

## **THE EVIDENCE FOR MITOCHONDRIAL RESPIRATION IN EXOSOMES FROM BRONCHIAL-ALVEOLAR LAVAGE**

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**Introduction.** A liquid biopsy is one of the promising diagnostic methods in respiratory oncology. Exosomes, small vesicles released from a variety of cells, carry organelles, however uncertainty about exosomal mitochondria exists. The study aimed to develop the method for exosomes isolation from bronchial-alveolar lavage (BAL) and test them for mitochondrial respiration. **Material and methods.** We analyzed BAL samples from 14 consecutive lung cancer patients from Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences. First, BAL was centrifuged to remove cells, cellular debris, and larger particles. Then exosomes were isolated from BAL supernatant using size exclusion chromatography on qEV columns (IZON). The purity of the exosomal fraction was tested by absorbance measurements, the evaluation of protein content and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. The exosomal fraction was then used for high-resolution respirometry with the use of O2k oxygraph (Oroboros). We have applied ROUTINE, LEAK, ETS and ROX protocol. **Results.** The eluted fractions showed differences in Na<sup>+</sup>/K<sup>+</sup>-. We were able to show mitochondrial respiration in exosomes. Following respiratory states were observed in exosomes: ROUTINE respiration represents physiological energy turnover in mitochondria; LEAK state compensates proton leak, proton slip, and circulation across inner mitochondrial membrane; electron transfer system capacity (ETS) - the maximum oxygen flux induced by optimal administration of uncoupler; residual oxygen consumption (ROX) reflects oxidative side reactions active after ETS inhibition.

**Conclusions.** Size exclusion chromatography of BAL yields exosomes and provides samples for biomarkers analyzes. Mitochondrial respiration can be observed in exosomes from BAL samples.