Streszczenie w języku angielskim (wraz z angielską wersją tytułu rozprawy)

Evaluation of the changes in the intestinal barrier permeability after hematopoietic stem cell transplantation.

Allogeneic hematopoietic cell transplantation (allo-HCT) is often the only curative procedure for various onco-hematologic and non-neoplastic diseases. One ofe its main limitations is the high mortality associated with the procedure, mainly due to the graft-versus-host disease (GVHD), especially the acute form (aGVHD), and infectious complications. In the last two decades, the intestinal barrier has gained increasing interest as the site of initiation and propagation of these complications.

The intestinal barrier is a complex structure consisting of a layer of epithelial cells covered with mucus inhabited by microorganisms, including bacteria, viruses, and fungi collectively referred to as the intestinal microbiome or intestinal microbiota. An intact intestinal barrier prevents the penetration of bacteria and toxins from the gastrointestinal tract into the bloodstream. On the other hand, intestinal permeability allows for the absorption of essential nutrients, water transport, and electrolytes. However, to date, there is a lack of methods for intestinal barrier evaluation that could be applied in clinical practice. The reference method used in scientific research is the sugar absorption test (SAT), based on the difference in the absorption pathways of lactose (paracellular transport) and mannitol (transcellular transport). So far, it has not been evaluated whether SAT can be replaced by the analysis of other markers of intestinal damage, such as zonulin (a marker of intestinal permeability) or calprotectin and beta-defensin-2 (markers of intestinal inflammation), to assess intestinal barrier permeability in the peri-transplantation period.

There is also a lack of data on characteristic disruptions in the metabolome, understood as the entirety of metabolites present in the body, occurring after allo-HCT, especially those that may be associated with the occurrence of aGVHD. The assessment of the metabolome is possible through a new "omic" technique called metabolomics, involving the identification and analysis of metabolites using techniques such as liquid or gas chromatography combined with mass spectrometry (GC-MS, LC-MS) or nuclear magnetic resonance (NMR) spectroscopy, and the analysis of obtained data using dedicated software.

The doctoral thesis consists of three thematically related scientific publications, aiming to evaluate the permeability of the intestinal barrier in patients undergoing allo-HCT using SAT and the analysis of markers of intestinal barrier damage. It also includes an assessment of changes in the metabolome in allo-HCT recipients, with a particular focus on patients diagnosed with aGVHD in the post-transplantation period.

The following detailed objectives were set:

1. Evaluation of intestinal barrier permeability using SAT.
2. Analysis of the relationship between intestinal barrier permeability and patient- and transplantation-related factors.
3. Analysis of the relationship between intestinal barrier permeability and the occurrence of post-transplantation complications (aGVHD, infectious complications, mucositis).
4. Assessment of intestinal barrier damage using the analysis of biomarker concentrations in feces (zonulin, calprotectin, beta-defensin-2).
5. Assessment of fecal markers of intestinal barrier damage as surrogates for intestinal barrier permeability.
6. Analysis of the relationship between the concentration of fecal markers of intestinal barrier damage and patient- and transplantation-related factors.
7. Analysis of the relationship between the concentration of fecal markers of intestinal barrier damage and the occurrence of post-transplantation complications (aGVHD, infectious complications, mucositis).
8. Evaluation of metabolic profiles of patients in the peri-transplantation period.
9. Comparison of metabolic profiles between patients who experienced aGVHD and other patients.

The publication cycle includes a review article and two original papers.

In the first paper titled "*Advances in Intestinal Barrier Preservation and Restoration in the Allogeneic Hematopoietic Cell Transplantation Setting"* (doi:10.3390/jcm10112508), a review of available literature on the intestinal barrier is conducted, focusing on its structure, normal functioning, evidence of how damage and increased intestinal permeability affect the outcomes of patients after allo-HCT. Methods for assessing intestinal permeability are discussed. Existing therapeutic strategies aimed at protecting the intestinal barrier, with a special emphasis on preserving and restoring normal gut microbiota, are described.

The goal of the next paper titled "*Increased Intestinal Permeability and Stool Zonulin, Calprotectin and Beta-Defensin-2 Concentrations in Allogenic Hematopoietic Cell Transplantation Recipients"* (doi: 10.3390/ijms232415962) was to evaluate intestinal barrier permeability and other potential markers of intestinal barrier damage in patients undergoing allo-HCT at the Clinic of Hematology, Transplantology, and Internal Medicine, Central Clinical Hospital of the University Clinical Center of Warsaw Medical University (CSK UCK WUM). Fifty-one patients were included in the study. Intestinal permeability was assessed

using SAT, and concentrations of zonulin, calprotectin, and beta-defensin-2 in feces were also evaluated during the peri-transplantation period. For the majority of patients undergoing allo-HCT, abnormal intestinal barrier permeability was demonstrated seven days before the start of the allo-HCT procedure, correlating significantly with a higher hematopoietic cell transplantation-specific comorbidity index (HCT-CI). After allo-HCT, a further increase in intestinal barrier permeability was observed in most patients. However, no correlation was observed between SAT results and the assessment of other markers of intestinal barrier damage (zonulin, calprotectin, and beta-defensin-2). In patients who developed aGVHD, significantly higher levels of calprotectin in feces were observed after allo-HCT compared to patients without this complication. The results of this study indicate that intestinal barrier damage develops before allo-HCT, intensifies in the post-transplantation period, and precedes further complications. However, other markers of intestinal barrier damage were not shown to be useful as surrogates for intestinal barrier permeability.

The aim of the next paper titled "*Altered lipid metabolism in patients with acute graft-versus-host disease after allogeneic hematopoietic cell transplantation"* (doi: 10.1016/j.leukres.2024.107435 was to assess the metabolic profiles of patients after allo-HCT who were diagnosed with aGVHD during the post-transplantation period, compared to patients without this complication. In clinical practice, the risk of developing GVHD is assessed individually based on clinical factors such as Human Leukocyte Antigen (HLA) and gender mismatch, older age of the donor and/or recipient, source of stem cells, donor alloimmunization, and the presence of antibodies against cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Using non-targeted metabolomic analysis methods, global changes in the metabolism of amino acids, carbohydrates, lipids, nucleotides, and bacterial-derived metabolites were evaluated in 38 patients undergoing allo-HCT at the Clinic of Hematology, Transplantation, and Internal Medicine CSK UCK WUM, of whom 15 developed aGVHD. Compared to other patients, those with aGVHD showed deregulation of metabolic profiles, which was evident as early as 7 days before the start of the allo-HCT procedure. Significant changes in lipid metabolism related to the bile acid transformation pathway and cholesterol synthesis were identified in this group of patients. Due to the pilot nature of the study, these results only suggest a potential association between lipid metabolism and the risk and/or development of aGVHD, requiring confirmation in further studies on the development of aGVHD, especially studies on prediction models for aGVHD.

In summary, the presented series of papers focuses on analyzing alterations of the intestinal barrier and the metabolome after allo-HCT, and their impact on the occurrence of

post-transplantation complications, with special emphasis on aGVHD. The results of the presented studies contribute to expanding knowledge regarding the influence of the intestinal barrier's status and the metabolome on the outcomes of patients undergoing allo-HCT. These findings form the basis for planning further studies aimed at developing prediction models for aGVHD and the development of therapeutic options targeting the protection of the intestinal barrier, optimizing medical care, and improving treatment outcomes in this vulnerable patient group.