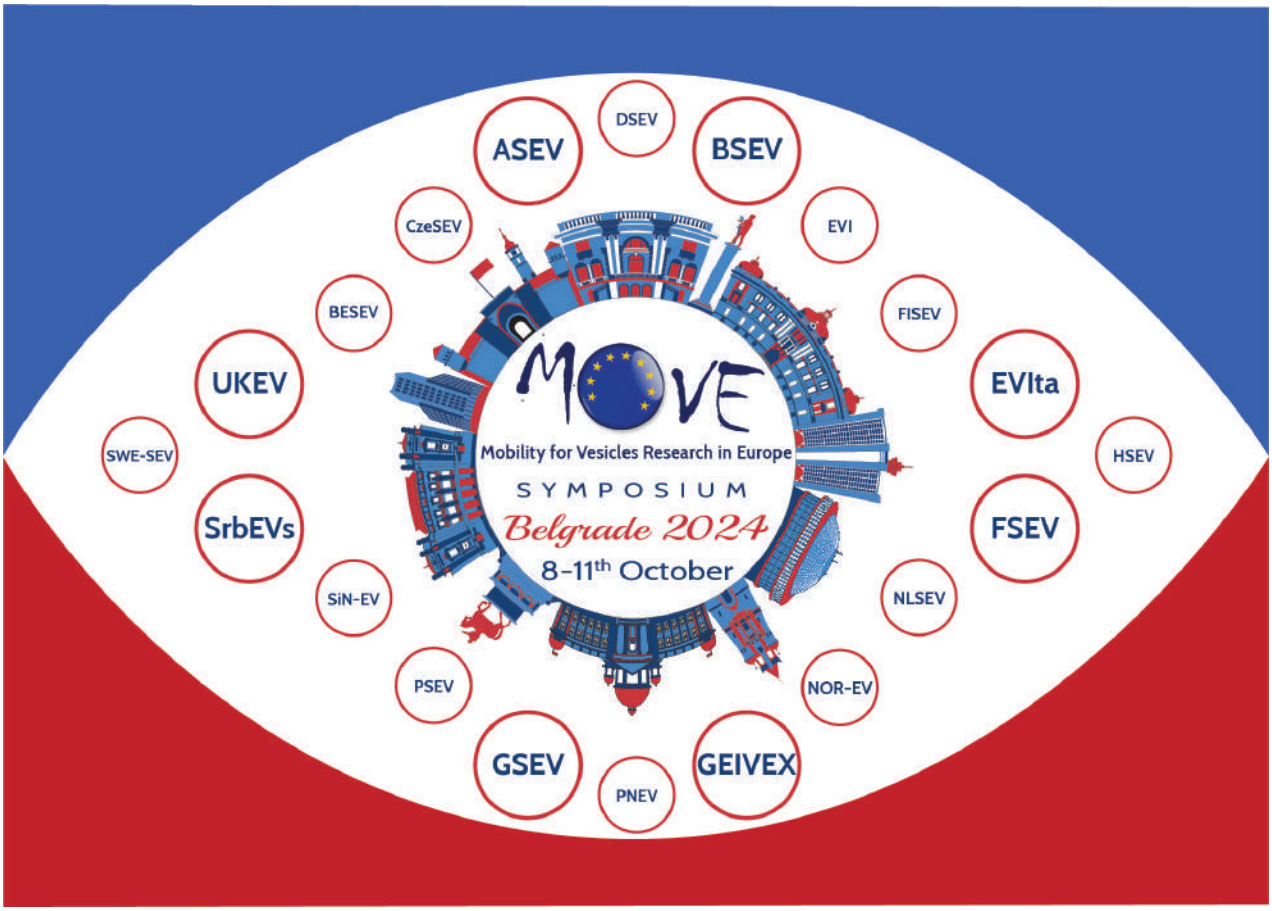
2nd MOVE Symposium



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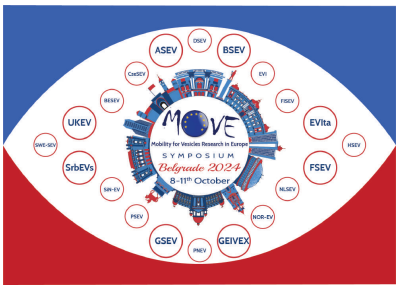

2nd MOVE Symposium

8-11 October 2024, Belgrade, Serbia

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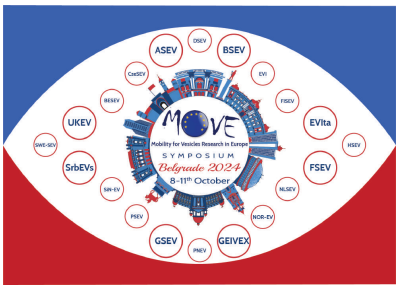

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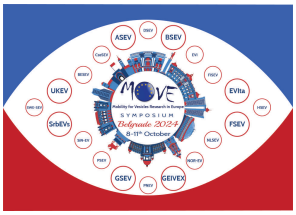

P-BM-11

Three methods of isolating EVs from pleural effusion samples of patients with advanced lung adenocarcinoma - potential applications in clinical practice?

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Introduction: Pleural effusion (PE) occurs in 17-23% of lung cancer (LCa) patients and it contains extracellular vesicles (EVs) from cancer cells, representing “liquid biopsy” of LCa. Isolated PE-EVs could be used for LCa diagnosis or monitoring its progresion/therapy. However, there is still no standard method for isolation of EVs from pleural fluid. The aim of this work is to compare PE-EVs isolation methods that could be employed both in research or clinical settings.

Methods: PE samples diluted in PBS (1:1) from patients with advanced non-small cell lung cancer (NSCLC) were utilized. Three methods for isolating EVs were employed: an in-house spherical porous methacrylate-based copolymer coupled with VHH antibodies (chromatography method-CH), ultracentrifugation (UC), and the Norgen Plasma/Serum Exosome Purification and RNA Isolation Mini Kit (Commercial kit-CK). For each EVs isolation method, efficiency was monitored in terms of the amount of starting sample, time required for vesicle isolation, yield, quality of the obtained isolates and overall cost.

Results: In terms of the amount of starting sample, the CH and CK have an advantage, allowing work with as little as 500µL, whereas UC required several milliliters of sample (in our case, due to the rotor type, 12mL minimum). The shortest isolation time was with the CK, followed by the CH, while UC took the longest. Vesicle isolates were cleanest when isolated by CH, followed by the CK, with the lowest purity obtained through UC. When comparing costs per sample, they were approximately: UC–23 euro/sample, CH–20 euro/sample, CK–20 euro/sample.

Conclusion: Depending on downstream analyses, the CH method proved to be the most effective for characterizing the vesicles or analyzing their proteome. For analyzing non-coding RNAs as potential biomarkers in lung cancer, due to the short isolation time of vesicles and the designation of the kit, the CK is the preferable choice.

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