Introduction

In recent years, an increasing number of studies have focused on microvesicles—fragments of the cell membrane that act as signal carriers released by various cell types. In the case of erythrocytes, these structures are referred to as erythrocyte-derived extracellular vesicles. While most current research has concentrated on vesicles secreted by tumor cells, relatively little is known about microvesicles derived from human erythrocytes. During the storage of erythrocytes under blood bank conditions, structural, biochemical, and functional changes occur. These changes may serve as markers of erythrocyte and microvesicle aging and are associated with transfusion-related complications. Currently, there is no standardized method for assessing the biological quality of stored RBCs beyond conventional biochemical markers. The presence and characteristics of erythrocyte-derived microvesicles may provide novel, clinically relevant biomarkers of RBC quality, which remains an unmet need in transfusion medicine.

Aim

This project aims to characterize erythrocyte-derived microvesicles as novel biomarkers of red blood cell aging and storage-related deterioration, ultimately contributing to safer transfusion practices and improved storage protocols. Specifically, we will investigate qualitative and quantitative changes in erythrocyte-derived microvesicles released during the storage of red blood cell concentrates (RBCs), in the context of erythrocyte aging under blood bank conditions. Analysis of the composition, biomarkers, number, and morphometric parameters of these microvesicles may serve as a valuable indicator of blood product quality and contribute to a better understanding of the mechanisms underlying various diseases. We hypothesize that changes in the quantity, morphology, and content of microvesicles will be closely dependent on the storage duration of erythrocytes.

Research description

In this study, erythrocyte-derived microvesicles will be isolated using an antibody recognizing glycophorin A (a specific marker of erythrocytes) and annexin V, which binds phosphatidylserine (an “eat-me” signal present on vesicle surfaces). This will enable their identification and analysis via flow cytometry—a rapidly advancing technique considered the "gold standard" for microvesicle detection. In addition, to obtain a more detailed and multidimensional view, we will employ spectral cytometry. This technique analyzes the complete emission spectra of fluorochromes, allowing the simultaneous assessment of multiple markers with exceptional precision and resolution. It will facilitate the creation of a detailed phenotypic map of microvesicles at various storage stages. Calibration beads of known size and concentration will be used to accurately determine the quantity and size of microvesicles. To acquire three-dimensional images of microvesicles and the spatial distribution of surface molecules, we will use confocal microscopy, while detailed ultrastructural analyses at the nanometer level will be performed using transmission electron microscopy (TEM). Beyond morphological and cytometric analyses, RNA profiling will be conducted using RNA-seq technology to identify and characterize RNA species (mRNA, miRNA, lncRNA) present in microvesicles and assess storage-related changes in their content. The project will also include proteomic analysis based on mass spectrometry techniques to determine the protein composition of the microvesicles. This will allow the identification of proteins involved in erythrocyte aging and potential biomarkers of RBC concentrate quality. Furthermore, we will assess the impact of microvesicles on the immune system. Microvesicles can be recognized and phagocytosed by monocytes due to the exposure of phosphatidylserine and other “eat-me” signals, which influence monocyte activation and cytokine secretion. Therefore, cytokine levels (both pro- and anti-inflammatory) will be measured using the immunoenzymatic ELISA method to accurately evaluate the immunomodulatory properties of the microvesicles.

Main results

The results of cytometric, proteomic, microscopic, and molecular analyses will enable the creation of a comprehensive picture of the changes occurring in erythrocyte-derived microvesicles during RBC storage. This will improve our understanding of erythrocyte aging and microvesicle interactions with immune cells, including mechanisms of monocyte phagocytosis. Our findings may lead to the development of predictive biomarkers for blood product quality, inform new guidelines for RBC storage, and open avenues for interventions aimed at mitigating transfusion-related immune complications.