A Panel of Multiple Markers Associated With Chronic Systemic Inflammation and the Risk of Atherogenesis Is Detectable in Asthma and Chronic Obstructive Pulmonary Disease

Tsu-Lan Wu,¹ Pi-Yueh Chang,¹ Kuo-Chien Tsao,¹ Chien-Feng Sun,¹ Lily L. Wu,² and James T. Wu^{2*}

¹Department of Pathology, Chang Gung Memorial Hospital, Taipei, Taiwan

²Department of Pathology and ARUP Laboratories, University of Utah Health Sciences Center, Salt Lake City, Utah

Asthma and chronic obstructive pulmonary disease (COPD) are both lung diseases involving chronic inflammation of the airway. The injury is reversible in asthma whereas it is mostly irreversible in COPD. Both patients of asthma and COPD are known at risk for cardiovascular disease (CVD) and type 2 diabetes (T2DM), nephropathy, and cancer. We measured multiple risk markers for atherogenesis in 55 patients with asthma and 62 patients with COPD. We wanted to know whether risk markers for atherogenesis corresponding to sequence of events of chronic

inflammation were also detectable in the airway inflammatory diseases. Elevation of almost all markers involving inflammation of the endothelial cells in the coronary artery were detectable in asthma and COPD involving the inflammation of the epithelial cell lining of the airway. Both the level and % elevation of all markers were found mostly higher in COPD, the more severe form of the lung disease. We believe that these markers are useful for predicting risk of developing clinical complications such as CVD. *J. Clin. Lab. Anal.* 21:367–371, 2007. © 2007 Wiley-Liss, Inc.

Key words: asthma; COPD; risk markers; atherogenesis; chronic systemic inflammation; oxidative and nitrosative stress

INTRODUCTION

Both asthma and chronic obstructive pulmonary disease (COPD) are inflammatory diseases involving epithelial cell lining of the airways (1–4). Inflammation in asthma is usually sporadic, with a pattern of infrequent, repeated acute inflammations, triggered by dust mites, mold, animal dander, cold air, exercise, and respiratory viruses (1). On the other hand, COPD such as emphysema and chronic bronchitis is due to long time, more frequent exposure to cigarette smoking. In general, the airway injury caused by inflammation in asthma is reversible whereas in COPD it is more severe and in most cases irreversible.

Development of cardiovascular disease (CVD) has been reported in patients with asthma (5,6) and with COPD (7,8). In fact, both airway inflammatory disorders have also been known at increased risk for developing insulin resistance and type 2 diabetes (T2DM) (9), nephropathy (10), and even cancer (11).

Therefore, as reported by Iribarren et al. (6) and Engstrom et al. (8), events such as focal plasma leakage and extensive leukocyte adherence, commonly found in patients with CVD involving endothelial cells, could also be found in patients with chronic inflammatory airway diseases. Therefore, we were interested to know whether risk markers for atherogenesis, such as proinflammatory cytokines, acute inflammation markers,

Abbreviations: COPD, chronic obstructive airway disease; ELISA, enzyme linked immunosorbent assay; IL-6, interleukin 6; CRP, C-reactive protein; CVD, cardiovascular disease; ICAM-1, intercellular adhesion molecule; uMA, urinary microalbumin; 3NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxydeoxyguanosine; SAA, serum amyloid A; VCAM-1, vascular cell adhesion molecule.

*Correspondence to: James T. Wu, ARUP, 500 Chipeta Way, Salt Lake City, UT 84108. E-mail: wuj@aruplab.com

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adhesion molecules, urinary microalbumin (uMA), and markers of oxidative and nitrosative stress, which have been identified to correspond to sequence of events of chronic inflammation (12), are detectable in chronic inflammatory lung diseases such as asthma and COPD. In fact, Wenzel (1) pointed out earlier, in his investigation, that events described for chronic inflammation in the coronary artery, such as stimulation of proinflammatory cytokines, expression of adhesion molecules, and recruitment of leukocytes, could also take place in the airway inflammation.

In this investigation we decided to measure these multiple risk markers for atherogenesis (12) in patients with either asthma or COPD. We have detected elevation of almost all risk markers in both patients with these lung diseases. In addition, we have also found that higher levels and higher % elevation of these risk markers were associated with COPD, the more severe form of airway inflammation. Our investigation also indicated that measuring multiple markers at the same time would improve the sensitivity of detection of the presence of inflammation. Therefore, we believe that simultaneously measuring these multiple markers will be useful for predicting the risk for the development of clinical complications such as CVD.

MATERIALS AND METHODS

Specimens

Both the serum and random urine were obtained from 55 patients diagnosed with asthma with mild to moderate status in stable condition. None had acute infection 6 months prior to the study. There were 35 females and 20 males at age ranging between 26 to 85 years old. Specimens were also collected from 62 patients with COPD. There were only nine females in this group. The age of this cohort ranged from 47 to 83 years old. All samples were stored at -70°C prior to analyses.

They were all outpatients in clinically stable condition at time of their hospital visit. Asthma was diagnosed clinically on the basis of a patient's medical history, physical examination, and assessment of the reversibility of airway obstruction. Patients with asthma were usually associated with symptoms such as wheezing, shortness of breath, and asthma attacks. COPD was defined by a single physiological variable, the forced expiratory volume in 1 sec (FEV1) to forced vital capacity (FVC) of less than 0.7 measured 20 min after the administration of albuterol. COPD and asthma were also defined by a history of smoking that exceeded and below 20 pack-years, respectively. Complications with other diseases such as diabetes, kidney disease, heart disease, etc. were not explored at that time.

Assays

Most markers were measured with in-house developed enzyme linked immunosorbent assay (ELISA) including C-reactive protein (CRP) with a highly sensitive assay (13), serum amyloid A (SAA) (14) adhesion molecules (15,16), uMA (17), 8-hydroxydeoxyguanosine (8-OHdG) (18), interleukin-6 (IL-6) (19), and 3-nitrotyrosine (3NT) (20). All in-house kits have been compared with a commercial kit with good to excellent correlation. Creatinine was determined by the colorimetric method on Hitachi 7600 autoanalyzer (Roche Diagnostics, Indianapolis, IN).

RESULTS

Acute Inflammation Markers

IL-6, CRP, and SAA usually rise acutely in response to acute inflammation. Their appearance also serves as an indication of a continuous presence of inflammatory risk factor(s). In this study we have found that elevated IL-6, CRP, and SAA were all detectable in both asthma and COPD (Fig. 1). Higher % elevation of CRP, SAA, and IL-6 were found in COPD (Fig. 1b). Regarding the level of these markers, CRP had a distinctively higher level in COPD (44 vs. 22%). Both levels of IL-6 and SAA were only slightly higher. Apparently among these acute inflammation markers, CRP is the best marker to indicate the severity of airway inflammation.

Adhesion Molecules

Appearance of adhesion molecules is usually a response of the innate immunity to cell injury by inflammatory risk factors (12) for the purpose of recruiting leukocytes. This event has been described in the endothelial cell dysfunction in the coronary artery (20–23). We have found in this investigation that all three adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and E-selectin, were all elevated in asthma and COPD. Both the level (log scale) and % elevation of all three adhesion molecules were higher in COPD (Fig. 1). Apparently, in addition to CRP, adhesion molecules are also sensitive markers to reflect disease severity in airway inflammation. Among these three adhesion molecules, VCAM-1 appeared to show the largest difference between a more severe form of COPD and the milder form of asthma (Fig. 1b). Therefore, if cost is a concern, only VCAM-1 needs to be measured to reflect the response to the chronic inflammation.

Urinary Microalbumin (uMA)

Detection of microalbuminuria in chronic inflammation is an indication that inflammation has spread

Fig. 1. Detection of multiple risk markers for atherogenesis of chronic inflammation in asthma and COPD. **a:** Mean $\pm$ standard deviation (SD) of all risk markers in log scale were plotted for asthma (A) and COPD (C). Values of mean were also listed on top of the bar figures. For most markers higher levels were found in COPD. Both 8-OHdG and 3NT were only slightly elevated. **b:** The % elevation of all risk markers were plotted in this figure for both asthma and COPD. Higher % elevation of all inflammatory markers, except IL-6, was associated with COPD. Elevation of both markers of oxidative stress was also detectable in asthma and COPD. Only 8-OHdG but not 3NT was higher in COPD. Upper cutoffs used: IL-6, 6.4 pg/mL; CRP, 3 mg/L; SAA, 7 μ g/mL; VCAM-1, 769 ng/mL; ICAM-1, 396 ng/L; E-selectin, 91 ng/L; uMA, 19 μ g/mg creatinine; 8-OHdG, 55 ng/L; and 3NT, 25 nmol/L.

systemically to the kidneys and has caused an increased urinary excretion or plasma albumin due to cell leakage (17). The presence of microalbuminuria also signals an increased risk for CVD (24). In this study we have detected microalbuminuria in both asthma and COPD (Fig. 1). Apparently the airway inflammation has also spread to the kidney. Again higher level and higher % elevation of uMA were found in COPD. However, it is interesting to note, based on these elevated levels of uMA, that not all patients with either asthma or COPD were associated with the advanced stage of nephropathy. The average level of uMA was way below 300 μ g/mg creatinine, a sign of the onset of the more advanced albuminuria. In other words, their impaired renal function was still in a reversible stage and can be reversed to normal by taking medication. Conceivably, uMA should be monitored in patients with asthma or COPD.

If uMA is found to be elevated, patients should be treated to prevent progression to the end-stage of renal failure.

Markers of Oxidative and Nitrosative Stress

Elevation of urinary 8-OHdG, a DNA marker of oxidative stress, has frequently been found in individuals (18) suffering from chronic systemic inflammation. Production of 8-OHdG is usually the downstream event of inflammation reaction. Like uMA, only a slight elevation of 8-OHdG was found in patients with asthma and COPD (Fig. 1a). Again, patients with COPD were associated with both a higher level and higher % elevation of 8-OHdG (Fig. 1).

Elevation of 3NT, a protein marker of both oxidative and nitrosative stress, is also an indication of the presence of increased concentration of peroxynitrite, a

powerful agent of nitrosative stress (20). Apparently, oxidative and nitrosative stress, commonly associated with chronic inflammation are also present in asthma and COPD. However, it is not clear why patients with COPD had a lower level and lower % elevation of 3NT. We speculate that this may have to do with the mild oxidative stress associated with benign lung diseases.

Advantage of Measuring Multiple Markers

We have found that none of the single markers corresponding to the individual sequence of events of the chronic inflammation had satisfactory sensitivity to reflect the presence of inflammation. On the other hand, these multiple markers are apparently complementary to each other. As shown in Fig. 2, measuring multiple markers at the same time increased the overall sensitivity. We selectively measured simultaneously several markers, best representing the individual event of the chronic inflammation, which have reached 100% sensitivity for COPD and 58% for asthma, much higher than that of any single marker. However, we believe it may take further investigation to find the best combination of multiple markers to reach maximal sensitivity for each clinical disorder.

DISCUSSION

There are several unique features associated with asthma and COPD in terms of their relationship with chronic inflammation. Both asthma and COPD are involved with the inflammation of the epithelial cell lining of the airway, which is different from the CVD, involving the endothelial cell of the blood vessel. There is another characteristic feature of the airway inflammation in asthma and COPD. The inflammatory attack in

the airway is intermittent, more like many repeated acute inflammations. The attack appears to be more frequent in COPD and less frequent in asthma. On the other hand, in CVD and T2DM, the chronic inflammation is generated by a continuous exposure to inflammatory risk factors such as abdominal fat, hyperglycemia, abnormal lipid profile, etc. (12).

Apparently, a sequence of risk markers for atherogenesis associated with the chronic inflammation identified in connection with the coronary artery are detectable in asthma and COPD. Detection of these markers in asthma and COPD may have to do with why both these lung diseases are at risk for CVD. It is not clear whether the detection of these markers in the airway inflammation has to do with the abundant blood capillaries surrounding alveoli in the lung and the systemic nature of chronic inflammation. However, we believe that the sequence of events of chronic inflammation are similar regardless of where the inflammation is initiated and takes place. In fact, we should also expect to find various products of lipid peroxidation in airway inflammation (12,25) as the downstream products of chronic inflammation. The association of higher level and greater % elevation of these markers in a more severe form of airway inflammation such as COPD indicates that these markers are useful to assess disease severity of lung diseases.

Detection of elevated CRP and SAA during the stable period of asthma suggests that both acute reactants did not return to normal between intermittent inflammatory attacks. As we have discussed above, IL-6 and SAA do not appear to be as good of a marker as CRP to reflect disease severity in lung diseases. However, we believe that they are still useful to indicate that the lung is continuously suffering from the airway inflammation.

It has frequently been reported in recent years, based on the histology and immunohistology, that it is difficult to clearly distinguish asthma from COPD (26). Considering that both lung diseases are the consequence of airway inflammation, the histological difference found between these two diseases may only be related to their difference in extent and severity of airway inflammation. Perhaps that measuring these multiple markers will provide a better differentiation between these two lung diseases. It also seems that measuring a panel of multiple markers in asthma and COPD, such as the combination of CRP, uMA, and VCAM-1, will provide sufficient sensitivity of risk assessment for atherogenesis and other inflammatory complications.

Fig. 2. Improved sensitivity for detecting chronic inflammation with multiple markers. Multiple markers were selected representing major events of chronic systemic inflammation. They were measured in both asthma and COPD. The panel reached 58% and 100% sensitivity in asthma and COPD, respectively.

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