Journal of Chromatography B, 1047 (2017) 97–105

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Exhaled breath condensate analysis from intubated newborns by nano-HPLC coupled to high resolution MS

A.S. Kononikhin[a,e,b,*,1], N.L. Starodubtseva[a,b,1], V.V. Chagovets[a], A.Y. Ryndin[a], A.A. Burov[a], I.A. Popov[b,e,c], A.E. Bugrova[a,c], R.A. Dautov[b], A.O. Tokareva[b,e], Y.L. Podurovskaya[a], O.V. Ionov[a], V.E. Frankevich[a], E.N. Nikolaev[b,c,e,d,*], G.T. Sukhikh[a]

[a] V. I. Kulakov Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Healthcare of the Russian Federation, Moscow, Russia
[b] Moscow Institute of Physics and Technology, Moscow, Russia
[c] Emanuel Institute for Biochemical Physics, Russian Academy of Sciences, Moscow, Russia
[d] Skolkovo Institute of Science and Technology, Skolkovo, Russia
[e] V.L. Talrose Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Moscow, Russia

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 1 November 2016
Accepted 21 December 2016
Available online 23 December 2016

Keywords:
Exhaled breath condensate (EBC)
HPLC
Proteomic
Metabolomics
Mass spectrometry
Newborns
Respiratory diseases
Pneumonia

ABSTRACT

Invasiveness of examination and therapy methods is a serious problem for intensive care and nursing of premature infants. Exhaled breath condensate (EBC) is the most attractive biofluid for non-invasive methods development in neonatology for monitoring the status of intubated infants. The aim of the study was to propose an approach for EBC sampling and analysis from mechanically ventilated neonates. EBC collection system with good reproducibility of sampling was demonstrated. Discovery-based proteomic and metabolomic studies were performed using nano-HPLC coupled to high resolution MS. Label-free semi-quantitative data were compared for intubated neonates with congenital pneumonia (12 infants) and left-sided congenital diaphragmatic hernia (12 infants) in order to define disease-specific features. Totally 119 proteins and 164 metabolites were found. A number of proteins and metabolites that can act as potential biomarkers of respiratory diseases were proposed and require further validation.

1. Introduction

Premature babies constitute a significant portion in the intensive care units. The important tasks are the correct and rapid diagnosis determination and the appropriate treatment. These are crucial for the differentiation of respiratory disorders of infectious and non-infectious genesis to use a suitable care strategy with and without antibiotics [1,2]. Inflammatory disorders of lungs greatly increase the risk of serious diseases such as neonatal chronic lung disease and bronchopulmonary dysplasia (BPD) [3–6]. Mainly invasive methods of examination and therapy of newborns is another serious problem in intensive care and therapy. Nowadays the "gold standard" of neonate pulmonary diagnostics remains extremely invasive methods, like microbiological evaluation of airway lavage and/or tracheal aspirate, immunological diagnostics, including markers in blood [7,8]. Easily accessible biofluids such as buccal scrapings [9] and urine [10–13] were proposed as non-invasive methods for monitoring the status of intubated infants.

Exhaled air and exhaled breath condensate (EBC) analysis become popular and relevant [14–18]. EBC is obtained by cooling exhaled breath and condensing the water vapor in a collecting tube at temperatures below water freezing point. EBC collection procedures in adults are strictly described in reliable guidelines, published in 2010 [17]. EBC contains different classes of compounds including high-mass molecules such as peptides and proteins and low molecular components, for instance, markers of oxidative stress [19].

Numerous studies of EBC biomarker search were performed for adults with various respiratory pathologies, such as asthma, gastroesophageal reflux, chronic obstructive pulmonary disease

* Corresponding authors at: Moscow Institute of Physics and Technology, Moscow, Russia.
E-mail addresses: alex.kononikhin@gmail.com (A.S. Kononikhin), ennikolaev@rambler.ru (E.N. Nikolaev).
[1] Both these authors have contributed equally to this work.

http://dx.doi.org/10.1016/j.jchromb.2016.12.036
1570-0232/

(COPD), lung cancer, non-small lung cancer, cystic fibrosis, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, interstitial lung diseases and obstructive sleep apnea [14,16,20]. Unfortunately, there is still a long way between EBC biomarker search and implementation of this biomarker test to routine clinical practice, due to poor reproducibility, sensitivity and specificity, absence of large validation surveys with multiple disorders for comparison, longitudinal studies and normal reference data.

Clinical application of EBC measuring in pediatrics was demonstrated by different researchers [21–28]. Comprehensive review on the EBC biomarkers in pediatrics was published in 2015 [29]. The authors of the review observed 84 original papers and concluded that due to the lack of EBC collection and standardized analysis methods, the proposed markers of inflammatory pulmonary diseases were far from clinical use [29]. EBC in young children and infants (1–30 months) collected during sedated sleep was shown to be feasible and safe [21]. Children with stable asthma were shown to produce more acidic EBC than healthy controls, the same trend was found in severe disease compared to mild stage [25,26]. Conflicting data on EBC hydrogen peroxide level in asthma may be associated with significant differences in research methodologies [30,31]. Among other potential biomarkers of asthmatic airway inflammation were the increase of asymmetric dimethylarginine (ADMA) [32], 8-isoprostane [33], cysteinyl leukotrienes [34], T helper 2 cytokines [31,35,36], matrix metalloprotease 9 (MMP-9) [35] and decrease of glutathione (GSH) [37] and T helper 1 cytokines [35,36]. Notably, cystic fibrosis markers included almost the same set of molecules: H_2O_2, eicosanoids (8-isoprostane), MMP-9 and cytokines [29]. Hence, a single biomarker does not provide a sufficient sensitivity and specificity in the diagnosis and monitoring of such complex conditions as asthma. Metabolomic profiling seems to be the only method to find potential biomarkers panel of inflammatory pulmonary diseases. This approach was applied by several research groups in asthma and cystic fibrosis diagnostic studies [27,28].

Non-invasive methods based on EBC are especially important in neonatology during intensive care and nursing of premature infants [38]. Only few studies were performed based on EBC from intubated neonates with limited number of compounds under consideration [39–41]. Hitka P et al. developed EBC collection method for hydrogen peroxide measurement in exhaled air from intubated neonates [41]. It was proposed as a biomarker of chronic lung disease (CLD) risk development in ventilated neonates with respiratory distress syndrome (RDS) as was reported by Cheah FC et al. [39]. Rosso MI et al. showed that glutathione status (reduced and oxidized glutathione) in EBC samples of ventilated newborns correlated with one of invasive tracheal aspirate samples, promising non-invasive control of premature lung [40]. Previously, low levels of GSH in the bronchoalveolar fluid of premature infants were observed during the development of BPD, one of the most common complications of extreme prematurity, resulting in infants' morbidity and mortality [42]. All the studies mentioned above were targeted and no comprehensive screening of proteomic and metabolomic features were provided. There is a need in the standardization of EBC collection used for clinical monitoring of neonates on respiratory support [39,40,43]. Deep screening study of newborns EBC by mass spectrometry (MS) and nuclear magnetic resonance (NMR) is necessary.

In the present study, an in-house made system for EBC collection from intubated preterm newborns in neonatal intensive care unit (NICU) was developed. Two groups of intubated neonates with respiratory infectious and non-infectious pathology were investigated to evaluate the method for clinical applicability. Infectious group included 12 infants with congenital pneumonia. The comparison group was presented by 12 newborns with left-sided congenital diaphragmatic hernia. The control group of healthy newborns was not included in the study because the proposed method of EBC collection was developed for intubated neonates.

Discovery-based proteomic and metabolomic analysis was performed using nano-HPLC coupled to high resolution MS. Label-free proteomic and metabolomic data were compared for intubated neonates with congenital pneumonia and left-sided congenital diaphragmatic hernia in order to define disease-specific features for further biomarkers search.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals, solvents and reagents were HPLC grade from Sigma-Aldrich (Gillingham, UK). Modified porcine trypsin (Promega, USA) was used for protein digestion.

2.2. Patient groups

The EBC samples were collected from 24 intubated newborns (4–20 days of life) in the NICU at the V. I. Kulakov Research Center for Obstetrics, Gynecology and Perinatology. Twelve infants with congenital pneumonia, and 12 infants with left-sided congenital diaphragmatic hernia (LCDH) were used as a comparison group. Other neonatal and prenatal complications were severe asphyxia at birth, intrauterine growth restriction, preeclampsia, neonatal seizures. Newborns' groups were comparable for sex, weight, ethnicity and maternal age. Preterm infants were 50.0% in the pneumonia group and 16.7% in the comparison group (Table 1).

Informed consent was obtained from the parents of all infants. The study has been approved by the investigational review board of V. I. Kulakov Research Center for Obstetrics, Gynecology, and Perinatology under the Law of the Russian Federation on Public Health Care.

Table 1
Demographical and clinical data on newborns. Means ±SDs are presented for continuous variables; counts, percentages.

Characteristics	Congenital pneumonia (n = 12)	Left-sided congenital diaphragmatic hernia (n = 12)
Male sex	8(66.7%)	6(50%)
Gestational age at birth, wk	32.1 ± 4.3	35.3 ± 2.8
Mode of delivery		
Vaginal	0(0%)	3(25.0%)
Cesarean section	12(100%)	9(75.0%)
Apgar score ≤7		
at 1 min	8(66.6%)	10(83.3%)
at 5 min	3(25.0%)	9(75.0%)
Antibiotic therapy at the time of the survey	10 (83.3%)	1 (8.3%)
Maternal age, years	32.5 ± 5.7	29.1 ± 4.2
Nulliparous	7(58.3%)	5(41.7%)
Twin gestation	4(33.3%)	2(16.7%)
Preeclampsia	6(50.0%)	2(16.7%)
Intrauterine growth restriction	4(33.3%)	1(8.3%)
Abruption	2(16.7%)	1(8.3%)
Prenatal maternal antibiotic exposure	7(58.3%)	3(25.0%)
Maternal use of antenatal corticosteroids	5(41.7%)	2(16.7%)
Severe asphyxia at birth	2(16.7%)	3(25.0%)
Neonatal seizures	2(16.7%)	1(8.3%)
Surfactant treatment (Curosurf, 120 mg)	5(41.7%)	1(8.3%)