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Procalcitonin and Proinflammatory Cytokines in Early Diagnosis of Bacterial Infections after Bronchoscopy

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Abstract

BACKGROUND: Fiberoptic bronchoscopy (FOB) guided bronchoalveolar lavage (BAL) remains as the chief diagnostic tool in respiratory disorders. 1.2-16% of patients frequently experience fever after bronchoscopy. To exclude the need for multiple antibiotic prescribing in patients with post-bronchoscopy fever, the presence of the self-limiting inflammatory responses should be excluded.

AIM: The current study was conducted to test the serum of patients undergoing bronchoscopy for some proinflammatory cytokines including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1beta (IL-1β), Interleukin-8 (IL-8) and Interleukin-6 (IL-6) and the value of Procalcitonin (PCT).

MATERIAL AND METHODS: Current case-control study was conducted at the National Research Institute of Tuberculosis and Lung Disease in Iran. Nineteen patients (48.72%) that attended with a reasonable sign for a diagnostic bronchoscopy from January 2016 to December 2017 were included in the case group. The control group consisted of 20 patients who underwent a simple bronchoscopy and without FOB-BAL. The laboratory findings for PCT concentrations and cytokine levels in the three serum samples (before FOB-BAL (t0), after 6 hr. (t1), and at 24 hr. past (t2) FOB-BAL) were compared between two groups.

RESULTS: The frequency of post-bronchoscopy fever was 5.12, and the prevalence of post-bronchoscopy infectious fever was 2.56%. PCT level was considerably higher in the patient with a confirmed bacterial infection when compared to other participants (p-value < 0. 05). Interestingly, IL-8 level in the bacterial infection proven fever patient was higher than in other patients ($p < 0.001$). IL-8 levels displayed a specificity of 72.7% and a sensitivity of 100%, at the threshold point of 5.820 pg/ml. PCT levels had a specificity of 84% and a sensitivity of 81%, at the threshold point of 0.5 ng/ml.

CONCLUSION: The present findings show that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. The current findings also illustrate that to monitor the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy.

Introduction

In numerous pulmonary and respiratory invasive techniques such as bronchoscopy are required to be applied. Bronchoscopy is generally a well-tolerated technique by most patients [1]. However, rare side effects such as severe arrhythmia, bleeding, pneumonia or pneumothorax are observed. Serious complications of bronchoscopic infections include spreading the infectious agents from one patient during bronchoscopy, transferring to the next procedures. Treatment for an invasive bacterial or viral infection is mandatory in such cases [2], [3].

However, one-third of patients develop fever, and sepsis-like syndrome after fiberoptic bronchoscopy (FOB) guided bronchoalveolar lavage (BAL) for yet unknown reasons and as a systemic

inflammatory response [2], [3], [4]. Therefore, patients with post-interventional fever should undergo further evaluations for observing the exact reason for the fever. The standard gold technique to detect systemic bacterial infection in patients with fever after bronchoscopy is blood cultures. However, such microbiological workup is time-consuming. In recent years, the usage of circulating proinflammatory mediators such as C reactive protein (CRP) and Procalcitonin (PCT) as alternative rapid predictive parameters have been broad [5], [6], [7].

In patients with sepsis serum levels are TNFα, IL-1β and IL-6 are increased [8]. Moreover, PCT is evaluated in response to intermediates or endotoxins released against bacterial infection (IL -6, TNF-α, IL-8, IL-1β), and is strongly associated with the amount and severity of bacterial infections.

Procalcitonin (PCT), a calcitonin hormone precursor, is produced by C cells in the thyroid gland, or by neuroendocrine cells in the lung and intestine, and its serum levels in healthy individuals are less than 0.15 ng/ml [7]. Expression of Procalcitonin is related to IFNγ, which is the released cytokine in response to viral infections is reduced. Therefore, PCT is a specific indicator of bacterial infections, and it may help to differentiate bacterial infections from viruses. PCT with a half-life of 25 to 30 hours has been described as a predictor of disease severity and antibiotic efficacy. Procalcitonin levels increase significantly in bacterial infections. In cases of viral infections or non-infectious febrile illnesses, procalcitonin levels are low or normal [9].

The production of PCT is associated with inflammation in response to inflammatory cytokines, which is characterised by a rapid increase in PCT levels. Endogenous systemic inflammatory responses stimulate alveolar macrophages to release increased cytokine concentrations. Some well-known inflammatory cytokines include Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1beta (IL-1β), Interleukin-8 (IL-8) and Interleukin-6 (IL-6) [10].

In inflammatory reactions, TNF increases and reaches its maximum value in 90 minutes. While IL-6 reaches its maximum value in 180 minutes. The levels of PCT in sputum and serum elevate only after 3 to 6 hours of inflammation and reaches to its highest peaks at less than 6 to 8 hours, following a pattern similar to that of acute bacterial infection [11].

In previous studies, non-pneumonia subjects, with a rise in temperature above 1°C, were compared to subjects without increasing the temperature after BAL. The non-pneumonia group showed an elevated serum PCT and IL-6 levels that were secreted within 12 hours and was resolved after 24 hours [5].

Considering the heterogeneous and scattered reports of changes in serum proxlotonin levels and inflammatory markers after bronchoscopy and BAL, it seems that further research is required in a non-randomized clinical trial in this regard. The current study was conducted to research this field.

Hence, in the current approach, we tested the prevalence of patients with fever because of infection after FOB-BAL at the interventional pulmonology ward. Next, to clinical signs and symptoms, we assessed PCT value in the serum sample, in three intervals. In parallel, to check the self-limiting inflammatory responses and to exclude the need for antibiotic prescribing in patients with post-bronchoscopy fever, we assessed the concentrations of the proinflammatory cytokines TNF-α, IL-1β, IL-8 and IL-6 in serum.

Material and Methods

Study Design

The current case-control study is a performed approach at the interventional pulmonology ward, National Research Institute of Tuberculosis and Lung Disease (NRITLD) of Iran. The research committee and the ethics of the Shahid Beheshti University of Medical Sciences (IR.SBMU.NRITLD.REC.1396.375) confirmed stages of the study. An expert methodologist estimated the least sample size of 20 individuals in each of case and control groups by taking the statistical assumptions and formula [12].

We screened every patient that attended with a reasonable sign for a diagnostic bronchoscopy during January 2016 for inclusion and exclusion criteria. We entered patients if they were above 18 years old and with no infectious diseases or pneumonia. We excluded individuals with cardiac arrhythmia and acute from the study. We included the patients with an urgent diagnosis of bronchoscopy in the case group. Patients who did not need immediate diagnostic bronchoscopy included in the control group. Hence, none of the patients experienced bronchoscopy without a necessary reason.

After receiving signed consent letters, thirty-nine patients with respiratory disorders joined the current approach. The case group comprised 19 patients (48.72%) an immediate need for diagnostic FOB-BAL. A group of 20 selected patients with precise diagnosis and no need for diagnostic FOB-BAL (51.28%) entered the study as the control group.

We documented the demographic and laboratory information and recorded axillary body temperature before FOB-BAL (t0), after 6 hr (t1), and at 24 hr past (t2) FOB-BAL. We documented fever when the body temperature was ≥ 38°C. Data collection including age, sex, the cause of bronchoscopy, underlying disease, corticosteroid use, antibiotic use, body temperature, performed laboratory tests findings before the study and the laboratory

findings at 6 and 24 hours after the intervention. Serum culture was performed to evaluate the bacterial contamination following bronchoscopy.

In this study, we considered positive fever patients with positive serum culture and concurrent elevation of PCT serum levels, as the pneumonia cases. The laboratory findings for PCT concentrations and cytokine levels in the three serum samples (before FOB-BAL (t0), after 6 hr. (t1), and at 24 hr. past (t2) FOB-BAL) were compared between two groups.

Blood sampling and FOB-BAL

Three arterial blood samples were collected in sterile and with no additive tubes before, 12 hr and 24 hr after BAL. After centrifuging at 3.56 G for 10 min, we stored serum at -70C° for further use on processing day. We performed BAL, along with the former guideline using a flexible fiberoptic bronchoscope (Olympus; Tokyo, Japan) [13].

Laboratory Assay

Solid phase enzyme-linked immunosorbent assay (ELISA) (Biosource, Camarillo, CA) detected plasma concentrations of cytokines based on the earlier protocols [14]. We assessed serum PCT value using Electrochemiluminescence immunoassay (ECLIA) method (Boditech, South Korea) based on the company instructions. We considered elevated Serum cytokine value when it was above the upper limit value of the control group (≥ 5 pg/ml for IL-6; ≥ 20 pg/ml for TNF-a ≥ 15 pg/ml for IL-1b; ≥ 29 pg/ml for IL-8). PCT serum values above 0.5 ng/ml defined positive values and a bacterial infection in the patient.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) (ver. 22.0; SPSS Inc. Chicago, IL, USA) software regulated the statistical analysis. Frequency, percentage, means (± standard deviation) or median (least- greatest) expressed the continuous variables. Also, for categorical variables, frequencies and percentages were the presented results. Spearman's rank correlation coefficient test showed the correlations between limits. Nonparametric Mann-Whitney U-test compared the comparisons between groups with continuous variables. Otherwise, the chi-square test analysed the comparisons between groups with categorical variables. Student's t-test and the Kruskal-Wallis test represented differences in the mean with a p-value of < 0.05 for a significant value.

Results

Demographic features

Table 1 represents the baseline demographic features in the study case and control groups. The mean age in the case group was 56 ± 16 (25 to 84 years) and 49 ± 15 in the control group (25-81 years).

Table 1: Baseline characteristics of the study patients

Characteristic	All patients	Case group	Control group
Number of patients, n (%)	(n = 39)	(n = 19, 48.72%)	(n = 20, 51.28%)
Age (yr), mean ± (SD) (interquartile range)		56 ± 16 (25-84)	49 ± 15 (25- 81)
Gender, n (%)	Male	12 (30.8%)	7 (17.9%)
	Female	7 (17.9%)	13 (33.3%)

The gender of the studied population was; 19 male (48.7%) and 20 female patients (51.3%). Two groups of case and control were similar in the frequency's distribution on the sex of participants (Pearson Chi-Square, p-value = 0.079). Post-bronchoscopy fever developed in two of 39 patients (5.12%) (Table2).

Table 2: The prevalence of post-bronchoscopic fever in study subjects

Body temperature (°C), mean (SD)	
Before bronchoscopy	36.8 (0.38)
After bronchoscopy, 6 h	37.4 (0.71)
After bronchoscopy, 24 h	36.9 (0.37)
Fever after bronchoscopy (Temp ≥ 38 °C), n (%)	2 (5.12)

The indications of FOB-BAL were for diagnosis of COPD in four cases, suspected of tuberculosis in one case, Lung cancer in 6 cases, Tracheostomy in one case and respiratory hypertension in seven subjects.

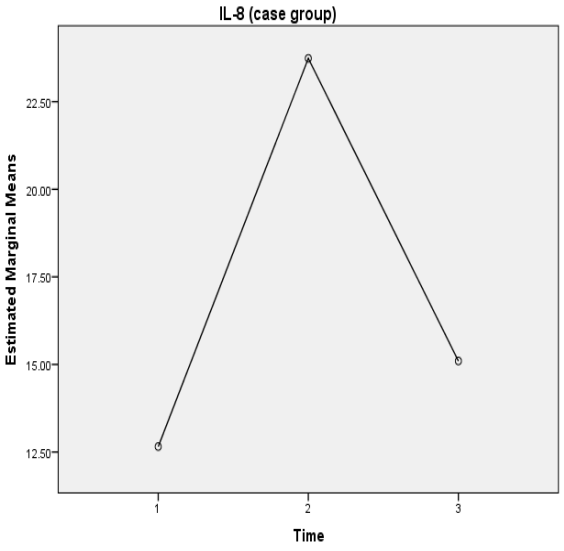

*Figure 1: Differences in IL8 concentration of case group during three intervals (P-value = 0.006); *: variance by Greenhouse-Geisser test was applied*

The concentration of IL-8, patients in the case

group, presented significantly increased, when compared with the control cases (254.2 vs 1731.5 pg/ml P < 0.0001). Concentrations of IL-8 significantly increased, during the three episodes of sampling (P-value = 0.006) (Figure 1). Elevation of IL8 did not relate to gender (value of P = 0. 833) (Figure 2).

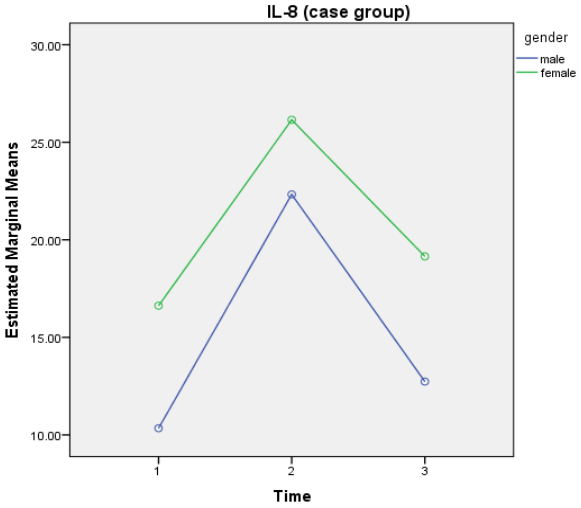

Figure 2: Correlations of IL8 concentration in three samples with sex in the case group (P-value=0. 833)

IL-6 concentrations have increased in the second specimens and reached the normal value in the third specimen. The increasing value of IL-6 in the second sample in the case group differed significantly from the mean value of IL-6 in the control group (19.38 vs 6.28 pg/ml P-value = 0.023, Mann-Whitney U test was applied) (Figure 3).

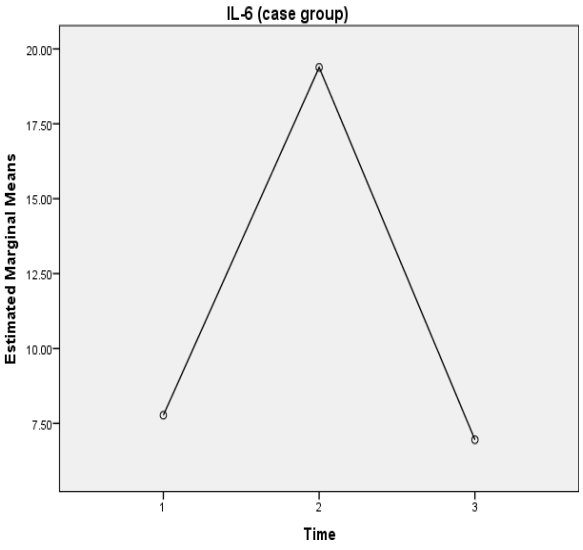

Figure 3: Differences in IL-6 level of case group in three samples (P-value = 0.023)

However, IL-6 levels did not represent significant differences in subjects with fever and without fever. Elevation of IL-6 did not relate to sex (value of P-value = 0. 593) (Figure 4). Also, in case patients with a temperature above 1°C after BAL, non-pneumonia and pneumonia subjects represented an increase in levels of interleukin 6 with no significant differences.

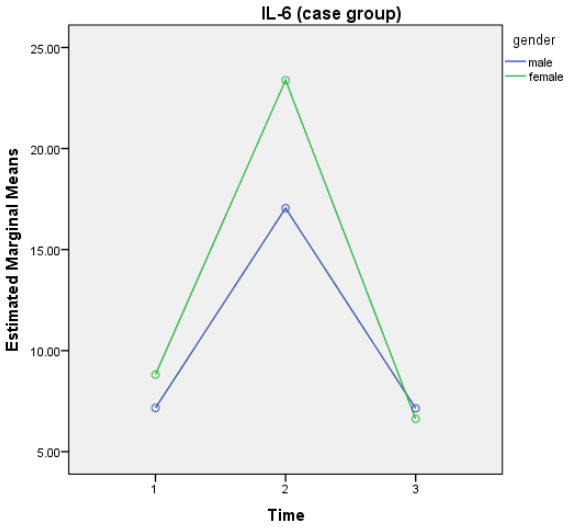

Figure 4: Correlations of IL-6 level in three samples with sex in the case group (P-value = 0. 593)

Levels of TNF-α and IL-1β decreased in the case group compared to the control group. IL-1β levels significantly decreased in the case group. However, this trend was not significant for TNF-α (P-value = 0.032 Mann-Whitney U test, P-value = 0.136, Mauchly's Test of Sphericity, respectively). Serum levels of TNFα and IL-1β were not related to the gender of the subjects (P-value = 0.833, P-value = 0.796 respectively) (Figure 5).

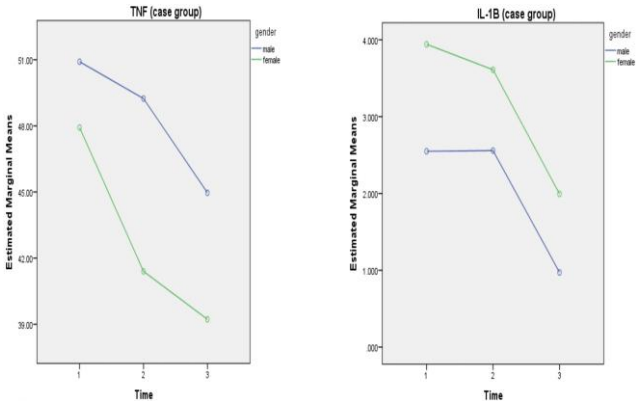

Figure 5: Correlations of TNF-α and IL-1β serum levels in three samples with the gender of the subjects (P-value = 0.833, P-value = 0.796 respectively)

Reduction of IL-1β level in the third sample was significantly different between the two groups (1.34 vs 3.40 pg/ml, P-value = 0.21). Also, TNF-α level was significantly different in all three samples of the case group compared to the control group (P-value < 0.001, Mann-Whitney U test).

Of two cases with fever after FOB-BAL, only one subject (5.3% of patients in case group) represented an elevated level of PCT in t2. PCT

represented none significant differences in the case group, neither regarding the gender of the participants or the interval of the sampling. According to the results, PCT values were normal in all 20 participants of the control group and every 3 samples of each. Laboratory findings for serum procalcitonin levels are presented in Table 3. Pearson correlation coefficient of PCT level and smoking did not represent a significant linear relationship. There was no relationship between the duration of bronchoscopy and PCT levels in any of the participants.

Table 3: Laboratory findings for serum procalcitonin levels

	Min.	Max.	Mean	Standard deviation
Procalcitonin	0.10	23.00	1.29	3.53

Current results represented that the levels of PCT in the fever positive patients with high levels of IL-6 and IL-8 are significantly higher than in patients with fever alone or in patients with isolated elevation of serum IL-6 and IL-8 protein levels (P < 0.01).

ESR levels represented an increase at t1 and reached the normal level at t2. The mean ESR level was not significantly different between the two cases and control groups at any of the three sampling times (P-value = 0.328, Mann-Whitney U test) (Figure 6).

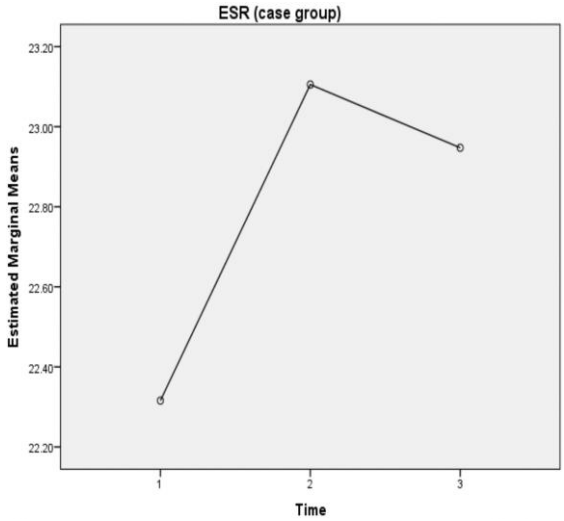

*Figure 6: ESR level in case group during three intervals (P-value > 05); *: Mann-Whitney U test*

Variations of ESR levels were similar in women and men of the case group (p-value = 0.651). As well, changes in ESR levels of three samples did not correlate with the sex of participants in the case group (p-value > 0. 05). Table 4 represents the laboratory findings of the study population.

Table 4: Laboratory findings of the study population

Group		N	Mean	Std. Deviation	p-value
ESR Time1	case	19	22.3158	10.53593	0.163*
	control	20	18.4500	6.05653	
ESR Time2	case	19	23.1053	10.51398	.072*
	control	20	18.4500	6.05653	
ESR Time3	case	19	22.9474	10.01928	.091*
	control	20	18.4500	6.05653	

*: Mann-Whitney U test.

The evaluated mean procedure time was 20.22 ± 5.62 minutes (15.00-30.00). The mean procedure time was not significantly different between the two cases and control groups and fever and unfevered cases (p-value > 0. 05).

The frequency of post-bronchoscopy fever was 5.12%. Microbiological analysis was positive for *Pseudomonas aeruginosa* in the serum culture of one subject (2.56%). Therefore, the percentage of unspecific fever after bronchoscopy was 2.56%.

PCT level was considerably higher in the patient with a confirmed bacterial infection when compared to other participants (p-value < 0.05). Interestingly, IL-8 level in the bacterial infection proven fever patient was higher than other patients (p < 0.001).

There was a moderate relationship between PCT and IL-6 concentrations in the fever cohort (Spearman rho = 0.463; p ≤ 0.001). However, there was no association between PCT and TNF-α or IL-1β, (p-value > 0. 05). We also found a strong correlation between elevated procalcitonin results with elevated IL-8 levels in the patient with positive serum culture when compared to the patients with negative culture, using exact Fisher test (P Value < 0.01).

At the threshold, the point of 3.706 pg/ml IL-6 concentrations showed a sensitivity of 100% and a specificity of 71.1%. IL-8 levels displayed a specificity of 72.7% and a sensitivity of 100%, at the threshold point of 5.820 pg/ml. Moreover, PCT levels had a specificity of 84% and a sensitivity of 81%, at the threshold point of 0.5 ng/ml. Finally, the best time to figure the diagnostic levels of procalcitonin and proinflammatory cytokines to predict a bacterial infection after bronchoscopy was 24 hours after the bronchoscopy.

Discussion

While a post-interventional fever can be due to bacterial or viral contamination, the use of antibiotics in such cases has been a matter of debate for years. Although prescribing antibiotics based on the observed fever after bronchoscopy is simple and practical, many authors believe that using these criteria results in antibiotic overuse, antibiotic-related adverse reactions and antibiotic-resistant bacteria without a thorough microbiologic study. On the other hand, serum culture and microbiologic studies are time-consuming and sometimes expensive.

PCT is a valuable alternative marker to diagnose bacterial infections because its serum values elevate as early as 3 to 4 hours after infection, much faster than other inflammatory markers such as ESR and C-reactive protein [15]. Current results show

a 5.12% frequency of post-bronchoscopy fever that is in line with earlier reports, presenting a prevalence of post-bronchoscopy fever in 1.2 – 16% of patients [2], [16].

Procalcitonin has also been a well-recognised marker for antibiotic therapy since 1993 [17], [8].

Bronchoscopy-BAL results in a vast acute phase reaction, such as peripheral neutrophilia and raised values of CRP [18]. About changes in IL-6 concentration, the findings of the current study were consistent with the results of previous studies [19], [20]. In the study by Krause et al., on 50 patients with and without BAL, IL-1β and IL-6 showed an elevation within 6 hours of operation [21].

With the concentrations of IL-8, the patients in the study group represented a significant elevation compared to the control group ($P < 0.0001$). This is in opposition to the reports by Huang et al. This contradiction is because of the differences in the population studied in two studies. Huang et al. observed over 50% elevation in neutrophils and over 7 times elevation in CRP value after bronchoscopy. However, they reported no changes in serum concentrations of IL-8 post-bronchoscopy. They performed their study on 28 healthy subjects to investigate the natural effects of Bronchoscopy with BAL [18].

In the current study, TNF-α concentrations elevated to a high peak at 24 hr. After bronchoscopy and decreases to an undetectable value by 48 hr. [18]. Here we applied the three collection steps of serum samples because of serum. TNF-α concentrations are detectable as early as 4 hr. after bronchoscopy [18]. Current results show that the prevalence of post-bronchoscopy infectious fever was 2.56%. The current findings like earlier studies emphasise on broader sterilisation to remove transmission of nosocomial infection during bronchoscopy operations [22].

In former studies, PCT was not reported as a valuable postoperative interpreter in immediate postnatal [23]. On the other hand, it was reported as a highly sensitive biomarker in the prediction of the severe community-acquired pneumonia [24].

However, the present study showed that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. The current findings illustrate that to check the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy. Some limitations of the study may be the lack of investigation of the impact of local anaesthesia. Therefore, we recommend further studies on this topic with some larger sample size and inclusion of different local anaesthesia to merge the findings of this study.

In conclusion, the present findings show that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. So, we recommend considering the results of procalcitonin besides the results of routine tests, in the protocol to start the antibiotic administration after bronchoscopy to reduce unnecessary antibiotic use in non-pneumonia individuals. The current findings also illustrate that to monitor the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy.

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