Increased levels of exhaled sICAM1, sVCAM1, and sE-selectin in patients with non-small cell lung cancer

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Received 5 March 2014; accepted 12 August 2014
Available online 20 August 2014

Summary

Aim: The mortality of lung cancer remains high and methods for early diagnosis are still lacking. Recently, exhaled breath condensate (EBC) has been considered a potential tool for obtaining biological information leading to a reliable diagnosis of non-small cell lung cancer (NSCLC).

Objective: This study assessed the potentials of exhaled and serum concentrations of soluble(s) forms of intercellular adhesion molecule 1 (sICAM1), vascular cell adhesion molecule 1 (sVCAM1), and E-selectin as biomarkers for diagnosis and predicting metastasis in NSCLC patients.

Methods: We enrolled 33 patients with NSCLC, 35 patients with chronic obstructive pulmonary disease (COPD) and 30 healthy controls. EBC and serum samples from subjects were collected at the time of diagnosis and, where applicable, 3 months after surgical treatment. Measurements of sICAM1, sVCAM1, and sE-selectin were determined by enzyme immunoassay.

Results: Concentrations of sICAM1, sVCAM1, and sE-selectin were significantly elevated compared to COPD patients and healthy controls. The exhaled and serum levels of sICAM1 and sVCAM1, but not sE-selectin, decreased significantly after tumor resection from pre-surgery levels. In addition, analyzed results showed a correlation between exhaled sICAM1 levels and disease progression of NSCLC patients.

Abbreviations: COPD, chronic obstructive pulmonary disease; EBC, exhaled breath condensate; ICAM1, intercellular adhesion molecule 1; NSCLC, non-small cell lung cancer; sICAM1, soluble intercellular adhesion molecule 1; sVCAM1, soluble vascular cell adhesion molecule 1; VCAM1, vascular cell adhesion molecule 1.

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http://dx.doi.org/10.1016/j.rmed.2014.08.003
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Introduction

Lung cancer still remains the most frequent cause of cancer-related death. Survival rates are poor despite substantial improvements in diagnosis and treatment [1,2]. Early diagnosis of this malignancy is a major challenge, as symptoms occur predominantly in the later stages [3,4]. To date, surgery is the only curative option, and adequate tumor extirpation can only be performed at its early stage. Thus, the development of effective screening methods for early diagnosis and prognosis has great clinical importance.

Recently, exhaled breath condensate (EBC), obtained by cooling exhaled air under spontaneous breathing conditions, has been proposed to monitor lung pathobiology. Various biomarkers in EBC have been identified and evaluated in inflammation and oxidative stress [5] and [6]. Many researchers have applied this technology to screen cancer biomarkers [7,8]. Moreover, nanomaterial-based sensors have been successfully developed to identify diagnostic and prognostic biomarkers in disease-related EBC [9,10].

Intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) are members of the immunoglobulin superfamily that have an important role in enabling the adhesion of host cells to cancer cells [11]. In normal lung tissue, ICAM1 is expressed mainly by bronchial and alveolar epithelial cells [12]. However, ICAM1 was shown to enhance the metastatic ability of malignant tumors [13] and is strongly expressed in all histological types of NSCLC [14]. In addition, high concentrations of the soluble form of ICAM1 (sICAM1) in the circulation have been reported in several malignant diseases, including lung cancer [15,16], and thus sICAM1 may be a reliable surrogate marker for predicting the efficacy of chemotherapy and prognosis in cancer patients.

VCAM1 is found in endothelial cells, dendritic cells, macrophages, renal parietal epithelial cells, synovial lining cells and reactive mesothelial cells [17]. Soluble forms of VCAM (sVCAM) have been identified, and elevated serum levels of sVCAM have been reported in a variety of malignant tumors [11,18–20]. Moreover, ICAM1 and VCAM1 signaling pathways are involved in the activation of GTPases, production of reactive oxygen species, and phosphorylation of target proteins, which cause functional disruption, cytoskeletal remodeling, and/or membrane fusion events [21].

E-selectin (also known as endothelial leukocyte adhesion molecule 1) is a transmembrane protein containing lectin-like and endothelial growth factor-like domains, followed by short cysteine-rich repeats. E-selectin is absent from normal skin, but is strongly expressed by dermal endothelium in both squamous cell and basal cell carcinomas [22,23]. Also, it is released in a soluble form (i.e., sE-selectin) by endothelial cells and is easily detected in sera [11,24,25].

At present, no study has documented the levels of sICAM1, sVCAM1, or sE-selectin in the EBC of patients with lung cancer. This study assessed whether these molecules in EBC and sera of NSCLC patients could have diagnostic and prognostic value.

Materials and methods

Characteristics of the patients

The study population consisted of 98 subjects: (a) 30 healthy individuals (controls); (b) 35 patients with chronic obstructive pulmonary disease (COPD); (c) 33 patients with NSCLC.

Subjects were consecutively enrolled at the Department of Thoracic Surgery and Department of Respiratory Medicine in our Hospital. Written informed consent was obtained from all subjects upon approval of the study by the Ethical Committee at the Second Affiliated Hospital of Nantong University. All the patients were enrolled in the study before pathological diagnosis and a complete physical examination (including lung X-ray) was performed, including neurologic; cardiopulmonary; and ear, nose, and throat examinations prior to treatments (surgery, radiation or chemotherapy). Alcohol assumption was prohibited for all subjects for 24 h before the procedure.

Following cytohistological diagnosis, patients with NSCLC underwent clinical examination, plain chest X-ray, a computed tomography (CT) scan of the chest, upper abdomen and brain, fiberoptic bronchoscopy, and bone scan. The diagnosis of NSCLC was confirmed either by bronchoscopic biopsy or by transthoracic needle aspiration.

EBC and sera collection

EBC and serum samples from NSCLC patients were collected at the time of diagnosis and 3 months after surgical treatment. Samples from COPD patients and healthy controls were collected at the time of diagnosis. Briefly, EBC was collected between 8 am and 10 am. Before collection, all subjects were required to do fast overnight, and refrain from smoking or drinking for 24 h. EBC was collected using a condenser (EcoScreen, Germany), which allowed for the noninvasive collection of non-gaseous components of the exhaled air. While wearing a nose clip patients and control subjects breathed through a mouthpiece and a two-way non-rebreathing valve (which also served as a saliva trap) at a normal frequency and tidal volume for a period of 10 min. If they felt saliva in their mouth, they were instructed to...
swallow it. The condensate, at least 1 mL, was collected on ice at −20 °C into 1.5 mL microcentrifuge tubes and immediately stored at −80 °C for subsequent analysis.

Peripheral blood samples were collected into sterile glass tubes in the morning after an overnight fast. The samples were allowed to coagulate at room temperature for 30 min and then were centrifuged at 2500 g for 20 min. The serum was separated and stored at −70 °C until analyzed. Before analysis, EBC and serum samples were thawed slowly and mixed gently.

Measurement of sICAM1, sVCAM1 and sE-selectin levels
Quantitative sandwich enzyme immunoassay kits (R&D systems, UK) were used to measure the concentrations of sICAM1, sVCAM, and sE-selectin in the EBC and sera. Results are reported as mean ± standard deviation (SD). The sensitivity levels of the tests for sICAM1, sVCAM, and sE-selectin were 0.25 ng/mL, 1.25 ng/mL, and 0.025 ng/mL, respectively. The intra- and inter-assay coefficients of variation for each were ≤7%, ≤8%, and ≤9%.

Statistical analysis
Results were statistically analyzed using the t-test and analysis of variance test. This was followed by the Wilcoxon signed-rank test. Statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA). A p-value < 0.05 was considered significant.

Results
Subjects’ characteristics
There were no significant differences in terms of age, gender, or smoking status among the NSCLC, COPD, and healthy control groups (Table 1). Staging in the NSCLC group revealed that 48.5% of the patients (16/33) had distant metastases. Thus, we divided the NSCLC patients into two groups: the first group (17/33) included patients without distant metastasis (stage I-III), whereas the second group included patients with distant metastasis, pleural dissemination, or metastasis to the contralateral lung (stage IV) [26].

Concentrations of soluble adhesion molecules in EBC and sera
In NSCLC patients, the concentrations of sICAM1, sVCAM1, and sE-selectin in both EBC and sera were significantly higher than in either the COPD or the healthy controls (p < 0.001 for all comparisons, Table 2 and Table 3).

In NSCLC patients, the concentration of sICAM1 in EBC was 63.4 ± 26.0 ng/mL (range: 23.3–120.2 ng/mL) and in sera 660 ± 271 ng/mL (232–1268 ng/mL). In the COPD patients the sICAM1 in EBC was 39.5 ± 16.9 ng/mL

Table 2  EBC levels of soluble adhesion molecules in NSCLC patientsa, COPD patientsb, and healthy individualsc, ng/mL.

<table>
<thead>
<tr>
<th></th>
<th>NSCLC Before resection</th>
<th>NSCLC After resection</th>
<th>COPD</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM1</td>
<td>63.4 ± 26.0a,b,c,e</td>
<td>44.0 ± 17.7</td>
<td>39.5 ± 16.9c</td>
<td>16.4 ± 4.6</td>
</tr>
<tr>
<td>sVCAM1</td>
<td>52.1 ± 21.5a,b,c,e</td>
<td>37.7 ± 14.0</td>
<td>33.7 ± 13.9</td>
<td>29.7 ± 10.9</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>6.96 ± 2.79a,b,c</td>
<td>5.87 ± 2.06</td>
<td>3.77 ± 1.48</td>
<td>3.67 ± 1.39</td>
</tr>
</tbody>
</table>

a n = 33.
b n = 35.
c n = 30.
d p < 0.001, compared with COPD patients.
e p < 0.001, compared with the healthy controls.
f p < 0.01, compared with NSCLC patients after tumor resection.
(15.3–77.4 ng/mL); sera was 388 ± 162 ng/mL (152–801 ng/mL). In the healthy controls, the sICAM1 in EBC was 16.4 ± 4.6 ng/mL (7.3–25.2 ng/mL), and in sera it was 172 ± 57 ng/mL (79.8–291 ng/mL).

The sVCAM1 concentration in the EBC of NSCLC patients was 52.1 ± 21.5 ng/mL (range 21.7–106.7 ng/mL), and that of sera was 733 ± 311 ng/mL (298–1435 ng/mL). In the COPD patients, sVCAM1 in the EBC was 33.7 ± 13.9 ng/mL (17.2–64.1 ng/mL), and was 404 ± 180 ng/mL (194–803 ng/mL) in sera. For the healthy controls, the sVCAM1 in the EBC was 29.7 ± 10.9 ng/mL (14.6–51.8 ng/mL), and was 324 ± 117 ng/mL (174–583 ng/mL) in sera.

The sE-selectin concentration in the EBC of NSCLC patients was 6.96 ± 2.79 ng/mL (range: 2.95–13.20 ng/mL), and in sera the sE-selectin was 74.0 ± 30.8 ng/mL (30.4–148 ng/mL). In the COPD patients, sE-selectin in the EBC was 3.77 ± 1.48 ng/mL (1.89–6.85 ng/mL), and in sera 40.8 ± 15.2 ng/mL (19.4–71.0 ng/mL). In the healthy controls the sE-selectin in the EBC was 3.67 ± 1.39 ng/mL (1.82–6.49 ng/mL), and 38.2 ± 14.2 ng/mL (18.9–66.7 ng/mL) in sera.

Correlation between concentrations of soluble adhesion molecules and distant metastasis

We found that the concentrations of sICAM1 in the EBC were strongly correlated with distant metastasis in the NSCLC patients (Fig. 1). In NSCLC patients with metastatic disease, the mean concentration of sICAM1 (76.6 ± 22.5 ng/mL) was significantly higher (p < 0.01) than that of NSCLC patients with no metastasis (51.0 ± 23.3 ng/mL; Fig. 1). However, there was no correlation between concentrations of either sVCAM1 or sE-selectin and the presence of distant metastasis in the NSCLC groups (Fig. 1). There were also no such correlations observed between the serum concentrations of sICAM1, sVCAM1, or sE-selectin and distant metastasis in the NSCLC groups (Fig. 2). Interestingly, we also observed that the sICAM1 level in the EBC was significantly higher in the NSCLC patients with lymph node metastasis and at late stage (IV) (Table 4), while the serum sICAM1 level was not associated with any clinical characteristics analyzed (Table 5).

Effects of tumor resection on the levels of soluble adhesion molecules in NSCLC patients

To study the effect of tumor resection on the levels of soluble adhesion molecules in all NSCLC patients (i.e., with or without metastasis), we measured and compared the concentrations of sICAM1, sVCAM1, and sE-selectin in the same patients before and 3 months after surgery.

In EBC, the levels of sICAM1 and sVCAM1, but not sE-selectin, decreased significantly after radical resection of the tumor compared to preoperative levels (p < 0.01, all). The pre- and post-surgery concentrations of sICAM1 were 63.4 ± 26.0 ng/mL and 44.0 ± 17.7 ng/mL, respectively; and those of sVCAM1 were 52.1 ± 21.5 and 37.7 ± 14.0 ng/mL (Table 2).

Similarly, in sera the levels of sICAM1 and sVCAM1, but not sE-selectin, decreased significantly after surgery (p < 0.01, all). The pre- and postoperative serum levels of

<table>
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<th>Table 3</th>
<th>Serum levels of soluble adhesion molecules in NSCLC patients&lt;sup&gt;a&lt;/sup&gt;, COPD patients&lt;sup&gt;b&lt;/sup&gt;, and healthy indivduals&lt;sup&gt;c&lt;/sup&gt;, ng/mL.</th>
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<tbody>
<tr>
<td></td>
<td>Before resection</td>
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<tr>
<td>sICAM1</td>
<td>660 ± 271&lt;sup&gt;d,e,f&lt;/sup&gt;</td>
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<tr>
<td>sVCAM1</td>
<td>733 ± 311&lt;sup&gt;d,e,f&lt;/sup&gt;</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>74.0 ± 30.8&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 33.
<sup>b</sup> n = 35.
<sup>c</sup> n = 30.
<sup>d</sup> p < 0.001, compared with the COPD patients.
<sup>e</sup> p < 0.001, compared with the healthy controls.
<sup>f</sup> p < 0.01, compared with NSCLC patients after tumor resection.

Figure 1 The correlation between EBC levels of soluble adhesion molecules and distant metastasis in patients with NSCLC. EBC samples from metastasis and no metastasis NSCLC patients were collected upon diagnosis. sICAM1, sVCAM1 and sE-selectin concentrations were measured using quantitative sandwich enzyme immunoassay. The correlation between EBC levels of soluble adhesion molecules and distant metastasis in patients with NSCLC were determined using Kruskal–Wallis analysis of variance (ANOVA). *A significant difference compared to the no metastasis group (p < 0.01). †A significant difference compared to the control group (p < 0.001). ‡A significant difference between no metastasis and controls (p < 0.01).
**Discussion**

Our data showed that sICAM1, sVCAM1, and sE-selectin were detected in the EBC and sera of all subjects, but significantly higher levels of sICAM1, sVCAM1, and sE-selectin were observed in the NSCLC patients compared to either COPD patients or healthy individuals. These results are consistent with previous studies [25,27]. These findings suggest that levels of sICAM1, sVCAM1, and sE-selectin may be used as diagnostic markers for lung cancer. Furthermore, the exhaled concentrations of sICAM1 were significantly higher in NSCLC patients with metastatic disease compared to those NSCLC patients without metastasis. This implies that sICAM1 in EBC can be used as a predictor of progression in NSCLC.

High expression of ICAM1 NSCLC indicates that ICAM1 plays a role in the inflammation and tumor growth induced by immune responses. Moreover, an in vitro study showed that the adherence of SCLC cell lines to cultured vascular endothelium is dependent on ICAM1 expression, suggesting a very important role of sICAM1 in the metastatic process [28]. In the present study, we found that the concentration of sICAM1 in EBC positively correlated with distant metastasis in NSCLC patients. This provides further evidence that sICAM1 may be involved in the development and progression of NSCLC [27,29].

It has been demonstrated that exhaled sVCAM1 has a role in cancer development. Mukae and colleagues [30] reported that VCAM1 might play a role in the generation of an effective pulmonary immune response. This is the first study to report elevated sVCAM1 levels in EBC from NSCLC patients. In lung cancer, VCAM1 on the surface of cancer cells binds to $\alpha$4$\beta$1 integrin on metastasis-associated macrophages, and thus triggers VCAM1-mediated activation of phosphoinositide 3-kinase growth and the survival pathway in the cancer cells [31]. sVCAM1 levels in EBC may have diagnostic, predictive, and prognostic value in NSCLC patients. A previous study showed that VCAM1 promoted pulmonary metastasis in a mouse model [32], but the role of VCAM1 in the metastasis of NSCLC has been rarely reported. In the present study, we found that concentrations of both exhaled and serum sVCAM1 was not associated significantly with distant metastasis of NSCLC. Moreover, the EBC and serum levels of sICAM1 and sVCAM1 significantly decreased after radical resection in NSCLC patients. One study reported that elevated sVCAM1 serum levels in patients with metastatic

<table>
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<th>Table 4</th>
<th>Association between EBC levels of soluble adhesion molecules and NSCLC patient clinical characteristics.</th>
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<tr>
<td></td>
<td>n</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>25</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
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<tr>
<td>Smoking history</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>10</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>23</td>
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<tr>
<td>Tumor size</td>
<td></td>
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<tr>
<td>&lt;3 cm</td>
<td>17</td>
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<tr>
<td>&gt;3 cm</td>
<td>16</td>
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<tr>
<td>Histology</td>
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<tr>
<td>Adenocarcinoma</td>
<td>13</td>
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<tr>
<td>Squamous carcinoma</td>
<td>17</td>
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<tr>
<td>Large cell carcinoma</td>
<td>3</td>
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<tr>
<td>Lymph node metastasis</td>
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<tr>
<td>Positive</td>
<td>10</td>
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<td>Negative</td>
<td>23</td>
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<tr>
<td>Tumor stage</td>
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<tr>
<td>I + II + III</td>
<td>17</td>
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<td>IV</td>
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</table>
Roselli et al. [37] observed that elevated concentrations of sE-selectin make it a potential target for therapeutic purposes. Moreover, the upregulation of E-selectin in lung cancer, compared with the limited expression in normal body tissues, suggests that ICAM1 and VCAM1 are likely involved in the development of asthma in childhood. Because both sICAM1 and sVCAM1 were elevated in NSCLC and asthma patients, it is necessary to use other biomarkers to differentiate NSCLC from asthma. Izbicka et al. [36] found that EGF and IL-8 were strong predictors of NSCLC but not asthma, which may improve the diagnosis of NSCLC.

Previous studies have shown that levels of sE-selectin are increased in benign lung disease and lung cancer [37,38]. Here we observed that the exhaled concentrations of sE-selectin were significantly higher in NSCLC patients. Moreover, the upregulation of E-selectin in lung cancer, compared with the limited expression in normal body tissues, makes it a potential target for therapeutic purposes. Roselli et al. [37] observed that elevated concentrations of sE-selectin were related to the presence of distant metastasis in patients with squamous lung cancer. However, we did not find a correlation between sE-selectin and the presence of distant metastasis in our patients with NSCLC. Different subtypes of lung cancer may have different pathophysiology, but the discrepancy in the role of sE-selectin in metastasis needs further investigation.

The patients in this study were not followed up prospectively, which prevented us from determining if sICAM1, sVCAM1, or sE-selectin are biomarkers for recurrence and overall survival in NSCLC. Nevertheless, this report could serve as a pilot study for exploring the roles of exhaled sICAM1, sVCAM1, or sE-selectin in the early diagnosis of suspected lung cancer when bronchoscope and other established diagnostic results are negative. In addition, this study serves as a firm rationale for further research with a larger population to determine the differences among TNM stages in the levels of sICAM1, sVCAM1, or sE-selectin in EBC and serum.

In summary, we found that the levels of sICAM1, sVCAM, and sE-selectin were significantly elevated in the EBCs and sera from patients with NSCLC, and serum levels of sICAM1 and sVCAM1, but not sE-selectin, significantly decreased after radical resection. More importantly, exhaled sICAM1 levels were associated with distant metastasis in NSCLC patients. These data suggest that levels of sICAM1, sVCAM, and sE-selectin can be used as potential biomarkers for the diagnosis or prognosis of patients with NSCLC.

### Conflict of interests
The authors declared no conflict of interests.

### References


