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Immunomagnetic molecular probe with UHPLC–MS/MS: A promising way for reliable bronchial asthma diagnostics based on quantification of cysteinyl leukotrienes

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ABSTRACT

A sensitive and precise method for simultaneous quantification of cysteinyl leukotrienes (=cys LTs) – leukotriene C_4 (=LTC_4), leukotriene D_4 (=LTD_4) and leukotriene E_4 (=LTE_4) – essential biomarkers of bronchial asthma present in exhaled breath condensate (=EBC) was developed. An immunomagnetic molecular probe was prepared by anchoring cysteinyl leukotrienes antibody on the surface of functionalized monodispersed magnetic particles and used to selectively isolate cys LTs from biological matrices – EBC, plasma and urine. Immobilization and the immunoaffinity capture procedures were optimized to maximize the amount of separated cys LTs, which were detected "off-beads" after acidic elution by UHPLC–ESI-MS/MS operated in a multiple reaction monitoring mode. The developed method was characterized with high precision ≤13.6% (intra-day precision determined as RSD) and ≤14.5% (inter-day precision determined as RSD), acceptable accuracy ≤18.5% (determined as RE), and high recovery of immunoseparation (≥93.1%) in aforementioned biological matrices. The applicability of the method was demonstrated on EBC, plasma and urine clinical samples of patients with various subtypes of bronchial asthma (occupational, steroid-resistant, moderate with and without corticosteroids therapy) and healthy subjects where reasonable differences in cys LTs concentration levels were found. Combining extremely selective immunomagnetic separation with highly sensitive and precise detection step, the developed method was used to aid diagnosis, predict the most effective therapy, and monitor the response to treatment. The detection of elevated inflammatory mediators (cys LTs) in EBC of subjects with relatively asymptomatic asthma and normal pulmonary function tests could offer a novel way for monitoring the lung inflammation and perhaps initiating treatment in an earlier stage.

1. Introduction

Bronchial asthma, defined as a chronic inflammatory disorder of the airways, is one of the top public health problems globally affecting both children and adults. Therefore, proper and early diagnostics and treatment is necessary. However, the diagnostics can be rather difficult. Currently, there is no precise physiological, immunological, or histological test for diagnosing asthma. In order to rule out other possible conditions, the lung functions are generally tested first in an asthma diagnosis process. Tests to measure the lung functions include spirometry test and peak expiratory flow rate test. In addition, methacholine or histamine challenge tests can evaluate the airways hyperactivity, typical for asthma, and fractional exhaled nitric oxide (FENO) test measures the level of eosinophilic inflammation in the airways. Unfortunately, this non-invasive test is negatively influenced in smokers. At present, the quantification of inflammation in the lungs may be based on invasive (open lung biopsy [1–3], bronchoalveolar lavage [2,4]) or *semi*-invasive methods (method of induced sputum [5,6]) and the measurement of inflammatory markers in plasma and urine, which are likely to reflect systemic rather than lung inflammation. A relatively novel method is the analysis of EBC, collected during normal tidal breathing, which reflects the composition of extracellular lung fluid. The measurements of metabolite products in the EBC are absolutely non-invasive and repeatable with a good potential to become the preferred alternative. New approaches are based on

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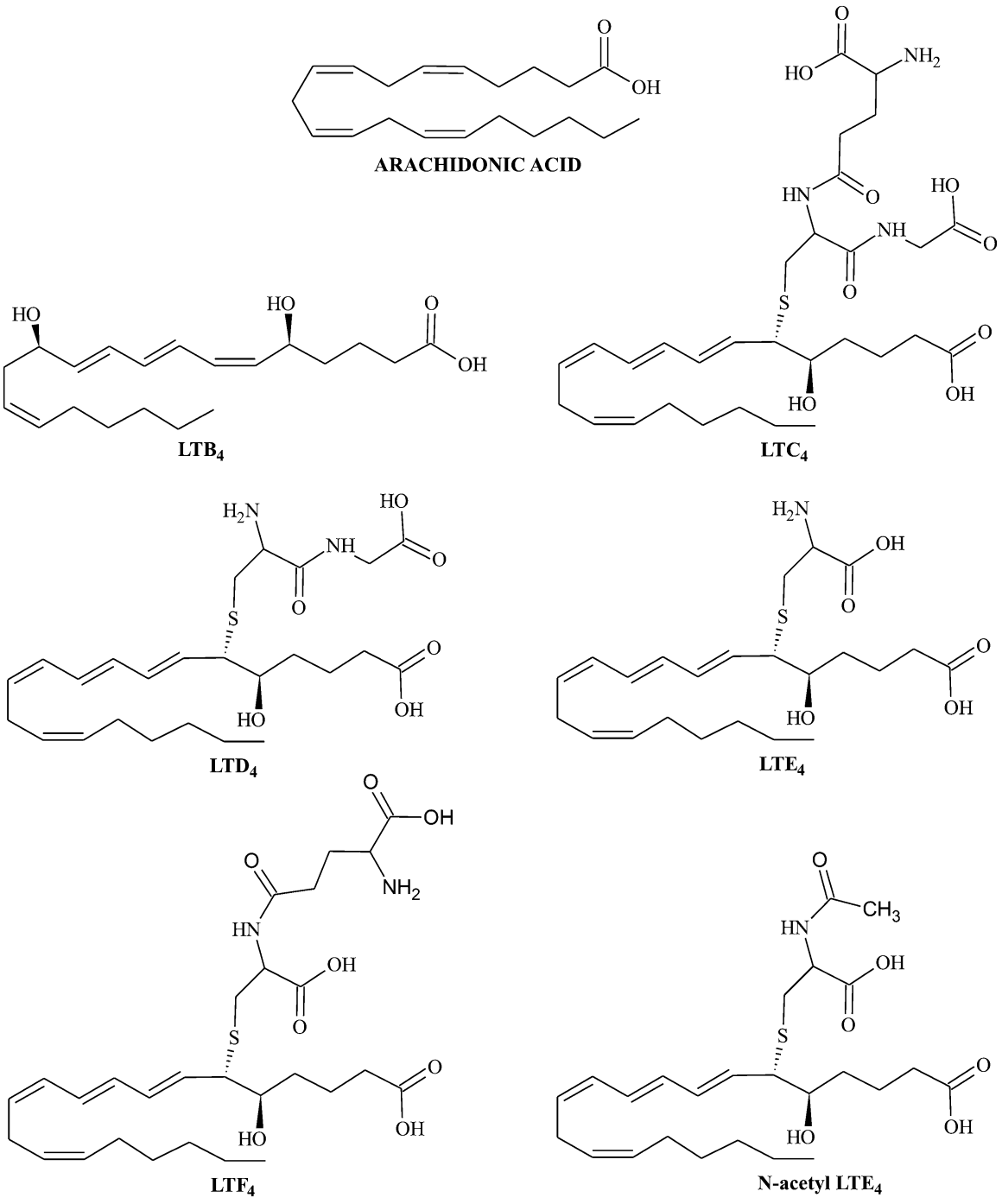

Fig. 1. Arachidonic acid derivatives – potential biomarkers of bronchial asthma.

attempts of identifying robust biomarkers which could be utilized in establishing the diagnosis of asthma [7–9]. The former studies investigated the predictive value of EBC pH for asthma, the latter researched hydrogen peroxide, nitrogen oxides (FENO is nowadays diagnostically/clinically used) [10], arachidonic acid derivatives, cytokines and others. Analysis of breath volatile compounds by electronic nose has been proposed as a new technique for assessing airway inflammation in respiratory medicine [11]. Arachidonic acid derivatives, especially cys LTs, have shown the most consistent results for the diagnosis of asthma [12]. Various inflammatory markers present in EBC have been investigated as possible asthma biomarkers and several independent studies [13–17] have indicated the presence of elevated levels of arachidonic acid derivatives (cys LTs, leukotriene B_4 (=LTB_4)) in EBC of asthma patients. Elevated concentrations of 8-iso-prostaglandin $F_{2\alpha}$, a marker of oxidative stress [18], have been reported in inflammatory respiratory diseases, including asthma [19], COPD [20], and cystic fibrosis [21], and in healthy smokers [22]. Therefore, measurements of these mediators are considered potentially effective in the establishment of asthma diagnosis in a large cohort. Leukotrienes are derived from arachidonic acid, which is also the precursor of prostaglandins (Fig. 1). There are two families of leukotrienes (LTB_4 and cys LTs – LTC_4, LTD_4, LTE_4 and metabolites of LTE_4 – leukotriene F_4 (LTF_4) and *N*-acetyl leukotriene E_4 (*N*-acetyl-LTE_4)). LTB_4 acts primarily when the inflammation is dependent on neutrophils such as cystic fibrosis, inflammatory bowel disease, and psoriasis. The second group