



# Sputum procalcitonin: a potential biomarker in stable bronchiectasis

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Sputum procalcitonin levels are higher in patients with stable bronchiectasis than in healthy controls. Sputum procalcitonin has the potential to be a biomarker of airway inflammation and infection in bronchiectasis. <https://bit.ly/3ivn7R9>

Cite this article as: Good W, Jeon G, Zeng I, et al. Sputum procalcitonin: a potential biomarker in stable bronchiectasis. *ERJ Open Res* 2021; 7: 00285-2021 [DOI: 10.1183/23120541.00285-2021].

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Received: 26 April 2021  
Accepted: 9 July 2021

## Abstract

Sputum procalcitonin is elevated in exacerbations of bronchiectasis. The primary aim of this study was to investigate whether sputum procalcitonin levels are higher in patients with stable bronchiectasis than in healthy controls. We also assessed differences in procalcitonin levels in spontaneously expectorated and induced sputum samples and their repeatability 1 week later.

Participants included were aged over 18 years and either had radiologically confirmed bronchiectasis or were healthy controls. Patients with bronchiectasis were clinically stable for at least 6 weeks and had spontaneous and induced sputum collected at visit 1 and again 7 days later. Only induced sputum samples were collected from healthy controls during visit 1. Sputum procalcitonin concentrations in sputum were measured.

In total, 30 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 (1.5 ng·mL<sup>-1</sup>, 95% CI 1.0–2.1 ng·mL<sup>-1</sup> versus 0.4 ng·mL<sup>-1</sup>, 95% CI 0.2–0.9 ng·mL<sup>-1</sup>; 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 (1.8 ng·mL<sup>-1</sup>, 95% CI 1.2–2.7 ng·mL<sup>-1</sup> versus 1.1 ng·mL<sup>-1</sup>, 95% CI 0.7–1.8 ng·mL<sup>-1</sup>) and visit 2 (1.5 ng·mL<sup>-1</sup>, 95% CI 1.0–2.5 ng·mL<sup>-1</sup> versus 1.2 ng·mL<sup>-1</sup>, 95% CI 0.8–1.6 ng·mL<sup>-1</sup>; p=0.001). Repeating spontaneous and induced sputum procalcitonin levels 1 week later produced similar concentrations (p=0.29, intraclass correlation coefficient (ICC)=0.76 and p=0.72, ICC=0.70, respectively).

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

## 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 (IL)-8 [11]. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6 and IL-1 $\beta$  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]. Conversely, 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 hypothesised 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 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.

## Methods

### 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 high-resolution computed tomography (HRCT) and who 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 exacerbations (defined as more than four 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 medication.

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 7 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 because the BSI was developed after this study was undertaken. Spirometry that included forced expiratory volume in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC) was performed according to the American Thoracic Society guidelines [18]. Blood samples were collected at all visits to measure procalcitonin, total white cell count, neutrophil count, concentration of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Procalcitonin levels were measured using the Vidas B.R.A.H.M.S. PCT assay (B.R.A.H.M.S. 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 20–60 min later, depending on the time taken to complete sputum induction and other study procedures. Induced sputum samples only 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 s, 1 min, 2 min, 4 min, 8 min) from an ultrasonic nebuliser (DeVilbiss 65; DeVilbiss Corporation, Somerset, PA, USA).

The test was stopped when 15.5 min (cumulative) of nebulisation time had elapsed. Whole specimens (sputum plus saliva) were examined and processed within 2 h of collection following European Respiratory Society recommendations [20, 21]. Specifically, the sputum sample was treated with dithiothreitol to break up the mucus and disperse the cells. The volume added was twice the weight of the sputum sample. The treated sample was rocked for 30 min on a rocker at 37°C. The sample was then filtered through a 48 mm nylon mesh to remove mucus and debris. A total cell count was performed on the filtered sample. Subsequently the sample was centrifuged for 10 min at 300–1900×g (300 rpm). The fluid phase component (supernatant) was then separated from the cell pellet and the level of procalcitonin measured immediately using the Vidas B.R.A.H.M.S. PCT assay. 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 five-point sputum colour chart (BronkoTest; Heredilab Inc., Salt Lake City, UT, USA) as soon as the sample was collected and prior to sputum processing [23].

The primary end-point was procalcitonin concentration in induced sputum. Secondary end-points 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 in those patients with bronchiectasis having an acute exacerbation (mean serum procalcitonin is reported to be 0.030 ng·mL<sup>-1</sup> versus 0.102 ng·mL<sup>-1</sup>), and that the mean sputum procalcitonin level in normal subjects will be much lower than the level in bronchiectasis patients.

Our recent pilot study showed that the sputum procalcitonin level has a log-normal distribution [15]. On the natural log scale, the mean±SD level of sputum procalcitonin is assumed to be -0.8±0.6 in patients with stable bronchiectasis. In the current study, 24 patients and 12 healthy controls were expected to provide >85% statistical power to detect a difference ≥0.7 units on the log scale at a 5% significance level, assuming both groups had a common standard deviation. The sample size of 24 patients would also control the margin of error in the mean log sputum procalcitonin level to within 0.2 units at a 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 two observations per subject would achieve a >85% power to detect an intraclass correlation coefficient (ICC) of 0.65, assuming the null hypothesised ICC was 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 are reported. ANCOVA was used to compare the level of sputum procalcitonin between the two groups, and was adjusted for age and gender. Mixed-effect repeated measure of analysis was used when the visit 1 and visit 2 procalcitonin measurements for patients with bronchiectasis were pooled. ANCOVA was also used to compare the level of sputum procalcitonin between male and female patients with bronchiectasis, and was adjusted for radiological score, BSI severity score and age. Repeatability of sputum and serum procalcitonin levels within patients with bronchiectasis was assessed by ICCs, derived from mixed-effect repeated measure of analysis. The Bland–Altman 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 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

In total, 30 patients with bronchiectasis and 15 healthy controls were recruited from July 2011 to September 2012. Table 1 summarises the baseline characteristics of the participants. The mean±SD age of patients with bronchiectasis and healthy controls was 67±11 years and 43±11 years, respectively. There were 21 female patients with bronchiectasis (70%) and 10 female healthy controls (67%). The mean±SD FEV<sub>1</sub> % predicted was lower in patients with bronchiectasis than in healthy controls (73.7±17% versus

TABLE 1 Baseline characteristics of participants

Characteristic	Bronchiectasis group	Healthy group
Subjects (n)	30	15
Age (years)	67±11	43±11
Gender (female)	21 (70%)	10 (67%)
Ethnic origin (%)		
European	19 (63%)	12 (80%)
Pacific	6 (20%)	0 (0%)
Maori	4 (13%)	1 (7%)
Other	1 (4%)	2 (13%)
<b>Spirometry</b>		
FEV <sub>1</sub> (L)	1.87±0.62	3.31±0.89
FEV <sub>1</sub> % pred	73.7±16.8	98.6±11.7
FVC (L)	2.71±0.89	4.06±1.05
FVC % pred	79.7±12.5	98.7±11.5
FEV <sub>1</sub> /FVC (%)	69.7±10.3	75.2±11.0
C-reactive protein (mg·L <sup>-1</sup> )	2.8 (2.0–3.9) <sup>¶</sup>	1.6 (1.0–2.4) <sup>¶</sup>
ESR (mm·h <sup>-1</sup> )	22.7 (17.7–29.1) <sup>¶</sup>	8.8 (6.4–12.0) <sup>¶</sup>
Radiological severity <sup>#</sup>	3 (2–4) <sup>‡</sup>	N/A
BSI severity score		N/A
Mild	10	
Moderate	13	
Severe	7	

Data are n (%) or mean±SD, unless otherwise stated. FEV<sub>1</sub>: forced expiratory volume in 1 s; FVC: forced vital capacity; ESR: erythrocyte sedimentation rate; BSI: Bronchiectasis Severity Index (range 0–24). <sup>¶</sup>: geometric mean (95% CI); <sup>#</sup>: median (IQR); <sup>‡</sup>: number of lobes with bronchiectasis.

98.6±12%). Patients with bronchiectasis had higher CRP and ESR levels than healthy controls. Patients with bronchiectasis had a median radiological score of three lobes and a BSI score of 6 points (moderate to severe severity).

#### Comparison of procalcitonin in induced sputum from patients with bronchiectasis and healthy controls

The geometric mean sputum procalcitonin level at visit one was higher in the bronchiectasis group than in the healthy control group (1.1 ng·mL<sup>-1</sup>, 95% CI 0.7–1.8 ng·mL<sup>-1</sup> versus 0.5 ng·mL<sup>-1</sup>, 95% CI 0.3–0.9 ng·mL<sup>-1</sup>; p=0.02; table 2). After adjusting for age and gender, the geometric mean sputum

TABLE 2 Sputum procalcitonin levels

	Spontaneous	Induced	p-value
<b>Participants with bronchiectasis</b>			
Visit one			0.001 <sup>¶</sup>
Median (IQR)	1.7 (1.1–4.0)	1.3 (0.5–2.0)	
Geometric mean (95% CI)	1.8 (1.2–2.7)	1.1 (0.7–1.8)	
Visit two			
Median (IQR)	2.4 (0.6–3.7)	1.2 (0.7–2.4)	
Geometric mean (95% CI)	1.5 (1.0–2.5)	1.2 (0.8–1.6)	
<b>Healthy participants</b>			
Visit one			
Median (IQR)		0.5 (0.2–1.2)	
Geometric mean (95% CI)		0.5 (0.3–0.9)	
<b>Procalcitonin concentration ratio between patients with bronchiectasis and healthy participants (95% CI)</b>		2.3 (1.2–4.4) <sup>#</sup>	0.02 <sup>#</sup>
		3.6 (1.5–8.6) <sup>‡</sup>	(0.006) <sup>‡</sup>

<sup>¶</sup>: comparison of spontaneous sputum and induced sputum results in the bronchiectasis group using repeated measures ANOVA that pooled visit 1 and visit 2 data; <sup>#</sup>: comparison of bronchiectasis and healthy groups using mixed-effect repeated measures ANOVA that pooled visit 1 and visit 2 measurements of patients with bronchiectasis without adjusting for age and gender; <sup>‡</sup>: adjusted for age and gender.

procalcitonin level was higher in the bronchiectasis group, although this was not statistically significant ( $1.4 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.9\text{--}2.2 \text{ ng}\cdot\text{mL}^{-1}$  versus  $0.6 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.3\text{--}1.3 \text{ ng}\cdot\text{mL}^{-1}$ ;  $p=0.09$ ). Using the pooled data from both visits 1 and 2, the geometric mean sputum procalcitonin level was significantly higher in the bronchiectasis group (mean ratio 3.6, 95% CI  $1.5\text{--}8.6$ ,  $p=0.006$ ).

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  $0.8 \text{ ng}\cdot\text{mL}^{-1}$  (95% CI  $0.5\text{--}1.3 \text{ ng}\cdot\text{mL}^{-1}$ ) in female patients and  $2.6 \text{ ng}\cdot\text{mL}^{-1}$  (95% CI  $1.3\text{--}5.6 \text{ ng}\cdot\text{mL}^{-1}$ ) in male patients ( $p=0.01$ ). In healthy participants, the geometric mean level of procalcitonin did not differ significantly between female ( $0.51 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.21\text{--}1.23 \text{ ng}\cdot\text{mL}^{-1}$ ) and male ( $0.53 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.23\text{--}1.26 \text{ ng}\cdot\text{mL}^{-1}$ ) participants.

#### 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 ( $1.8 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $1.2\text{--}2.7 \text{ ng}\cdot\text{mL}^{-1}$  versus  $1.1 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.7\text{--}1.8 \text{ ng}\cdot\text{mL}^{-1}$ ) and visit 2 ( $1.5 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $1.0\text{--}2.5 \text{ ng}\cdot\text{mL}^{-1}$  versus  $1.2 \text{ ng}\cdot\text{mL}^{-1}$ , 95% CI  $0.8\text{--}1.6 \text{ ng}\cdot\text{mL}^{-1}$ ) ( $p=0.001$ ) (table 2, figure 1). The mean ratio between induced and spontaneous sputum was  $-0.65$  ( $p=0.002$ ) for visit 1 and  $-0.84$  ( $p=0.23$ ) for visit 2.

Patients with bronchiectasis had a lower proportion of sputum macrophage cells (8%, IQR 3–19% versus 38%, IQR 30–44%) and higher total cell counts ( $1.71\times 10^9\cdot\text{mL}^{-1}$ , IQR  $0.32\text{--}6.00\times 10^9\cdot\text{mL}^{-1}$  versus  $0.36\times 10^9\cdot\text{mL}^{-1}$ , IQR  $0.21\text{--}2.24\times 10^9\cdot\text{mL}^{-1}$ ) compared to healthy controls. The median induced sputum volume was similar in the patients with bronchiectasis and healthy controls (1.8 g, IQR 1.1–2.8 g versus 1.7 g, IQR 1.0–2.6 g). A higher proportion of neutrophil cells was seen in patients with bronchiectasis, with a median percentage sputum neutrophil count for patients with bronchiectasis of 88% (IQR 71–94%) and 51% (IQR 64–94%) for healthy controls ( $p=0.0001$ ). Both induced and spontaneous sputum procalcitonin were significantly associated with total cell count, with correlation coefficients for induced sputum of 0.75 ( $p<0.001$ ) and 0.52 ( $p=0.005$ ), and for spontaneous sputum of 0.57 ( $p=0.001$ ) and 0.56 ( $p=0.002$ ), for visits 1 and 2, respectively. Induced sputum procalcitonin was weakly associated with sputum neutrophil counts at visit 1 (correlation coefficient 0.38,  $p=0.04$ ) but was not significant at visit 2 (correlation coefficient 0.12,  $p=0.56$ ). Spontaneous sputum procalcitonin was not associated with

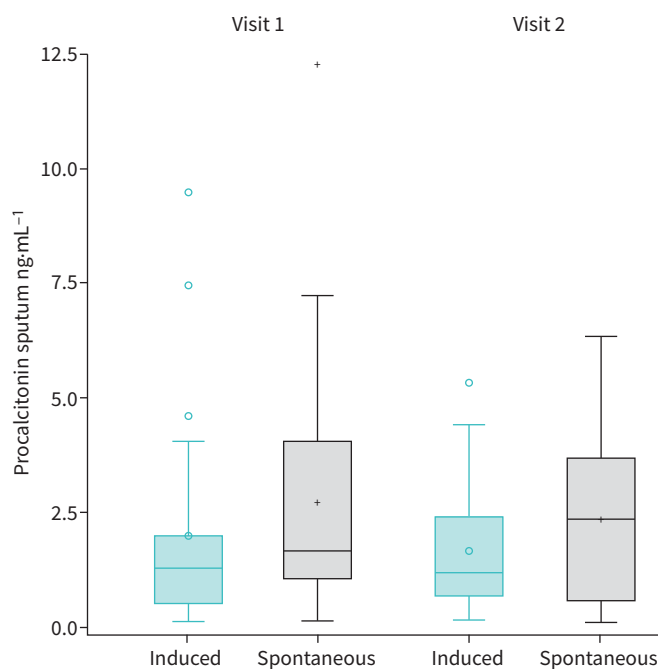


FIGURE 1 Sputum procalcitonin levels in patients with bronchiectasis at visit 1 and 2.

neutrophil counts at either visit (visit 1 correlation coefficient 0.10,  $p=0.59$ ; visit 2 correlation coefficient 0.25,  $p=0.24$ ).

#### Repeatability of sputum procalcitonin levels after 1 week

Procalcitonin levels in spontaneous sputum at baseline were similar to those obtained 1 week later ( $p=0.29$ ) (table 2). The 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 1 week later ( $p=0.72$ ). The unadjusted and adjusted ICCs were 0.70 and 0.57, respectively.

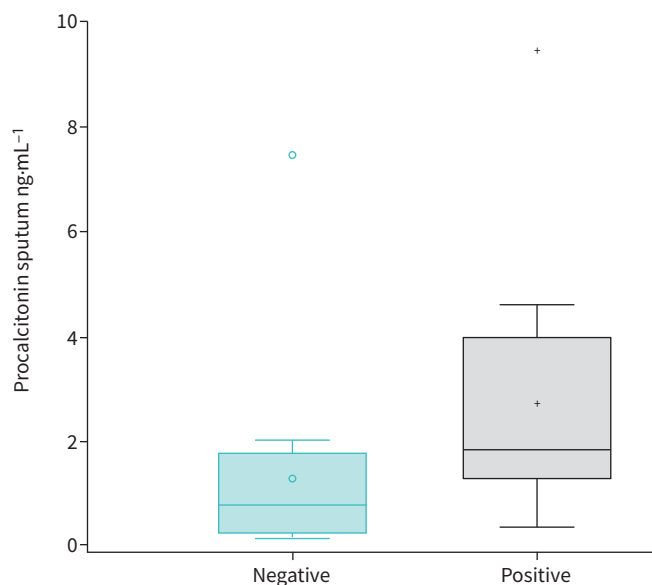
#### Comparison of sputum and systemic inflammatory markers including serum procalcitonin

The systemic markers in blood were compared between patients with bronchiectasis and healthy controls. In both groups, all participants had a serum procalcitonin level  $<0.05 \text{ ng}\cdot\text{mL}^{-1}$  at baseline. The ESR and CRP levels in blood were significantly higher in patients with bronchiectasis than in healthy controls; the median ESR level for patients with bronchiectasis was  $24 \text{ ng}\cdot\text{mL}^{-1}$  (IQR  $14\text{--}39 \text{ ng}\cdot\text{mL}^{-1}$ ) and for healthy controls was  $8 \text{ ng}\cdot\text{mL}^{-1}$  (IQR  $5\text{--}11 \text{ ng}\cdot\text{mL}^{-1}$ ) ( $p=0.0007$ ).

#### Association between sputum procalcitonin and disease indicators

Induced sputum procalcitonin had a moderate positive correlation with sputum colour (correlation coefficient 0.56,  $p=0.002$ ) and sputum volume (correlation coefficient 0.47,  $p=0.01$ ). Similarly, spontaneous sputum procalcitonin had a moderate positive correlation with sputum volume (correlation coefficient 0.42,  $p=0.02$ ). Neither spontaneous nor induced sputum procalcitonin concentrations were associated with lung function or ESR. There was a weak association with induced sputum procalcitonin and CRP (correlation coefficient 0.25,  $p=0.19$ ).

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 *Haemophilus influenzae* (11 samples). For patients with positive sputum bacterial culture results, the geometric mean of induced procalcitonin was  $1.9 \text{ ng}\cdot\text{mL}^{-1}$  (95% CI  $1.2\text{--}3.2 \text{ ng}\cdot\text{mL}^{-1}$ ) ( $n=14$ ), and for patients with negative sputum bacterial culture results, the geometric mean of procalcitonin was  $0.7 \text{ ng}\cdot\text{mL}^{-1}$  (95% CI  $0.4\text{--}1.3 \text{ ng}\cdot\text{mL}^{-1}$ ) ( $n=16$ ) based on baseline visit 1 (figure 2). Induced sputum procalcitonin level was significantly associated with sputum bacterial culture results at both visits, with an OR of predicting a positive culture result of 2.7 (95% CI  $1.2\text{--}7.9$ ,  $p=0.03$ ) for visit 1 and 3.2 (95% CI  $1.1\text{--}14.3$ ,  $p=0.07$ ) for visit 2.



**FIGURE 2** Induced sputum procalcitonin and bacteria cultural results at visit 1. Negative indicates sputum bacteria culture negative, and positive indicates sputum bacteria culture positive.

## Discussion

Our study demonstrated that sputum procalcitonin levels were higher in patients with stable bronchiectasis than in healthy controls. We have shown 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 hospitalisation for an infective exacerbation of their airway disease [15]. Procalcitonin levels during these exacerbations were much higher than in this stability study and we hypothesise 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 was initially collected spontaneously and then induced during the same visit. We demonstrated that the sputum procalcitonin concentrations were significantly higher in spontaneous samples than 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 difference 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 a type 1 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, has no gender variation [26]. Despite this, there is increasing interest in 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].

This study has several limitations. This was a small study, with only 30 participants with bronchiectasis and 15 healthy controls. Larger studies are clearly important in the validation of this biomarker. The control group was also not well matched with 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 IL-8. Assessing the relationship of sputum procalcitonin with 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 *P. aeruginosa* infection in the bronchiectasis group also limits the generalisability 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 with other airway inflammatory markers are now needed.

Provenance: Submitted article, peer reviewed.

Support statement: This research was undertaken with a grant from the Auckland Medical Research Foundation. This funding source was not involved in the study design, analysis, interpretation or writing-up of the study submitted. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: None declared.

## References

- 1 Menéndez R, Sibila O. Pathophysiology, immunology, and histopathology of bronchiectasis. *In: Bronchiectasis*. New York, Springer, 2018; pp. 51–64.
- 2 Boaventura R, Shoemark A, Chalmers J. Pathophysiology. *In: Chalmers J, Polverino E, Aliberti S, eds. Bronchiectasis (ERS Monograph)*. Sheffield, European Respiratory Society, 2018; pp. 8–28.
- 3 Chalmers JD, Goeminne P, Aliberti S, et al. The bronchiectasis severity index. An international derivation and validation study. *Am J Respir Crit Care Med* 2014; 189: 576–585.
- 4 Chalmers JD, Aliberti S, Filonenko A, et al. Characterization of the “frequent exacerbator phenotype” in bronchiectasis. *Am J Respir Crit Care Med* 2018; 197: 1410–1420.
- 5 Hill AT, Haworth CS, Aliberti S, et al. Pulmonary exacerbation in adults with bronchiectasis: a consensus definition for clinical research. *Eur Respir J* 2017; 49: 28596426.
- 6 Martínez-García MA, Soler-Cataluna J-J, Perpiñá-Tordera M, et al. Factors associated with lung function decline in adult patients with stable non-cystic fibrosis bronchiectasis. *Chest* 2007; 132: 1565–1572.
- 7 Becker K, Nylen E, White J, et al. Clinical review 167: procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. *J Clin Endocrinol Metab* 2004; 89: 1512–1525.
- 8 Becker KL, Snider R, Nylen ES. Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. *Br J Pharmacol* 2010; 159: 253–264.
- 9 Müller B, White JC, Nylén ES, et al. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. *J Clin Endocrinol Metab* 2001; 86: 396–404.
- 10 Matwyoff G, Prah J, Miller R, et al. Immune regulation of procalcitonin: a biomarker and mediator of infection. *Inflamm Res* 2012; 61: 401–409.
- 11 Wei J, Verity A, Garle M, et al. Examination of the effect of procalcitonin on human leucocytes and the porcine isolated coronary artery. *Br J Anaesth* 2008; 100: 612–621.
- 12 Liappis AP, Gibbs KW, Nylen ES, et al. Exogenous procalcitonin evokes a pro-inflammatory cytokine response. *Inflamm Res* 2011; 60: 203–207.
- 13 Muller B, Becker KL. Procalcitonin: how a hormone became a marker and mediator of sepsis. *Swiss Med Wkly* 2001; 131: 595–602.
- 14 Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. *BMC Med* 2011; 9: 107.
- 15 Good W, Mooney S, Zeng I, et al. Sputum procalcitonin levels in patients admitted to hospital with acute exacerbations of bronchiectasis. *Health Sci Rep* 2020; 3: e203.
- 16 Loebinger M, Shoemark A, Berry M, et al. Procalcitonin in stable and unstable patients with bronchiectasis. *Chron Respir Dis* 2008; 5: 155–160.
- 17 Chalmers JD. Bronchiectasis: phenotyping a complex disease. *COPD* 2017; 14: S12–S18.
- 18 Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.
- 19 Gibson PG, Wlodarczyk JW, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998; 158: 36–41.
- 20 Hamid Q, Kelly M, Linden M, et al. Methods of sputum processing for cell counts, immunocytochemistry and *in situ* hybridisation. *Eur Respir J* 2002; 20: 19s–23s.
- 21 Djukanović R, Sterk P, Fahy J, et al. Standardised methodology of sputum induction and processing. *Eur Respir J* 2002; 20: 1s–2s.
- 22 Stockley RA, Bayley D, Hill SL, et al. Assessment of airway neutrophils by sputum colour: correlation with airways inflammation. *Thorax* 2001; 56: 366–372.
- 23 Simpson JL, Lochrin A, Wood LG, et al. Bronko Test sputum colour as a marker of neutrophilic bronchitis in adults with asthma. *Am J Respir Crit Care Med* 2016; 193: A1440.
- 24 Dandona P, Nix D, Wilson MF, et al. Procalcitonin increase after endotoxin injection in normal subjects. *J Clin Endocrinol Metab* 1994; 79: 1605–1608.
- 25 Good W, Lata J, Vandal A, et al. How do we know what works? Clinical trial end-points and quality of life assessment. *In: Chalmers J, Polverino E, Aliberti S, eds. Bronchiectasis (ERS Monograph)*. Sheffield, European Respiratory Society, 2018; pp. 99–132.
- 26 Chalmers JD, Moffitt KL, Suarez-Cuartin G, et al. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. *Am J Respir Crit Care Med* 2017; 195: 1384–1393.
- 27 Deleaux I, Andre M, Colombier M, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? *Ann Rheum Dis* 2003; 62: 337–340.



- 28 Tangedal S, Aanerud M, Persson LJ, *et al.* Comparison of inflammatory markers in induced and spontaneous sputum in a cohort of COPD patients. *Respir Res* 2014; 15: 138.
- 29 Pizzichini M, Popov TA, Efthimiadis A, *et al.* Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996; 154: 866–869.
- 30 Pavord I, Pizzichini M, Pizzichini E, *et al.* The use of induced sputum to investigate airway inflammation. *Thorax* 1997; 52: 498–501.
- 31 Schuetz P, Bretscher C, Bernasconi L, *et al.* Overview of procalcitonin assays and procalcitonin-guided protocols for the management of patients with infections and sepsis. *Expert Rev Mol Diagn* 2017; 17: 593–601.
- 32 Vidailac C, Yong VF, Jaggi TK, *et al.* Gender differences in bronchiectasis: a real issue? *Breathe (Sheff)* 2018; 14: 108–121.
- 33 Smith A, Choi J-Y, Finch S, *et al.* Sputum pregnancy zone protein (PZP) – a potential biomarker of bronchiectasis severity. *Eur Respir J* 2017; 50: Suppl. 61, OA1969.