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SHORT REPORT

Endothelin-1 in stable bronchiectasis

L. Zheng[#], G. Tipoe*, W-K. Lam[#], J.C.M. Ho[#], I. Shum[#], G.C. Ooi[+], R. Leung[#], K.W.T. Tsang[#]

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ABSTRACT: Endothelin (ET)-1 has been suggested to promote neutrophil adhesion to endothelium, migration to inflamed areas, and release of elastase. ET-1 might therefore play a role in the pathogenesis of bronchiectasis, a chronic inflammatory and infective airway disease which is still poorly understood.

Thirty five patients with stable bronchiectasis (20 females, mean age±SD 49.1±15.0 yrs) and 18 control subjects (8 females, 49.4±11.3 yrs) were recruited prospectively. The ET-1 levels in serum and sputum were measured by commercially available enzyme linked immunosorbent assay (ELISA) kits.

Patients with *Pseudomonas aeruginosa* in their sputum had a significantly higher serum level of ET-1 (median 25.8, interquartile range 13–43.9 pg·mL^{-1}) than patients without *P. aeruginosa* (0, 0–10.5 pg·mL^{-1}; p=0.0004) and healthy control subjects (4.6, 0–16.3 pg·mL^{-1}; p=0.002). However, patients with and without *P. aeruginosa* infection had no significant difference in sputum ET-1 level (p=0.15). There was no correlation between serum or sputum ET-1 levels with the serum and sputum levels of the interleukin (IL)-1β, IL-8 and tumour necrosis factor (TNF)-α; the number of bronchiectasis lung lobes; and spirometry. Serum ET-1 level correlated with 24 h sputum volume for the bronchiectasis patients (r=0.51, p=0.002).

The results, therefore, suggest a significant pathogenic role for endothelin-1 among *Pseudomonas aeruginosa*-infected patients with bronchiectasis. Further studies should be performed to evaluate the clinico-pathological correlation and expression of endothelin-1 in bronchiectasis.

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University Depts of [#]Medicine, *Anatomy and [+]Diagnostic Radiology, The University of Hong Kong, Hong Kong SAR, China.

Correspondence: K.W.T. Tsang, University Dept of Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China. Fax: 852 28725828

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Endothelin (ET)-1 is the most potent vasoconstrictor known [1] and has a wide range of biological activities in the respiratory tract [2–4]. Bronchiectasis is a chronic inflammatory and infective airway disease characterized by irreversible dilatation of the bronchi and, in many cases, by persistent production of purulent sputum. Neutrophils are the predominant cells found in the bronchiectatic airways. This neutrophil recruitment into the airways is partly mediated by airway pro-inflammatory cytokines including interleukin (IL)-1β, IL-8 and tumour necrosis factor (TNF) α [5].

Human airway epithelial and endothelial cells and macrophages can all produce ET-1 [6–8], which promotes neutrophil adhesion to endothelial cells, migration to areas of inflammation and release of elastase *in vitro* [9–11]. The authors hypothesized that ET-1 might contribute to neutrophil trafficking into the airways, and play a significant role in the pathogenesis of bronchiectasis. The authors have, therefore, performed this study to investigate the levels of ET-1 in serum and sputum (sol), and correlated these with clinical and laboratory parameters in patients with steady state bronchiectasis.

Methods

Subject recruitment

Consecutive patients with proven bronchiectasis, diagnosed by high resolution computed tomography (HRCT), were recruited with written informed consent between March 1998 and February 1999. Inclusion criteria included: absence of asthma or other systemic diseases; no alteration in medication and dosage for at least 3 months; and "steady state" bronchiectasis as described previously [12]. Healthy control subjects, who were without respiratory, cardiovascular, gastrointestinal, renal and neurological diseases were recruited with verbal consent. Spirometry was measured with a SensorMedics 2200 (SensorMedics, Yorba Linda, CA, USA) package. Thoracic HRCT was performed within 12 months of the study, using a General Electric Hispeed Advantage Scanner (GE Medical Systems, Milwaukee, WI, USA). The number of lung lobes (including lingula) affected by bronchiectasis, as evident by the bronchial segment or subsegment being larger than the accompanying artery, was determined for each patient [13].

Serum and sputum collection

Fresh sputum was collected from bronchiectasis patients within 1 h of physiotherapy, and was used for all assessment. Sputum was ultracentrifuged (100,000×g for 30 min at 4°C) to obtain the sol which, similar to serum, was stored at -70°C until assay [12]. The volume of a 24 h sputum specimen was determined, to the nearest 0.5 mL, as the mean of a three consecutive day collection [12].

Determination of serum and sputum endothelin-1, interleukin-8, interleukin-1β, and tumour necrosis factor α levels

Undiluted serum and sputum sol levels of ET-1, IL-8, IL-1β, and TNFα levels were measured, within the same

day for each mediator, using commercially available enzyme linked immunosorbent assay kits (Biotra ELISA System; Amersham, Little Chalfont, Buckinghamshire, UK for ET-1; and R&D Systems, Minneapolis, MN, USA). The sensitivity of the assay was <24 pg· mL^{-1} for ET-1, <1 $pg{\cdot}mL^{-1}$ for IL-1β, 2 $pg{\cdot}mL^{-1}$ for IL-8 and <5 $pg{\cdot}mL^{-1}$ for TNFα. The level of a mediator in a particular specimen was adopted as the mean of the duplicate specimens after referring to the standard curve. The coefficient variabilities of ET-1 level measurement, according to the manufacturer, were 3.5–6.6% within assay and 16.8–20.3% between assays.

Determination of sputum leukocyte density and elastase level

Sputum leukocyte density was determined by haemocytometry with the fresh sputum specimens serially diluted with phosphate buffered saline. Sputum sol elastase activity was determined as the mean of triplicate specimens. Briefly, a standard curve was constructed on the rate of change in optical density (at 410 nm) after incubating known concentrations of elastase solutions (Sigma, Poole, Dorset, UK) with the chromogenic substrate succinyl-L-alanyl-L-alanine-p-nitroanilide (Sigma). By comparing with the standard curve, the rate of change in optical density determined at 410 nm was converted into elastase activity.

Microbiological assessment of sputum

Fresh sputum was obtained for microbiological evaluation [14]. Standard microbiological procedures were employed to identify all the sputum bacteria and classify them into pathogens (*Pseudomonas aeruginosa* and other species, *Haemophilus influenzae*, *Moraxella catarrhalis*, other Gram negative bacilli, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and Mycobacteria) or commensal bacteria (*Neisseria* spp., α-haemolytic streptococci, diptheroids, and coagulase-negative staphylococci) [12].

Statistical analysis

Comparisons between groups were made using the non-parametric Mann-Whitney rank order test. Correlations were evaluated by Spearman′s rank method. A p-value <0.05 was taken as statistically significant.

Results

Patient demographic data and clinical characteristics

Patient demographic data and clinical characteristics are shown in table 1. There was no difference in age, forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) between patients with and without *P. aeruginosa* infection (p>0.05). However, the number of bronchiectatic lung segments (p=0.03) and 24 h sputum volume (p=0.01) were higher among *P. aeruginosa*-infected patients compared with patients without *P. aeruginosa*.

Serum and sputum sol endothelin-1 levels, sputum leukocyte density, and sputum elastase levels

Patients with *P. aeruginosa* infection had a higher serum level of ET-1 compared with healthy control subjects (p=0.002), and their counterparts (p=0.0004; fig. 1; table 1).

Table 1. – Clinical characteristics, and levels of mediators in sputum and serum in subjects

Parameter	Control	Bronchiectasis patients		
		All	PA-infected	Non-PA-infected
n	18	35	18	17
No. of females	8	20	10	10
Clinical				
Mean age±SD yrs	49.4±11.3	49.1±15.0	48±16.0	50±14.0
Mean±SD FEV_1 % pred	-	61.5±28.8	53.4±27.9	70.6±27.8
Mean±SD FVC % pred	-	75.2±25.9	69.1±22.1	81.9±28.9
No. of bronchiectatic lung lobes	-	3.4±1.3	3.9±1.4*	2.9±1.1
Median (range) 24 h sputum volume mL	-	22 (3–145)	25 (8–145)*	16 (3–58)
Sputum bacterial isolates				
PA n	-	18	18	0
Commensals n	-	9	0	9
Haemophilus influenzae n	-	4	0	4
Mycobacterium chalonae n	-	1	0	1
Staphylococcus aureus n	-	2	0	2
Streptococcus pneumoniae n	-	1	0	1
Median (interquartile range) mediator levels				
Serum ET-1 $pg{\cdot}mL^{-1}$	4.6 (0–16.3)	11.8 (0–30.6)	25.8 (13–43.9)*,+	0 (0–10.5)
Sputum ET-1 $pg{\cdot}mL^{-1}$	-	31.1 (12.9–47.0)	34.9 (18.0–53.6)	20.3 (4.9–41.1)
Serum IL-1β $pg{\cdot}mL^{-1}$	Undetectable	Undetectable	Undetectable	Undetectable
Sputum IL-1β $ng{\cdot}mL^{-1}$	-	14.5 (2.3–28.1)	20.6 (5.4–38.4)*	2.6 (1.2–21.0)
Serum IL-8 $pg{\cdot}mL^{-1}$	50.6 (33.2–79.7)	54.3 (34.0–55.2)	45.2 (33.8–54.7)	57.7 (32.5–147.9)
Sputum IL-8 $ng{\cdot}mL^{-1}$	-	19 (6.8–44.8)	23.4 (12.0–47.3)	8.9 (4.3–40.7)
Serum TNFα $pg{\cdot}mL^{-1}$	Undetectable	Undetectable	Undetectable	Undetectable
Sputum TNFα $pg{\cdot}mL^{-1}$	-	113.3 (37.8–358.7)	141.1 (100–361)	60.3 (10–326)

No sputum was available from control subjects. * and $^{+}$: p<0.05 when compared with patients who had no *Pseudomonas aeruginosa* (PA) infection and controls respectively.

There was no difference in the sputum levels of ET-1 between *P. aeruginosa* and non-*P. aeruginosa*-infected bronchiectasis patients (p=0.15; fig. 2). Sputum leukocyte density was higher in *P. aeruginosa*-infected (median 48.7, interquartile range 15.7–97.7×10^6·mL^{-1}) than in non-*P. aeruginosa*-infected patients (9.1, 1.5–25.5×10^6·mL^{-1}; p=0.02). Sputum sol elastase level was higher in *P. aeruginosa*-infected (222.6, 18.3–267.5 unit·mL^{-1}) than non-*P. aeruginosa*-infected patients (8.7, 0.6–186.7 unit·mL^{-1}; p=0.03).

Serum and sputum interleukin-8, interleukin-1β and tumour necrosis factor α levels

Table 1 depicts the IL-8, IL-1β and TNFα levels in serum and sputum of patients with and without *P. aeruginosa*-infection. Sputum IL-1β levels were higher among *P. aeruginosa*-infected patients compared with their counterparts (p=0.03). Sputum IL-8 and TNFα levels were not different between these two subgroups of bronchiectasis patients (p>0.05). There was no difference in the serum levels of IL-8 between the controls, and the patient groups (p>0.05). Both serum IL-1β and TNFα levels were undetectable in patients with bronchiectasis and normal controls.

Correlation analysis

There was no correlation between serum or sputum ET-1 levels with the serum and sputum levels of the aforementioned pro-inflammatory mediators, number of lung lobes affected by bronchiectasis, FEV_1 % predicted, and FVC % pred (data not shown; p>0.05). However, serum, but not sputum, ET-1 levels correlated with 24 h sputum volume for the bronchiectasis patients (r=0.51, p=0.002, and r= 0.29, p=0.87, respectively). This correlation was only present in the subgroup of patients (r=0.43, p=0.04) who had *P. aeruginosa* infection but not their counterparts (r=0.23, p=0. 19). Amongst the bronchiectasis patients infected by *P. aeruginosa*, but not their counterparts, sputum IL-8 levels correlated significantly with sputum leukocyte density (r=0.57, p=0.02). However, there was no correlation between sputum IL-8 with the other cytokine levels.

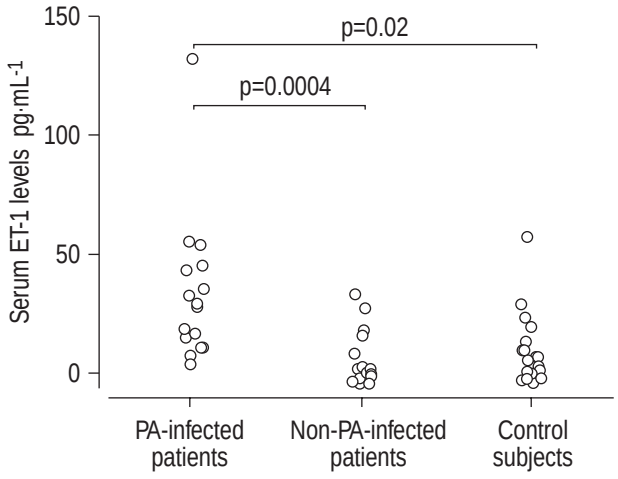

Fig. 1. – A scatterplot of serum endothelin (ET)-1 level in healthy control subjects (n=18) and patients with bronchiectasis (with (n=18) or without (n=17) *Pseudomonas aeruginosa* (PA) infection).

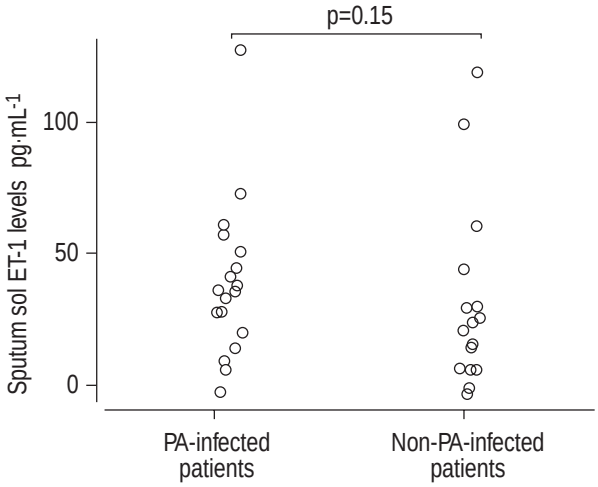

Fig. 2. – A scatterplot of sputum endothelin (ET)-1 level in bronchiectasis patients with (n=18) and without (n=17) *Pseudomonas aeruginosa* (PA) infection.

Discussion

The results of the study showed that serum level of ET-1, but not the other pro-inflammatory mediators evaluated (IL-8, IL-1β and TNFα), was significantly raised among patients with *P. aeruginosa* infection compared with patients without *P. aeruginosa* infection and healthy controls. However, there were no significant differences between *P. aeruginosa* infected and non-*P. aeruginosa*-infected patients in their sputum levels of ET-1, IL-8 and TNFα (table 1). Serum, but not sputum, ET-1 levels correlated with 24 h sputum volume in the entire cohort of bronchiectasis patients although this correlation did not exist for non-*P. aeruginosa*-infected patients. Sputum levels of IL-1β, elastase activity, leukocyte density, 24 h sputum volume and the number of lung lobes affected by bronchiectasis were significantly higher in *P. aeruginosa*-infected than in non-*P. aeruginosa*-infected patients [14]. Sputum IL-8 level correlated with sputum leukocyte density in *P. aeruginosa*-infected patients but not their counterparts. Interpretation of ET-1 sputum level was hindered by the lack of sputum data from control subjects. However, sputum ET-1 levels in the bronchiectasis patients (median 31.1, interquartile range 12.9–47.0 pg·mL^{-1}; table 1) appear to be lower than those reported in patients with cystic fibrosis (77.6, 29–122.8 pg·mL^{-1}), but higher than in those with chronic obstructive pulmonary disease (16.4, 6.8–38.2 pg·mL^{-1}) [15].

Although ET-1 has been implicated in the pathogenesis of asthma, fibrosing alveolitis and pulmonary hypertension [2–4], little is known of its role in bronchiectasis. The chemotactic and elastase-inducing properties of ET-1 might help to activate recruited neutrophils, and lead to further airway damage in bronchiectasis [9–11]. The higher serum but not sputum levels of ET-1 amongst bronchiectasis patients with *P. aeruginosa* infection suggests that ET-1 might predominantly act at peri-vascular rather than intra-bronchial sites. This is further supported by the correlation between serum, but not sputum, levels of ET-1 with 24 h sputum volume, which reflects "leukocyte trafficking" into the bronchiectasis airways. Alternatively, there might have been an increase in sputum

level of ET-1 among *P. aeruginosa*-infected patients, but the ET-1 could have undergone degradation by activated neutrophils *in vivo* [16]. As *P. aeruginosa*-infection occurs frequently in patients who have severe bronchiectasis [14], the increased serum ET-1 detected in *P. aeruginosa*-infected patients could merely reflect disease severity independent of *P. aeruginosa* infection. This is a less likely explanation as ET-1 levels did not correlate with the number of lung segments affected by bronchiectasis or spirometry. It is therefore likely that the high levels of ET-1 detected in *P. aeruginosa*-infected patients genuinely reflects an up-regulation of ET-1 expression by *P. aeruginosa* infection. Other bacterial products such as *Escherichia coli* lipolysaccharide and pro-inflammatory cytokines, such as IL-1β and TNFα also up-regulate ET-1 expression in human bronchial epithelium [6].

Migration of neutrophils from the intravascular compartment to an inflammed tissue requires interaction of neutrophils with vascular endothelium and the presence of chemoattractants which serve as homing triggers. ET-1 up-regulates the expression of adhesive molecules, CD18 and CD11b on neutrophil surface, and enhances neutrophil adhesion to pulmonary endothelium [9, 11, 17]. The findings of an elevated serum ET-1 in *P. aeruginosa*-infected patients, but not the other pro-inflammatory cytokines, suggests an important role for ET-1 in the endothelial recruitment of neutrophils in bronchiectasis. Moreover, IL-8 and IL-1β are highly potent neutrophil chemotactic factors [8] whose production by human airway epithelial cells is up regulated by *P. aeruginosa* products [18]. The results show that there was a correlation between sputum IL-8 levels and leukocyte density among the *P. aeruginosa*-infected patients. The data, therefore, suggest that the extensive airway recruitment of neutrophils in bronchiectasis patients infected by *P. aeruginosa* might be due to high levels of ET-1 in circulation, and IL-8 and IL-1β peri-bronchially.

The authors′ original data show that endothelin-1 expression was increased amongst patients with severe bronchiectasis who had *Pseudomonas aeruginosa* infection. This was also accompanied by simultaneous increase in sputum leukocyte density and sputum elastase activity in these patients. The results, therefore, suggest a significant pathogenic role for endothelin-1 in *Pseudomonas aeruginosa*-infected bronchiectasis. It is possible that peri-vascular endothelin-1, up-regulated by *Pseudomonas aeruginosa* infection, could collaborate with the intra-bronchial actions of interleukin-1β and interleukin-8 to initiate or maintain the heavy neutrophil trafficking, which is an essential step in the pathogenesis of bronchiectasis.

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