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Neutrophil CD11b and soluble ICAM-1 and E-selectin in community acquired pneumonia

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Neutrophil CD11b and soluble ICAM-1 and E-selectin in community acquired pneumonia. P. Glynn, R. Coakley, I. Kilgallen, S. O'Neill. ©ERS Journals Ltd 1999.

ABSTRACT: It was hypothesized that there would be an upregulation of systemic neutrophil CD11b expression in pneumonia. Expression of CD11b and concentrations of soluble intercellular adhesion molecule (ICAM)-1 and E-selectin were evaluated as potential surrogate markers of the severity of pneumonia. Possible age-related immunosenescence in relation to neutrophil CD11b expression in elderly patients with pneumonia was examined for.

In patients with community-acquired pneumonia (n=36) neutrophil CD11b expression was measured by flow cytometry and soluble ICAM-1 and E-selectin concentrations by enzyme-linked immunosorbent assay.

An upregulation of neutrophil CD11b expression and increased soluble adhesion molecule concentrations on admission were confirmed, but the concentrations did not correlate with patient Acute Physiology and Chronic Health Evaluation II scores. Neutrophil CD11b expression was similar between elderly (age range 70–100 yrs) and younger (age range 18–70 yrs) patients with pneumonia.

In conclusion, there is evidence of neutrophil and endothelial cell activation in pneumonia as indicated by upregulation of CD11b and increased soluble intercellular adhesion molecule and E-selectin, however, they do not appear to be good surrogate markers of severity of infection. Advanced age does not influence adhesion molecule expression in pneumonia.

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A key component of the inflammatory response to infection is leukocyte-endothelial cell interaction mediated by constitutive and inducible leukocyte and endothelial cell adhesion proteins expressed on their cell surfaces. These molecules can be grouped into three distinct families based on their molecular structure. The β_2 integrin CD11b/CD18 is expressed on resting neutrophils, but after stimulation by inflammatory mediators a large intracellular pool can be mobilized to the cell surface [1]. Intercellular adhesion molecule (ICAM)-1, a member of the immunoglobulin supergene family, is expressed on a variety of leukocytes and endothelial cells [2]. Release of cytokines at sites of inflammation causes cell activation and upregulation of ICAM-1 [3]. A soluble form circulates in the blood of normal healthy individuals, but elevated levels have been reported in a variety of inflammatory states [4]. E-selectin, a member of the selectin family is only expressed on activated endothelium stimulated by inflammatory cytokines such as lipopolysaccharide (LPS), tumour necrosis factor (TNF)-α and interleukin (IL)-1 [5]. Its soluble form has also been found in healthy individuals and in a number of disease states, including sepsis [6]. Increased circulating quantities of soluble forms of these adhesion molecules may be due either to cytokine-induced increased expression and shedding or to proteolytic cleavage of membrane-bound receptors [7, 8]. Both of these molecules may therefore serve as markers of endothelial activation or damage, particularly E-selectin as it is confined to activated endothelium, but they may also have a biological role to play in the inflammatory response. There are a number of potential ways in which they could do this, such as by binding to leukocyte receptors and thus preventing their adherence to the endothelium or by modification of expression of other adhesion molecules, an example being the potential upregulation of CD11b by E-selectin [9].

Study of adhesion molecules has been limited to date in pneumonia, a condition in which intense neutrophil recruitment to the lung is critically dependent on these proteins. It is known that neutrophils and monocytes are primed to produce more reactive oxygen species in pneumonia [10] as a means of killing bacteria, and that neutrophil byproducts may mediate endothelial damage, potentially leading to complications such as adult respiratory distress syndrome (ARDS). It is well established that there is increased basal expression of CD11b on peripheral blood neutrophils [11] and on alveolar neutrophils [12] in ARDS. However it is not known to what extent upregulation of surface neutrophil adhesion molecules occurs in community-acquired pneumonia or to what extent the endothelium is activated or even damaged in this condition, characterized by intense parenchymal neutrophilic inflammation. This could have important implications since a significant number of such infections which fail to resolve are complicated by ARDS. No studies to the authors' knowledge have examined the

relationship between CD11b and circulating adhesion molecules in sepsis or examined soluble adhesion molecules as surrogate markers of severity of sepsis in pneumonia.

In performing this study, the primary hypothesis was that CD11b would be upregulated on peripheral blood neutrophils in pneumonia. Another aim was to determine whether the degree of neutrophil CD11b upregulation and concentrations of the soluble adhesion molecules ICAM-1 and E-selectin could be related to clinical status, and evaluate them as potential surrogate markers of the severity of infection. Finally, it was hypothesized that CD11b expression may fail to be activated optimally in elderly patients with pneumonia, due to age-related immunosenescence. There is some data in healthy elderly populations demonstrating impairment of neutrophil chemotaxis, phagocytosis, oxidative metabolism and intracellular killing in these individuals [13, 14]. Therefore, this possible age-related impairment of neutrophil function in pneumonia was explored.

Materials and methods

Study subjects

Thirty-six patients admitted to hospital for management of community-acquired pneumonia were studied. Pneumonia was defined as an acute respiratory tract infection associated with clinical and radiological evidence of lung consolidation, which was previously unrecorded, and for which no other cause was found. Patients were excluded if they were immunocompromised, or suffering from tuberculosis. Patients who had received antibiotic treatment in the community (n=11) were included only if there was ongoing clinical evidence of sepsis on admission such as pyrexia or leukocytosis, in the presence of lung consolidation. These patients did not differ on statistical analysis from those who were antibiotic naive as regards the degree of pyrexia or leukocytosis on admission.

Study design

The clinical data recorded on admission included temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure, and a number of laboratory measurements of relevance including white cell counts, urea, creatinine, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels. Acute Physiology and Chronic Health Evaluation (APACHE) II scores were calculated for each patient. Blood samples for laboratory assays were taken within 12 h of admission, and where possible prior to administration of systemic antibiotics. Measurement of neutrophil CD11b expression and soluble ICAM-1 and E-selectin concentrations in the blood was performed in all patients and 33 age-matched healthy control subjects.

Methods

Neutrophil CD11b expression. A whole blood assay was used for the measurement of neutrophil CD11b expression in 30 of these patients and 24 healthy-age matched control subjects. Phycoerythrin-conjugated anti-CD11b antibody (10 μL; Becton Dickinson, Cowley, Oxford, UK) was added to 50 μL of heparinized whole blood and incubated at room temperature in the dark for 15 min. Erythrocytes were lysed by the addition of lysing solution (Becton Dickinson) for 20 min, and after centrifugation at $300 \times g$ for 5 min the cell pellets were washed with phosphate-buffered saline (PBS; Sigma, Poole, Dorset, UK) and centrifuged again at $300 \times g$ for 5 min. Flow cytometry was performed immediately with a fluorescence-activated cell sorter (FACScan; Becton Dickinson, Mountain View, CA, USA). Forward and sideward scatter were used to identify the neutrophil population and to gate out other cells and debris. Ten thousand events were counted per sample. Fluorescence emitted at 580 nm was detected. Nonspecific staining/fluorescence was adjusted in all cases to an arbitrary value of 1×10^1 which was subsequently subtracted from the fluorescence of stained samples and the final figure was expressed as mean fluorescence intensity (MFI) to quantify CD11b receptor density. All experiments were carried out in the presence of an isotype control.

Soluble intercellular adhesion molecule-1 and E-selectin

Serum was obtained by centrifugation of whole blood at $700 \times g$ for 10 min, and stored at -80°C. Immunoenzymatic assays (R&D Systems Europe Ltd, Abingdon, Oxon, UK) were used for quantification of soluble ICAM-1 and soluble E-selectin in samples. All samples were assayed in duplicate.

From the original 36 patients, 10 patients, in whom there was clinical evidence of resolution of pneumonia, were randomly selected for repeat assays of neutrophil CD11b expression, soluble ICAM-1 and E-selectin between days 7 and 14 of admission using the methods described above.

Analysis

Calculations were performed using the statistical software package GraphPad Prism Version 2.0 (GraphPad Software, Inc., San Diego, CA, USA). The Mann–Whitney test was used for analysis of nonparametric data. Paired admission and recovery data was analysed using the paired Student′s t-test. Pearson′s correlation coefficient was used for correlation between CD11b and the soluble adhesion molecules, and between these measurements and the individual clinical and laboratory parameters mentioned above and APACHE II scores. For analysis of data from "elderly" *versus* "young" patients, a cut-off point of ≥60 yrs was used to define a patient as "elderly" and <60 yrs to define one as "young".

Results

Thirty-six patients were studied in total. Table 1 shows the clinical and laboratory data pertaining to these patients. Significant differences between "elderly" and "younger" patients with respect to a number of these parameters are indicated in table 2. APACHE II scores ranged 2–34, indicating a spectrum of severity of illness.

Table 1. – Clinical and laboratory data in all patients (n=36) with community-acquired pneumonia

Age >60 yrs %	67
T_{max} >38°C %	33
f_R >30 breaths·min^{-1} %	14
Pulse rate >100 beats·min^{-1} %	31
Systolic BP >90 mmHg %	5.5
Diastolic BP <60 mmHg %	5.5
WCC >12 × 10^9 cells·mL^{-1} %	67
P_{O_2} <8.0 kPa %	19
Urea >7 mmol·L^{-1} %	50
Creat >150 mol·L^{-1} %	19
*ESR >50 mm·h^{-1} %	72
$^{+}$CRP >10 mg·dL^{-1} %	83
APACHE II score mean±SD	12±8
Positive microbiological data n	
Blood culture$^{\#}$	1 (*S. pneumoniae*)
Sputum culture‡	1 (*S. aureus*)
Pleural fluid culture§	4 (sterile, pH <7.2)

T_{max}: maximum temperature; f_R: respiratory frequency; BP: blood pressure; WCC: white cell count; P_{O_2}: oxygen tension; Creat: creatinine; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; APACHE: Acute Physiology and Chronic Evaluation; *S. pneumoniae*: *Streptococcus pneumonia*; *S. aureus*: *Staphylococcus aureus*. *: n=29; $^{+}$: n=12; $^{\#}$: n=26; ‡: n=16; §: n=4. (1 mmHg=0.133 kPa.)

Two patients required mechanical ventilation in an intensive care unit (ICU) and three patients, in whom a decision not to ventilate was made owing to advanced age and the presence of comorbid illnesses, died due to respiratory failure. No patient met the criteria for severe sepsis or septic shock. Microbiological data were limited, as the primary aims of the study were not to identify an aetiological organism. In those patients in whom samples were taken, the positive results are included in table 1. Eleven patients had received prior antibiotics in the community, but they had ongoing evidence of sepsis and their white cell count and maximum temperature on admission were similar to those who had not received prior antibiotic therapy (p>0.05).

Neutrophil CD11b expression and soluble ICAM-1 and E-selectin concentrations were all significantly higher on admission compared to healthy control subjects (fig. 1). A histogram comparing neutrophil CD11b expression in a representative patient and control subject is shown in figure 2.

There was no significant correlation between CD11b expression and either ICAM-1 or E-selectin concentrations, but there was a positive correlation between ICAM-1 and E-selectin (r=0.4, p<0.05). Elderly patients had a similar expression of CD11b to younger patients (table 2). Similarly, concentrations of the soluble adhesion molecules in pneumonia did not vary with increasing patient age (table 2).

There was no correlation between neutrophil CD11b expression or concentrations of either soluble adhesion molecule and patient white cell count, ESR urea, temperature, respiratory frequency, or APACHE II scores. CRP levels correlated positively with soluble ICAM-1 concentration only (Pearson′s r=0.61, p<0.05), but not with soluble E-selectin or CD11b expression.

The 10 randomly selected patients in whom adhesion molecule concentrations were repeated on recovery were similar on statistical analysis to all the others in the study in terms of age, temperature, white cell count, APACHE II score, soluble ICAM-1, soluble E-selectin, and CD11b expression on admission. Soluble E-selectin, when measured again in these patients between days 7 and 10 after inpatient treatment fell to control levels (85.6±10 ng·mL^{-1} *versus* 62±8.3 ng·mL^{-1}, p<0.05), but ICAM-1 levels and neutrophil CD11b expression did not fall (fig. 3).

Discussion

This study demonstrated upregulation of neutrophil CD11b expression and increased soluble adhesion molecule concentrations in pneumonia, but only soluble ICAM-1 levels fell to normal within 10 days of treatment. The inflammatory response in sepsis is critically dependent on neutrophil activation and adhesion to the endothelium, facilitating leukocyte recruitment to the site of inflammation, such as the lung in pneumonia.

Studies have established that concentrations of soluble ICAM-1 and E-selectin correlate with disease severity and outcome in sepsis. Patients with systemic inflammatory response syndrome (SIRS) have higher levels of soluble ICAM-1, E-selectin and vascular cell adhesion molecule (VCAM)-1 than control subjects, and those with SIRS plus organ dysfunction have significantly higher levels of E-selectin than those with uncomplicated SIRS [15]. SESSLER *et al.* [16] demonstrated similar findings for soluble ICAM-1 in sepsis with shock, showing higher levels of ICAM-1 in nonsurvivors.

Neutrophil CD11b expression is increased in septic shock [17], but this is not specific for sepsis, as it also occurs in post-traumatic ARDS [18]. ROSENBLOOM *et al.* [19] showed upregulated CD11b in SIRS due to infection and correlation between mean neutrophil CD11b expression, IL-6 levels and Goris scores of organ failure. Adhesion molecules have been less thoroughly studied in pneumonia, a condition critically dependent on neutrophil recruitment to the lung, and occasionally complicated by ARDS in which neutrophil-mediated toxicity may be important [20]. The aims of the present study were to demonstrate upregulation of CD11b expression in community-acquired pneumonia, and to study the relationship

Table 2. – Clinical data, neutrophil CD11b expression, soluble intercellular adhesion molecule (sICAM)-1, and soluble E-selectin in patients over 60 yrs (n=25) and under 60 yrs (n=11) of age with community-acquired pneumonia

Age range yrs	WCC × 10^9 cells·mL^{-1}	Temp °C	Pulse rate	f_R	Urea mmol·L^{-1}	PMN CD11b MFI	sICAM-1 ng·mL^{-1}	sE-selectin ng·mL^{-1}
60–100	17.8	37.8	104^{+}	26	11.8*	427±80	609±64	120.7±12
15–60	16	38.1	87	21	6.5	423±69	553.7±67	133.5±19

Values are presented as mean±SEM. WCC: white cell count; Temp: temperature; f_R: respiratory frequency; PMN: polymorphonuclear; MFI: mean fluorescent intensity. *: p<0.05; $^{+}$: p<0.005.

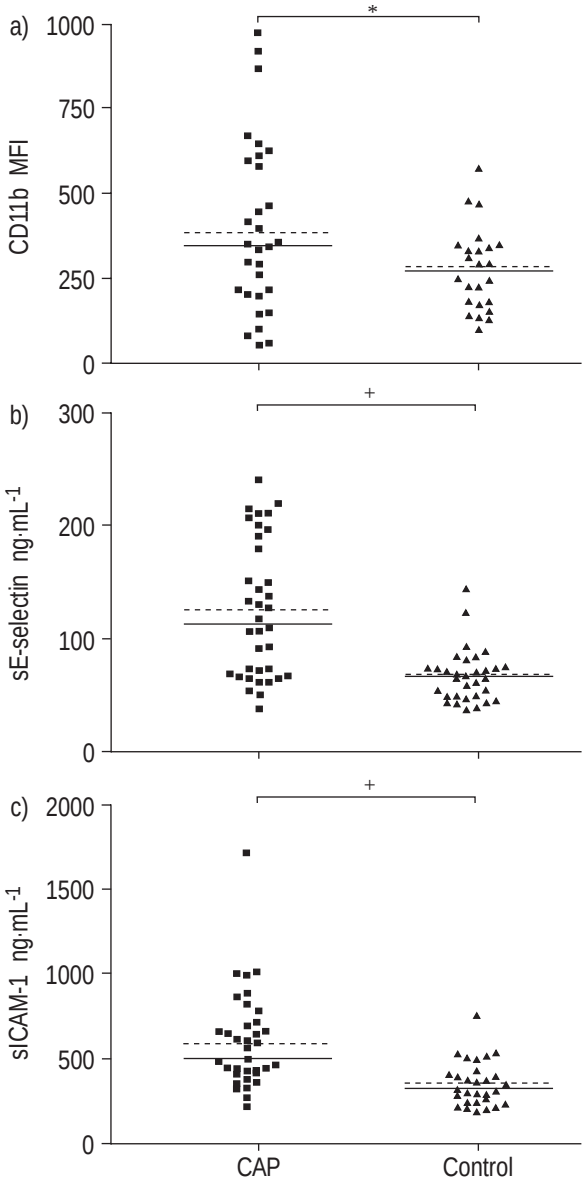

Fig. 1. – Peripheral blood neutrophil CD11b expression (a), soluble E-selectin concentrations (b), and soluble intercellular adhesion molecule (sICAM)-1 concentrations (c) in community-acquired pneumonia (CAP) patients compared to control subjects. —— : medians; - - - - : means. MFI: mean fluorescent intensity. *: $p<0.05$; $^{+}$: $p<0.0001$.

between CD11b, the soluble adhesion molecules ICAM-1 and E-selectin, and the clinical status of the patient. An increased CD11b expression on admission was confirmed, even in the absence of complications such as septic shock, ARDS or multiorgan failure. Upregulation of peripheral blood neutrophil CD11b expression in pneumonia probably reflects neutrophil activation secondary to *in vivo* exposure to inflammatory stimuli.

No correlation was found between CD11b concentrations and the severity of illness as measured by APACHE II scores. In this population, peripheral blood CD11b expression does not appear to be a useful surrogate marker for severity of infection, although comparing local neutrophil adhesion molecule expression in the marginated pool in the lung to systemic neutrophil activation may

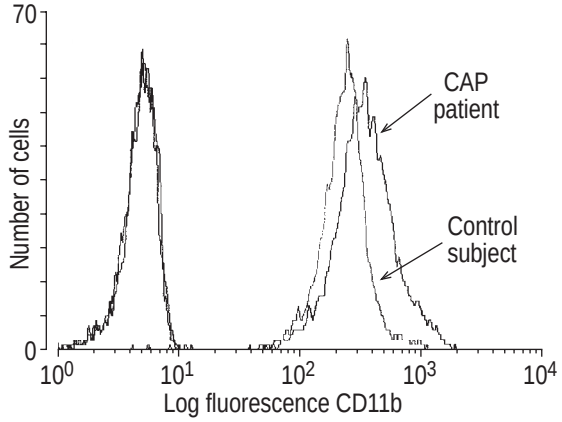

Fig. 2. – Histogram showing neutrophil CD11b expression in a representative control subject and a patient with community-acquired pneumonia (CAP), measured as emitted fluorescence of labelled sample at 580 nm. The two superimposed curves on the left are virtually indistinguishable, representing cellular autofluorescence of patient and control (both $<10^1$). Cellular autofluorescence of both samples $<10^1$.

reveal a compartmentalized response. Compartmentalization of the inflammatory response in pneumonia to the site of infection within the lung has been established for TNF-α, IL-lβ, IL-6, IL-8 and neutrophil elastase [21, 22]. In the inflamed lung, an expanded marginated pool of activated neutrophils may have upregulated CD11b expression. Recruitment of this subpopulation of activated neutrophils away from the systemic circulation to the lung would explain the weak correlation found between circulating neutrophil CD11b expression and the severity of infection.

The adhesion molecules E-selectin and ICAM-1 have not previously been studied in immunocompetent patients with pneumonia. Soluble ICAM-1 levels are elevated in association with pneumonia in neutropenic patients, but soluble E-selectin and soluble VCAM-1 do not rise [23]. SHIJUBO *et al.* [24] failed to demonstrate elevated circulating ICAM-1 in patients with bacterial and mycoplasma pneumonia studied as a control group. However, it is unclear how these patients differed from the present patients in terms of demographic data, need for hospitalization, or timing of the measurements during infection.

Study of circulating molecules can give us indirect evidence of the degree of endothelial activation or damage in pulmonary sepsis. In fact in other infections accompanied by a massive systemic inflammatory response, they appear to be good surrogate markers for severity of sepsis and may predict outcome [25]. In the present study, elevated soluble ICAM-1 and E-selectin have been demonstrated in community-acquired pneumonia uncomplicated by septic shock or multiorgan failure. However, a drawback of this study is the shortage of patients at the severe end of the spectrum of infection. This may partly account for the weak correlation between CD11b expression, ICAM-1 and E-selectin concentrations and APACHE II scores or other indices of sepsis, such as features of SIRS. However, soluble ICAM-1 concentrations did correlate positively with CRP level, a parameter which has been identified as a good clinical marker of the severity of pneumonia [26]. It is speculated that a study limited to those with severe community-acquired pneumonia may

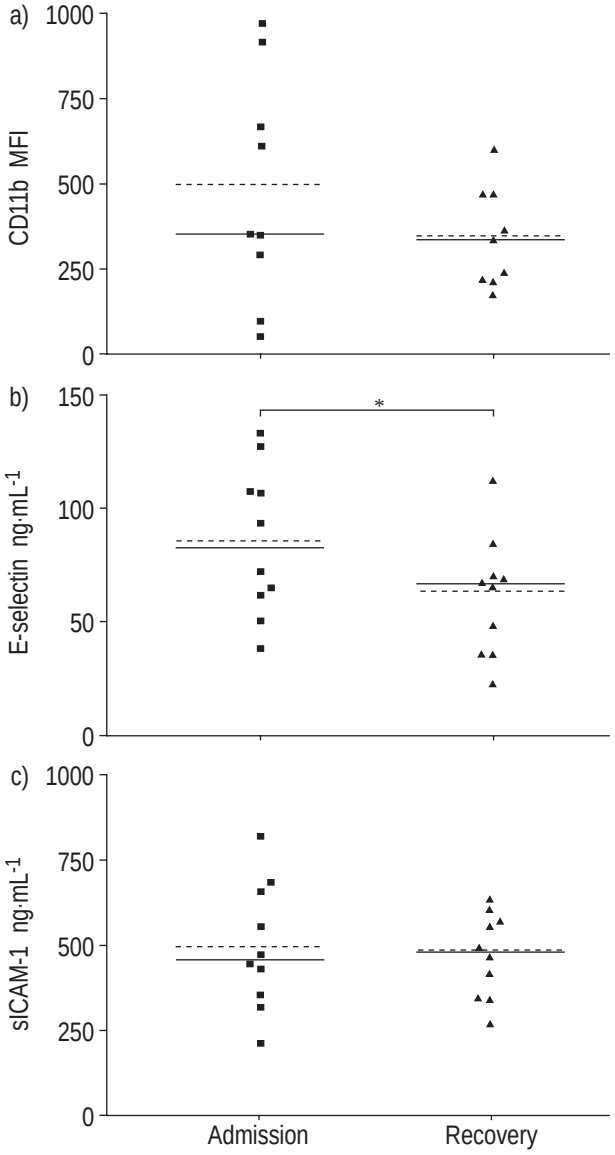

Fig. 3. – Neutrophils CD11b expression (a), soluble E-selectin concentration (b), and soluble intercellular admission molecule (sICAM)-1 concentration (c) at admission and recovery. —— : median; - - - - : mean. MFI: mean fluorescent intensity. *: $p<0.05$.

have demonstrated more relevant findings in this respect. Increased soluble ICAM-1 and E-selectin concentrations may indicate activation of the host inflammatory response to infection, in particular endothelial activation, and possibly also endothelial damage. It is postulated that just as there are mechanisms to limit the inflammatory cytokine cascade in sepsis [27], the shedding of these biologically active molecules may protect the endothelium from further neutrophil adherence and damage by a number of possible mechanisms. Soluble ICAM-1 may compete with membrane-bound forms for leukocyte adhesion molecules preventing attachment of leukocytes to endothelium [7]. Soluble E-selectin can upregulate neutrophil CD11b function [9] and may promote interaction between neutrophil and endothelium. In fact just as there are complex interactions between other soluble mediators such as cytokines and cell-bound adhesion molecules [28], there may also be important interactions between soluble and surface adhesion molecules. The precise source of these molecules in serum is unknown as ICAM-1 is expressed widely on a variety of cells including lymphoid and endothelial cells [2], and E-selectin only on activated endothelial cells [5].

E-selectin fell to normal within 14 days of treatment in a number of survivors who were retested, but ICAM-1 levels remained elevated. These patients were a randomly selected group in whom clinical resolution of infection had occurred. In terms of age, clinical features of infection on admission, and adhesion molecule expression on admission, patients in this group were similar to those in whom recovery data were not obtained, and therefore it is felt that they were representative of the group as a whole. The data suggest a dampening down of the endothelial inflammation after early resolution of infection, however, interpretation is limited owing to the small number in this subgroup. However, ICAM-1 concentrations remain elevated agreeing with data on persistently elevated ICAM-1 in neutropenic patients with pneumonia [23]. CD11b expression also remained elevated from admission to discharge.

The final question raised was whether elderly patients with pneumonia fail to activate neutrophils, potentially impairing host defence. Those >60 yrs of age were compared to patients <60 yrs of age for expression of neutrophil CD11b and concentrations of soluble ICAM-1 and E-selectin, and no significant difference was found. Elderly patients in the study displayed ample ability to mount a septic response to infection, contrary to previous findings [29]. There is no evidence of impaired neutrophil activation in the elderly in pneumonia. Boldt *et al.* [30] have also shown that soluble adhesion molecule levels in critically ill elderly and younger patients do not differ.

In summary, this study demonstrated upregulation of neutrophil CD11b and increased concentrations of circulating intercellular adhesion molecule-1 and E-selectin in community-acquired pneumonia. These findings reflect neutrophil and endothelial cell activation and possibly endothelial damage in pneumonia. Age has no influence on neutrophil CD11b expression or concentration of soluble intercellular adhesion molecule and E-selectin in pneumonia.

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