

MICROBIOME AND ITS IMPACT ON CARDIOVASCULAR AND RENAL HEALTH

MIKROBIOM I NJEGOV UTICAJ NA KARDIOVASKULARNO I BUBREŽNO ZDRAVLJE

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Abstract

The human microbiome, comprising various microorganisms, is crucial in health preservation and disease development. Recent technological advancements have revealed the microbiome's pervasive presence, challenging traditional notions of bodily sterility. While the microbiome inhabits various body parts, the intestinal tract emerges as a hotspot, hosting over a thousand bacterial species, with *Firmicutes* and *Bacteroidetes* dominating the landscape.

Dysbiosis, disruptions in this delicate balance, is linked to various pathological intestinal and extraintestinal conditions, including cardiovascular or chronic kidney disease. This article explores the association between the gut microbiome and cardiovascular and renal pathology development. Cardiovascular diseases, a global health challenge, involve atherosclerosis influenced by inflammation. The gut microbiome contributes to inflammation by producing uremic toxins, causing „leaky gut“, impacting metabolic health and triglyceride metabolism in the liver.

The focus then shifts to specific uremic toxins, such as indoxyl sulfate, P-cresyl sulfate, and trimethylamine-N-oxide, exploring their origins, mechanisms of toxicity, and clinical implications. These toxins have been linked to renal dysfunction, cardiovascular morbidity, and atherosclerotic progression. The article discusses potential therapeutic options, including oral adsorbents, sulfotransferase inhibitors, and gut microbiome modulation. While promising, these interventions require further investigation through large-scale, randomized studies to elucidate their efficacy and safety.

Understanding the intricate interplay between the gut microbiome, cardiovascular health, and uremic toxin synthesis is crucial for developing personalized therapeutic approaches to manage these toxins and mitigate their impact on cardiovascular and renal health.

Keywords:

gut microbiome,
inflammation,
atherosclerosis,
uremic toxins

Sažetak

Ljudski mikrobiom se sastoji od različitih vrsta mikroorganizama i može imati važnu ulogu u očuvanju zdravlja, ali i razvoju bolesti. Napredak tehnologije omogućio je da bolje shvatimo sastav ljudskog mikrobioma, kao i njegovu zastupljenost u ljudskom telu, čak i u regijama koje su prethodno smatrane sterilnim. Crevni mikrobiom je možda i najgušće naseljena regija ljudskog tela mikroorganizmima, među kojima dominiraju bakterijski tipovi *Firmicutes* i *Bacteroidetes*.

Disbioza predstavlja poremećaj sastava crevnog mikrobioma i može biti u osnovi različitih crevnih bolesti, ali i sistemskih poremećaja poput bolesti srca i krvotoka ili hronične bolesti bubrega, a dokazivanje moguće veze između mikrobioma i ovih poremećaja jedna je od osnovnih tema našeg preglednog rada. Ateroskleroza se nalazi u osnovi kardiovaskularnih bolesti – vodećeg svetskog zdravstvenog problema. Sam proces ateroskleroze je donekle uzrokovan i povezan sa zapaljenjem. Crevni mikrobiom može doprineti zapaljenskom sindromu na nekoliko načina: povećanom produkcijom uremijskih toksina - razgradnih produkata metabolizma aminokiselina, povećanjem propustljivosti crevne sluznice za bakterije i komponente čelijskog zida bakterija, poremećajem metabolizma ugljenih hidrata i triglicerida u jetri.

Uremijski toksini, indoksil-sulfat, p-krezil-sulfat i trimetilamin N-oksidi, ističu se kao najdominantniji po pitanju kardiovaskularne i bubrežne toksičnosti, te se radom definiše njihovo poreklo i ukazuje na moguće mehanizme njihovog nepovoljnog dejstva na organizam. Članak razmatra moguće terapijske opcije, uključujući oralne adsorbente, inhibitore sulfotransferaza, kao i taktike izmene crevnog mikrobioma. Iako obećavajući, ovi terapijski modaliteti zahtevaju dalje ispitivanje kroz velike, randomizovane studije u cilju boljeg sagledavanja njihove efikasnosti i bezbednosti.

Razumevanje složenog odnosa između crevnog mikrobioma i kardiovaskularnog zdravlja, posredovanog uremijskim toksinima, može biti ključno za dalji razvoj terapijskih pristupa koji bi mogli za cilj imati poboljšanje zdravlja.

Ključne reči:

crevni mikrobiom,
inflamacija,
ateroskleroza,
uremijski toksini

Introduction

Microbiome and Its Role in Health Preservation and Disease Development

The human microbiome, a dynamic and complex consortium of bacteria, fungi, viruses, parasites, and archaea, establishes symbiosis with the host during early childhood. The microbiome's composition is individualized and ever-changing, influenced by genetics, environmental conditions, delivery methods, breastfeeding, childhood medications, and diet. Recent technological advancements have unraveled the complexity of the microbiome, providing insights into its presence throughout the body and challenging conventional notions of sterile bodily regions, such as urine, gastric, or lung mucosa (1). While the microbiome inhabits various body parts, the intestinal tract emerges as a hotspot, hosting over a thousand bacterial species, with *Firmicutes* and *Bacteroidetes* dominating the landscape. This microbial community is pivotal in health maintenance and disease prevention (2).

What are the functions of the gut microbiome? One of its crucial roles is in food digestion. The gut microbiome bacteria can digest fibers our body cannot process, synthesizing short-chain fatty acids (SCFA) such as butyrate, propionate, and acetate (3). These SCFAs are the primary energy source for the colon mucosa's covering cells, making them essential in maintaining the intestinal barrier's continuity (4). The symbiotic relationship

between the gut microbiome and the immune system is a cornerstone of human health. The microbiome acts as an educator, enhancing immunotolerance primarily through T-regulatory lymphocytes. Simultaneously, the immune system acts as a sentinel, safeguarding against enteropathogenic microorganisms (5). Research in rodent models underscores the importance of specific bacterial families, notably *Lactobacillus*, in developing the central nervous system and cognitive functions (6). Recent scientific endeavors have directed attention towards the gut-brain axis, unraveling the significance of the gut microbiome in conditions such as irritable bowel syndrome (6).

The stability of the gut microbiome is inherently vulnerable to external environmental conditions and endogenous factors. Hormone composition, metabolites, and the nervous system intricately influence the delicate balance of the microbiome. Disruptions in this equilibrium, called dysbiosis, can contribute to the onset or progression of various gastrointestinal diseases. Recently, there has been increasing discussion about the role of the gut microbiome in the development of extraintestinal pathological conditions such as obesity, diabetes mellitus, cardio- and cerebrovascular morbidity, or even chronic kidney disease (7).

Aim

The aim of this text is to examine the relationship between the gut microbiome, uremic toxins, cardiovascular pathology, and renal dysfunction, with particular

emphasis on potential microbiome-targeted therapeutic approaches.

Association between the Gut Microbiome and the Development of Cardiovascular Pathology

Cardiovascular diseases (CVDs) represent a formidable global health challenge, claiming 18 million lives annually (8). Atherosclerosis, a central pathophysiological process in CVD, is influenced by many factors, including genetics, immunity, and lipid metabolism (9). Inflammation, however, emerges as a common thread, and recent research delves into the role of the gut microbiome in fueling this inflammatory cascade (2). The digestive system and its accompanying microbiome represent a significant source of inflammation due to direct infection or an inflammatory process in the intestine. For example, infections with enteropathogenic *Escherichia coli* or *Bacteroides fragilis* are simultaneously associated with increased local inflammation in inflammatory bowel disease and an elevated risk of cardiovascular morbidity in this patient group (10). Increased intestinal "leakiness" emerges as a pivotal mechanism underlying the development of metabolic syndrome and insulin resistance. The influx of bacterial metabolic breakdown products, especially lipopolysaccharides, into the systemic circulation directly stimulates macrophages, triggering the synthesis of proinflammatory interleukins. This cascade of events contributes to the development and progression of the atherosclerotic process (11).

The influx of bacterial metabolic breakdown products and proinflammatory interleukins into the systemic circulation can also alter triglyceride metabolism in the liver parenchyma, increasing their deposition within hepatocytes. Liver steatosis can be discussed when this percentage exceeds 5% of the total liver mass. As a prevalent pathological condition affecting nearly a third of the world's population, it is intricately linked to the gut microbiome (12). Studies demonstrate a direct correlation between liver steatosis and the development of insulin resistance, ultimately contributing to the progression of the atherosclerotic process (13).

Association between the Gut Microbiome and Renal Function

Chronic kidney disease (CKD) stands as a significant global health concern, affecting approximately 13.4% of the world's population, with a rising prevalence (14). The multifactorial nature of CKD involves chronic inflammation, stimulation of the renin-angiotensin-aldosterone axis, proteinuria, and recurrent episodes of acute kidney injury. Therapeutic options for controlling the progression of CKD are limited and currently involve using renin-angiotensin-aldosterone system inhibitors, mineralocorticoid antagonists, and, more recently, sodium-glucose cotran-

sporter 2 (SGLT2) inhibitors (15).

Beyond traditional risk factors, the intricate relationship between CKD and the gut microbiome adds a layer of complexity to the understanding of renal function. Chronic kidney disease profoundly impacts the composition of the gut microbiome. Elevated levels of serum nitrogen compounds, especially urea, along with alterations in intestinal transit and dietary adjustments recommended for CKD patients, contribute to a reduction in the diversity of the gut microbiome. This dysbiosis is characterized by an overabundance of protein-fermenting strains, such as *Enterococcaceae*, *Clostridiaceae*, or *Methanobacteriaceae*, and a reduction in anti-inflammatory and SCFA-producing bacterial families, such as *Bifidobacteriaceae*, *Lactobacillus*, or *Faecalibacterium*, marking a shift in the delicate balance of the gut microbial community (16).

Furthermore, in patients with CKD, the accumulation of over 90 different substances produced through protein fermentation at the level of the intestinal mucosa is noticeable. These gut-produced uremic toxins can trigger a cascade of inflammation and oxidative stress, significantly contributing to the progression of atherosclerosis and worsening renal function (17).

Uremic Toxins of the Digestive Tract

The term uremia refers to a collection of symptoms and signs observed in individuals with chronic kidney disease (CKD) resulting from accumulating specific metabolites - uremic toxins - due to their reduced renal clearance. So far, around 150 different uremic toxins produced at the level of the gastrointestinal mucosa have been identified. Most are chemically classified as phenols, indols, or amines. Their toxicity manifests at the level of various organ systems, significantly affecting the function of renal tubular cells, osteoblasts, endothelial cells, or lymphocytes. This is further emphasized by the fact that most of these toxins strongly bind to plasma proteins, making their removal challenging even with dialysis regimens (18).

Indoxyl Sulfate

Indoxyl sulfate (IS) is a uremic toxin with a molecular mass of 213 g/L. It circulates bound to plasma proteins, with a free fraction of about 10%. It is produced through the fermentation of tryptophan, which, under the influence of gut flora, transforms into indole and is absorbed into the portal circulation. In the liver, it combines with a sulfate molecule, forming IS. The average serum level of IS ranges from 0.7 - 6.3 $\mu\text{mol/L}$ (18).

It is a substance with pronounced nephron- and cardiovascular toxicity. In the 1990s, preclinical studies by Niwa and colleagues, followed afterward by Miyazaki, demonstrated significant toxic effects of IS, stimulating local inflammation in the proximal tubules of mouse kidneys, leading to subsequent glomerulosclerosis of the corresponding nephrons (19,20). Regarding clinical studies, Wu demonstrated a faster and more frequent progression to dialysis in patients with higher levels of IS in a study on

pre-dialysis chronic kidney disease patients (21).

Vascular toxic effects of IS have been proven in pre-clinical and clinical studies. In a study on human umbilical veins removed after delivery, Dou showed that local application of IS hinders the proper regeneration of endothelial cells of blood vessels (22). Yamamoto demonstrated that IS supplementation in mice significantly worsens the atherosclerotic process, leading to increased instability of atherosclerotic plaques due to aggravated local inflammation and smooth muscle cell hyperplasia in blood vessels (23). In the clinical setting, Sato showed a clear correlation between IS levels and the degree of diastolic dysfunction (24). At the same time, Hsu linked IS levels to more pronounced calcification of atherosclerotic plaques in the coronary vessels of stage III-V CKD patients (25). In preclinical studies, possible effects of IS levels on bone metabolism, cognitive function, and uremia symptoms have been demonstrated, but these need validation in more extensive clinical trials.

P-Cresyl Sulfate (PCS)

The P-Cresyl Sulfate with a molecular mass of 108.1 g/mol, is a uremic toxin formed during the fermentation of amino acids tyrosine and phenylalanine. This process is carried out by gut microbiota producing tyrosinase or tyrosine-phenolase, bacterial families *Enterococcaceae*, *Clostridiaceae*, *Staphylococaceae*, and *Enterobacteriaceae*. After synthesis, PCS binds to albumin and circulates through the bloodstream to peripheral tissues. Only 5% of PCS is found in the plasma as the free fraction. In cases of renal impairment, its plasma level progressively increases. The usual total PCS level in serum ranges from 15 - 35 $\mu\text{mol/L}$ (18). Experimental studies on mice elucidate its significant nephrotoxic effects, primarily through the stimulation of the renin-angiotensin-aldosterone system and induction of renal interstitial fibrosis (26). Several clinical studies have investigated the role and significance of PCS levels in patients with chronic kidney disease. A clinical study by Rossi correlated PCS levels with inflammation markers, oxidative stress parameters, and kidney function, emphasizing its role as a mediator of renal dysfunction and systemic inflammation in CKD patients (27). As early as 2006, Bammens and colleagues, in their study, demonstrated a direct correlation between PCS levels and cardiovascular morbidity in the population of patients with chronic kidney disease, which was later explained by Watanabe in his *in vitro* study, demonstrating PCS's proven toxicity on mouse endothelial and smooth muscle cell cultures (28, 29).

Trimethylamine-N-oxide (TMAO)

Trimethylamine-N-oxide a small organic molecule with a mass of 75.1 Daltons, arises from the digestion of choline and lecithin in the gastrointestinal mucosa. Studies on the human microbiome reveal specific bacterial families capable of synthesizing TMAO - *Deferribacteraceae*, *Anaeroplasmataceae*, *Prevotellaceae*, and *Enterobacteriaceae*. Consumption of foods rich in these compounds, such as red meat, eggs, or dairy products, influences the serum

TMAO level. The average TMAO level in serum is 0 - 2.6 $\mu\text{mol/L}$ (30). TMAO gained attention during the 1990s after studies demonstrated its direct correlation with cardiovascular pathology. Despite its known association with chronic kidney disease, the precise mechanisms by which TMAO influences atherosclerotic processes remain elusive. Theories abound, from Seldin's theory about direct activation of the NF- κ B signaling pathway to Wang's about disrupted cholesterol metabolism and Zhu's theory of increased platelet activation (31, 32). The elevation of TMAO levels in CKD patients, up to 40 times higher than those without chronic comorbidities, prompted Kaysen and colleagues to investigate further, proving its role as a predictor of susceptibility to coronary artery disease, hospitalizations, and all-cause mortality in this population (33).

Potential Therapeutic Options for Reducing Uremic Toxin Levels

Studies demonstrating the direct nephron- and cardiovascular toxicity of uremic toxins imply the need for developing potential therapeutic strategies to reduce their levels. Several therapeutic modalities have been investigated, but oral adsorbents will be presented here - sulfotransferase inhibitors and synbiotics.

An oral adsorbent, AST-120 (kremlin), represents a therapeutic strategy for treating CKD patients in select regions - Japan, Korea, and the Philippines. Comprising spherical carbon-based particles with high adsorptive capacity, especially for indole-based substances like IS, AST-120 has shown positive effects in smaller retrospective studies. However, the results of the large-scale, randomized, placebo-controlled EPICC-1 study involving 2035 patients failed to demonstrate a statistically significant impact on the dynamics of kidney disease progression (34).

Sulfotransferase (SULT), a liver enzyme, enriches potentially toxic uremic toxins with a sulfate group. Inhibition of SULT, mainly through phytochemical polyphenols like Quercetin, Meclofenamate, and Resveratrol, presents a potential avenue to reduce the production of uremic toxins such as IS and PCS. While experimental evidence supports the potential efficacy of SULT inhibitors, additional randomized studies are warranted to dissect their effects on the intricate interplay between the gut microbiome and uremic toxins (35).

Using drugs like ibuprofen, which bind to the same sites on albumin as uremic toxins, is a potential strategy. However, the adverse effects of nonsteroidal anti-inflammatory drugs in chronic kidney disease patients present challenges. A study with the plant *Salvia miltiorrhiza* in dialysis patients showed promising results, but additional prospective studies are needed (36).

Modifying the gut microbiome by supplementing prebiotics, probiotics, or synbiotics is a compelling therapeutic avenue. So far, nine studies have been published with the aim of investigating the effectiveness of pre-, pro-, or synbiotic supplements in the population of patients with chronic kidney disease (Table 1) (37-45).

Table 1. The characteristics of the studies aimed at examining the effect of pre-, pro- or synbiotic preparations

Study	Year	Population	Treatment	Study period (weeks)	Measurement*
Nakabayashi ³⁷	2011	9	Mixed Probiotics	2	1,2,3,4
Guida ³⁸	2014	30	Mixed Probiotics	4	1,3,4,5,6,7
Viramontes-Hörner ³⁹	2014	22	Mixed probiotics	8	1,2,5,6,7,8,9
Sirich ⁴⁰	2014	40	Mixed probiotics	6	3
Cruz-Mora ⁴¹	2014	18	Synbiotic	8	10
Wang ⁴²	2015	39	Mixed probiotics	24	1,2,8,9,11
Poesen ⁴³	2016	40	Prebiotic	4	3
Rossi ⁴⁴	2016	31	Synbiotic	6	1,2,3,5,6,7,8,9,10,12
Borges ⁴⁵	2018	33	Synbiotic	18	1,2,5,6,7,10,12

*1 - Creatinine; 2 - Urea; 3 - Uremic Toxins; 4 - Gastrointestinal symptoms; 5 - Albumine; 6 - Lypids; 7 - Glucose; 8 - Tumour-necrosis factor α; 9 - Interleukin 6; 10 - Microbiome; 11 - Endotoxin; 12 - C-reactive protein

The most comprehensive of all, the SYNERGY study by Megan Rossi and colleagues, with a randomized prospective design and strict control of antibiotic use and diet, has demonstrated the potential of the combination of pre- and probiotics to significantly reduce the levels of Indoxyl sulfate and p-Cresyl sulfate statistically (44). On the other hand, the study by Borges and colleagues did not show a significant effect of synbiotic use in terms of reducing the levels of uremic toxins. Conversely, in the population of patients treated with synbiotics, a statistically significant increase in the level of Indoxyl sulfate was observed. What sets this study apart is that it is based on a population of hemodialysis patients, and the composition of probiotic cultures differed, primarily in the use of probiotics based on *Streptococcus thermophilus* (45).

The meta-analysis by McFarlane included all the studies conducted. It stated that preliminary studies demonstrated heterogeneous results and only a limited number of studies monitored the effects of therapy on changes in stool microbiome composition. The imperative is to conduct prospective, placebo-controlled studies to elucidate the intricacies of gut flora modulation (46). Such studies are critical for comprehending the efficacy and safety of these therapeutic interventions, paving the way for personalized approaches in managing uremic toxins and their impact on human health.

Conclusion

In conclusion, the intricate interrelationship between the human microbiome and health is undeniable, with the gut microbiome emerging as a central player in maintaining homeostasis and influencing disease development. This article delves into the critical roles of the gut microbiome in food digestion, immune system modulation, and its impact on cardiovascular and renal health. The exploration of uremic toxins, such as Indoxyl Sulfate, P-Cresyl Sulfate, and Trimethylamine-N-oxide, highlights their role in renal dysfunction and cardiovascular morbidity, bridging the gap between gut health and systemic diseases. The potential therapeutic options discussed, from

oral adsorbents to gut microbiome modulation, offer promising avenues for intervention. However, the complexity of these interactions necessitates further research through rigorous, large-scale studies to ascertain their efficacy and safety. As we unravel the mysteries of the microbiome, personalized approaches to managing uremic toxins may hold the key to advancing cardiovascular and renal health.

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