

The European Cancer Information System (ECIS) estimates that more than one million cancer-related deaths occurred in EU nations in 2023. Research on the relationship between cancer and human body composition indicated that around 30% of cancer-related deaths is the result of cancer-associated cachexia (CAC). Also known as a wasting syndrome, CAC is characterized by general weakness and muscle mass loss with or without loss of fatty tissue. It is brought on by intractable loss of appetite, metabolic abnormalities, and inflammation, which are all associated with cancer. Since current therapies and increased calorie intake are ineffective in treating CAC, a better understanding of the disease's underlying mechanisms may help find more effective therapies.

Our team believes that there is still not enough understanding of the causes of cancer-associated cachexia, and that improvement in the knowledge in this regard could lead to earlier CAC diagnosis and better prognosis for millions of patients globally. In our preliminary studies we established a novel association between lung cancer metastases in the pleura (the membrane covering the lungs) and skeletal muscle loss. In metabolomic studies, our team further discovered that pleural locations of cancer metastatic deposits was marked in the blood by an elevated levels of a protein called periostin, reduced levels of cholic acid and elevated acetyl-carnitines.

In this project we intend to further explore the mechanism of cachectic processes, which cancer patients encounter during progression of cancer. How do we hope to accomplish this? We were able to collect clinical information in a cohort of 250 lung cancer patients who underwent surgery with curative intent and who unfortunately relapsed with cancer after an average follow-up time of 14 months. We accessed the radiological computed tomography (CT) scans from those patients and using artificial intelligence-driven software were able to assess the skeletal muscle and adipose changes between the onset of cancer and the time of cancer progression. In this way, we were able to delineate a group of patients with loss of skeletal muscle with or without accompanying loss of adipose tissue, i.e., the group with features of cancer-associated cachexia and the group who did not experience wasting of the body compartments despite progression of the cancer.

In the proposed study, we aim to examine patients' blood samples, looking at 5400 proteins and more than 10.000 blood metabolites to identify potential indicators of cachexia. Given an association between elevated periostin in the context of pleural lung cancer metastasis, and association between this location of cancer relapse with loss of skeletal muscle, we are interested to examine the ability of periostin to induce in normal cells the secretion of proteins that can cause catabolic muscle wasting. To do this, we will grow *in-vitro* muscle, blood vessels and connective tissue cells, known as myoblasts, endothelium, and fibroblasts, respectively, with addition of periostin. We will analyse the global gene expression changes in these cells by looking at RNA products via total RNA-sequencing. We also plan to determine alterations in these cell lines that happened on metabolic levels to track the effects of periostin on the cell metabolism. Ultimately, we will cross-check the results of patients' blood proteomic and metabolomic screens and *in-vitro* analyses to identify proteins characteristic of cancer-cachexia that are also under regulation of periostin.

Having identified potential markers of CAC, we will implant the selected proteins into mice to see if these indeed can cause cachectic phenotype in animals. This will be accomplished by assessment of changes in mouse muscle with the use of repeated ultrasonographic measurement. We will also analyse changes in the composition of mouse blood proteins. Furthermore, the presence of inflammatory infiltrate markers in muscle, adipose and brain tissue will be investigated. In parallel, we will use a mouse model with cancer growing in pleura, i.e., mesothelioma model and another mouse cancer model of lung cancer spreading to pleura, with analogous analyses planned to be performed.

As a result of this project, we expect to substantially increase the understanding of the cellular and systemic mechanisms that contribute to the development of cancer-associated cachexia and to identify markers that might be used to detect early signs of cachexia and which could be targeted to treat wasting syndrome in cancer patients.