

Up-regulation of circulating adhesion molecules in bronchiectasis

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ABSTRACT: Adhesion molecules are expressed on the surface of endothelial cells and leukocytes and are responsible for mediating the migration of intravascular leukocytes into inflamed tissue. Intensive recruitment of neutrophils into the airways occurs in bronchiectasis, although little is known about the role of adhesion molecules in this process.

The authors, therefore, determined serum levels of E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1 in stable bronchiectasis patients (n=37) and healthy control subjects (n=17), and evaluated their relationship with clinical markers of disease severity in bronchiectasis.

Serum levels of E-selectin, ICAM-1 and VCAM-1 in bronchiectasis patients were significantly higher than those in control subjects (p=0.02, <0.0001 and 0.0002 respectively). Both E-selectin and ICAM-1 levels were inversely related to forced expiratory volume in one second (FEV₁)% predicted (r=-0.57, p<0.001; and r=-0.53, p=0.001 respectively), and FVC% predicted (r=-0.52, p=0.002; and r=-0.46, p=0.005). This was not the case for VCAM-1 levels. There was a correlation between serum ICAM-1 levels and 24 h sputum volume (r=0.34, p= 0.04). Serum E-selectin and ICAM-1, but not VCAM-1, levels showed correlation with the number of lung lobes affected by bronchiectasis (r=0.35, p=0.04 and r=0.34, p=0.04 respectively).

These original observations strongly suggest that E-selectin, intercellular adhesion molecule-1 and Vascular adhesion molecule-1 could play a significant role in the pathogenesis of bronchiectasis.

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Bronchiectasis is a chronic infective and inflammatory airway disease characterized pathologically by permanent abnormal dilation of the bronchi, and clinically by recurrent purulent sputum production. Intense neutrophil influx into the airways occurs in bronchiectasis [1, 2] which could be harmful when neutrophil products, particularly elastase, are released in response to bacterial infection [3–6]. There is increasing evidence to suggest that the recruitment of neutrophils contributes to continued airway damage in bronchiectasis [3–6]. Adhesion molecules, expressed on the surface of endothelial cells and leukocytes [7–9], mediate migration of leukocytes from the vascular compartment into inflamed tissue.

There are three families of adhesion molecules which mediate this process, namely the selectins (including E-, L- and P-selectins), the integrins (CD11/CD18), and the immunoglobulin superfamily (including intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1)) [10–13]. Selectins initiate the adhesive cascade by triggering free-flowing intravascular leukocytes to roll along the endothelium at sites adjacent to extravascular sources of chemokines and other chemoattractants [8]. Leukocytes, mediated by the binding of integrins to endothelial immunoglobulin superfamily,

adhere firmly onto endothelial cells, change shape, and migrate between endothelial cells [8]. After crossing the endothelial basement membrane, leukocytes migrate to the inflamed sites through the extravascular matrix under the effects of established chemotactic gradients [14].

The adhesion molecules, E-selectin, ICAM-1 and VCAM-1, have been implicated in the pathogenesis of several airway diseases, such as asthma, cystic fibrosis (CF), and diffuse panbronchiolitis [15–17]. E-selectin is produced exclusively by activated endothelium [11, 12] while ICAM-1 is expressed on endothelium, epithelium, fibroblasts and leukocytes [18]. VCAM-1 is also widely distributed on endothelial, macrophage and dendritic cells [19]. Unlike ICAM-1, endothelial cells [19, 20] do not normally express VCAM-1. The expression of these adhesion molecules are up-regulated following exposure to pro-inflammatory cytokines including interleukin-1 (IL-1), tumour necrosis factor- α (TNF- α), and bacterial lipopolysaccharide (LPS) [11–13, 18–20], which are abundant in the airways of patients with bronchiectasis [1, 21]. The soluble isoforms of these adhesion molecules can be found in the circulation, and have been increasingly recognized as markers of inflammation and endothelial activation [22].

To address the role of adhesion molecules in the pathogenesis of bronchiectasis, the authors compared the levels of circulating E-selectin, ICAM-1 and VCAM-1 in bronchiectasis patients with controls. The relationship between those circulating adhesion molecules with clinical markers of disease severity in bronchiectasis was also examined.

Methods

Patients

Patients with proven bronchiectasis, diagnosed by high resolution computed tomography (HRCT), were recruited from the specialist respiratory clinics of the University of Hong Kong with verbal informed consent. Inclusion criteria included: absence of asthma or other unstable systemic diseases; no alteration in medication for at least 3 months; and "steady state" bronchiectasis. The latter was defined as the absence of significant (<20%) alteration in the 24 h sputum volume, forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), or changes in respiratory symptoms for three consecutive weeks [21]. Spirometry (FEV₁ and FVC), expressed as % predicted (% pred), was measured between 10:00 and 12:00 h with a SensorMedics 2200 package (SensorMedics, Yorba Linda, USA). Thoracic HRCT was performed, within the previous 12 months, using a General Electric Hispeed Advantage Scanner (GE Medical Systems, Milwaukee, WI, USA) to perform 1 mm thick sections at 10 mm intervals in the supine position. 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 [23]. Healthy control subjects who were asymptomatic for respiratory, cardiovascular and other systemic diseases were also recruited with verbal consent. All the procedures and assessment processes involved in this study had approval from the institutional ethics committee.

Microbiological assessment of sputum and sputum collection

Patients received chest physiotherapy before being instructed by a research physician to produce fresh sputum for microbiological evaluation as described previously [24]. Fresh sputum was collected in sterile clear plastic pots between 10:00 and 11:00 h and directly inoculated onto agar media within 1 h of collection using a standard plastic loop. Standard microbiological procedures were employed to identify all the sputum bacteria and classify them into pathogens (*Pseudomonas aeruginosa*, and species *Haemophilus influenzae*, *Moraxella catarrhalis*, other Gram negative bacilli, *Staphylococcus pneumoniae*, *Staphylococcus aureus*, and Mycobacteria) or commensal bacteria (*Neisseria* spp., α -haemolytic streptococci, diptheroids, and coagulase-negative staphylococci).

The following media were employed: blood agar (Oxoid CM271; Oxoid, Basingstoke, UK), with 5% defibrinated horse blood, MacConkey agar, chocolate agars supplemented with 18.9 U·mL⁻¹ bacitracin (Sigma, St. Louis, USA), mannitol salt agar (Oxoid CM85) and cetrimide-nalidixic acid agar (Oxoid CM559 and SR102). The blood

agar plate was incubated under anaerobic conditions. All other plates were incubated at 37°C in 5% CO₂ and the selective plates with negative results were re-incubated and re-examined daily for 4 days before disposal.

The volume of a 24 h sputum specimen was determined to the nearest 0.5 mL, as the mean of three consecutive day collections (09:00–09:00 h) as described previously [21]. Briefly, 24 h sputum collections were made by the patients at home in clear sterile plastic pots (60 mL) and stored at 4°C. All patients received expectoration-aiding chest physiotherapy twice daily, at home which was provided by the spouse or another designated family member.

Determination of serum E-selectin, intercellular and vascular adhesion molecule-1 levels

Serum was obtained from each subject and stored at -70°C until assay. Serum E-selectin, ICAM-1 and VCAM-1 levels were determined by using commercially available enzyme linked immunosorbent assay (ELISA) test kits (R&D System, Minneapolis, USA) as recommended by the manufacturer. Specimens were diluted 1:20 for E-selectin, 1:10 for ICAM-1, and 1:50 for VCAM-1 before assay. All measurements were performed in duplicate and the mean was taken as the result for each patient. The sensitivity of the assay was <0.1 ng·mL⁻¹ for E-selectin, <0.04 ng·mL⁻¹ for ICAM-1, and 0.5 ng·mL⁻¹ for VCAM-1. The coefficients of variation of the assays (CV%), according to the manufacturer, were 3–5% within assays and 5–10% between assays. Two ELISA kits were used for the assay in total for each of the adhesion molecules. The intra-assay CV% was <6%, and interassay CV% was <15% in this study, and the concentrations of the control samples fell within the ranges specified by the manufacturer.

Statistical analysis

All data were expressed as median and range unless stated otherwise. 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 of <0.05 was considered to be statistically significant. The analysis was performed using the Statistical Analysis System package (SAS Institute Inc., NC, USA).

Results

Patient demography and clinical characteristics

Subject demography and clinical characteristics are shown in table 1. Thirty-seven patients with bronchiectasis (15 females; mean age \pm SD 47.4 \pm 15.9 yrs; range 22–79 yrs) and 17 control subjects (7 females; 48.1 \pm 10.2 yrs; 19–59 yrs) were recruited between January 1998 and May 1999. There was no significant difference in age (p=0.50) or gender distribution (p=0.96) between control and patient groups.

Table 1. – Clinical characteristics of patients in steady state bronchiectasis

Parameter	Control	Patients
Subjects n	17	37
Age yrs	48.1±10.2	47.4±15.9
Females	7 (41.2%)	15 (40.5%)
FEV1% pred	-	60.6±29.7
FVC% pred	-	74.2±26.8
Number of bronchiectatic lung lobes	-	3.4±1.4
24 h Sputum volume mL*	-	20 (3–145)
Sputum leukocyte density ×10 ⁶ cells·mL ⁻¹	-	22.8 (0.05–198)
Sputum bacterial isolates n		
<i>Pseudomonas aeruginosa</i>	-	21
Commensals	-	8
<i>Haemophilus influenzae</i>	-	4
<i>Mycobacterium chelonae</i>	-	1
<i>Staphylococcus aureus</i>	-	2
<i>Streptococcus pneumoniae</i>	-	1

Data are presented as mean±SD or absolute number with percentage or range in parenthesis. *: median (range). FEV1% pred: forced expiratory volume in one second per cent predicted; FVC: forced vital capacity % predicted. There was no significant difference in the age and gender distribution between the control and bronchiectasis subjects.

Circulating E-selectin, intercellular and vascular adhesion molecule-1 levels

The levels of E-selectin, ICAM-1 and VCAM-1 for patients and control subjects are shown in table 2. Serum levels of E-selectin, ICAM-1 and VCAM-1 in bronchiectasis patients were significantly higher than those of control subjects ($p=0.02$, <0.0001 and 0.0002 respectively) (fig. 1). Patients with *Pseudomonas aeruginosa* infection had significantly higher circulating ICAM-1, but not VCAM-1 or E-selectin levels, when compared to their counterparts (table 3).

Correlation analysis

The results of the correlation analysis between the levels of adhesion molecules themselves, and with clinical markers of disease severity in bronchiectasis, are shown in table 4, and fig. 2 and 3. Among patients with bronchiectasis, serum levels of E-selectin correlated with serum ICAM-1 levels ($r=0.58$, $p<0.001$). Both E-selectin and ICAM-1 levels were inversely related to FEV1% pred

Table 2. – Serum levels of E-selectin, intercellular and vascular adhesion molecule-1 in bronchiectasis and healthy subjects

Adhesion molecule	Controls	Patients
Subjects n	17	37
E-selectin ng·mL ⁻¹	45.4 (23.9–92.8)	61.9 (22.0–204.5)*
ICAM-1 ng·mL ⁻¹	90.9 (61.7–152.2)	166.1 (87.8–425.7)*
VCAM-1 ng·mL ⁻¹	404.1 (250.8–671.1)	811 (132–3750)*

Data are presented as median (range). *: $p<0.05$ when compared to healthy control subjects. ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular adhesion molecule-1.

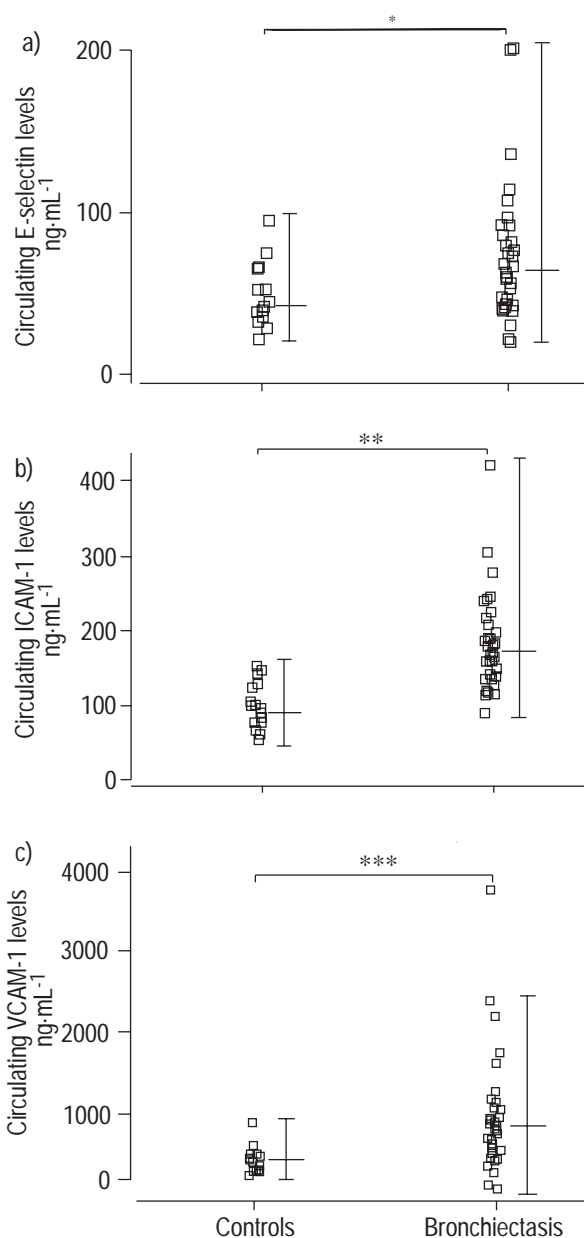


Fig. 1. – Dot plots showing the levels, median and range of: a) E-selectin; b) intercellular adhesion molecule; and c) vascular adhesion molecule-1 in bronchiectasis and control subjects. *: $p=0.015$; **: $p=0.0001$; ***: $p=0.0002$. Vertical lines represent median and range.

($r=-0.57$, $p<0.001$; and $r=-0.53$, $p=0.001$ respectively), and FVC% pred ($r=-0.52$, $p=0.001$; and $r=-0.46$, $p=0.005$ respectively). In addition, there was a correlation between serum ICAM-1 levels and 24 h sputum volume ($r=0.34$, $p=0.04$). There was no significant correlation between serum VCAM-1 levels and clinical parameters of disease severity, namely FEV1% pred ($r=0.15$, $p=0.38$), FVC% pred ($r=0.10$, $p=0.58$), number of bronchiectasis lung lobes ($r=0.05$, $p=0.79$), or 24 h sputum volume ($r=-0.23$, $p=0.19$), among the bronchiectasis patients. There was a correlation between selectin E-selectin and ICAM-1, but not VCAM-1, with the number of lung lobes affected by bronchiectasis ($r=0.35$, $p=0.04$ and $r=0.34$, $p=0.04$ respectively).

Table 3. – Serum levels of adhesion molecules in bronchiectasis patients with and without *Pseudomonas aeruginosa* in sputum

	<i>P. aeruginosa</i> infection	Non- <i>P. aeruginosa</i>	p-value
E-selectin ng·mL ⁻¹	66.1 (21.9–204.5)	58.97 (27.38–137.80)	0.27
ICAM-1 ng·mL ⁻¹	179.9 (112.7–425.7)	146.5 (87.78–223.14)	0.02*
VCAM-1 ng·mL ⁻¹	751 (210–2312)	877 (132–3750)	0.36

Data are presented as median (range). *: $p < 0.05$ when compared to *P. aeruginosa* infection. *P. aeruginosa*: *Pseudomonas aeruginosa*; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular adhesion molecule-1.

Discussion

The results of this study show that the serum levels of adhesion molecules: ICAM-1, VCAM-1, and E-selectin are up-regulated in patients with stable bronchiectasis compared to healthy control subjects. Serum levels of E-selectin and ICAM-1 showed good correlation whilst they correlated negatively with FEV₁ and FVC% pred among patients with bronchiectasis. Serum levels of these adhesion molecules also correlated with clinical param-

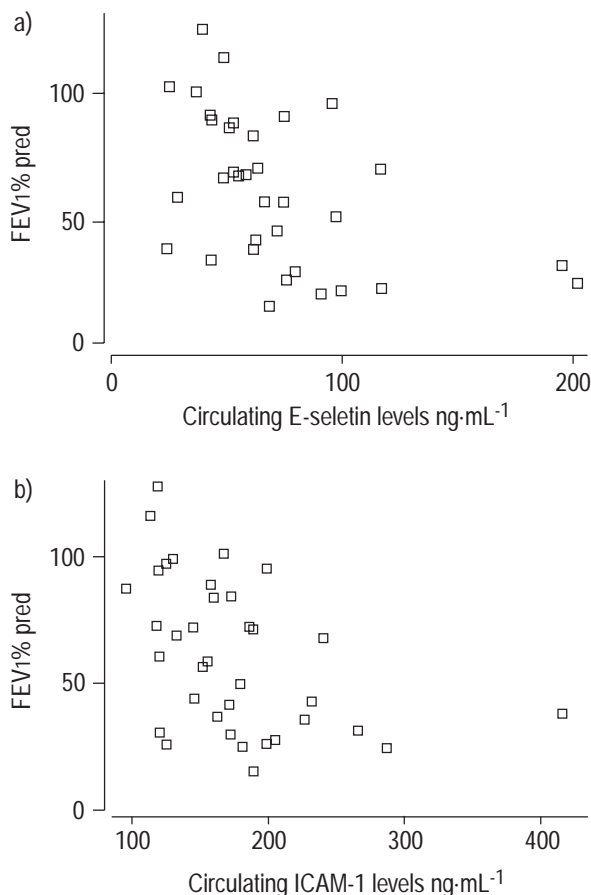


Fig. 2. – Scatter plots showing the relationship between FEV₁% predicted with a) serum E-selectin, $r = -0.57$, $p < 0.001$; and b) serum intercellular adhesion molecule-1 levels, $r = -0.53$, $p = 0.001$.

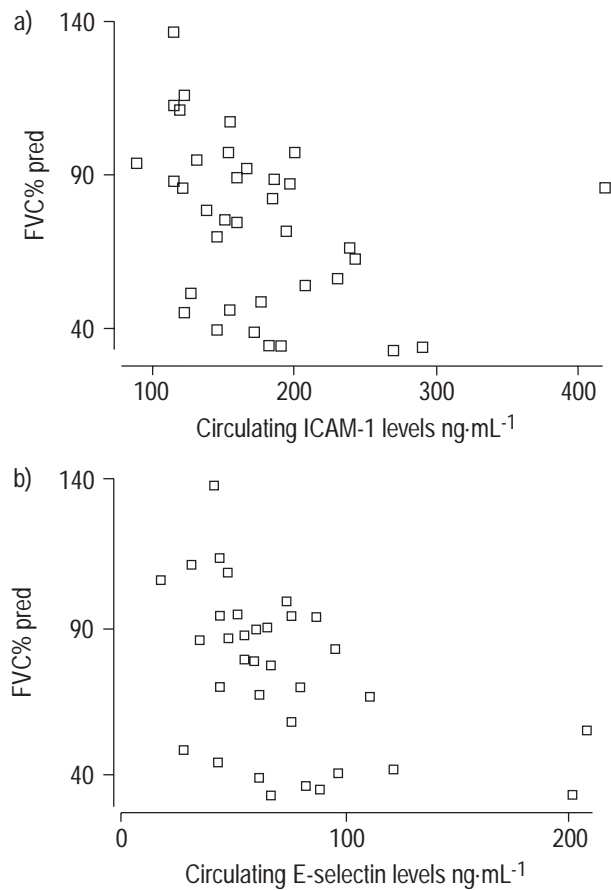


Fig. 3. – Scatter plots showing the relationship between FVC% predicted with a) serum intercellular adhesion molecule-1 levels, $r = -0.46$, $p = 0.005$; and b) serum E-selectin, $r = -0.52$, $p = 0.002$.

eters of disease severity in bronchiectasis. ICAM-1 levels showed correlation with the 24 h sputum volume in the patients with bronchiectasis. In addition, serum E-selectin and ICAM-1 levels showed correlation with the number of lung lobes affected by bronchiectasis. There was, however, no correlation between serum levels VCAM-1 with E-selectin, ICAM-1, or other clinical parameters of disease severity in bronchiectasis, namely the 24 h sputum volume, FEV₁% pred, and FVC% pred.

Intensive neutrophil recruitment into the airways and their subsequent migration into the airway lumen, clinically manifested as sputum production, are hallmarks in the pathogenesis of bronchiectasis [1, 2]. This is associated with a prolific production in pro-inflammatory mediators, such as IL-1, IL-8, TNF- α , and leukotriene B₄ (LTB₄) in the bronchiectatic airways [1, 21]. While the role of these pro-inflammatory mediators in the recruitment of neutrophils into the tracheobronchial tree has recently been established [1, 22, 25–27], little is known about the interaction between neutrophils and endothelial cells in the pathogenesis of bronchiectasis.

Although this study showed an up-regulation in serum E-selectin, ICAM-1 and VCAM-1 in patients with bronchiectasis, the source of these adhesion molecules remains obscure. It is probable that up-regulation of these adhesion molecules occurs in vascular endothelium within the bronchiectatic airways. The trigger(s) for this up-regulation is

Table 4. – Relationship between markers of disease severity and circulating levels of E-selectin, intercellular and vascular adhesion molecule-1 in 37 patients with stable bronchiectasis

	FEV ₁ % pred		FVC % pred		24 h volume		Sputum leukocyte density		Number of lobes with bronchiectasis	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
E-selectin ng·mL ⁻¹	-0.57	0.000	-0.52	0.002	0.18	0.31	0.20	0.26	0.35	0.04
ICAM-1 ng·mL ⁻¹	-0.53	0.001	-0.46	0.005	0.34	0.04	0.16	0.37	0.34	0.04
VCAM-1 ng·mL ⁻¹	0.15	0.384	0.10	0.58	-0.23	0.19	-0.08	0.68	0.05	0.80

Data shown are Spearman's rank correlation coefficients. FEV₁% pred: forced expiratory volume in one second per cent predicted; FVC% pred: forced vital capacity per cent predicted; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular adhesion molecule-1.

unknown but could be extravascular bacterial products and/or epithelial derived factor(s), particularly the aforementioned pro-inflammatory cytokines [11, 13, 18–20]. The correlation between circulating levels of E-selectin and ICAM-1 in bronchiectasis, but not in the control subjects, suggests that they could be up-regulated by the same agent(s). Migration of leukocytes from the circulation to inflamed tissue is complex and is mediated by multiple factors, including adhesion molecules, chemoattractants (such as IL-8), and some pro-inflammatory cytokines (such as TNF- α , IL-1 β , and LTB₄). The lack of correlation between serum adhesion molecules and sputum leucocyte density suggests that other factors are also involved in the migration of neutrophils into bronchiectatic airways. The authors, have also previously shown that sputum leukocyte output had no correlation with sputum levels of IL-1, IL-8, LTB₄ and TNF- α [28]. However, the current study demonstrated a correlation between serum ICAM-1 with the 24 h sputum volume (table 3). These observations suggest that factors derived from endothelium could play a more important role in the recruitment of leukocytes than intraluminal pro-inflammatory cytokines in bronchiectasis.

Adherence of circulating intravascular leukocytes to endothelium is a key event that occurs at the vicinity of inflamed tissues [7–9]. Neutrophils express CD11/CD18 on their surfaces, which change in conformation upon activation, to enable the recognition of endothelial ligands [9]. Endothelial cell surface ICAM-1 is one of the ligands for neutrophil CD11/CD18, and mediates CD11/CD18-dependent adhesion to endothelium [18, 29, 30]. E-selectin recognizes Sialy-Lewis X, which is another neutrophil ligand [31]. Adhesion of neutrophils to E-selectin increases its CD11/CD18 expression, which in turn augments adhesion of neutrophils to endothelium [32]. Several studies have demonstrated that CD11/CD18, E-selectin and ICAM-1 are important determinants in neutrophil trafficking [29–34]. Up-regulation of circulating E-selectin and ICAM-1 is found in the airways of patients with asthma and cystic fibrosis [15, 16], where marked airway infiltration by eosinophils or neutrophils occurs. Antibodies against E-selectin and ICAM-1 block neutrophil extravasation in rat lungs [33], and inhibit neutrophil trafficking into the inflamed lungs of a rabbit [34]. VCAM-1 is involved in the recruitment of eosinophils, monocytes/macrophages and lymphocytes into the lungs [19, 20]. The demonstration of an upregulation of circulating ICAM-1, VCAM-1 and E-selectin in this study, therefore, suggests that endothelial activation, probably situated within the airway, occurs in bronchiectasis [11–13, 19–21].

Although VCAM-1 levels were significantly higher among patients with bronchiectasis ($p=0.0002$) compared to control subjects, unlike E-selectin and ICAM-1, the levels had no correlation with any of the disease severity markers (table 3). This could suggest that endothelial VCAM-1 might not be involved in the recruitment of neutrophils to the bronchiectatic airways [19, 20]. There is also a mononuclear cell infiltration, including CD4⁺ T cells and CD68⁺ macrophages, into the bronchiectatic airways, although the role of these cells in the pathogenesis is unclear [35]. The up-regulation of circulating VCAM-1 among bronchiectasis patients could be secondary to stimuli on the airway endothelium by bacterial derived LPS, or local inflammatory cytokine such as TNF- α and IL-1 production [1]. It is also possible that *in vivo* VCAM-1 up-regulation serves to recruit non-neutrophil leukocytes, namely monocytes and lymphocytes, into the bronchiectasis airways, while ICAM-1 and E-selectin recruit the neutrophils [19, 20, 29–34].

The observations showing the correlation between circulating levels of adhesion molecules with clinical parameters, namely forced expiratory volume in one second, forced vital capacity, and sputum volume, in this study, suggest that adhesion molecules could play an important role in the pathogenesis of bronchiectasis.

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