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Are leukocytes in asthmatic patients aging faster? A study of telomere length and disease severity

To the Editor:

Asthma is a chronic inflammatory disease characterized by episodic and reversible airflow obstruction, airway hyperresponsiveness, and airway wall remodeling. It is common among older adults, and it is estimated that 4% to 13% of adults older than 65 years have asthma,[1] suggesting that aging could be a risk factor and contribute to the clinical outcome of asthma in the elderly.

The normal aging process involves cellular senescence, a state of permanent growth arrest that limits tissue renewal. Cellular senescence can be characterized as either replicative senescence or stress-induced premature senescence and involves the shortening of telomeres.[2] Telomeres are terminal regions of chromosomes containing repeats of TTAGGG that protect DNA from damage.[3] When telomeres critically shorten, cells become susceptible to senescence or apoptosis, indicating that telomere length is a feature of cellular aging. Telomerase plays an important role in telomere maintenance, cell proliferation, and immortality by preventing the shortening of telomeres.[4] Telomerase contains 2 main subunits: a telomerase RNA component (TERC) and the catalytic subunit of telomerase reverse transcriptase (TERT). TERT is a core functional component of telomerase activity, and the potential roles of TERT expression and/or activity in disease pathogenesis have become a focus of active investigation in cancer, aging, and metabolic and cardiovascular diseases.[4]

Studies investigating telomere length in respiratory diseases have demonstrated correlations between telomere shortening and disease outcome. Patients with chronic obstructive pulmonary disease (COPD) have shorter telomeres in circulating leukocytes than do age-matched healthy control subjects.[5] Other studies reported a significant relationship between telomere length and airflow obstruction in patients with COPD[6] and as a risk factor for idiopathic pulmonary fibrosis.[7] While these studies suggest that analysis of telomere length is a predictor of disease progression in COPD and idiopathic pulmonary fibrosis, telomere length and telomerase expression in asthma remain unexplored. We hypothesized that cellular senescence is a marker for disease outcome in asthma and is related to asthma severity. Our aim was to investigate whether differences exist in telomere length and telomerase expression between asthmatic adults and healthy control subjects.

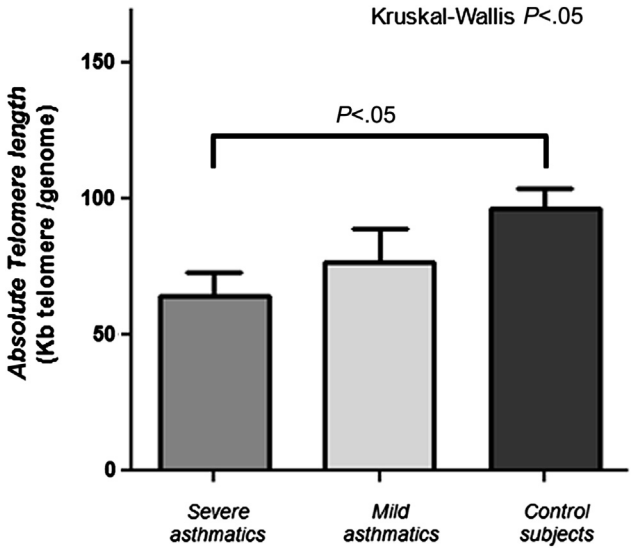

FIG 1. Comparison of absolute telomere length of peripheral blood between patients with severe asthma (n = 8), patients with mild asthma (n = 6), and control subjects (n = 15) ($P < .05$, Kruskal-Wallis; $P < .05$, patients with severe asthma vs control subjects). Kruskal-Wallis test and Dunn *post hoc* test were used for across-group comparison and between-group comparison, respectively. Results are expressed as mean ± SE.

We studied 15 healthy and 14 asthmatic adults aged 25 to 60 years who were nonsmokers. Asthmatic adults consisted of 6 with mild and 8 with severe asthma. For comparison, we also studied 7 patients with COPD aged 58 to 77 years. All patients with COPD were classified as Global Initiative for Chronic Obstructive Lung Disease (GOLD) II according to the current GOLD criteria (GOLD 2011). The mean age of study subjects was as follows: adults with severe asthma, 52.63 ± 2.12 years; adults with mild asthma, 40.83 ± 4.42 years; control subjects, 37.80 ± 2.07 years; patients with COPD, 68.14 ± 3.08 years. We used blood and bronchial biopsy tissues stored at the Tissue Bank of the Respiratory Health Network of the Fonds de la Recherche en Santé du Québec. A hospital research ethics committee approved the study protocol, and written consent was obtained from all subjects. To evaluate telomere length, genomic DNA of peripheral leukocytes was isolated from peripheral blood by using the FlexiGene DNA kit (Qiagen, Toronto, Ontario, Canada). Absolute telomere length was measured by determining the number of TTAGGG hexamer repeats by using quantitative real-time polymerase chain reaction.[8] We performed immunohistochemistry on paraffin-embedded bronchial biopsies to evaluate the localization and expression of human TERT (hTERT) protein by using a rabbit polyclonal antibody to hTERT (Santa Cruz Biotechnology, Paso Robles, Calif; sc-7212).

Telomere length measurements in peripheral blood cells can provide information about the replicative history of cells and the clinical value of telomere length assessment in asthmatic patients. This appears to be reflected in patients with severe asthma whose peripheral blood cells had significantly shorter telomeres than those of control subjects ($P < .05$, multiple comparison test after Kruskal-Wallis) (Fig 1). The mean telomere length (kb telomere/genome) was 64.3 ± 8.9 in patients with severe asthma, 76.4 ± 12.4 in patients with mild asthma, 77.9 ± 10.2 in patients with COPD, versus 96.3 ± 7.5 in control subjects. Telomere

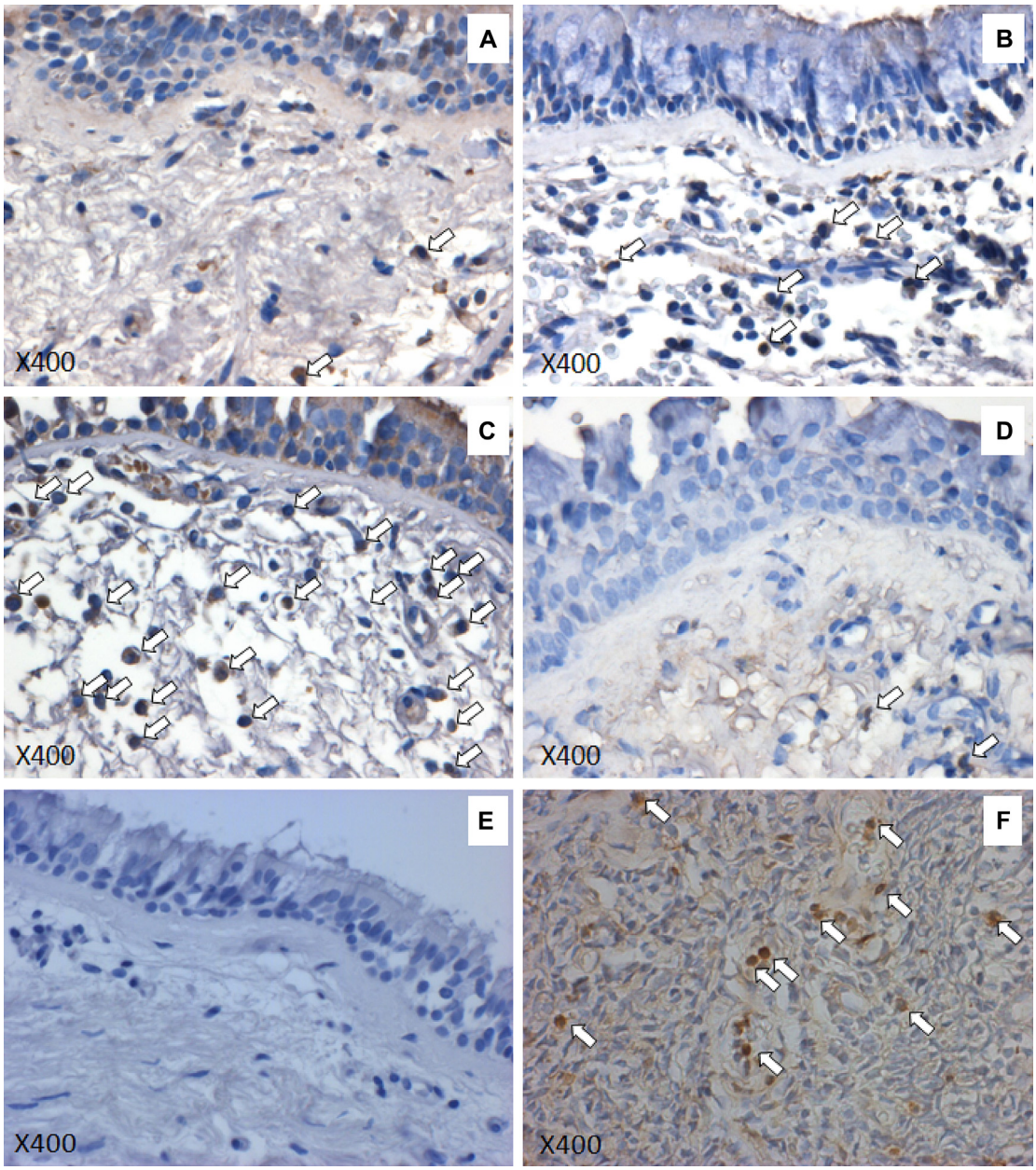

FIG 2. Representative photomicrographs showing immunohistochemical staining for hTERT in bronchial biopsies. **A,** Patient with severe asthma. **B,** Patient with mild asthma. **C,** Healthy control. **D,** Subject with COPD. **E,** Negative control. **F,** Tonsil tissue. *White arrowheads* point to immunopositive cells (*brown* stain) for hTERT expression. Original magnification ×400.

length decreased with age in asthmatic patients (data not shown). A decrease in absolute telomere length in circulating leukocytes in asthmatic patients could reflect increased inflammatory/immune cell turnover leading to a progressive decline in telomere reserves, and thus telomere shortening. As total leukocytes are a diverse cell population, it would be of interest in future studies to investigate whether telomere length was equally affected among all leukocyte cell types. We also analyzed the telomere length of peripheral blood cells and PBMCs. No statistical difference in telomere length was found between peripheral blood cells and PBMCs (data not shown).

As telomerase modulates telomere length, we next performed immunohistochemistry for the evaluation of hTERT protein expression. Fig 2 shows representative immunohistochemical staining of hTERT in the submucosa of bronchial biopsies of patients with severe asthma, patients with mild asthma, control subjects, and patients with COPD. Biopsies from patients with severe or mild asthma revealed a fewer number of immunostained cells positive for hTERT than did biopsies from control subjects. In contrast, hTERT expression was minimal in biopsies from patients with COPD. The mean number of hTERT immunopositive cells in bronchial biopsies from control subjects (30.83 ± 7.83) was significantly higher when compared with biopsies from patients with mild (4.62 ± 1.43) or severe (2.01 ± 0.41) asthma or patients with COPD (1.59 ± 0.57) ($P < .001$, Kruskal-Wallis multiple comparison test with Bonferroni correction) (see