The impact of the gut microbiota on the treatment and complications in patients with multiple myeloma
Streszczenie w języku angielskim

Gut microbiota contains the community of microorganisms (bacteria, archaea, viruses, fungi, and other microbes) that inhabit the gastrointestinal tract of a human or other organism. On the other hand, gut microbiome is the collective genetic material of all the microorganisms present in the gut, including their genes and gene products.

In recent decades, the development of research on the gut microbiota has led to a breakthrough understanding of the role played by the community of microorganisms inhabiting the intestines in shaping the immune response and in the etiopathogenesis of many diseases, including hematologic malignancies. The first observations indicating a link between elements of the microbiota and the immune system appeared as early as the beginning of the 21st century, yet only with the advent of next-generation sequencing (NGS) techniques and metagenomics it became possible to precisely characterize the species-level and functional diversity of the gut microbiota in patients.

In the case of multiple myeloma (MM), a malignancy originating from plasma cells, studies of the gut microbiota are still at an early stage. The work conducted so far has concerned analysis of microbiome changes under the influence of therapy and the impact of those changes on patient prognosis. In MM patients who are younger and in good clinical condition, consolidation of the achieved remission with high-dose chemotherapy with subsequent administration of autologous hematopoietic stem cells (auto-SCT) is an established standard treatment.

This doctoral dissertation consists of a cycle of four thematically related scientific publications, showing the comprehensive influence of the gut microbiota on disease progression, treatment outcomes and complications—particularly complications associated with auto-SCT.

The role of the gut microbiota in shaping the immune response is well documented in inflammatory bowel diseases and autoimmune disorders. Numerous experimental studies have demonstrated that short-chain fatty acids (SCFAs), fermentation products of bacteria from the genera *Clostridium* and *Bacteroides*, play a key role in maintaining intestinal-barrier integrity, stimulate the production of anti-inflammatory cytokines (including IL-10) and support the differentiation of regulatory T cells (Tregs), which counteracts excessive activation of Th17 lymphocytes. Conversely, chronic dysbiosis is often marked by a decline in SCFA-producing bacteria and an increase in bacteria from the genera *Enterobacteriaceae* or *Streptococcus*. This leads to chronic mucosal inflammation, excessive production of pro-inflammatory cytokines (including IL-6, TNF-α) and potential stimulation of clonal B-cell proliferation. In MM this is

particularly important, because disease development is preceded by MGUS (monoclonal gammopathy of undetermined significance), in which clonal plasma cells undergo further mutations in immunoglobulin heavy-chain variable regions (IgH) in response to external antigens. A hypothesis has been put forward that bacterial antigens present in the gastrointestinal lumen may initiate or accelerate disease progression.

The publication cycle contains two review papers and two original research articles.

In the first paper, entitled „*Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma*" (https://doi.org/10.1038/s41598-024-82589-z), the impact of colonization of the gastrointestinal tract by antibiotic-resistant bacteria (ARB) before auto-SCT on the risk of infectious complications after the procedure was demonstrated. According to current literature, ARB colonization is a marker of a depleted gut microbiota that, as other studies suggest, may be involved in the development and progression of plasma-cell dyscrasias. The paper analyzes 138 patients who underwent a total of 141 auto-SCT procedures. Patients colonized with ARB in the gastrointestinal tract had a markedly higher risk of infection after auto-SCT compared with the non-colonized group (52% vs 26%, P = 0.02). Although not statistically significant, a tendency toward shorter overall survival (OS) was observed in the colonized group.

The next paper entitled "*Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation*" (https://doi.org/10.1038/s41598-021-02002-x), presents the role of using ice-cream as prophylaxis against oral mucositis after auto-SCT. The authors carried out a retrospective analysis of 74 patients, demonstrating that eating ice-cream during the infusion of high-dose melphalan significantly reduces the incidence of oral mucositis (OM). The method is better tolerated than traditional rinsing of the mouth with cold fluids. The incidence of OM in the ice-cream group was 28.84%, whereas in the group that did not receive ice-cream it was 59.09%. The likely reduction in OM frequency shortened hospital stays and lowered the cost of treating complications. This study more broadly highlights the role of gastrointestinal-mucosal integrity in the incidence of complications after auto-SCT. The gut microbiota is one of the key factors in maintaining the blood-intestinal barrier.

The subsequent study, entitled "*The Role of the Crosstalk Between Gut Microbiota and Immune Cells in the Pathogenesis and Treatment of Multiple Myeloma*" (doi: 10.3389/fimmu.2022.853540), details the role of the gut microbiota and its changes throughout life, with particular emphasis on its influence on the formation of the immune system. It focuses especially on chronic antigenic stimulation of B lymphocytes as a mechanism in MM

pathogenesis. A complex system of interactions among B lymphocytes, the microbiota, dendritic cells, macrophages, neutrophils, plasma cells and intestinal epithelial cells is presented. This publication summarizes the existing knowledge about factors initiating MM development, underscoring the role microorganisms play in that process.

The next paper, entitled "*The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias*" (doi: 10.3389/fonc.2021.741376), aimed to show the factors responsible for the progression of plasma-cell dyscrasias along the pathway from MGUS, through SMM (smoldering myeloma), ultimately to MM. In addition, it highlighted the impact of the gut microbiota on therapeutic responses in MM patients. The role of the microbiota in MM development and progression, treatment response in MM patients, and the potential role of fecal microbiota transplantation (FMT) as a procedure that might modify progression risk or resensitize treatment-resistant cells was discussed.

Integration of the results of the original studies and the summaries of current knowledge in the review papers presents a coherent model of the multistage influence of the gut microbiota on the course of MM. The first stage is shaping the gut microbiome at the earliest stages of life and its influence on immune-system functioning throughout life. The second is chronic antigenic stimulation of B lymphocytes by bacterial antigens and the creation of a microenvironment conductive to MGUS development and progression from MGUS to MM. The third is promotion of a specific profile of secreted cytokines and metabolites during treatment, influencing therapy outcomes and patient prognosis. The fourth is the potential impact of modifying the gut microbiota on reducing auto-SCT complications or even on prophylaxis of progression from MGUS to MM.

In summary, this series of studies contributes to understanding the role of the gut microbiota in MM pathogenesis, treatment and its complications, and points to practical interventions that can markedly improve treatment outcomes and patients' quality of life. The works presented above combine basic research with clinical application of their results.

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