ENGLISH VERSION: METHODICAL APPROACHES TO COLLECTING AND ANALYZING THE EXHALED BREATH CONDENSATE*

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Currently, one of the promising non-invasive diagnostic methods in pulmonology, cardiology, endocrinology, oncology is the study of the exhaled breath condensate (EBC). A significant limitation of the study of EBC in clinical practice is the absence of generally accepted standards for its collection and study of the content of biological substances containing ultra low concentrations in it. The growing scientific and practical interest in this issue led to the organization of the target group American Thoracic Society and the European Respiratory Society and the development of recommendations for the collection of condensate. We have studied and analyzed the recommendations of ATS / ERS and developed an algorithm for sampling EBC.

Key words: exhaled breath condensate, ATS/ERS recommendations biomarkers, condensate collection algorithm.

Non-invasive diagnostics is one of the important directions in modern medicine. The relevance of the problem is due to the impact minimization methods of collecting the material for analysis, when a patient does not have to experience pain, physical and emotional discomfort. It is also due to the safety of the study because it excludes the possibility to contract blood-borne or toolborne infections. Non-invasive diagnostic methods can be used, on the one hand, in outpatient settings, which ensures their widespread use; on the other hand - in patients at the intensive care unit, because the severity of patient's condition is not a contraindication for their conducting. Currently, the study of exhaled breath condensate (EBC) is one of the promising non-invasive methods in pulmonology, cardiology, endocrinology and oncology. EBC contains numerous components, the main of which is exhaled condensed liquid: it constitutes 99% of the volume of EBC. Only a small fraction of it contains nonvolatile molecules that can be hydrophilic and hydrophobic. During condensation, volatile, water-soluble particles are adsorbed along with the liquid. EBC does not contain cells, but there are many different biologically active substances that are produced by the cellular elements of the airway mucosa, which has already been substantiated [1], [2], [3].

A significant limitation of EBC study in clinical practice is the absence of generally accepted standards for its collection and examination of its content of biological substances, which contain ultra-low concentrations.

The main ways to solve the problem are standardization and development of unified approaches to the collection and analysis of EBC. On resolving these major issues, we will obtain more evidence and effectiveness in the application of this diagnostic method. A burst of scientific and practical interest to this issue led to organization of the target group of the American Thoracic Society and the European Respiratory Society in 2001, with the aim of development of recommendations for condensate collecting, which were published in 2005 [1] with revisions and amendments in 2012 [3] and 2013 [4]. At this stage, the existing problems are solved by following the ATS/ERS recommendations in EBC:

- standardization of terminology;
- studying the composition of EBC;
- description of the procedure for condensate collecting.

The result of implementing the ATS/ERS recommendations is the further development in this direction of noninvasive diagnostics of diseases:

- serial production of special condensate collection devices such as EcoScreen® (Jaeger Tonnies Hoechberg, Germany); R-Tube® (Respiratory Research, Inc., USA), and accessories - mouthpieces, saliva collecting frame, nasal clamps, and others.
- the commonly accepted conditions for carrying out the procedure of collecting EBC have been formulated: the time of condensate collecting, the duration of the procedure, the amount of condensate, the temperature of cooling during the collection of condensate and the temperature for storage of samples, the intensity of exhalation, the maneuver of exhalation, control of contamination with saliva, body position during the procedure, preparing the patient for the procedure (rinsing the mouth, abstinence from food and smoking).
- new substrates have been isolated in EBC: interleukins, cytokines, chemokines, eicosanoids, erythropoietin, adenosine, DNA and others.

Analyzing the modern recommendations, we developed the algorithm of the procedure for EBC collecting.

Specifications:

1. In order to collect EBC, it is permissible to use commercial devices or self-made products. The best way to carry out the procedure is to use commercial models of condensers, since this allows us to avoid many problems. Commercial models such as EcoScreen®, Jaeger, Anacon have an unidirectional valve that prevents accidental cooling of the air from the condenser during inhalation, which is important for preventing mixing of air. R-Tube® is individual, it does not need to be disinfected. They exclude the possibility of the release of detergent residue into EBC, which also affects the composition of condensate. The device must have a salivary collecting frame with a mouthpiece, as it is important to control continuously the contamination of the EBC with saliva, as it has already been proven that contamination with saliva may affect the level of various mediators in EBC. At the same time, the use of filter is not recommended because it can become a trap for molecules contained in exhaled air. As a cooling component, one can use ice, ethanol, liquid nitrogen, and frozen metal tubes.

In the case of using a self-made device, it is necessary to specify the detailed information about the appliance (saliva collecting frame, its resistance, material of the condensation surface, cooling method, condenser temperature, and their stability during the assembly period).

The device is assembled before the procedure begins.

2. Preparing the patient for the procedure:

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- The study should be conducted in the morning (8.00-12.00), on an empty stomach or at least 8 hours after a light breakfast. When re-examining on another day, it is preferable to conduct the study at the same time of day.

- The patient must not smoke and drink alcohol within 24 hours [6].

- The patient must not take medications 12 hours before the procedure.

- Before launching the study, it is necessary to comprehensibly explain the patient the method of conducting, to instruct the patient, and in case of need (for children, elderly patients) to demonstrate the procedure, paying attention to the intensity, duration of exhalation, periods of rest, control of contamination with saliva).

- It is necessary to carry out the study at rest, at least 30 minutes after physical exertion. Before study, the patient must rest for 10-15 minutes.

- One should conduct research in clothing that does not compress the thorax and the upper respiratory tract. It is required to remove any foreign objects (dentures, chewing gum, etc.) from the oral cavity.

- The patient is offered to carefully remove the traces of possible contamination (saliva, cosmetics, food remains) from the area of lips with a gauze cloth dipped in distilled water, and then is asked to rinse the mouth for three times with warm boiled water.

3. Condensate collection:

- Put the patient in a comfortable sitting position, in the calm atmosphere, with the feeling of maximum comfort.

- Conduct the study with the use of a nasal clamp during exhalation, the clamp is removed by the patient at each inhalation through the nose.

- During the study, the patient must tightly hold a mouthpiece with his/her lips, but must not close the mouthpiece with the tongue or teeth during exhalation.

- On the average, 10–15 minutes are required to collect 1-2 ml of condensate, under condition of the correct, regular, not too slow exhalation through the mouth, inhalation only through the nose, without exhausting a patient. When collecting condensate, the flow of exhaled air is always streamed directly to the chilled chamber where it is collected in the form of condensate.

- If necessary, a re-examination can be done after a rest in 30 minutes or postponed to another day. *

4. Storage and analysis of condensate

- Samples of CVD should be frozen immediately after collection and stored at -70°C until the analyzes. If it is planned to measure more than one marker, samples should be collected in different test tubes, in order to avoid further freezing / defrost cycles that can destroy biological substances.

- Most mediators that are detected in EBC are contained in ultra-low concentrations, and the measured values have a high variability. The potential solution to this problem is concentration of samples, which allows us to increase the sensitivity and reproducibility of the method. For this purpose, lyophilization, resuspension and vacuum evaporation may be used, depending on the nature of the biomarkers to be studied.

*Note * There are currently no studies showing the effect of respiratory depression, the effect of cough (both random and induced) during the procedure on the concentration of biomarkers in EBC. The volume of EBC does not depend on the functional parameters of the lungs, including the forced expiratory volume in 1 second (FEV1) and forced vital capacity of the lungs (FVCL) in healthy subjects and patients with broncho-obstructive disease. There is currently no data indicating a change in the concentration of markers in EBC when changing the airway gauge, there is no data on the potential impact of race, gender and body position during the collection of EBC. **

Conclusions

1. Currently, due to the lack of national recommendations for the EBC in Ukraine, it is necessary to follow the ATS / ERS recommendations [1].

2. Applying the above-mentioned algorithm, developed on the basis of the ATS / ERS recommendations, one can significantly enhance the implementation of this method for study in different fields of medicine and standardization of biomarkers analysis.

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