7 (7.3-13.2)	9.7 (5.5-10.3)	*0.237*	*0.729*	*0.012**

Table (6): nCD64, CRP, TLC, and ETA cultures correlation in all three-sample sets

1st sample	nCD64		2nd sample	nCD64		3rd sample	nCD64	
	r	P value		r	P value		r	P value
CPR (P)	0.147	0.367	CPR (P)	0.008	0.959	CPR (P)	-0.416	0.139
TLC (P)	-0.235	0.145	TLC (P)	**-0.471**	**0.002***	TLC (P)	**-0.573**	**0.032***
ETA culture [S]	NA	NA	ETA culture [S]	0.231	0.151	ETA culture [S]	-0.204	0.484

NA: not available

Table (7): MDR percentage among patients' three ETA sample sets

MDR	1st Sample	2nd sample	3rd sample	P value		
				1 vs 2	1 vs 3	2 vs 3
No	16(40%)	14(35%)	4(28.6%)	0.527	0.414	0.9
Yes	24(60%)	26(65%)	10(71.4%)			

Discussion

This study was conducted on 40 patients with isolated traumatic brain injury with GCS ≤ 8 who mechanically ventilated at time of admission to Intensive Care Unit, Minia Health Insurance Hospital. Each patient was subjected to three times sets of samples; at the time of admission, after 3 days of admission, and after 7 days of admission. The primary goal of this study was to assess the role of CD64 in the diagnosis of VAP in TBI in the intensive care unit (ICU) and monitoring antimicrobial treatment, the secondary goal was to detect the prevalence of commonly causing organisms of

VAP. CD64 % was significantly higher at the second time sample set (after 3 days of empirical treatment) when compared with the first (time of admission) and third sample time sets (after treatment with specific antibiotics according to the antimicrobial sensitivity tests (AST). ETA culture gave positive results in all patients at the first time sample set while, 90% and 85.8% in the second and third time sets respectively.

VAP is a concerted iatrogenic pulmonary infection in critically ill mechanically ventilated patients [12].

Quick finding of the causative agent lead to decrease the morbidity and avoid exaggerated or inappropriate antibiotic use that lead to increase the emergence of antimicrobial resistance [13].

Endotracheal intubation may cause injury and suppression the mechanisms of airway clearance [14]. Therefore, the patient's lower respiratory tract (LRT) is at high risk of bacterial colonization or infection [15].

Specimens as ETA is an ordinarily used specimen for diagnosis of lower respiratory tract infections with relative insensitivity and minimal specificity for bacterial diagnosis [16]. ETA can be useful for identification patients with high risk for developing VAP [17]. The source of pathogens causing VAP in mechanically ventilated patients can be significantly due to bacterial colonization of ventilator loop [18].

In the present study, 40 patients, 32(80%) males and 8(20%) females admitted to ICU at Minia Health Insurance Hospital with higher frequency among males, which may be referred to behavioral habits such as smoking among males. These results were in similar to the results obtained by Vijay Dalela [19], who found that, among of 200 patients with LRTI 66% were males, and 34% were females.

Liu et al. [20] have shown that smoking was the strongest predictor of VAP development. They found that the incidence of VAP in smoker was 4.37 times that of VAP in non- smokers. This is because the long-term smoking leads to suppression of pulmonary macrophage function resulting in decreased bacterial clearance, which makes the lungs more susceptible to pathogenic strains.

Three times sets of samples planned to obtained, after exclusion of bacteremia and UTI, but due to death fate, only 14 patients completed the third set of samples. In the 1st time set of samples (40 patients), routine ETA culture was done before starting empirical treatment, and revealed colonization by Klebsiella pneumoniae and Acinetobacter baumanii in 10 patients (25%) were the most predominant organisms. These results were in concomitant with Lu et al. (21), who found that Klebsiella and Acinetobacter baumanii were the most prevalent isolates in a study conducted on 25 neonatal VAP patients referring to the nosocomial nature of these pathogens especially in ICUs.

On the other hand, these results were in disagreement with Kabak et al. [22] who found that staph. aureus was the most prevalent isolates in (56.8%) from ETA samples of 125 VAP patients, and this may be explained by long hospital length, time on mechanical ventilation, mortality, and the time of VAP onset development which may influenced by colonization pattern. Also these results of the current study were mismatched with Khan et al. [23], who found that, Streptococcus pneumonia was the most predominant pathogen in LRTIs specimens (51.7%), followed by Staphylococcus aureus (48.3%), and this may be referred to variation of bacterial distribution in geographic areas.

Both Klebsiella and Acinetobacter baumanii, in the 1st time set of samples, were MDR isolates as they were resistant to many antimicrobials as β-lactam antibiotics, aminoglycosides and quinolones. Two isolates were sensitive to Meropenem, this was in agreement with a study conducted by Hadiseh Hosamirudsai [24], on 132 patients in ICU, found that 72.7% of isolates were MDR and this predominance of MDR isolates may explained by prolonged antibiotic use, long ICU stay and prolonged intubation time.

Results of the 2nd time set samples (on 40 patients) and after empirical treatment with (Ceftriaxone and clarithromycin) for 3 days. Streptococcus viridans was the most predominant pathogen isolated in 8 patients (20%) (True infection not colonization as signs and symptoms suggesting LRTIs, poor immunological state, increased inflammatory markers as CRP and TLC plus the significant colony count). Strept. viridans were sensitive to both linezolid and vancomycin and resistant to penicillin and oxacillin. These results were in agreement with Laurens. et al [25], who also demonstrated that strept. viridans were the most prevalent in ICU patients. Moreover, this explained by changing intubation of ventilated patients after 3 days of mechanical ventilation, which may displace most of upper respiratory tract normal flora during intubation. In parallel with sterpt. viridans, Klebsiella pneumonaie was also isolated in the 2nd set of samples in 8 patients (20%). Although Klebsiella pneumonia is considered as an upper respiratory tract colonizer, it was considered a significant pathogen as its presence was accompanied by persistent clinical manifestations and/or

significant colony count. The unchanged number of isolated Klebsiella pneumoniae in the second set after using empirical treatment, and the increased number of isolated Acinetobacter (15%) at the 2nd set of samples, denoting poor response to the empirical antibiotic treatment.

Only 14 patients completed the 3rd time set of samples and their results were; Klebsiella pneumoniae was the most predominant organism isolated from ETA cultures (28.6%), similarly coinfection with Klebsiella pneumoniae and Acinetobacter baumanii was (28.6%), and both of them were MDR and sensitive to meropenem. These results were in concomitant with Khanal. et al [26] who found that among of 167 bacterial isolates isolated from 187 tracheal aspirate samples Acinetobacter spp. (32.9%) and Klebsiella pneumonia (25.1%) were the predominant isolates with 68.8% of these isolates were MDR. Explanation of this, referred to the nature of these two pathogens as a major colonizer of oropharynx, aspiration with contaminated secretions and poor immune response of those patients. The predominant organisms varied among the three sets of samples, and this may be related to the poor infection control measures at ICU, low immune state of the patients and the inappropriate usage of empirical treatment. In addition to accession of new pathogens along hospital stay.

Regarding nCD64 results in this study, there was a slight increase of nCD64 %, in all cases in the three sets, in comparison to control group but not reaching the threshold of good positive percentage [ranging from (8.7- 9.7%), +ve threshold >20%]. This may have explained by the poor immune response of patients, absence of bacteremia that is a major cause for high nCD64, in addition to the nature of nCD64 expression, which dramatically decreased within 48 hours, and be back to normal levels within 7 days and thus antibiotic umbrella throughout the current study, could affect nCD64 expression levels.

Results of nCD64 percentage show non-significant difference at the 1st time set of samples when compared with the 2nd set with a p-value of (0.237) indicating poor effect of the empirical antibiotics in get rid of infections. nCd64 % was significantly increased at the 2nd time set when compared with the 3rd set with a p-value of (0.012). This may be referred to the better effect of the specific antibiotics therapy

that can significantly affect the expression of nCD64 and this was in agreement with Gros et al., [27] who found the same effect of antibiotic use on nCD64 expression. nCD64 results show a high sensitivity rate (100%), NPV (100%), poor specificity (50%) and PPV (80%).These results were in agreement with Rudensky., et al [28] who reported that nCD64 have a higher sensitivity (94.7%), with poor specificity (46.5%). Also in studies conducted by Dilli et al. [29] and Ng et al. [30] found similar results of high sensitivity and low specificity of nCD64 in neonates with early onset pneumonia. This could be explained by rapid expression of nCD64 in the presence of stimulating factors like bacterial cell wall components, IFN-γ, and GCSF. nCD64 expression will decrease ultimately within 48h and be back to normal baseline levels after 7 days if these stimulators were removed or after efficient antibiotic therapy [31]. There was no significant correlation between nCD64 results and TLC in the 1st set of samples, that was in concomitant with Bassunoi et al [32] who found that there were no significant correlations between TLC and nCD64 expression in early onset neonatal sepsis.

This study revealed that there was no significant correlation between nCD64 results and CRP titers in all sample sets. Also, there was no significant correlation between nCD64 and ETA culture results in 2nd sample and 3rd sample with a p-value of (0.239, 0.356 respectively), and this may be assigned to the nature of nCD64 expression in case of bacterial infection and the absence of bacteremia.

Conclusion

In critically ill patients, with traumatic brain injury on mechanical ventilator in ICU, Using of ETA cultivation, routine inflammatory markers and clinical presentation are dependable tools for VAP diagnosis; and nCD64 percentage was a useful tool for diagnosis VAP and monitoring of antibiotic response.

Limitations

Firstly, Tracheal aspiration was done using a sterile catheter for suctioning but it more likely to collect biofilm-forming organisms colonizing the catheter plastics, so elusive results suggesting the patient has pneumonia caused by one of these colonizing pathogens. Secondly, saline effect on specimen quality will

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vary depending on transport time and rapid processing which may damage or kill certain fastidious pathogens over time. In addition, saline leads to bacterial dilution inside the specimen, which could affect in culture results. Thirdly, the small sample size and collection from single medical center.

Recommendations:
Bronchoscope usage for sample collection is superior to tracheal aspiration. Large multi-center projects with randomized controlled trials are ensured to confirm and expand understanding the role of risk factors for the VAP development. Find out patients with high risk of VAP development and exploration of early administration of antimicrobial therapy. Lastly, changing the used empirical treatment protocols according to culture and sensitivity results in each hospital is highly recommended.

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