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Sputum procalcitonin – a potential biomarker in stable bronchiectasis

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Summary at a Glance: Our study demonstrated that sputum procalcitonin levels were higher in patients with stable bronchiectasis compared to healthy-controls. It is also a repeatable measurement in both spontaneous and induced sputum specimens. Sputum procalcitonin has the potential to be a biomarker of airway inflammation and infection in bronchiectasis

ABSTRACT

Introduction/Aim – Sputum procalcitonin has been demonstrated to be elevated in exacerbations of bronchiectasis. The primary aim was to investigate whether sputum procalcitonin levels were higher in patients with stable bronchiectasis compared with healthy-controls. We also assessed differences in procalcitonin levels in spontaneously expectorated and induced sputum samples and their repeatability one week later.

Methods – Participants included were aged over 18 years and had either radiologically confirmed bronchiectasis or were healthy-controls. Patients with bronchiectasis were clinically stable for at least six weeks and had both spontaneous and induced sputum collected at visit one and again, seven days later. Only induced sputum samples were collected from healthy-controls during visit one. Sputum procalcitonin concentrations in sputum were measured.

Results – Thirty patients with bronchiectasis and 15 healthy-controls were enrolled in this observational study. In the pooled data from visit 1 and 2, the geometric mean procalcitonin level in induced sputum was significantly higher in the bronchiectasis group than in the healthy-control group (1.5ng/mL [95%CI 1.0-2.1] versus 0.4ng/mL [95%CI 0.2-0.9], mean ratio: 3.6 [95% CI 1.5- 8.6], *p=0.006*). Mean procalcitonin level was higher in spontaneous sputum than in induced sputum at visit 1 (1.8ng/mL [95%CI 1.2-2.7] versus 1.1ng/mL [95%CI 0.7-1.8]) and visit 2 (1.5ng/mL [95%CI 1.0-2.5] vs 1.2 ng/mL [95%CI 0.8-1.6], *p-value=0.001*). Repeating spontaneous and induced sputum procalcitonin levels one week later produced similar concentrations (p-*value=0.29*; intraclass correlation co-efficient (ICC)=0.76 and *p-value=0.72*; ICC=0.70 respectively).

Conclusion – Sputum procalcitonin is increased in patients with stable bronchiectasis and has potential as a biomarker of airway inflammation and infection in bronchiectasis.

Keywords: Bronchiectasis, Procalcitonin, Infection, Biomarker, Sputum

INTRODUCTION

Bronchiectasis is a chronic, debilitating disease characterised by productive cough, dyspnoea and repeated respiratory infections (1). Exacerbations often require admission to hospital and prolonged courses of intravenous and oral antibiotics (2). These factors adversely impact on quality of life and survival (3-6).

Procalcitonin is a prohormone for calcitonin that is normally secreted by cells in the thyroid (7). Bacterial infection induces gene expression and release of procalcitonin from many tissues throughout the body, including the lungs (8, 9). Previous studies have demonstrated that procalcitonin is not dependent on release from white blood cells; and parenchymal cells have been suggested as the main source of extrathyroidal procalcitonin expression during bacterial infection (10). Procalcitonin is proinflammatory and induces inflammation through increased surface markers CD16 and CD14 on neutrophils and lymphocytes, similar to the action of interleukin-8 (11). Tumour necrosis factor-alpha, IL-6 and IL-1β also demonstrate a dose-dependent increase to procalcitonin in whole human blood (12). This response appears to create a self-perpetuating inflammatory cascade, with TNF-alpha, a known potent stimulant of procalcitonin (10). Studies have established the value of serum procalcitonin in distinguishing bacterial infections from viral infections (13, 14).

Sputum procalcitonin, which is present in higher concentrations in sputum than in blood during an infective exacerbation of bronchiectasis (15), could be a better marker to guide antibiotic treatment in patients with airway diseases. In patients with bronchiectasis, serum procalcitonin levels are characteristically low during acute exacerbations requiring hospitalisation and when patients are clinically stable in the outpatient setting (15, 16). It is likely that sputum procalcitonin will remain elevated in clinically stable patients, given the extensive literature demonstrating infiltration of the airways with inflammatory cells and the abundant neutrophils, inflammatory cells and cytokines occurring in bronchiectasis (2). Furthermore, patients often have persistent bacterial infection and colonisation (17).

We hypothesized that levels of *sputum* procalcitonin in patients with stable bronchiectasis would be higher than those in healthy subjects. The clinical utility of sputum procalcitonin is also dependent on the repeatability of the test and the ability of the patient to produce an adequate sputum sample for assessment.

Our primary aim was to evaluate the levels of sputum procalcitonin in patients with stable bronchiectasis and healthy-controls. We also aimed to assess the repeatability of the test, and whether spontaneously expectorated sputum produces similar results to induced sputum.

METHODOLOGY

Study design and patients

We conducted a single centre, prospective, observational study at Middlemore Hospital in Auckland, New Zealand. Participants aged 18 years or older were eligible to participate if they had non-cystic fibrosis bronchiectasis or were healthy participants.

We recruited patients with bronchiectasis who had a diagnosis defined by a high-resolution computed tomography (HRCT) scan, and were able to expectorate sputum spontaneously for testing. Exclusion criteria were: respiratory or systemic infection requiring anti-inflammatory or antibiotic management within 6 weeks of recruitment; frequent exacerbators (defined as more than 4 exacerbations in the past year); *Pseudomonas aeruginosa* culture positive in sputum; cystic fibrosis; primary ciliary dyskinesia; or hypogammaglobulinaemia. Healthy participants were eligible for inclusion if they had no significant acute or chronic medical conditions, had never smoked, and were not taking regular medications.

The study was approved by the Northern Regional Ethics Committee, New Zealand. All participants provided written informed consent.

Procedures

Patients with bronchiectasis were assessed at baseline and seven days later. Healthy participants had only one clinic visit. Patients with bronchiectasis completed a daily symptom diary card. Radiological scores for the patients with bronchiectasis were calculated by counting the number of bronchiectasis-affected lobes on their most recent HRCT scan (minimum score of 1 and maximum score of 6). Similarly, the Bronchiectasis Severity Index (BSI) was calculated at baseline for patients with bronchiectasis (3). This was undertaken retrospectively as the BSI was developed after this study was undertaken. Spirometry which included Forced Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC) was performed according to the American

Thoracic Society guidelines (18). Blood samples for the measurement of procalcitonin, total white cell count (WCC), neutrophil count, concentration of C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were collected at all visits. Procalcitonin levels were measured using the Vidas BRAHMS PCT assay (Brahms GmbH, Hennigsdorf, Germany).

Spontaneous and induced sputum samples were collected at both visits in patients with bronchiectasis. Spontaneous sputum was collected first followed by induced sputum collected between 20 – 60 minutes afterwards, depending on time taken to complete sputum induction and other study procedures. Only induced sputum samples were collected from healthy participants. Sputum was induced with hypertonic saline according to the method of Gibson et al. (19). Hypertonic saline (4.5%) was inhaled for doubling time periods (30 sec, 1 min, 2 min, 4 min, 8 min) from an ultrasonic nebulizer (DeVilbiss 65; DeVilbiss Corporation, Somerset, PA). The test was stopped when 15.5 cumulative minutes of nebulisation time had elapsed. Whole specimens (sputum plus saliva) were examined and processed within two hours of collection following European Respiratory Society recommendations (20, 21). Specifically, the sputum sample was treated with dithiothreitol (DTT) in order to break up the mucus and disperse the cells. The volume added was two times the weight of the sputum sample. The treated sample was rocked for 30 minutes on a rocker at 37°C. The sample was then filtered through a 48mm nylon mesh to remove mucus and debris. A total cell count (TCC) was performed on the filtered sample. Subsequently the sample was centrifuged for 10 minutes at 300-1900xg (300rpm). The fluid phase component (supernatant) was then separated from the cell pellet and tested immediately for procalcitonin measurements using the Vidas BRAHMS PCT assay (Brahams GmbH, Hennigsdorf, Germany). Sputum cellular analysis included a differential cell count from 400 non-squamous cells. Sputum samples were cultured for respiratory pathogens according to the standard hospital protocol. Sputum weight and colour were recorded for each sample (22). Sputum colour was assessed by study investigators using a 5-point sputum colour chart (BronkoTest; Heredilab Inc, Salt Lake City, Utah, USA) as soon as the sample was collected and prior to sputum processing (23).

The *primary endpoint* was procalcitonin concentration in induced sputum. Secondary endpoints included procalcitonin concentration in spontaneous sputum, sputum neutrophil count, serum procalcitonin level, CRP and ESR.

Statistical analysis

Sample size

Based on two published observational studies (16, 24) and data from a recent pilot study(15), we assumed that the mean level of sputum procalcitonin in patients with stable bronchiectasis is 70% lower than the mean level of those patients with bronchiectasis having an acute exacerbation (mean serum procalcitonin is reported to be 0.030 ng/mL vs. 0.102 ng/mL), and the mean sputum procalcitonin level in normal subjects will be much lower than the level of bronchiectasis patients.

Our recent pilot study showed that the sputum procalcitonin level is log-normal distributed.(15) On the natural log scale, the mean level (std) of sputum procalcitonin is assumed to be -0.8 (0.6) in patients with stable bronchiectasis. Twenty four patients and 12 healthy-controls were expected to provide >85% statistical power to detect a difference ≥0.7 unit on the log scale at 5% significant level, assuming both groups have a common standard deviation. The sample size of 24 patients will also control the margin of error in the mean log sputum procalcitonin level to be within 0.2 units at 95% confidence level. After adjusting for a 15% possible withdrawal rate, 30 patients with bronchiectasis and 15 healthy-controls were planned for the study. The proposed 30 patients with 2 observations per subject will achieve a >85% power to detect an intraclass correlation coefficient of 0.65, assuming the null hypothesized intra class correlation coefficient is 0.2.

Primary analysis

Central tendency (geometric mean and median) and variability (95% confidence interval and interquartile range) of serum and sputum procalcitonin levels in patients with bronchiectasis and healthy-controls were reported. Analysis of covariance were used to compare the level of sputum procalcitonin between the two groups, and were adjusted for age and gender. Mixed effect repeated measure of analysis was used when the patients with bronchiectasis visit 1 and visit 2 procalcitonin measurements were pooled. Analysis of covariance were also used to compare the level of sputum procalcitonin between male and female patients with bronchiectasis, and were adjusted for radiological score, BSI severity score and age. Repeatability of sputum and serum procalcitonin levels within patients with bronchiectasis were assessed by intra-class correlation

coefficients, derived from mixed effect repeated measure of analysis. Bland Aland method was also used to investigate the agreement between repeated tests.

Secondary analysis

Correlation coefficients were used to assess the associations between sputum procalcitonin and disease indicators and also between sputum procalcitonin and inflammation indicators. Logistic regression was used to derive the association (odds ratios) of sputum procalcitonin and sputum bacterial culture.

RESULTS

Patients

Thirty patients with bronchiectasis and 15 healthy participants were recruited from July 2011 to September 2012. *Table 1* summarises the baseline characteristics of the participants. The mean age of patients with bronchiectasis and healthy-controls was 67 (SD 11) years and 43 (SD 11) years respectively. There were 21 (70%) female patients with bronchiectasis and 10 (67%) female healthy participants. Mean percentage of FEV_1 percent predicted (FEV_1%) was lower in patients with bronchiectasis than in healthy participants (73.7% [SD 17] vs 98.6% [SD 12]). Compared to healthy participants, patients with bronchiectasis had higher CRP and ESR levels. The patients with bronchiectasis had a median radiological score of 3 lobes and a BSI score of 6 points (moderate to severe severity).

***Table 1* – Baseline characteristics of participants**

Characteristic	*Bronchiectasis Group (n = 30)*	*Healthy Group (n = 15)*
Age (years)	67 (11)	43 (11)
Gender (women)	21 (70%)	10 (67%)
Ethnic Origin (%)		
European	19 (63%)	12 (80%)
Pacific	6 (20%)	0 (0%)
Māori	4 (13%)	1 (7%)
Other	1 (4%)	2 (13%)
Spirometry		
FEV_1 (L)	1.87 (0.62)	3.31 (0.89)
FEV_1 %	73.7 (16.8)	98.6 (11.7)
FVC (L)	2.71 (0.89)	4.06 (1.05)
FVC %	79.7 (12.5)	98.7 (11.5)
FEV_1/FVC (%)	69.7 (10.3)	75.2 (21.0)
C-reactive protein (mg/L)	2.8 (2.0-3.9) †	1.6 (1.0-2.4) †
ESR (mm/hr)	22.7 (17.7-29.1) †	8.8 (6.4-12.0) †

Radiological severity median(IQR)	3 (2-4) *	N/A
BSI Severity Score Mild Moderate Severe	 10 13 7	N/A

Data are n (%) or mean (SD), unless otherwise stated. FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; † Geometric mean (95% confidence interval). *Median (IQR). ESR = erythrocyte sedimentation rate; Radiological score = number of lobes with bronchiectasis; BSI = Bronchiectasis Severity Index (range 0-24).

Comparison of procalcitonin in induced sputum for patients with bronchiectasis and healthy participants

Geometric mean sputum procalcitonin level in the bronchiectasis group at visit 1 was higher than in the healthy group (1.1 ng/mL [95% CI 0.7-1.8] vs 0.5 ng/mL [95% CI 0.3-0.9]; *p-value=0.02*) (*Table 2*). After adjusting for age and gender, geometric mean sputum procalcitonin level was higher in the bronchiectasis group, although this was not statistically significant (1.4ng/mL [95% CI 0.9-2.2] vs. 0.6ng/mL [95% CI 0.3-1.3], *p-value=0.09*). Using the pooled data from both visit 1 and 2, the geometric mean sputum procalcitonin level was significantly higher in the bronchiectasis group (mean ratio: 3.6 [95% CI 1.5-8.6], *p-value=0.006*).

Table 2. Sputum procalcitonin levels

	Spontaneous	Induced	*P-value*
Participants with bronchiectasis			
Visit one	Median (IQR) 1.7 (1.1,4) Geometric mean (95% C.I) 1.8 (1.2, 2.7)	Median (IQR) 1.3 (0.5,2) Geometric mean (95% C.I) 1.1 (0.7,1.8)	0.001†
Visit two	Median (IQR) 2.4 (0.6, 3.7) Geometric mean (95% C.I) 1.5 (1.0, 2.5)	Median (IQR) 1.2 (0.7,2.4) Geometric mean (95% C.I) 1.2 (0.8, 1.6)	
Healthy participants			
Visit one		Median (IQR) 0.5 (0.2,1.2) Geometric mean (95% C.I) 0.5 (0.3,0.9)	
*Procalcitonin concentration **ratio** between patients with bronchiectasis and healthy participants*		2.3 [95% CI 1.2-4.4]* 3.6 [95% CI 1.5 – 8.6]**	0.02* (0.006)**

† Comparison of spontaneous sputum and induced sputum results in the bronchiectasis group using repeated measures analysis of variance that pooled visit 1 and visit 2 data.

*Comparison of bronchiectasis and healthy groups using mixed effect repeated measures analysis of variance that pooled visit 1 and visit 2 measurements of patients with bronchiectasis without adjusting for age and gender; ** Adjusted for age and gender.

Female patients with bronchiectasis had lower levels of procalcitonin in induced sputum than male patients. This gender difference was present even after controlling for radiological score, BSI severity score and age. The adjusted geometric mean procalcitonin level was 0.8ng/mL (95% CI 0.5-1.3) in female patients and 2.6ng/mL (95% CI 1.3-5.6) in male patients (*p-value=0.01*). In healthy participants, the geometric mean level of procalcitonin did not differ significantly in female (0.51 ng/mL; 95% CI 0.21-1.23) and male participants (0.53 ng/mL; 95% CI 0.23-1.26).

Comparison of sampling method and analysis of sputum cell count for patients with bronchiectasis

Geometric mean procalcitonin level was higher in spontaneous sputum than in induced sputum at both visit 1 (1.8 ng/mL [95% CI 1.2-2.7] vs 1.1 ng/mL [95% CI 0.7-1.8]) and visit 2 (1.5 ng/mL [95% CI 1.0-2.5] vs 1.2 [95% CI 0.8-1.6]) (*p-value=0.001*) (*Table 2, Figure 1)*. The mean ratio between induced and spontaneous method was -0.65 (*p-value=0.002* – Visit 1) and -0.84 (*p-value=0.23* – Visit 2).

Patients with bronchiectasis demonstrated a lower proportion of sputum macrophage cell counts (8%; IQR 3-19 versus 38%: IQR 30-44), and higher total cell counts (1.71; IQR 0.32–6 versus 0.36: 0.21–2.24), compared to healthy-controls. The median induced sputum volume was similar in the patients with bronchiectasis and healthy-controls (1.8g; IQR 1.1–2 .8 versus 1.7g; IQR: 1–2.6). Higher proportion of neutrophil cell counts were seen in the patients with bronchiectasis with the median percentage sputum neutrophil counts for patients with bronchiectasis were 88% (IQR: 71–94) and 51% (IQR: 64–94) for healthy-controls (*p-value=0.0001*). Both induced and spontaneous sputum procalcitonin were significantly associated with total cell count; induced sputum correlation co-efficient 0.75 (*p-value<0.001*) and 0.52 (*p-value=0.005) and* spontaneous sputum correlation co-efficient 0.57 (*p-value=0.001*) and 0.56 (*p-value=0.002*), for visits 1 and 2 respectively. Induced sputum procalcitonin was weakly associated with sputum neutrophil counts at visit 1 (correlation coefficient (corr.) 0.38, *p-value=0.04*) but was not significant at visit 2 (corr: 0.12, p=0.56). Spontaneous sputum procalcitonin was not associated with neutrophil counts at either visit (visit 1 corr: 0.10, *p-value=0.59*; visit 2 corr: 0.25, *p-value=0.24*).

Repeatability of sputum procalcitonin levels after one week

Procalcitonin levels in spontaneous sputum at baseline were similar to those obtained one week later (*p-value=0.29*) (*Table 2*). The intraclass correlation co-efficient (ICC) was 0.76. After accounting for variations of age, gender, and total cell count, the ICC was 0.66. Similarly, procalcitonin levels in induced sputum at baseline were not significantly different from levels obtained one week later (p-*value=0.72*). The unadjusted and adjusted ICCs were 0.70 and 0.57 respectively.

Comparison of sputum and systemic inflammatory markers including serum procalcitonin

The systemic markers in blood were compared between patients with bronchiectasis and healthy-controls. In both groups, all participants had a serum procalcitonin < 0.05ng/mL at baseline. The ESR and CRP level in blood were significantly higher in patients with bronchiectasis than in healthy controls; median ESR for patients with bronchiectasis was 24 ng/mL (IQR: 14-39 ng/mL) and median ESR for healthy-controls was 8 ng/mL (IQR: 5-11 ng/mL) (*p-value=0.0007*).

Association between sputum procalcitonin and disease indicators

Induced sputum procalcitonin had a moderate positive correlation with sputum colour (correlation co-efficient 0.56, *p-value=0.002*) and sputum volume (correlation co-efficient 0.47, *p-value=0.01*). Similarly, spontaneous sputum procalcitonin had a moderate positive correlation with sputum volume (correlation co-efficient 0.42, *p-value=0.02*). Both spontaneous and induced sputum procalcitonin concentrations had no association with lung function or ESR. There was a weak association with induced sputum procalcitonin and CRP (corr: 0.25, *p-value=0.19*).

Positive sputum bacterial culture results in patients with bronchiectasis were recorded in 15 spontaneous and 14 induced sputum samples. The positive sputum bacterial culture samples were primarily due to *H. influenzae* (11 samples). For positive sputum bacterial culture patients, the geometric mean of induced procalcitonin was 1.9 ng/mL (95% CI 1.2-3.2) (n=14), and for sputum bacterial culture negative patients, the geometric mean of procalcitonin was 0.7 ng/mL (95% CI 0.4-1.3) (n=16) based on the baseline visit 1 *(Figure 2)*. Induced sputum procalcitonin was significantly associated with sputum bacterial culture results at both visits, with odds ratio of

predicting a positive culture result 2.7 (95% CI 1.2-7.9 – *p-value=0.03*); 3.2 (95% CI 1.1-14.3 – *p-value=0.07*), for visit 1 and 2 respectively.

DISCUSSION

Our study demonstrated that sputum procalcitonin levels were higher in patients with stable bronchiectasis compared to healthy-controls. We showed that sputum procalcitonin measurement is repeatable, which is an important characteristic of an effective biomarker (25). These findings suggest that sputum procalcitonin may be a useful biomarker of the local inflammatory response to infection in the airways of patients with bronchiectasis.

Our previous research showed that sputum procalcitonin levels were increased in patients with bronchiectasis requiring hospitalization for an infective exacerbation of their airways disease (15). Procalcitonin levels during these exacerbations were much higher than in this stability study and we hypothesize that procalcitonin levels may increase and decrease depending on clinical stability. This is the case with neutrophil elastase, which is an important marker of airway inflammation in bronchiectasis (26). Identifying changes in sputum procalcitonin between stable and exacerbation states may provide clearer evidence of the presence of an infective exacerbation of bronchiectasis and help to guide antibiotic treatment (5, 27).

Our study also assessed whether the sampling method affects the procalcitonin concentration (28). Sputum procalcitonin was initially collected spontaneously and then induced during the same visit. We demonstrated that the sputum procalcitonin concentrations were significantly higher in spontaneous samples compared to induced samples. This was the case for both visit 1 and visit 2 and indicates a diluting effect of hypertonic saline. A key reason why this may have occurred relates to the sputum processing that was undertaken. In our study, we analysed whole sputum samples (sputum and saliva), rather than selecting sputum plugs. Previous research has shown that using unselected samples can lead to a 'dilution effect' resulting from the associated salivary component (29, 30), and this can be augmented by the use of hypertonic saline during induction. Sputum 'plug' selection is now the preferred technique to mitigate this issue (20). Despite the variation,

spontaneous sputum samples demonstrated elevated procalcitonin levels and this approach is appealing given the ease of sampling and minimal risks associated.

Our study also found that there was a gender difference in sputum procalcitonin levels with males having significantly higher concentrations. This was present even after controlling for radiological score, BSI severity and age. We are unable to explain this sputum procalcitonin gender difference but the small size of our study and low percentage of male participants could have resulted in type one error. Serum procalcitonin has been extensively investigated and no previous studies have demonstrated a gender difference (31). Similarly, the major airway inflammatory marker in bronchiectasis, neutrophil elastase, also has no gender variation (26). Despite this, there is increasing interest into gender differences evident in bronchiectasis. Females more commonly have bronchiectasis and typically have worse disease with poorer clinical outcomes (32). Additionally, an oestrogen-regulated anti-proteinase hormone has recently been identified and correlates with airway inflammatory markers (33).

There are several limitations of this study. Firstly, this is a small study with only 30 participants with bronchiectasis compared to 15 healthy-controls. Larger studies are clearly important in the validation of this biomarker. The control group was also not well matched in regards to age and ethnicity and we are therefore unable to confidently rule out any effect of age or ethnicity on sputum procalcitonin levels. There are other well-established airway inflammatory biomarkers, including neutrophil elastase and cytokines such as interleukin 8. Assessing the relationship of sputum procalcitonin to these biomarkers warrants further study. The processing of the whole sputum specimen in our study highlights the issue of dilution when sputum is induced. The exclusion of *Pseudomonas aeruginosa* infection in the bronchiectasis group also limits the generalizability of our study to some extent and further studies assessing the impact of different bacteria on sputum procalcitonin would be useful. Finally, a longitudinal study investigating the changes of sputum procalcitonin could be undertaken to see if sputum procalcitonin levels do in-fact increase and decrease during periods of infection and stability.

CONCLUSION

Sputum procalcitonin is elevated in patients with stable bronchiectasis compared to healthy-controls and is a repeatable measurement in both spontaneous and induced sputum specimens. Sputum procalcitonin has the potential to be a biomarker of airway inflammation and infection in bronchiectasis and future studies assessing dynamic changes with exacerbations and the relationship to other airways inflammatory markers are now needed.

Conflict of Interest: There is no conflict of interest relating to this study.

Disclosure statement: *Dr William Good* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Figure 1: *Sputum Procalcitonin levels in patients with bronchiectasis at visit1 and 2.*

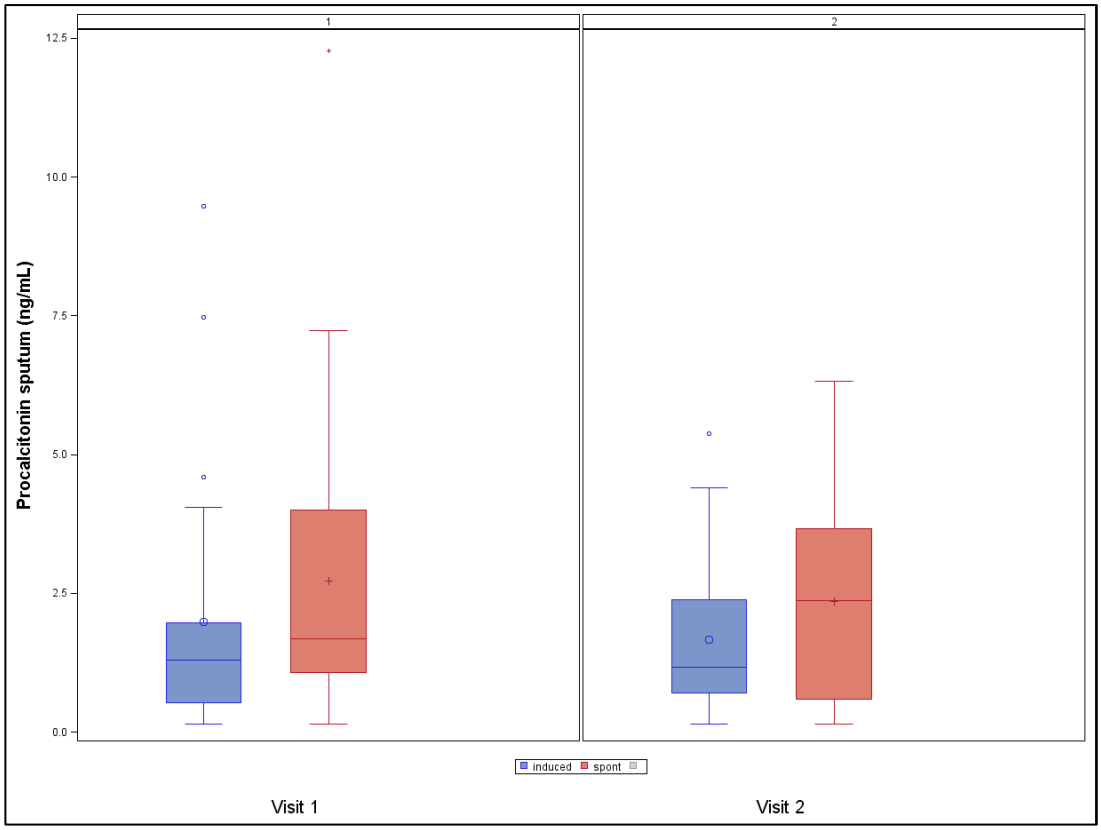
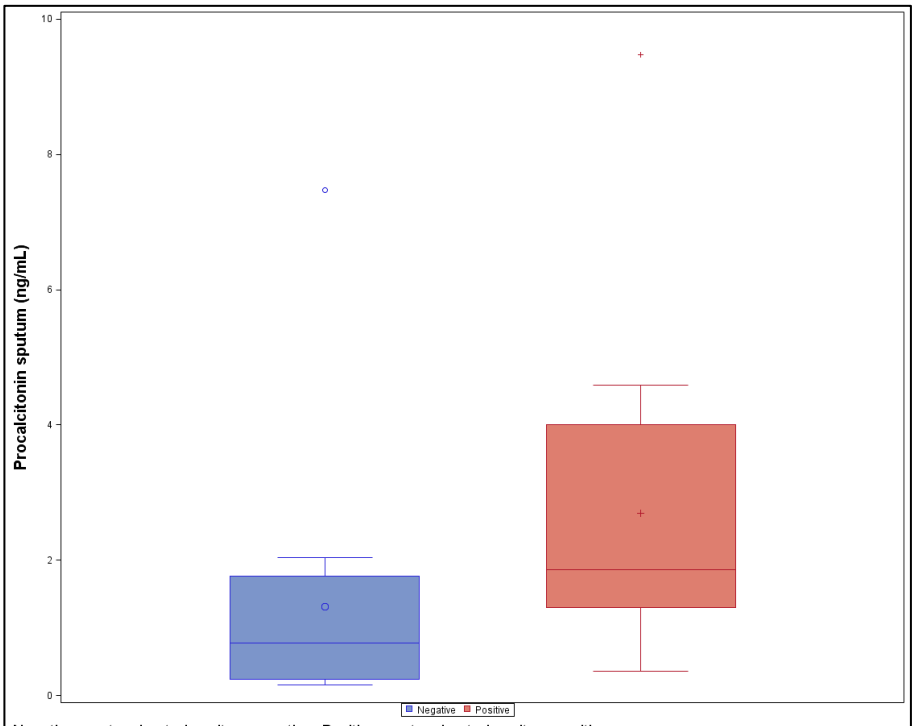


Figure 2. *Induced sputum Procalcitonin and bacteria cultural results at visit 1.*



Negative: sputum bacteria culture negative; Positive: sputum bacteria culture positive