lek. Albert Michał Stec

Rola zaburzeń bariery jelitowej i metabolitów bakteryjnych w twardzinie układowej – implikacje kliniczne i terapeutyczne.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

Promotor: prof. dr hab. n. med. Lidia Rudnicka

Katedra i Klinika Dermatologiczna

Warszawski Uniwersytet Medyczny

Kierownik Kliniki: prof. dr hab. n. med. Lidia Rudnicka

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WYKAZ PUBLIKACJI STANOWIĄCYCH PRACĘ DOKTORSKĄ

Lp.	Artykuł	Impact Factor	Punkty MEiN
1.	**Stec A**, Sikora M, Maciejewska M, Paralusz-Stec K, Michalska M, Sikorska E, Rudnicka L.: Bacterial Metabolites: a Link between Gut Microbiota and Dermatological Diseases. *International Journal of Molecular Sciences* 2023; 24(4):3494. https://doi.org/10.3390/ijms24043494	6,208	140
2.	**Stec A**, Maciejewska M, Paralusz-Stec K, Michalska M, Giebułtowicz J, Rudnicka L, Sikora M.: The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis. *Journal of Inflammation Research* 2023;16:1895-1904. https://doi.org/10.2147/JIR.S409489	4,631	140
3.	**Stec A**, Maciejewska M, Zaremba M, Paralusz-Stec K, Michalska M, Rudnicka L, Sikora M.: The Clinical Significance of Serum Biomarkers of the Intestinal Barrier in Systemic Sclerosis: a Cross-Sectional Study. *Journal of Personalized Medicine* 2023; 13(4):678. https://doi.org/10.3390/jpm13040678	3,508	70
	Łącznie	14,347	350

Spis treści

1. Wykaz stosowanych skrótów

ACR *(ang. American College of Rheumatology)* - Amerykańskie Kolegium Reumatologiczne

BMI *(ang. Body Mass Index)* - wskaźnik masy ciała

CRP *(ang. C-reactive protein)* - białko C-reaktywne

DLCO *(ang. Diffusing Capacity of the Lungs for Carbon Monoxide)* - zdolność dyfuzyjna płuc dla tlenku węgla

ELISA *(ang. Enzyme-Linked Immunosorbent Assay)* - test immunoenzymatyczny

EULAR *(ang. European League Against Rheumatism)* - Europejskie Towarzystwo Reumatologiczne

HPLC-MS *(ang. High-Performance Liquid Chromatography - Mass Spectrometry)* - wysokosprawna chromatografia cieczowa w połączeniu ze spektrometrią masową

I-FABP *(ang. Intestinal fatty-acid binding protein)* – jelitowe białko wiążące kwasy tłuszczowe

IL-6 *(ang. Interleukin-6)* - interleukina 6

ILD *(ang. Interstitial Lung Disease)* - choroba śródmiąższowa płuc

IQR *(ang. Interquartile Range)* - rozstęp międzykwartylowy

LPS *(ang. Lipopolysaccharide)* - lipopolisacharyd

LVEF *(ang. Left Ventricular Ejection Fraction)* - frakcja wyrzutowa lewej komory serca

mRSS *(ang. modified Rodnan Skin Score)* - zmodyfikowana skala oceny zmian skórnych Rodnana

NT-proBNP *(ang. N-terminal pro-brain natriuretic peptide)* - N-końcowy fragment mózgowego peptydu natriuretycznego typu B

SCTC-DI *(ang. Scleroderma Clinical Trials Consortium Damage Index)* - wskaźnik uszkodzeń Scleroderma Clinical Trials Consortium

TGF-β1 *(ang. Transforming Growth Factor-β1)* - transformujący czynnik wzrostu beta 1

TLR-4 *(ang. Toll-like receptor 4)* - receptor Toll-podobny 4

TMA *(ang. trimethylamine)* – trimetyloamina

TMAO *(ang. trimethylamine N-oxide)* - N-tlenek trimetyloaminy

TNF *(ang. Tumor Necrosis Factor)* - czynnik martwicy nowotworów

2. Streszczenie

Rola zaburzeń bariery jelitowej i metabolitów bakteryjnych w twardzinie układowej – implikacje kliniczne i terapeutyczne.

2.1. Wprowadzenie

Wpływ mikrobioty jelitowej na homeostazę człowieka jest obecnie tematem intensywnie badanym. Dysbioza, czyli zaburzenie w składzie mikrobioty jelitowej, jest obserwowana w wielu chorobach, w tym także z zakresu dermatologii, między innymi w takich jednostkach jak łuszczyca, toczeń rumieniowaty układowy, atopowe zapalenie skóry oraz twardzina układowa. Wpływ dysbiozy na występowanie i przebieg chorób dermatologicznych opisuje koncepcja osi jelito-skóra, według której zmieniona mikrobiota może wpływać na dermatozy poprzez uwalniane substancje (metabolity). Efekt ten może być potęgowany poprzez występujące z dysbiozą uszkodzenie bariery jelitowej, które przyczynia się do zwiększonej przepuszczalności jelit. Sprzyja to translokacji metabolitów, antygenów oraz fragmentów komórek bakteryjnych do krwioobiegu, co poprzez stymulację układu immunologicznego może prowadzić do zaostrzenia objawów chorób skóry.

Twardzina układowa (ang. systemic sclerosis, SSc) jest autoimmunologiczną chorobą tkanki łącznej, często o ciężkim przebiegu. Patogeneza choroby nie jest w pełni poznana, jednak uważa się, że kluczową rolę odgrywają w niej zaburzone mechanizmy naprawy tkanki łącznej w odpowiedzi na uszkodzenie. Polegają one na niekontrolowanej produkcji białek macierzy pozakomórkowej, głównie kolagenu typu I, przez chorobowo zmienione fibroblasty i prowadzą do włóknienia skóry i narządów wewnętrznych. Zajęcie narządów wewnętrznych przez proces chorobowy powoduje wystąpienie swoistych objawów, między innymi śródmiąższowej choroby płuc, nadciśnienia płucnego, zaburzeń motoryki przewodu pokarmowego oraz niewydolności serca z powodu włóknienia mięśnia sercowego.

Zmiany mikrobioty jelitowej w przebiegu twardziny układowej charakteryzują się zwiększonym występowaniem bakterii z rodzajów *Fusobacterium*, *Desulfovibrio*, *Ruminococcus* i *Lactobacillus*, natomiast zmniejszonym występowaniem bakterii z rodzaju *Faecalibacterium.* Ponadto bardziej wyrażona dysbioza została zaobserwowana u osób

ze współistniejącymi objawami – chorobą śródmiąższową płuc i zaburzeniami motoryki przełyku.

Najnowsze badania przedkliniczne wskazują, że zarówno szkodliwe metabolity bakterii jelitowych, jak i substancje ulegające translokacji wskutek zwiększonej przepuszczalności jelit, mogą nasilać procesy włóknienia uwrażliwiając fibroblasty na działanie czynników profibrotycznych.

2.2. Cele pracy

1. Określenie potencjalnych nieprawidłowości w stężeniu metabolitu dysbiotycznej mikrobioty jelitowej – N-tlenku trimetyloaminy (ang. trimethylamine N-oxide; TMAO) w twardzinie układowej oraz potencjalnego związku z wystąpieniem typowych objawów narządowych choroby.

2. Ocena stanu bariery jelitowej w twardzinie układowej i określenie związku stężenia markerów przepuszczalności jelit z aktywnością i objawami choroby, w tym w szczególności z występowaniem śródmiąższowej choroby płuc oraz zaburzeniami motoryki przełyku.

3. Analiza potencjalnych różnic w stężeniu markerów przepuszczalności jelit u pacjentów z różnym czasem trwania twardziny układowej.

2.3. Materiał i metody

Do badania włączono 50 pacjentów z twardziną układową, którzy spełniali kryteria klasyfikacyjne ACR/EULAR. Grupę kontrolną stanowiło 30 ochotników dopasowanych pod względem płci, wieku i wskaźnika BMI. Badanie przedmiotowe oraz badania dodatkowe do oceny stopnia nasilenia zmian narządowych przeprowadzono zgodnie z rekomendacjami Polskiego Towarzystwa Dermatologicznego. Nasilenie stwardnienia skóry oceniano przy użyciu zmodyfikowanej skali Rodnana (mRSS). Poziom TMAO w osoczu został oznaczony z użyciem wysokosprawnej chromatografii cieczowej sprzężonej ze spektrometrem mas (HPLC-MS). Stężenia wybranych markerów bariery jelitowej oznaczono w surowicy metodą testu immunoenzymatycznego (ELISA). Poziom istotności statystycznej przyjęto dla $p < 0,05$.

2.4. Wyniki

U pacjentów z twardziną układową w porównaniu z grupą kontrolną wykazano istotnie statystycznie wyższe stężenie N-tlenku trimetyloaminy (TMAO) (283,0 ng/ml (rozstęp międzykwartylowy [IQR]: 188,5-367,5) vs. 205,5 ng/ml, (IQR 101,0-318,0); $p < 0,01$). Stwierdzono istotnie wyższe stężenie TMAO w podgrupie pacjentów ze śródmiąższową chorobą płuc w porównaniu do podgrupy bez tego powikłania (302,0 ng/ml (IQR 212,0-385,5) vs. 204,0 ng/ml (IQR 135,5-292,0); $p < 0,01$). W podgrupie pacjentów z towarzyszącym zaburzeniem motoryki przełyku (wykrytym w badaniu kontrastowym przełyku) wykazano istotnie wyższy poziom TMAO w porównaniu do podgrupy bez zaburzeń motoryki (289,75 ng/ml (IQR 213,75-387,5) vs. 209,5 ng/ml (IQR 141,5-315,0); $p = 0,026$). Ponadto stężenie TMAO wykazywało istotną ujemną korelację ze zdolnością dyfuzyjną płuc dla tlenku węgla (DLCO), będącą markerem restrykcji spowodowanej śródmiąższową chorobą płuc (rho = -0,53; $p = 0,013$). Dodatkowo współczynnik korelacji Spearmana wykazał istotną statystycznie ujemną korelację z badaną w echokardiografii frakcją wyrzutową lewej komory (LVEF) (rho = -0,39; $p < 0,01$) oraz istotną statystycznie dodatnią korelację ze stężeniem N-końcowego propeptydu natriuretycznego typu B (NT-proBNP) (rho = 0,41; $p < 0,001$). Zaobserwowano również istotną statystycznie dodatnią korelację pomiędzy stężeniem TMAO i wskaźnikiem uszkodzenia narządów w przebiegu twardziny (SCTC-DI) (rho = 0,78; $p < 0,001$).

Ocena bariery jelitowej wykazała istotnie wyższy poziom lipopolisacharydów bakteryjnych (LPS), będących wykładnikiem translokacji elementów komórek bakteryjnych ze światła jelita do krwioobiegu, w surowicy pacjentów z twardziną układową w porównaniu do osób w grupie kontrolnej (232,30 pg/mL (IQR 149,00-347,70) vs. 161,00 pg/mL (IQR 83,92-252,20); $p < 0,05$). Podgrupa pacjentów z krótszym okresem trwania choroby (mniejszym bądź równym 6 lat) charakteryzowała się istotnie wyższym poziomem lipopolisacharydów bakteryjnych i klaudyny-3 w porównaniu do podgrupy pacjentów z dłuższym okresem trwania choroby (powyżej 6 lat): LPS: (280,75 pg/mL (IQR 167,30-403,40) vs. 186,00 pg/mL (IQR 98,12-275,90); $p < 0,05$); klaudyna-3: (16,99 ng/mL (IQR 12,41-39,59) vs. 13,54 ng/mL (IQR 10,29-15,47); $p < 0,05$). Ponadto zaobserwowano, że w podgrupie z krótszym okresem trwania choroby pacjenci ze współwystępującą śródmiąższową chorobą płuc charakteryzowali się istotnie wyższym stężeniem LPS w porównaniu do pacjentów bez tego

powikłania (385,55 pg/mL (IQR 266,90-506,50) vs. 217,75 pg/mL (IQR 157,25-280,75); $p < 0,05$). Dodatkowo w grupie pacjentów z twardziną układową współwystępowanie zaburzeń motoryki przełyku wiązało się z istotnie niższym stężeniem LPS w surowicy w porównaniu do pacjentów z prawidłowym pasażem przełykowym (188,05 pg/mL (IQR 102,31-264,40) vs. 283,95 pg/mL (IQR 203,20-356,30); $p < 0,05$).

2.5. Wnioski

1. Stężenie metabolitu mikrobioty jelitowej – N-tlenku trimetyloaminy (ang. trimethylamine N-oxide; TMAO) w surowicy jest istotnie wyższe u pacjentów z twardziną układową w porównaniu do grupy kontrolnej. Występowanie niektórych objawów choroby, m. in. śródmiąższowej choroby płuc oraz zaburzeń motoryki przełyku, wiąże się ze szczególnie podwyższonym stężeniem TMAO.

2. Metabolity mikrobioty jelitowej mogą być łącznikiem między dysbiozą jelitową i zajęciem narządów w przebiegu twardziny układowej. Modulacja metabolitów pochodzących z bakterii jelitowych może stanowić nowe podejście terapeutyczne w leczeniu twardziny układowej.

3. Stężenie markera przepuszczalności jelitowej – lipopolisacharydów (ang. lipopolysaccharides; LPS) w surowicy jest istotnie wyższe u pacjentów z twardziną układową w porównaniu do grupy kontrolnej; podgrupa pacjentów o krótszym czasie trwania choroby (czas mniejszy lub równy 6 lat) charakteryzuje się istotnie statystycznie większym stężeniem markerów przepuszczalności jelitowej, LPS i klaudyny-3, w porównaniu do podgrupy o dłuższym czasie trwania choroby (powyżej 6 lat), co sugeruje występowanie zwiększonej przepuszczalności jelit na wczesnym etapie choroby.

4. Niższe stężenie markerów przepuszczalności jelitowej (LPS i Klaudyny-3) u pacjentów z dłuższym okresem trwania choroby (powyżej 6 lat) w porównaniu do chorych o krótszym przebiegu choroby (czas mniejszy lub równy 6 lat) może być spowodowane współwystępującymi zaburzeniami wchłaniania wynikającymi z zajęcia przewodu pokarmowego.

3. Summary

The role of intestinal barrier disruption and bacterial metabolites in systemic sclerosis: clinical and therapeutic implications.

3.1. Introduction

The influence of intestinal microbiota on human homeostasis is currently an intensively researched topic. Dysbiosis, i.e. a change in the composition of the intestinal microbiota, is observed in many diseases, including those in the field of dermatology, such as psoriasis, systemic lupus erythematosus, atopic dermatitis, and systemic sclerosis. The impact of dysbiosis on the occurrence and course of dermatological diseases is described by the concept of the gut-skin axis, according to which altered microbiota may affect dermatoses through produced substances (metabolites). This effect may be enhanced by the disruption of the intestinal barrier that often occurs with dysbiosis, which contributes to increased intestinal permeability. This promotes the translocation of metabolites, antigens, and fragments of bacterial cells into the bloodstream, which, by stimulating the immune system, can lead to exacerbation of the symptoms of skin diseases.

Systemic sclerosis (SSc) is an autoimmune connective tissue disease, which course is often severe. The pathogenesis of the disease is not fully understood, but it is believed that impaired connective tissue repair mechanisms in response to cellular damage play a key role. Impaired repair mechanisms are characterized by uncontrolled production of extracellular matrix proteins, mainly type I collagen, by pathologically changed fibroblasts and lead to fibrosis of the skin and internal organs. Involvement of internal organs by the disease process causes specific complications, including interstitial lung disease, pulmonary hypertension, gastrointestinal motility disorders, and heart failure due to myocardial fibrosis.

Changes in the intestinal microbiota in the course of systemic sclerosis are characterized by an increased presence of bacteria of the genera *Fusobacterium*, *Desulfovibrio*, *Ruminococcus,* and *Lactobacillus*, and a decreased presence of bacteria of the genus *Faecalibacterium*. In addition, more pronounced dysbiosis was observed in patients

with coexisting organ involvement: interstitial lung disease and esophageal motility disorders.

The latest preclinical studies indicate that both harmful metabolites of intestinal bacteria and substances undergoing translocation due to increased intestinal permeability may intensify fibrosis processes by sensitizing fibroblasts to profibrotic factors.

3.2. Objective

1. The aim of this study was to determine potential abnormalities in the concentration of the dysbiotic gut microbiota metabolite: trimethylamine N-oxide (TMAO) in systemic sclerosis, as well as its potential association with the occurrence of typical organ-related symptoms of the disease.

2. Evaluation of the intestinal barrier in systemic sclerosis and determination of the relationship between the concentration of gut permeability markers and disease activity and symptoms, particularly with the occurrence of interstitial lung disease and esophageal motility disorders.

3. Analysis of potential differences in the concentration of gut permeability markers among patients with different durations of systemic sclerosis.

3.3. Material and methods

The study involved 50 patients with systemic sclerosis who met the ACR/EULAR classification criteria. The control group consisted of 30 volunteers matched for sex, age, and body mass index (BMI). Physical examination and additional tests to assess the severity of organ lesions were performed in accordance with the recommendations of the Polish Society of Dermatology. The severity of skin induration was assessed using the modified Rodnan scale (mRSS). Plasma TMAO levels were determined using high-performance liquid chromatography coupled to mass detection (HPLC-MS). Concentrations of selected markers of the intestinal barrier were determined in the serum by the enzyme immunoassay (ELISA). The p-value of < 0.05 was considered statistically significant.

3.4. Results

Compared to the control group, patients with systemic sclerosis showed a statistically significantly higher concentration of trimethylamine-N-oxide (TMAO) (283.0 ng/ml

(interquartile range [IQR] 188.5-367.5) vs. 205.5 ng/ml (IQR 101.0-318.0); $p < 0.01$). A significantly higher TMAO concentration was found in the subgroup of patients with interstitial lung disease compared to the subgroup without lung involvement (302.0 ng/ml (IQR 212.0-385.5) vs. 204.0 ng/ml (IQR 135.5-292.0); $p < 0.01$). The subgroup of patients with concomitant esophageal motility disorders (detected by contrast examination of the esophagus) showed significantly higher levels of TMAO compared to the subgroup with normal motility (289.75 ng/ml (IQR 213.75-387.5) vs. 209.5 ng/mL (IQR 141.5-315.0), $p = 0.026$). In addition, TMAO concentration showed a significant negative correlation with the diffusion lung capacity for carbon monoxide (DLCO), a marker of restriction due to interstitial lung disease (rho= -0.53; $p = 0.013$). In addition, Spearman's correlation coefficient showed a statistically significant negative correlation with the left ventricular ejection fraction (LVEF) measured in echocardiography (rho = -0.39; $p < 0.01$) and a statistically significant positive correlation N-terminal B-type natriuretic propeptide (NT-proBNP) concentration (rho = 0.41; $p < 0.001$). There was also a statistically significant positive correlation between TMAO concentration and scleroderma organ damage index (SCTC-DI) (rho = 0.78; $p < 0.001$).

The assessment of the intestinal barrier showed a significantly higher level of bacterial lipopolysaccharides (LPS), which is an indicator of the translocation of bacterial cell elements from the intestinal lumen into the bloodstream, in the serum of patients with systemic sclerosis compared to those in the control group (232.30 pg/mL (IQR 149.00-347.70) vs. 161.00 pg/mL (IQR 83.92-252.20); $p < 0.05$). The subgroup of patients with a shorter duration of disease (less than or equal to 6 years) had significantly higher levels of bacterial lipopolysaccharides and claudin-3 compared to the subgroup of patients with a longer duration of disease (greater than 6 years): LPS: (280.75 pg/mL (IQR 167.30-403.40) vs. 186.00 pg/mL (IQR 98.12-275.90); $p < 0.05$), claudin-3: (16.99 ng/mL (IQR 12.41-39.59) vs. 13.54 ng/mL (IQR 10.29-15.47); $p < 0.05$). In addition, it was observed that in the subgroup with a shorter duration of disease, patients with interstitial lung disease had significantly higher LPS levels compared to patients without involvement of the lungs (385.55 pg/mL (IQR 266.90-506.50) vs. 217.75 pg/mL (IQR 157.25-280.75); $p < 0.05$). In addition, in the group of patients with systemic sclerosis, the coexistence of esophageal motility disorders was associated with significantly lower serum LPS levels compared

to patients with normal esophageal passage (188.05 pg/mL (IQR 102.31-264.40) vs. 283.95 pg/mL (IQR 203.20-356.30); $p < 0.05$).

3.5. Conclusions

1. The concentration of the gut microbiota metabolite: trimethylamine N-oxide (TMAO) in the serum is significantly higher in patients with systemic sclerosis compared to the control group. The presence of certain symptoms of the disease, including interstitial lung disease and esophageal motility disorders, is associated with particularly elevated levels of TMAO.

2. Gut microbiota metabolites may serve as a link between gut dysbiosis and organ involvement in systemic sclerosis. Modulating the metabolites derived from gut bacteria may represent a novel therapeutic approach in the treatment of systemic sclerosis.

3. The concentration of the gut permeability marker: lipopolysaccharides (LPS) in the serum is significantly higher in patients with systemic sclerosis compared to the control group. A subgroup of patients with a shorter disease duration (less than or equal to 6 years) is characterized by significantly higher levels of gut permeability markers, LPS and claudin-3, compared to the subgroup with a longer disease duration (above 6 years), suggesting increased gut permeability at an early stage of the disease.

4. Lower levels of intestinal permeability markers (LPS and Claudin-3) in patients with longer disease duration (more than 6 years) compared to patients with shorter disease duration (less than or equal to 6 years) may be due to co-occurring malabsorption resulting from the disease affecting the gastrointestinal tract.

4. Wstęp

Wpływ mikrobioty jelitowej na homeostazę człowieka jest obecnie tematem intensywnie badanym. Dysbioza, czyli zaburzenie w składzie mikrobioty jelitowej, jest obserwowana w wielu chorobach, w tym także z zakresu dermatologii, między innymi w takich jednostkach jak łuszczyca (1), toczeń rumieniowaty układowy (2), atopowe zapalenie skóry (3) oraz twardzina układowa (4). Wpływ dysbiozy na występowanie i przebieg chorób dermatologicznych opisuje koncepcja osi jelito-skóra, według której zmieniona mikrobiota może wpływać na rozwój i przebieg dermatoz (5). Jednym z proponowanych mechanizmów takiej interakcji jest wpływ substancji wydzielanych przez dysbiotyczną mikrobiotę jelitową (metabolitów). Mogą one wpływać zarówno na komórki skóry oraz barierę naskórkową, jak i modulować reakcje układu immunologicznego, którego dysfunkcja jest podłożem wielu chorób dermatologicznych (5, 6). Efekt ten może być potęgowany poprzez występujące z dysbiozą uszkodzenie bariery jelitowej, które przyczynia się do zwiększonej przepuszczalności jelit (7). Sprzyja to translokacji metabolitów, antygenów oraz fragmentów komórek bakteryjnych do krwioobiegu, co poprzez stymulację układu immunologicznego może prowadzić do zaostrzenia objawów chorób dermatologicznych (6, 8).

Twardzina układowa (ang. systemic sclerosis, SSc) jest autoimmunologiczną chorobą tkanki łącznej, często o ciężkim przebiegu (9). Choroba charakteryzuje się postępującym włóknieniem skóry i narządów wewnętrznych (9). Częstość występowania twardziny szacuje się na około 17,6 przypadków na 100 tysięcy mieszkańców. Ponadto choroba charakteryzuje się pięciokrotnie częstszym występowaniem wśród kobiet w porównaniu do mężczyzn (10).

Patogeneza choroby nie jest w pełni poznana, jednak uważa się, że kluczową rolę odgrywają w niej zaburzone mechanizmy naprawy tkanki łącznej w odpowiedzi na uszkodzenie. Polegają one na niekontrolowanej produkcji białek macierzy pozakomórkowej, głównie kolagenu typu I, przez chorobowo zmienione fibroblasty i prowadzą do włóknienia skóry i narządów wewnętrznych (11). Zajęcie narządów wewnętrznych przez proces chorobowy powoduje wystąpienie swoistych objawów, między innymi śródmiąższowej choroby płuc, nadciśnienia płucnego, zaburzeń motoryki przewodu pokarmowego oraz niewydolności serca będącej efektem włóknienia mięśnia sercowego (9).

Zmiany mikrobioty jelitowej w przebiegu twardziny układowej charakteryzują się zwiększonym występowaniem bakterii z rodzajów *Fusobacterium*, *Desulfovibrio*, *Ruminococcus* i *Lactobacillus*, natomiast zmniejszonym występowaniem bakterii z rodzaju *Faecalibacterium w* porównaniu do zdrowej populacji (12-14). Dodatkowo zmiany te są bardziej wyrażone u osób ze współistniejącym zajęciem narządowym – chorobą śródmiąższową płuc i zaburzeniami motoryki przełyku (13, 15). Dostępna literatura wskazuje, że dysbioza może być obserwowana już od wczesnych stadiów choroby (13). Zaobserwowano również, że dysbiotyczna mikrobiota jelitowa w twardzinie układowej wiąże się ze zmienionymi właściwościami metabolicznymi w porównaniu do osób zdrowych (16). Implikuje to możliwość ekspozycji pacjentów na inne spektrum substancji produkowanych przez bakterie jelitowe. Ekspozycja ta może być dodatkowo zwiększona poprzez występujące w twardzinie zmiany naczyniowe i zajęcie przewodu pokarmowego prowadzące do zwiększonej przepuszczalności jelitowej (17-19).

Substancjami produkowanymi przez mikrobiotę jelitową mogą być drobnocząsteczkowe produkty jej metabolizmu (20). Znanych jest kilka grup metabolitów mikrobioty jelitowej, które mogą działać zarówno korzystnie na homeostazę (między innymi krótkołańcuchowe kwasy tłuszczowe i pochodne tryptofanu), jak i negatywnie (pochodne amin czwartorzędowych, z których najlepiej poznanym jest N-tlenek trimetyloaminy) (6, 20).

N-tlenek trimetyloaminy (TMAO) to substancja produkowana wskutek utleniania metabolitu mikrobioty jelitowej, trimetyloaminy (TMA), zachodzącego w wątrobie. TMA powstaje z dostarczanych wraz z pożywieniem amin czwartorzędowych m. in. choliny, L-karnityny i fosfatydylocholiny (21). Wzrost stężenia TMAO we krwi często związany jest z dysbiozą (22) i obserwowany jest w wielu jednostkach chorobowych takich jak choroby sercowo-naczyniowe (23, 24), łuszczycowe zapalenie stawów (25) i reumatoidalne zapalenie stawów (26). TMAO wykazuje wielokierunkowe działanie prozapalne. Metabolit ten indukuje wytwarzanie prozapalnych cytokin, takich jak TNF, IL-6 i białko C-reaktywne (CRP) przez komórki układu odpornościowego (6). Ostatnie badania przedkliniczne wykazały, że TMAO może potencjalnie wpływać na patogenezę twardziny układowej poprzez indukowanie uszkodzenia śródbłonka, nasilenie działania cytokin związanych z procesem włóknienia, zwiększenie odkładania kolagenu, hamowanie zależnego od śródbłonka rozszerzania tętnic i hamowanie uwalniania tlenku azotu w śródbłonku naczyń (27-29). Ponadto dostępna

literatura sugeruje, że zwiększone stężenie TMAO może być związane ze zwiększonym ryzykiem i cięższym przebiegiem zajęcia narządowego w twardzinie układowej. Zwiększone stężenie TMAO we krwi obserwowane było u pacjentów cierpiących na nadciśnienie płucne, które występuje u ok. 15% pacjentów z twardziną układową (30-32). Wyższe stężenie TMAO związane było również z gorszym przebiegiem nadciśnienia płucnego ocenionym na podstawie klas czynnościowych zaproponowanych przez Światową Organizację Zdrowia (31). Badania przeprowadzone na mysim modelu włóknienia serca wykazały, że TMAO może nasilać włóknienie mięśnia sercowego, a tym samym zmniejszać frakcję wyrzutową i kurczliwość serca (33). Do tej pory nie zbadano stężenia TMAO we krwi pacjentów z twardziną układową oraz zależności pomiędzy TMAO a specyficznymi objawami choroby.

Czynnikiem, który może modulować wpływ mikrobioty jelitowej na przebieg twardziny układowej jest bariera jelitowa. Jest to specyficzna zdolność śluzówki jelita do selektywnego wchłaniania substancji odżywczych, elektrolitów i wody, przy jednoczesnym zatrzymywaniu w świetle jelita substancji zaburzających homeostazę (7, 34). Bariera jelitowa składa się z kilku elementów, z których kluczowymi dla zachowania selektywnej przepuszczalności wydają się być komórki nabłonka jelit (enterocyty) i połączenia pomiędzy nimi (7, 34). Uszkodzenie tych połączeń powoduje zwiększenie przepuszczalności śluzówki jelit dla potencjalnie szkodliwych substancji – antygenów, alergenów i fragmentów komórek bakteryjnych.

Dostępne badania podają, że przepuszczalność jelit w twardzinie układowej jest zwiększona (18, 19). Technika użyta we wspomnianych badaniach, test absorpcji oligosacharydów, jest metodą czasochłonną i trudną do zastosowania w praktyce klinicznej (35). Alternatywą dla tej metody są nowe biomarkery bariery jelitowej, które mogą być oznaczane z krwi pobranej w trakcie rutynowych badań. Zaletami są łatwość użycia oraz możliwość uzyskania informacji o wielkości translokacji bakteryjnej i stanie połączeń pomiędzy enterocytami. Spośród dostępnych biomarkerów najlepiej udokumentowanymi w ocenie bariery jelitowej są jelitowe białko wiążące kwasy tłuszczowe (I-FABP), klaudyna-3 oraz lipopolisacharydy bakteryjne (LPS).

I-FABP jest białkiem występującym niemal wyłącznie w cytoplazmie enterocytów w jelicie cienkim (36). W fizjologicznych warunkach translokacja tego markera do krążenia jest

minimalna, natomiast uszkodzenie komórek nabłonka jelitowego powoduje wyraźny wzrost poziomu I-FABP we krwi (36).

Klaudyna-3 jest białkiem wchodzącym w skład połączeń ścisłych pomiędzy enterocytami. Uszkodzenie tych połączeń powoduje wzrost stężenia klaudyny-3 w surowicy, co może być wykorzystane do oceny stanu bariery jelitowej (37-39).

LPS jest elementem tworzącym zewnętrzną błonę komórkową bakterii Gram-ujemnych (35). Zwiększenie jego stężenia we krwi świadczy o zachodzącej translokacji elementów komórek bakterii ze światła jelita do krwioobiegu, a co za tym idzie zwiększonej przepuszczalności śluzówki jelit na tle zaburzeń bariery jelitowej (35). Dodatkowo LPS jest substancją wysoce immunogenną o udokumentowanym wpływie pobudzającym na układ immunologiczny, co może prowadzić do zaostrzenia przebiegu chorób o mechanizmie autoimmunizacyjnym (40). Nowe badania przedkliniczne sugerują, że LPS poprzez sygnalizację związaną z receptorem toll-podobnym 4 (TLR4), będącym receptorem dla LPS, może wpływać na mechanizmy molekularne związane z włóknieniem obserwowanym w twardzinie układowej. W biopsjach skóry i płuc pacjentów chorujących na twardzinę układową stwierdzono zwiększone występowanie TLR4 na fibroblastach, co może sugerować zwiększoną podatność na niekorzystne działanie LPS (41). Ponadto wykazano, że LPS może zwiększać ekspresję genów macierzy pozakomórkowej, między innymi kolagenu, w fibroblastach skóry i znacząco zwiększać ich zdolność do rozpoczęcia odpowiedzi profibrotycznej po prowokacji transformującym czynnikiem wzrostu beta 1 (TGF-β1), cytokiną znaną jako główny mediator włóknienia w twardzinie układowej (41, 42).

5. Cel i założenia pracy

1. Określenie potencjalnych nieprawidłowości w stężeniu metabolitu dysbiotycznej mikrobioty jelitowej – N-tlenku trimetyloaminy (ang. trimethylamine N-oxide; TMAO) w twardzinie układowej oraz potencjalnego związku z wystąpieniem typowych objawów narządowych choroby.

2. Ocena stanu bariery jelitowej w twardzinie układowej i określenie związku stężenia markerów przepuszczalności jelit z aktywnością i objawami choroby, w tym w szczególności z występowaniem śródmiąższowej choroby płuc oraz zaburzeniami motoryki przełyku.

3. Analiza potencjalnych różnic w stężeniu markerów przepuszczalności jelit u pacjentów z różnym czasem trwania twardziny układowej.

6. Kopie opublikowanych prac

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Review

Bacterial Metabolites: A Link between Gut Microbiota and Dermatological Diseases

Albert Stec [1], Mariusz Sikora [2,*], Magdalena Maciejewska [1], Karolina Paralusz-Stec [1], Milena Michalska [3], Ewa Sikorska [4] and Lidia Rudnicka [1]

[1] Department of Dermatology, Medical University of Warsaw, Koszykowa 82A, 02-008 Warsaw, Poland
[2] National Institute of Geriatrics, Rheumatology and Rehabilitation, Spartańska 1, 02-637 Warsaw, Poland
[3] Department of General, Vascular and Transplant Surgery, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland
[4] Department of Experimental and Clinical Physiology Center for Preclinical Research, Medical University of Warsaw, Banacha 1b, 02-097 Warsaw, Poland
* Correspondence: drmariuszsikora@gmail.com

Abstract: Dysbiosis has been identified in many dermatological conditions (e.g., psoriasis, atopic dermatitis, systemic lupus erythematosus). One of the ways by which the microbiota affect homeostasis is through microbiota-derived molecules (metabolites). There are three main groups of metabolites: short-chain fatty acids (SCFAs), tryptophan metabolites, and amine derivatives including trimethylamine N-oxide (TMAO). Each group has its own uptake and specific receptors through which these metabolites can exert their systemic function. This review provides up-to-date knowledge about the impact that these groups of gut microbiota metabolites may have in dermatological conditions. Special attention is paid to the effect of microbial metabolites on the immune system, including changes in the profile of the immune cells and cytokine disbalance, which are characteristic of several dermatological diseases, especially psoriasis and atopic dermatitis. Targeting the production of microbiota metabolites may serve as a novel therapeutic approach in several immune-mediated dermatological diseases.

Keywords: microbiota; gut–skin axis; dysbiosis; inflammation; immune-mediated inflammatory diseases; intestinal microbial metabolites; SCFA; TMAO; probiotics; fecal microbiota transplantation

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1. Introduction

The intestinal microbiota are the microorganisms colonizing the gastrointestinal tract, which include more than a thousand distinct species of bacteria, viruses, fungi, and protozoa [1]. The impact of the gut microbiome on human homeostasis has been widely studied, especially its ability to modulate inflammatory responses. The quantitative and qualitative changes in the intestinal microbiota's composition have been observed in many dermatological conditions, such as psoriasis [2–4], systemic lupus erythematosus [5], atopic dermatitis [6,7], and systemic sclerosis [8,9]. This condition is called dysbiosis, and its impact on human health is still not clearly understood. In contrast to the normobiotic state, altered exposure of the host to diverse microbial stimuli may be linked with dysbiosis. Available data suggest that it can be a significantly harmful environmental factor, especially in genetically vulnerable hosts whose endogenous systems, which control inflammation, are already compromised [10].

There are several main mechanisms by which the microbiota could play a role in autoimmunity and stimulation of the immune response: molecular mimicry, an impaired intestinal barrier that may promote translocation of bacterial elements or even whole bacteria, direct interactions with immune cells in Peyer's patches, and an altered abundance of microbial metabolites with immunomodulatory functions [10–13]. To date, only some groups of metabolites have been discovered and extensively studied. The most relevant are

those that significantly impact the immune system physiology, such as short-chain fatty acids (SCFAs), tryptophan metabolites, and amine derivatives including trimethylamine N-oxide (TMAO) [10,14,15]. These are found to exert particular effects at the cellular and even systemic level by interacting with different receptors in immune and skin cells [16–18].

Understanding how microbial metabolites affect the skin and immune cells could be a milestone in deciphering the concept proposed by novel studies regarding the functional link between the intestine and skin, which is called the gut–skin axis [19].

2. Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are the most studied substances among gut bacterial metabolites. This group consists of aliphatic carboxylic acids with fewer than six carbon atoms. In humans, the three most abundant aliphatic carboxylic acids, which have the highest impact on homeostasis, are acetic, propionic, and butyric acid [16].

SCFAs are produced mostly in the colon due to the fermentation of indigestible dietary fiber mainly by two anaerobic bacterial phyla: *Bacteroides* and *Firmicutes* [20]. Some of the produced SCFAs are absorbed by colonocytes and utilized as an energy source. The rest of the SCFA pool is absorbed into the circulation and exerts systemic effects via four known pathways: interaction with the free fatty acid receptor 2 (FFAR2, also known as GPR43), the free fatty acid receptor 3 (FFAR3, also known as GPR41), the free fatty acid receptor GPR109a, and inhibition of histone deacetylase [16,20].

FFAR2, FFAR3, and GPR109a are G-protein-coupled receptors, and they are expressed in various tissues, including adipocytes, intestinal epithelial cells, pancreatic beta-cells, spleen, and immune cells such as M2 macrophages, neutrophils, eosinophils, and mast cells [16]. As inhibitors of histone deacetylases (HDACs), SCFAs cause an increase in the expression of certain genes in an epigenetic manner. There is a substantial body of evidence confirming that these substances not only act as histone deacetylase inhibitors but can also modify histones directly via so-called histone propionylation and butyrylation [21].

SCFAs' abilities to modulate the immune system could be beneficial in the prevention, modulation, or prognosis of dermatological disorders resulting from immune system dysregulation. Usami et al. proved that SCFAs, especially butyrate, inhibit the activity of NF-κB in mononuclear cells, which is a transcriptional factor controlling the expression of multiple genes involved in the inflammatory response. Furthermore, it has been shown that SCFAs can markedly reduce the secretion of TNF via the mentioned cells [22]. In macrophages, butyrate reduces the expression of inflammatory cytokines, such as TNF, MCP-1, and IL-6, via HDAC inhibition [23]. In T cells, SCFAs are potent regulators of differentiation and cytokine production. It is known that SCFAs can increase the pool of Treg lymphocytes in an FFAR2- and HDAC-dependent manner [24–26]. Additionally, signaling through GPR109a and FFAR2 in dendritic cells (DCs) can lead to the induction of the differentiation of Foxp3$^+$ Treg lymphocytes [11,27]. SCFAs also impact B cells. Through HDACs' inhibitory properties, they can modulate gene expression epigenetically. Specifically, butyrate and propionate decrease the expression of B cells' *Aicda* and *Prdm1* genes in humans and mice, the products of which are key regulators of antibody class switching and differentiation of B cells [28]. It has been observed that SCFA-fed mice are characterized by decreased IgG1, IgA, and IgE antibody levels, a dampened response to allergens by plasmocytes, and a reduced number of local and systemic class-switched B cells, antibody-forming cells, and class-switched antibodies [28]. The level of SCFAs has also shown a positive correlation with the number of regulatory B cells in rheumatoid arthritis [29]. All the mentioned facts suggest that SCFAs can dampen the immune response and may prevent autoimmune diseases. Valerate, which is a less-studied compound from the SCFA group, also displays tolerogenic properties. It induces IL-10 production in both B and T CD4$^+$ cells and suppresses Th17 lymphocytes via a reduction in IL-17 production and downregulation of Th17-associated genes. It also mediates the generation of Breg cells [30,31].

In contrast to this result, SCFAs can boost the generation of Th1 and Th17 cells during active immune responses and increase the cytotoxic activity and production of IL-17 via CD8 T cells [23]. These discrepancies in the function of SCFAs can be explained by the host state. In a steady state, SCFAs seem to present tolerogenic properties, but in acute immune responses, they stimulate the response of the immune system to fight the infection more effectively.

2.1. Atopic Dermatitis and Hypersensitivity Reactions

There is mounting evidence that SCFAs can interfere with allergy response on many levels. The most crucial function of SCFAs in the pathogenesis of hypersensitivity is their ability to improve intestinal and epithelial barriers. A disrupted epithelial barrier creates favorable conditions for allergen ingress and further sensitization, which is a pivotal feature of atopic dermatitis (AD) [32]. It has been observed in a mouse model of AD that orally administered SCFAs, particularly butyrate, can decrease transepidermal water loss (TEWL) and increase the level of cholesterol and ceramides, particularly ester-linked omega-hydroxy ceramides, in the stratum corneum [33]. The deficiency of these ceramides is linked with AD [34]. What is more, the administration of butyrate was found to affect keratinocytes directly by promoting terminal differentiation. Butyrate-treated human epithelial keratinocytes have displayed increased numbers of "late" differentiation markers such as involucrin, filaggrin, and calmodulin-like skin protein as well as "early" differentiation markers such as desmoglein-1, keratin-1, and keratin-10 [33].

Interestingly, there is ample evidence that AD is characterized by gut dysbiosis, especially the depletion of butyrate-producing bacteria [35–38]. The reduction in butyrate-producing *Bacteroides fragilis* has been found to correlate with increased total IgE, egg-IgE, and milk-IgE [38]. Moreover, the microbiota in AD produce fewer SCFAs, particularly propionate and butyrate, which have been assessed in fecal samples [38,39]. Reduced exposure to SCFAs early in life may be one of the factors responsible for the development and course of the disease, which seems to confirm the work of Roduit et al. They found that in a one-year-old pediatric population, a lower fecal level of propionate and butyrate was associated with a markedly higher prevalence of atopic diseases diagnosed at the age of 6 [40]. Other studies seem to confirm these results by showing that lower fecal butyrate levels in one-year-old patients are associated with the development of eczema [41–43]. Additionally, decreased exposure to valerate is supposedly correlated with a lower prevalence of eczema in children [44,45]. Dysbiosis and decreased SCFA formation might be an effect of prenatal and early-life exposure to antibiotics. As reported by recent large cohort studies, both prenatal and early-life exposures to antibiotics are linked with an increased risk of developing AD [46,47]. A study on a mouse model of AD showed corresponding results. Administration of antibiotics before sensitization was associated with a more severe disease course, increased expression of IL-4, increased levels of IgE, decreased levels of acetate, propionate, and butyrate, and decreased Foxp3$^+$ Treg cells [48]. Like in psoriasis, Treg lymphocytes have a significant role in the pathogenesis of atopic diseases [49], which could be at least partially improved by SCFAs.

The tolerogenic effect of SCFAs may be responsible for the acquisition of allergen tolerance. The pathogenesis of human food allergy includes an increased number of intestinal Th2 cells and type 2 innate lymphoid cells (ILC2s), which generate cytokines including IL-4, IL-5, and IL-13 after exposure to food allergens [50]. It is well known that IL-4 actively promotes B cells' development into plasma cells that synthesize IgE [51]. Cross-linking of allergen-specific IgE on mast cells via FcεRI can elicit degranulation of mast cells after exposure to a particular allergen, which results in an allergic reaction [51]. Many studies report that SCFAs have a direct impact on the majority of cells involved in allergic responses. In mast cells, SCFAs act epigenetically by altering the signaling cascade mediated by FcεRI, which can dampen allergic responses [52–54]. These findings are supported by the fact that fecal concentrations of acetate, propionate, and butyrate are lower in patients suffering from IgE-mediated food allergies [55]. Interestingly, like in the case of atopic

dermatitis, lower exposure to SCFAs in early childhood might result in the development of food allergy, and also IgE-associated diseases such as asthma [40,42,45]. Moreover, it seems that the protective effect of SCFAs is distinctively observable in IgE-related conditions, contrary to IgE-independent sensitizations [56]. However, sodium butyrate (SB) injected subcutaneously or administered topically suppressed both the elicitation phase and ongoing hypersensitivity reaction in a mouse model sensitized to 2,4,6-trinitro-1-chlorobenzene, which is model for contact dermatitis [57,58]. Sections from areas exposed to SB were characterized by an increased number of Treg lymphocytes, and greater expression of IL-10 and the Foxp3 transcription factor compared to sections from the control tissue [57]. The immunomodulatory effect of SB is histone-acetylation-dependent. Moreover, similar findings were observed in human skin biopsies; after exposure to SB, more Treg cells, increased transcription of Foxp3 and IL-10 genes, and decreased transcription of the IL-6 gene were observed [57]. The oral administration of butyrate or propionate can alleviate symptoms in the mouse model of allergic airway disease [40,52] and food allergy [59]. Additionally, some studies also suggest that exposure to butyrate and propionate may lead to a reduction in IL-4 secretion, which can potentially restore the skin barrier and suppress the allergic response [60].

2.2. Psoriasis

Schwarz et al. provide substantial information about the function of SB, which is the counterpart of the butyrate produced by the microbiota on skin immune cells, and its therapeutic potential in skin disorders. They observed that the injection or topical administration of SB can almost completely reverse imiquimod-induced psoriasis-like lesions in the mice. SB was also able to downregulate inflammatory response, which was manifested by decreased expression of IL-17 and enhanced expression of IL-10 and Foxp3. The anti-inflammatory effect was dependent on Treg lymphocytes [61]. The next part of the study included patients with psoriasis. Compared to the controls, the patients had reduced Treg activity, which was partially restored using SB. Moreover, upregulation of the Foxp3 factor and activation of skin Treg cells were observed. Additionally, in the studied skin, SB reduced the expression of IL-6 and IL-17 and upregulated the expression of IL-10 [61]. Similar results were observed by Krejner et al. They found that in skin samples of psoriatic patients, the expression of FFAR2 and GPR109a were decreased, and the administration of SB upregulated these receptors, reduced the expression of IL-6, IL-17, and increased the expression of IL-10 [62]. Interestingly, some of the drugs used in psoriasis management are potent agonists of these receptors; e.g., dimethyl fumarate exhibits a high affinity to the GPR109a receptor [63,64]. Cyclosporine, another antipsoriatic drug, may increase the intestinal uptake of certain SCFAs, especially butyrate [65].

2.3. Connective Tissue Diseases

SCFAs may impact lupus pathogenesis. In systemic lupus erythematosus (SLE) patients, gut dysbiosis, along with a decrease in butyrate-producing bacteria and altered SCFA concentrations, can be observed [66,67]. Oral administration of SCFAs, especially butyrate, in both lupus-prone mouse models MRL/Faslpr/lpr and NZB/W F1 can alleviate symptoms of the disease and can reduce dysbiosis by increasing microbiota diversity [28,68]. In comparison to the control group, the mice that were administered butyrate and propionate orally did not demonstrate IgG1/IgG2a kidney deposition, glomerular damage, or skin lesions [28]. Furthermore, this intervention decreased the number of autoantibodies to dsDNA, RNP/Sm, anti-RNA, histones, and nuclei, and reduced the number of plasmacytes [28].

Systemic sclerosis (SSc) is also characterized by dysbiosis, particularly in butyrate-producing genera, but to date, there are no data on human subjects regarding the influence of SCFAs on SSc [69,70]. However, promising results have been obtained in mice. In the bleomycin-induced systemic sclerosis model, the administration of sodium butyrate causes reduced collagen deposition and α-SMA expression in the skin [71]. Additionally, SB has

shown efficacy in reducing lung fibrosis and gut dysbiosis. The in vitro part of this study reported that SB can dampen the profibrotic response induced by TGF-β1 in human dermal fibroblasts (HDFs) [71].

3. Tryptophan Metabolites

In the gastrointestinal tract, dietary proteins are broken down into amino acids. Among them, tryptophan has ample evidence of being utilized by intestinal microbiota. Tryptophan can be metabolized in three general pathways: the kynurenine, serotonin, and indole pathways (Figure 1). The latter is known to be closely associated with microbiota metabolism [72]. Many genera of intestinal bacteria, such as *Clostridium*, *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*, are known to produce tryptophan metabolites [73]. For microorganisms, tryptophan can act as a substrate for the production of cofactor for NAD [74]. Moreover, some tryptophan microbial derivates, e.g., tryptophol and indole lactic acid, exhibit the potential to shape microbiota by acting as quorum-sensing molecules [75] or via their antibacterial and antifungal properties [76–78].

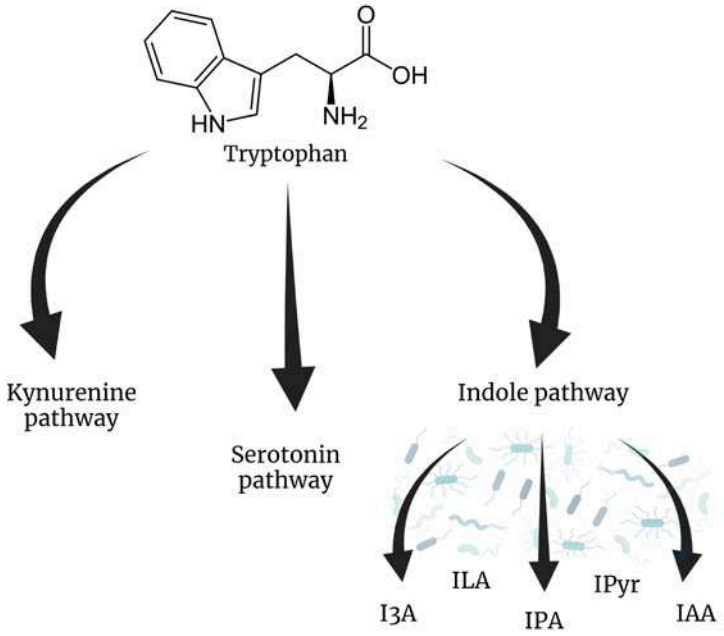

Figure 1. Three pathways of tryptophan metabolism. The indole pathway is mostly associated with intestinal microbiota metabolism. Various compounds produced in this pathway are considered beneficial to human health. I3A—indole-3-aldehyde, ILA—indole lactic acid, IPA—indole propionic acid, IPyr—indole pyruvate, IAA—indole acetic acid. Created with BioRender.com.

Recent studies have revealed that circulating microbial tryptophan catabolites may affect the homeostasis of the human body. Despite the multidirectional function of compounds derived in the indole pathway, including increasing GLP-1 production [79] and stimulating intestinal motility, activation of the aryl hydrocarbon receptor (AhR) by these substances seems to be their most important role in the context of dermatological diseases [17,80]. This receptor is widely expressed, especially in the skin, intestines, and lungs. In the skin, the AhR is extensively expressed in fibroblasts, keratinocytes, Langerhans cells, melanocytes, sebocytes, mast cells, and lymphocytes [81]. It is known that some microbial tryptophan metabolites, especially indole-3-carbaldehyde (I3A), can stimulate the AhR on keratinocytes [82], Langerhans cells [83], melanocytes [84], and fibroblasts [85]. The effect of stimulation seems dose- and ligand-dependent. Different ligands trigger the interaction of the AhR with different transcriptional molecules, thereby inducing several biological effects [81].

Stimulation of the AhR in the skin can cause multidirectional reactions. In keratinocytes of atopic dermatitis patients, AhR activation was found to be related to the upregulation of filaggrin and loricrin, i.e., key proteins which build the skin barrier. Restoration of the skin barrier can be observed as a decrease in TEWL [80,86]. Moreover, activation of the AhR by tryptophan metabolites can improve wound healing and decrease scar formation by upregulating metalloproteinases and suppressing type I collagen and fibronectin expression, directly on dermal fibroblasts [87]. The stress-alleviating effect of the AhR pathway was also found in response to UV [88]. Indole pyruvate (IPyr), one of the bacterial metabolites of tryptophan, was found to exert a protective effect on keratinocytes exposed to UVB, in which the amounts of secreted IL-1b and IL-6 were similar to those of unirradiated cells [89]. Additionally, in a mouse model, an increase in TEWL and expression of IL-1b after irradiation was less marked in the group which had received IPyr topically [89]. Furthermore, indole-3-propionic acid (IPA), also known as the microbiota metabolite, can reduce oxidative stress in response to exogenous stressors [90]. It was found that the indole derivatives, indole lactic acid (ILA) and indole-3-carbaldehyde (I3A), might be involved in the induction of tolerance to allergens, especially food allergens. Due to the stimulation of the AhR, these compounds were able to reprogram intestinal $CD4^+$ cells to known tolerogenic double-positive $CD4^+CD8\alpha\alpha^+$ cells [91].

Interestingly, the therapeutic effect of coal tar is related to the stimulation of AhR, which induces epidermal differentiation, neutralizes reactive oxygen species, restores filaggrin expression, and improves skin barrier proteins [92]. The importance of the AhR pathway in dermatology was emphasized by the discovery of the novel drug tapinarof, which acts as an AhR agonist [93]. Tapinarof has passed phase 3 of a clinical trial in psoriasis, and it has gained FDA approval for plaque psoriasis treatment [94,95]. Tapinarof is also undergoing several phase 3 clinical trials in adults and children with atopic dermatitis [95,96]. The promising effect of activation of the AhR suggests that tryptophan metabolites could exert similar therapeutic effects and could also be a useful tool in the management of dermatological diseases.

3.1. Atopic Dermatitis and Hypersensitivity Reactions

In the context of atopic diseases, there are a growing number of studies describing the function of tryptophan microbiota metabolites. Fang et al. revealed that administration of *Bifidobacterium longum* probiotics in atopic dermatitis patients can significantly decrease clinical symptoms measured in SCORAD and DLQI scales compared to a placebo. In the follow-up, the study group was characterized by lower IgE levels. Moreover, the responders were characterized by a significant increase in the serum levels of bacterial tryptophan metabolites, indole lactic acid (ILA), and indole-3-carbaldehyde (I3A) after the intervention. Additionally, fecal I3A was higher. It needs to be emphasized that a significant negative correlation was found between I3A level disease severity as measured with SCORAD and DLQI [97]. The following parts of the experiment confirmed the results in the mouse model and revealed that oral administration of I3A attenuated the activity of disease measured in the reduction in skin thickness, IgE levels, and concentrations of interleukins associated with the Th2 axis (TSLP, IL-4, IL-5) [97]. Another study, conducted by Yu et al., has reported corresponding results. Firstly, skin concentrations of I3A were significantly lower in patients with atopic dermatitis both in lesioned and non-lesioned skin [82]. The following part of the study confirmed the mentioned results in the mouse model. The topical and oral administration of I3A was effective in alleviating symptoms, e.g., skin thickness or itch, and induced a marked reduction in IL-4, IL-5, IL-6, IL-13, IL-22, and TSLP expression in skin cells. A decrease in TSLP expression after I3A treatment was also observed directly in keratinocytes. Interestingly, the effects of I3A administration included inhibition of inflammatory cell infiltration [82]. The decrease in TSLP expression found in both studies seems to be crucial in improving symptoms of atopic dermatitis. This cytokine is known as an orchestrating factor of the Th2 response and is responsible for the induction of atopy-related cytokines including IL-4, IL-5, IL-6, and IL-13 [98]. Another tryptophan

metabolite, formylindolocarbazole (FICZ), can be synthesized from bacterial-derived indole-3-acetaldehyde (I3A) or indole-3-pyruvate (I3Pyr), especially under UV light [88,99]. FICZ can stimulate filaggrin expression and its abundance in human keratinocytes [86,100] and keratinocytes of a mite-induced AD-like NC/Nga murine model [100]. The mentioned model is an established model of AD [101,102]. Additionally, topical FICZ treatment reduced transepidermal water loss (TEWL) and significantly improved dermatitis [100]. IL-4-induced filaggrin downregulation in mRNA levels was reversed [100]. Skin epithelial barrier damage, manifested by increased TEWL and a decreased amount of filaggrin, is associated with AD pathogenesis. Allergens passing through damaged skin can stimulate immune cells and, in consequence, further damage the epithelial barrier, constituting a vicious circle of exposure [32].

Additionally, hypersensitivity reactions are associated with specific Trp metabolite alteration. Allergic subjects have decreased levels of indole-3-butyric and indole-3-lactic acids [103]. Additionally, new reports show that direct administration of some Trp metabolites can be useful in the management of delayed-type hypersensitivity [104]. Counterintuitively, FICZ in this study was related to the exacerbation of delayed-type hypersensitivity in a mouse model [104]. However, the evidence is not robust, and additional studies should be carried out to clarify the bacteria-derived indoles in allergy and their potential use in dermatology.

3.2. Lupus

By far, most reports of tryptophan microbiota metabolites have come from work on SLE. In SLE patients, an increased kynurenine-to-tryptophan ratio was observed [105–107], which exhibited a strong positive correlation with neopterin, a marker of immune activation [106]. Furthermore, the higher kynurenine-to-tryptophan ratio was associated with a worse working memory and poor visuospatial processing test results, severe fatigue, active disease, decreased complement, and anemia [105–107]. SLE was also linked with decreased indole-3-propionic acid [108]. The mentioned results suggest accelerated Trp metabolism in the kynurenine pathway at the expense of the indole pathway, which may originate from intestinal microbiota disturbances [109]. The mouse studies show similar shifts in Trp metabolism [109,110]. Correspondingly to humans, kynurenine seems to favor pro-inflammatory T-cell phenotypes in the lupus-prone mouse model [109]. Moreover, it was observed that a diet low in tryptophan was associated with slowing down the progression of the disease, which prevented the development of anti-dsDNA IgG, injury of the kidneys, increased Treg cells activity, and decreased mTOR kinase activation [109,110]. This suggests that interventions based on decreasing the dietary intake of tryptophan may be useful in the management of the disease.

4. Trimethylamine (TMA) and Trimethylamine N-oxide (TMAO)

Various compounds of this group are products of intestinal microflora degradation of ingested small molecules with a quaternary amine group, e.g., choline, L-carnitine, or phosphatidylcholine, which are abundant in eggs, liver, dairy products, and peanuts [15]. Trimethylamine N-oxide (TMAO) is the most recognizable and studied of all monoamines. TMAO is produced by the bacterial metabolite trimethylamine (TMA), which is oxidized by the liver. Mouse studies have reported that the intestinal microbiota is essential to TMA formation, and the production of TMA and TMAO can be almost completely suppressed by broad-spectrum antibiotics [111,112]. There is ample evidence that some genera of intestinal bacteria are associated with higher TMAO concentrations in plasma. For example, bacterial genera such as *Clostridia*, *Proteus*, *Shigella*, and *Aerobacter* are involved in the production of TMA, especially the strains *Anaerococcus hydrogenalis*, *Escherichia fergusonii*, *Clostridium hathewayi*, *Clostridium asparagiforme*, *Edwardsiella tarda*, *Proteus penneri*, *Clostridium sporogenes*, and *Providencia rettgeri*. Microbiota rich in the *Prevotella* genus, which is related to dysbiosis caused by a high-fat diet, is associated with higher plasma TMAO levels. What is more, the

link between microbiota characterized by a higher level of the Bacteroides genus and lower levels of this metabolite in plasma has been proved [15,113].

Compared to previous substances, specific receptors for TMAO are still poorly known. TMAO is reported to bind to Protein Kinase RNA-Like ER Kinase (PERK) at physiological concentrations and to induce the FoxO1 transcription factor, which is the key factor in metabolic syndrome [18]. The harmful effect of this compound on the human organism is attributed to the acceleration of atherogenesis due to endothelial dysfunction, induction of oxidative stress, insulin resistance, and impairment of lipid metabolism [114]. There have been multiple studies describing its pro-inflammatory effect and a high TMAO level linked to persistent low-grade inflammation. The subjects characterized by high plasma TMAO levels have an elevated concentration of TNF, sTNF-R p75, and sTNF-R p55 [115]. Moreover, higher levels of TMAO in plasma are associated with overexpression of pro-inflammatory cytokines such as TNF, IL-6, and C-reactive protein, and decreased expression of anti-inflammatory cytokine IL-10 [15,116–119]. By induction of NLRP3 inflammasome activation, TMAO contributes to an increase in the levels of IL-1β and IL-18, which are crucial cytokines in various dermatological and rheumatological conditions [118,120]. Inhibition of the mentioned inflammasome supposedly decreases the detrimental effect of TMAO [121]. Because of NLRP3 inflammasome activation, lysosomal dysfunction and redox dysregulation of the cell occur, which results in the production of reactive oxygen species (ROS). Reactive oxygen species can damage the endothelium and disrupt the microenvironment of various organs, e.g., the intestine or the skin. Simultaneously, they can accelerate cellular senescence [120,122]. Additionally, it has been observed that TMAO can activate macrophages and monocytes, especially in the NLRP3 inflammasome pathway [118,121]. It is known that TMAO may enhance macrophage infiltration, and it may cause M1 polarization as well as Th1 and Th17 differentiation [121]. A higher TMAO concentration is associated with insulin resistance [123,124]. Furthermore, it is a factor of increased cardiovascular risk and accelerating atherosclerosis, and is also associated with a worse prognosis of the mentioned conditions [125,126].

Dermatological Context

In dermatological conditions, the impact of TMAO still remains elusive. However, more and more studies are being published on this subject, and the results are encouraging. Sikora et al. found that patients suffering from psoriasis have significantly greater TMAO concentrations in plasma compared to control subjects. Moreover, increasing TMAO concentrations were associated with a greater abundance of conditions characteristic of metabolic syndrome such as hyperlipidemia, hypertension, obesity, non-alcoholic fatty liver disease, and a higher cardiovascular risk as measured with various scales, e.g., SCORE, Framingham Risk Score, or AHA/ACC. Furthermore, TMAO was an independent predictor of increased cardiovascular risk in patients with psoriasis, even after adjustment for classical risk factors [127]. Consistent results obtained in the study by Sun et al. confirm elevated TMAO levels in patients with psoriasis. Additionally, a significant correlation between TMAO and PASI scores and elevated concentrations of the TMAO precursor betaine in the psoriatic group have been found [128]. One of the most common complications of psoriasis is psoriatic arthritis (PsA). So far, there has been one study describing the role of circulating TMAO in PsA. According to the authors, there are significant positive correlations between TMAO levels and the severity of the disease, as measured by both skin and joint activity scores. Additionally, a positive correlation between TMAO levels and CRP levels has been found, which supports the proinflammatory role of TMAO [129].

TMAO concentration was increased in HS patients and correlated with disease severity ($r = 0.57$). This association was still evident after adjusting for common confounding covariates [130]. Both psoriasis and hidradenitis suppurativa are considered to increase cardiovascular risk and have similar comorbidities, including diabetes, hypertension, dyslipidemia, and inflammatory bowel diseases [131,132]. All the facts mentioned above

suggest that an elevated TMAO level may not only affect the course of inflammatory skin diseases but can also modify their comorbidities.

TMAO impacts the pathogenesis of autoimmune- and immune-mediated diseases. Elevated TMAO levels affect patients with systemic lupus erythematosus (SLE), one of the most recognizable autoimmune diseases [133]. In a recent study, González-Correa et al. found that increased TMA and TMAO levels (5- and 8-fold, respectively) similarly occurred in a mouse model of SLE compared to the control group. Furthermore, they also discovered that a reduction in TMAO levels induced by the use of the trimethylamine lyase inhibitor 3,3-dimethyl-1-butanol (DMB), which inhibits bacterial synthesis of TMA, is related to a marked reduction in both TMA and TMAO and a less severe course of the disease in the animal model [134]. Decreased TMAO levels induced by DMB are associated with a reduction in proteinuria and inhibition of the development of hypertension induced by IMQ. It is also connected with the partial prevention of the increased plasma anti-dsDNA autoantibodies and IFNα mRNA levels, and normalization of T-cell imbalance. Additionally, strong positive correlations have been found between TMAO and systolic blood pressure and anti-dsDNA in plasma. DMB treatment has restored nrf2 levels and downstream antioxidant enzymes, suggesting *nfr2* downregulation is distinctive for higher TMAO levels and can contribute to oxidative stress in this fashion [134]. Despite these interesting results, the exact mechanism remains elusive.

To date, TMAO levels in systemic sclerosis (SSc) have not been studied yet. It is only known that elevated levels of the TMAO precursor, betaine, have been observed in this disorder [135]. However, recently, some engaging results have appeared from pre-clinical studies on the impact of TMAO on fibrosis in systemic sclerosis and skin physiology. TMAO can induce myofibroblast differentiation [136]. In isolated human fibroblasts, TMAO substantially increased cellular F-actin fibers and the expression of the genes responsible for the production of procollagen I and fibronectin [136]. Furthermore, skin fibroblasts exposed to TMAO were characterized by an increase in the secretion of TGF-β1, fibronectin, and procollagen I [136]. Additionally, the skin of SSc patients was characterized by 1.69- to 4.29-fold-higher FMO3 expression compared to control skin [137–139], which suggests that cell autonomous upregulation of FMO3 in SSc fibroblasts might contribute to their elevated collagen production underlying fibrosis.

In graft-versus-host disease (GVHD), in which pathogenesis resembles an autoimmune disease, TMAO can aggravate severity and mortality. This effect is caused by additional stimulation of autoreactive allogenic T cells by TMAO and can be seen in greater cytokine expression by these cells, especially IFN-γ, IL-17, and transcription factors STAT4 and STAT3 [121].

In spite of the promising results of the mentioned studies, more data are necessary to understand the role of bacterial metabolite TMAO and take clinical advantage of this knowledge.

The most important results of studies on metabolites in dermatological diseases are summarized in Table 1 at the end of the review.

5. Future Perspectives

The field of intestinal microbiota metabolites is rapidly developing. A comprehensive description of available data on lesser-known metabolites is beyond the scope of this review. However, it is worth mentioning bile salts, the group of secondary bile acids modified in the gut by microbiota. These compounds are recognized as anti-inflammatory factors, and their positive effects can potentially decrease the severity of psoriasis and atopic dermatitis [140–143].

The mentioned studies emphasize the role of the microbiome and its metabolites in the pathogenesis of various conditions. Consequently, modifying microbiota seems to be a promising therapeutical option (Figure 2).

Treatment alternatives may include direct modifications of microbiota, indirect modifications, or only supplementation of beneficial metabolites (Figure 2). Direct modifications

include the administration of probiotics or fecal microbiota transplantation (FMT). Indirect modification can be focused on the administration of dietary elements, so-called prebiotics, which can be metabolized by bacteria for nutritional purposes. Prebiotics can favor some beneficial bacterial strains and be precursors of health-promoting bacterial metabolites. One novel approach involves the direct use of beneficial bacterial metabolites, especially SCFAs. Health supplements composed of beneficial metabolites are termed postbiotics. The mentioned methods in the context of dermatological conditions are briefly discussed below.

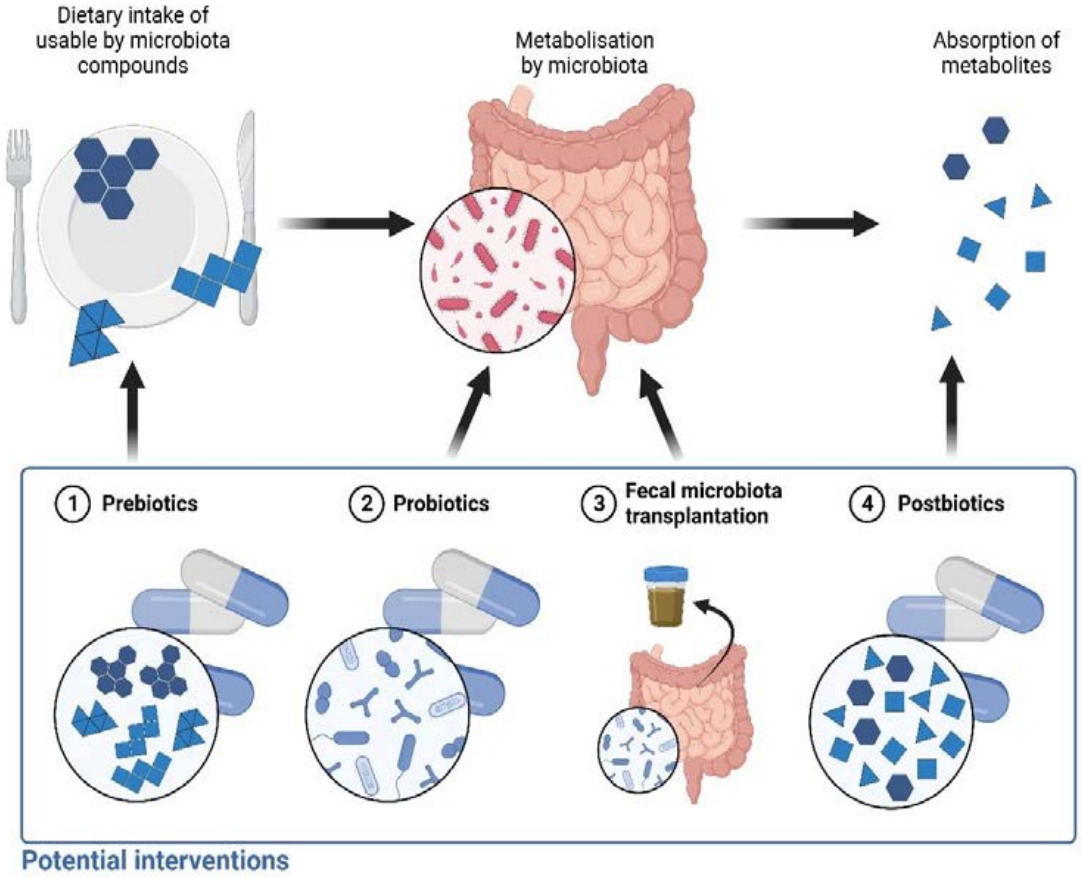

Figure 2. Production of microbiota metabolites and potential treatment options at various steps of this process. Ingested food is rich in compounds that can be utilized by intestinal microbiota. End products of microbiota metabolism are absorbed into the circulation. 1. Prebiotics, e.g., fructooligosaccharides and inulins, can be supplemented to achieve growth of beneficial microorganisms and an increase in production of eligible metabolites. 2. Probiotics. 3. Fecal microbiota transplantation is carried out with the aim to replace pathogenic bacteria with health-promoting ones. 4. Postbiotics consist of favorable metabolites of intestinal microbiota. Created with BioRender.com.

5.1. Prebiotics

Prebiotics are dietary ingredients that support the growth or activity of beneficial bacteria. This group consists of various dietary-derived substances such as galactooligosaccharides, fructooligosaccharides, fiber, beta-glucans, pectins, gums, resistant starch, etc. [144]. Supplementation of such substances can potentially be useful in the management of dysbiosis [145]. In addition, human milk oligosaccharides support infants' development of a balanced gut microbiota composition; this is interpreted by some as a risk factor for allergies [146]. The beneficial impact of prebiotics can be a result of an increase in the production of health-promoting bacterial metabolites, i.e., SCFAs [147].

Benefits from the supplementation of prebiotics are most marked in atopic dermatitis. Prebiotic treatment significantly decreased the incidence of AD in patients, according to

a meta-analysis of 22 clinical trials using prebiotics in newborns to avoid allergies [148]. Although some trials have indicated encouraging outcomes, there is little information available about the effectiveness of prebiotics in the treatment of AD. However, two modestly sized randomized controlled trials reported an improvement in symptoms of the disease [149,150].

In the context of allergy, there is some evidence of the beneficial role of prebiotics in the prevention of such diseases [151]. In line with this, the World Allergy Organization advises prebiotic supplementation in newborns who are not exclusively breastfed, emphasizing that the quality of the evidence is poor [152].

For autoimmune diseases, the evidence is even more scarce. However, studies in psoriasis and systemic lupus erythematosus found that supplementation of synbiotics containing fructooligosaccharides can alleviate disease symptoms and improve inflammatory status [153,154].

Despite the limited data, prebiotics have an excellent safety profile with no notable adverse effects reported in the revised literature.

5.2. Probiotics

Probiotics are living microorganisms that, when given in sufficient quantities, have a positive impact on the host organism [155]. The effects of probiotics seem to be strain- and dose-dependent, and with a multitude of bacterial genera, these aspects make research particularly hard to interpret [156]. Despite this knowledge, recommendations published by the most recognized scientific societies often consider the effect of probiotics as a group, neglecting their wide heterogeneity. However, recent studies report that this group potentially can be applied in many fields of dermatology.

Several large-cohort randomized controlled trials have investigated the value of oral probiotics for the treatment and prevention of AD. A recent meta-analysis of eleven randomized controlled trials including 2572 infants found that intake of *Lactobacillus rhamnosus* during pregnancy and thereafter significantly lowered the risk of developing AD when assessed at 2 years out and 6–7 years out (RR 0.60 and RR 0.62, respectively) [157]. What is more, the results from other meta-analyses support the use of oral probiotics for the treatment of AD both in children and adults [158–160]. The mixture of strains *Bifidobacterium animalis* subsp *lactis* CECT 8145, *Bifidobacterium longum* CECT 7347, and *Lactobacillus casei* CECT 9104 was associated with the best results in the pediatric population, and the mixture of *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 has the best results in adults [158–160]. Similar to AD, some probiotics may be effective in the prevention and treatment of allergies [161,162].

The beneficial effects of probiotics in autoimmune- and immune-mediated diseases appear to be connected to a decline in pro-inflammatory indicators, such as the production of IL-6 and CRP or the activation of Th1 and Th17 cells [163]. Additionally, recent studies imply that an anti-inflammatory effect of probiotics may result from their ability to improve gut permeability [164]. In contrast to findings in allergy and atopic dermatitis, the impact of this group on the prevention of autoimmunity is still poorly understood, despite the recent research suggesting that probiotics may also be helpful in this indication. Administration of some bacterial strains has been associated with protection against induced encephalitis in mouse models [165]. In the context of the treatment, available human research indicates that certain bacterial strains may be helpful in several dermatological disorders, such as psoriasis, SLE, and SSc.

Several randomized controlled studies have investigated the effectiveness of oral probiotics for the treatment of psoriasis. In one of them, administration of a multi-strain mixture including *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, and *Bifidobacterium longum* with 1.8×10^9 colony-forming units (CFUs) led to a significant decrease in PASI, Psoriasis Symptom Scale, and DLQI scores compared to the placebo group (MD −5.55, −4.84 and −9.87, respectively) [166]. Additionally, the intervention was linked to a notable reduction in inflammatory markers, including hs-CRP and IL-6, as well

as an increase in total antioxidant capacity [166]. The use of *Streptococcus salivarius* K-12 was also associated with a significant reduction in psoriasis symptoms [167]. The PASI 75, PASI 90, and PASI 100 response was achieved in 84%, 75%, and 55% of patients in the study group, respectively, compared to 42.8%, 30%, and 12.4% in the control group [167].

Apart from the improvement in PASI, a multi-strain preparation contributed to a decrease in inflammatory markers and an enhanced gut barrier, which was measured as lipopolysaccharide (LPS) translocation [168].

Probiotics have also been shown to have a beneficial impact on SLE in a recent randomized, double-blind, placebo-controlled trial [154]. The intervention included the administration of a preparation consisting of fructooligosaccharides and a mixture of *Lactobacillus* and *Bifidobacterium* strains. In the study group, after 60 days of supplementation of the preparation, significantly lower hs-CRP levels and a decrease in the IL-6 and SLE disease activity index 2K was noticed [154]. Interestingly, significant increases in butanoate or butyrate metabolism were seen in the post-synbiotic group and pre-synbiotic group compared to the pre-intervention-administered preparation group and the placebo group, according to functional prediction based on gut microbiota profiling [154].

Probiotics are not only beneficial in decreasing the severity of a disease, but they can also alleviate gastrointestinal symptoms. Despite having no effect on disease activity, probiotics were linked to an improvement in reflux symptoms in SSc patients [169].

5.3. Fecal Microbiota Transplantation

FMT is the most direct way of restoring the balance of the gut microbiota. Most data in dermatology come from FMT in atopic dermatitis. AD therapy with FMT has shown promising results. Zhou et al. reported a case of complete remission of AD in a 31-year-old female patient with concomitant functional constipation after FMT. It has also been noticed that the clinical improvement was accompanied by changes in the gut microbiota, especially a decrease in the abundance of bacteria involved in metabolic disorders and those that promote inflammation [170]. Due to the case report character of this study, the conclusions on the efficacy of FMT for treating AD are limited. A cross-over pilot study that included nine participants with moderate or severe AD showed a marked reduction in symptoms as measured by SCORAD score. The average SCORAD score decreased by approximately 85%. A 50% and 75% decrease in the SCORAD score was obtained by seven and six patients, respectively [171]. Furthermore, during the follow-up period, the frequency of weekly topical corticosteroid use decreased by 90% [171]. It was also observed that the higher the similarity between the microbiome of donors and the patients after FMT, the better the clinical improvement [171]. A study carried out by Zou et al. showed the long-term safety of FMT in AD in children. In a group of six children with AD, they observed the peak SCORAD score decrease after 3 months after FMT, followed by a gradual increase after the 6th month [172]. Three clinical remissions (defined as SCORAD ≤ 5 or a decrease of more than 30 points) and two clinical improvements (defined as a decrease in the SCORAD index of more than 10 points) were observed then. What is notable about the mentioned studies is the fact that no adverse events were noted and FMT was considered a safe procedure.

Promising results for the use of FMT in SLE come from a study by Huang et al. FMT was performed in 19 patients with SLE failing to respond to usual therapy for 8 weeks. After the intervention, there was observed a significant reduction in the mean SLEDAI-2K score from 9.45 ± 3.97 to 6.61 ± 4.43 at baseline. At the primary endpoint of week 12 after FMT, SRI-4 response was reached by 42.12% of patients, and a significant decrease in the level of serum anti-dsDNA antibodies was observed [173]. Furthermore, the microbiota of the patients increased in diversity, especially in SCFA-producing genera, and fecal SCFAs concentrations rose [173]. These results suggest that an induced increase in exposure to SCFAs can be at least partially related to clinical improvement.

In contrast, in research on the impact of FMT on SSc, no significant difference in SCFA concentrations after FMT was noticed [174]. However, FMT was found to relieve gastrointestinal symptoms, which are common complications of SSc [174].

Despite primary success in the case of a patient successfully treated with FMT [175], less encouraging results have been provided by Kragsnaes et al. in their study on FMT in psoriatic arthritis [176]. The study group consisted of 15 and 16 volunteers in FMT and placebo groups, respectively. FMT was found to slightly increase treatment failure, defined as a worsening of symptoms that required enhancement of the treatment [176].

Data on other immune-mediated skin conditions are limited. For psoriasis, there is a case report of a 36-year-old man who experienced an improvement in received the body surface area (BSA), PASI, dermatology life quality index (DLQI), intestinal symptoms, and serum level of TNF after FMT [177].

What is notable about the studies described above is that the FMT procedure was considered safe and the noticed adverse events were mild and transient, i.e., diarrhea, bloating, and abdominal pain 170–173,176]. Two major adverse effects in the form of duodenal perforation and laryngeal spasm were observed only in the SSc study [174]. Of note, adverse events were significantly method-dependent and endoscopic microbiota transplantation was associated with greater complications than transplantation with the use of capsulated microbial concentration [170–173,176].

It is worth noticing that there is still little information available on donor matching, which appears to be the most important factor in the context of the therapeutic success of FMT. The donor is chosen intuitively by the exclusion of potentially contagious infections rather than based on specific tests, which could potentially indicate the most adequate combination of donor and recipient. Additionally, there are currently no relevant standards for screening donor microbiota. The more we know about factors influencing the response to FMT, the better clinical results can be achieved.

5.4. Postbiotics

It is still barely known how the administration of bacterial metabolites affects human health. However, there is a whole range of supplements available on the market, especially those based on sodium butyrate. Even now, there are no data on the use of such supplements in dermatology. Available human studies prove that oral administration of SCFAs is safe and can be beneficial for health. In metabolic syndrome, after 4 weeks of oral administration of 4 g of butyrate, a dampened response of macrophages to oxLDL, which manifested as decreased TNF and IL-6 production, was observed [178].

Encouraging results come from randomized, placebo-controlled studies on type 2 diabetes, which is a common complication of certain dermatological diseases, e.g., psoriasis and psoriatic arthritis [179,180]. Butyrate taken in 600 mg daily doses significantly decreased oxidative stress by increasing the total antioxidant capacity (TAC) and activity of superoxide dismutase (SOD) of the blood [181]. Furthermore, butyrate exerted an anti-inflammatory effect by reducing the amount of hs-CRP, TNF expression, fasting blood glucose, and inflammasome-associated proteins, which suggests that butyrate can suppress low-grade inflammation associated with diabetes [182,183]. Similarly to butyrate, propionate supplementation was found to decrease fasting glucose and improve insulin sensitivity and the lipid profile in obese adults [184–186]. Additionally, propionate administration was related to an increase in resting energy expenditure and lipid oxidation in fasted humans [187].

Interestingly, in the case of Trp metabolites, there are several human studies available, especially on the effects of supplementation of indole-3-carbinol (I3C). I3C is considered a dietary product rather than a bacterial metabolite. However, it is known that indole-3-carbinol can be converted into indole-3-aldehyde and vice versa [188,189]. It has been found that supplementation of I3C is an effective and valuable adjuvant treatment, especially in HPV-associated infections, i.e., recurrent respiratory papillomatosis and vulvar intraepithelial neoplasia [190,191]. The administration of I3C's precursor, 3,3-Diindolylmethane, has been found to induce regression of cervical intraepithelial neoplasia [192]. Although these results are promising, no human studies have been conducted on the effects of other indole metabolites.

Table 1. A review of studies available on the subject of the impact of intestinal microbiota metabolites on dermatological conditions.

References	Disease	Studied Model	Main Findings
Short-chain fatty acids (SCFAs)			
Schwarz et al. [61]	Psoriasis	imiquimod-induced psoriasis-like skin inflammation mouse model, skin biopsies from patients with psoriasis	Topical administration of sodium butyrate (SB) reduced symptoms of psoriasis-like skin inflammation Topical SB increased the number and activity of Treg cells, IL-10 transcription, and decreased IL-17 transcription in the skin of a mouse model SB increased the transcription of IL-10 and decreased the transcription of IL-6 and IL-17 in human skin biopsies
Krejner et al. [62]	Psoriasis	skin biopsies from patients with psoriasis	Ex vivo treatment with SB caused a reduction in IL-17 and IL-6, and an upregulation of IL-10 transcription in skin biopsies Skin with psoriasis has decreased expression of GPR109a and GPR43, SB upregulates these receptors
Rodríguez-Carrio et al. [67]	Systemic lupus erythematosus	21 SLE patients, 25 healthy individuals	Fecal acetate and propionate are higher in patients with SLE compared to controls Dysbiosis in SLE patients
Sanchez et al. [28]	Systemic lupus erythematosus	MRL/lpr and NZB/W F1 lupus-prone mice	Oral mixture of sodium butyrate and sodium propionate reduced local and systemic antibody responses Orally administered SCFAsT reduced lupus skin lesions and kidney pathology
He et al. [68]	Systemic lupus erythematosus	MRL/lpr lupus-prone mice	Reduction in microbial diversity in the SLE mouse model Oral administration of SB reduced renal histopathological changes and increased microbiota diversity
Patrone et al. [69]	Systemic sclerosis	18 SSc patients, 9 healthy subjects	Dysbiosis manifested as a decrease in butyrate-producing genera more prominent in patients with gastrointestinal involvement
Park et al. [71]	Systemic sclerosis	bleomycin-induced fibrosis mouse model of SSc, human dermal fibroblasts	SB administered orally or subcutaneously reduced bleomycin-induced dermal and lung fibrosis SB treatment inhibits TGF-β1-induced fibrotic responses in human dermal fibroblasts
Reddel et al. [35]	Atopic dermatitis	19 children with AD and 18 healthy individuals	AD was characterized by dysbiosis, especially manifested in the depletion of butyrate-producing bacteria
Nylund et al. [36]	Atopic dermatitis	28 infants with atopic dermatitis and 11 healthy infants	Less severe eczema was associated with increased butyrate-producing bacterial abundance and microbiome diversity
Song et al. [39]	Atopic dermatitis	90 patients with AD and 42 volunteers without AD	Decreased fecal level of butyrate and propionate in AD patients Some subspecies of *Faecalibacterium prausnitzii* are linked with AD
Lee et al. [38]	Atopic dermatitis	234 patients with mild to severe AD, 112 non-AD subjects	Diversity of the microbiota in moderate to severe AD was significantly lower than in non-AD Disordered gut microbiota development in AD was associated with dysregulated SCFA production
Roduit et al. [40]	Atopic dermatitis	301 one-year-old children	Children with the highest fecal levels of butyrate and propionate were less prone to atopic sensitization and were less likely to develop asthma between the ages of 3 and 6 Food allergies and allergic rhinitis were less common in children with the highest butyrate levels

Table 1. *Cont.*

References	Disease	Studied Model	Main Findings
Cheng et al. [42]	Atopic dermatitis	75 infants	Low fecal butyric acid was associated with an increased risk of developing atopic dermatitis, food sensitization, and wheezing up to 8 years old
Gio-Batta et al. [44]	Atopic dermatitis	65 infants	A lower level of valeric acid at 3 years of age was associated with a higher prevalence of atopic eczema at the age of 8 years
Gio-Batta et al. [45]	Atopic dermatitis	110 one-year-old children	Eczema at 13 years of age was inversely correlated with the amount of fecal valeric acid at 1 year of age
Folkerts et al. [52]	Allergy	Human mast cells	Propionate and butyrate inhibited IgE- and non-IgE-dependent human mast cell degranulation
Schwarz et al. [57]	Contact dermatitis	sensitized C57BL/6J mice	Sodium butyrate (SB) administered topically or subcutaneously inhibited both the elicitation phase and ongoing contact hypersensitivity response SB induced the anti-inflammatory response via an increase in the number of skin Treg cells and an increase in IL-10 transcription
Trompette et al. [33]	Atopic dermatitis	atopic dermatitis-like skin inflammation mouse model	Fermentable fiber-rich diet or orally administered sodium butyrate alleviate systemic allergen sensitization and disease severity Oral butyrate stimulates terminal differentiation of epidermal keratinocytes and promotes skin barrier function
		Tryptophan metabolites	
Tsuji et al. [86]	Atopic dermatitis	normal human epidermal keratinocytes	The activation of AHR by tryptophane metabolite significantly increased filaggrin expression FICZ reversed the IL-4-induced downregulation in transcription and protein levels of filaggrin
Aoki et al. [89]	UVB-induced skin damage	HR-1 mice, HaCaT keratinocytes	Topical application of indole-3-pyruvate reduced the severity of UVB-induced skin lesions, the augmentation of dermal thickness, and transepithelial water loss Suppression of the overproduction of IL-1b and IL-6 in response to UVB radiation in a mouse model Indole-3-pyruvate improved the survival rate and reduced the expression of IL-1b and IL-6 in UVB-exposed HaCaT keratinocytes
Fang et al. [97]	Atopic dermatitis	87 patients with atopic dermatitis, sensitized female C57BL/6 mice	*Bifidobacterium longum* probiotic treatment increased serum and fecal indole-3-carbaldehyde, significantly reduced AD symptoms Indole-3-carbaldehyde displayed a significant negative correlation with atopic dermatitis severity measured in both SCORAD and DLQI Oral administration of indole-3-carbaldehyde alleviated AD-like skin lesions in sensitized mice
Yu et al. [81]	Atopic dermatitis	19 patients with AD, 19 healthy volunteers, sensitized C57BL/6 and BALB/c mice	Decreased indole-3-aldehyde was observed in both lesional and non-lesional skin of AD patients Topical and orally administered indole-3-aldehyde attenuated MC903-induced AD-like dermatitis in mouse and decreased expression of IL-4, IL-5, IL-6, IL-13, and TSLP Topically administered indole-3-aldehyde reduced inflammatory cell infiltration in mice

Table 1. *Cont.*

References	Disease	Studied Model	Main Findings
Kiyomatsu-Oda et al. [100]	Atopic dermatitis	NC/Nga mice, HaCaT cells and normal human epidermal keratinocytes (NHEKs)	Tryptophan metabolite FICZ improved symptoms of AD-like dermatitis, decreased TEWL, restored filaggrin expression, reduced the number of infiltrated mast cells, and reduced expression of IL-22 and IFN-γ genes in a mouse model FICZ upregulated expression and abundance of filaggrin in HaCaT and NHEKs cells
Singh et al. [104]	Delayed-type hypersensitivity	Sensitized C57BL/6 mice	Topical administration of indole-3-carbinol and 3,3′-diindolylmethane alleviated symptoms, triggered induction of Tregs, and suppressed Th17 cells of delayed-type hypersensitivity in a mouse model FICZ exacerbated disease in a mouse model and suppressed Treg cells
Shinde et al. [108]	Systemic lupus erythematosus	48 patients with active SLE, 24 patients with SLE in remission, and 20 control subjects	Serum indole-3-propionic acid was significantly higher than in the control group
Trimethylamine N-oxide (TMAO)			
Sikora et al. [127]	Psoriasis	72 patients with psoriasis and 40 matched controls	In patients with psoriasis, serum TMAO was significantly higher than in the control group TMAO was found to be an independent predictor of cardiovascular risk
Sun et al. [128]	Psoriasis	180 patients with psoriasis, 60 healthy controls	Psoriatic patients had significantly higher serum levels of TMAO compared to controls TMAO had a positive correlation with PASI score
Coras et al. [129]	Psoriatic arthritis	38 patients with psoriatic arthritis	Serum TMAO demonstrated a significant correlation with indicators of disease activity for the skin and peripheral joints Patients had increased serum TMAO levels compared to controls
Barea et al. [130]	Hidradenitis suppurativa	35 patients with hidradenitis suppurativa and 35 matched controls	The level of circulating TMAO correlated positively with the HS Sartorius score also after adjustment for confounding factors Serum TMAO levels and PhA were the two primary indicators of the clinical severity of HS based on a linear regression model
Li et al. [133]	Systemic lupus erythematosus	17 patients with SLE and 17 healthy controls	Serum levels of trimethylamine N-oxide (TMAO) were found to be elevated in lupus patients compared to controls
González-Correa et al. [134]	Systemic lupus erythematosus	Imiquimod-induced mouse model of SLE	Plasma TMAO concentrations were significantly elevated in the serum of active systemic lupus erythematosus patients
Wu et al. [121]	Graft-versus-host disease	C57BL/6 and BALB/c mice	Induced by oral administration elevation of plasma TMAO was associated with worse course and survival of graft-versus-host disease

6. Conclusions

Taking into consideration that various microbiota metabolites have opposite functional properties, an approach based on multi-metabolite analysis and correlation with metagenomic analysis of the gut microbiota appears to be a particularly interesting direction for future research.

Intestinal microbiota metabolites have a significant impact on the pathogenic processes in dermatological diseases. SCFAs and indole-derived metabolites seem to act via their

immunomodulatory and anti-inflammatory properties. Amine derivatives, particularly TMAO, can accelerate the progression of diseases and contribute to the development of complications because of their pro-inflammatory activity. Thus, modifications of microbiota, which may alter the metabolite concentrations, are a promising therapeutic option for several inflammatory dermatological diseases, potentially exhibiting a very good safety profile.

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6.2. Stec A, Maciejewska M, Paralusz-Stec K, Michalska M, Giebułtowicz J, Rudnicka L, Sikora M.: The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis. Journal of Inflammation Research 2023;16:1895-1904. https://doi.org/10.2147/JIR.S409489

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ORIGINAL RESEARCH

The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis

Albert Stec[1], Magdalena Maciejewska[1], Karolina Paralusz-Stec[1], Milena Michalska[2], Joanna Giebułtowicz[3], Lidia Rudnicka[1], Mariusz Sikora[4]

[1]Department of Dermatology, Medical University of Warsaw, Warsaw, Poland; [2]Department of General, Vascular and Transplant Surgery, Medical University of Warsaw, Warsaw, Poland; [3]Department of Bioanalysis and Drugs Analysis, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland; [4]National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland

Correspondence: Mariusz Sikora, National Institute of Geriatrics, Rheumatology and Rehabilitation, Spartańska 1, Warsaw, 02-637, Poland, Tel +48 22 670 91 00, Fax +48 22 844 77 97, Email drmariuszsikora@gmail.com

Background: Systemic sclerosis (SSc) is a rare immune-mediated connective tissue disease characterized by fibrosis of the skin and internal organs, whose pathogenesis is not fully understood. Recent studies have revealed dysbiosis in patients with systemic sclerosis and have indicated the possible role of the microbiota and its metabolites in the pathogenesis of the disease. Trimethylamine N-oxide (TMAO) is a compound produced by dysbiotic microbiota observed at higher concentrations in several autoimmune diseases.

Objective: To determine concentrations of the bacteria-derived metabolite TMAO in patients with systemic sclerosis and to assess possible correlation between TMAO and a specific manifestation of the disease.

Patients and Methods: The study included 63 patients with SSc and 47 matched control subjects. The concentration of TMAO was measured with high-performance liquid chromatography.

Results: Plasma TMAO level was significantly increased in patients with SSc (283.0 [188.5–367.5] ng/mL versus 205.5 [101.0–318.0] ng/mL; $p < 0.01$). An increased concentration of TMAO was observed in patients with concomitant interstitial lung disease (ILD) (302.0 ng/mL [212.0–385.5] ng/mL versus 204.0 [135.5–292.0] ng/mL; $p < 0.01$) and esophageal dysmotility (289.75 [213.75–387.5] ng/mL versus 209.5 ng/mL [141.5–315.0] ng/mL; $p < 0.05$) compared to patients without these complications. Furthermore, TMAO concentration exhibited significant correlation with markers of heart involvement (left ventricle ejection fraction, NT-proBNP), marker of ILD severity and Scleroderma Clinical Trials Consortium Damage Index.

Conclusion: The concentration of TMAO, gut microbiota-associated metabolite, is increased in systemic sclerosis, particularly in patients with advanced organ involvement. This is the first study evaluating plasma TMAO in systemic sclerosis. Bacterial metabolites may be a link between dysbiosis and organ involvement in the course of the disease. Modulation of gut bacterial-derived metabolites may represent a new therapeutic approach in the management of systemic sclerosis.

Keywords: gut microbiota, systemic sclerosis, trimethylamine N-oxide, bacterial metabolites, dysbiosis, damage index

Introduction

Systemic sclerosis (SSc) is an immune-mediated connective tissue disease. It is characterized by fibrosis of the skin, internal organs (eg, lungs, gastrointestinal tract) and vascular abnormalities. Based on the extent of skin involvement, SSc is commonly classified into two main subsets: diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc).[1–3] To date, the pathology of systemic sclerosis remains not fully elucidated and there is a lack of established biomarkers of SSc severity and organ involvement.[4,5] Early intervention with immunomodulators in patients with SSc may lead to better outcome.[6,7]

Recently, there has been a growing number of studies presenting alteration in intestinal microbiota (dysbiosis) in patients with systemic sclerosis and its impact on the disease. Gut microbiota profiling in systemic sclerosis revealed significant changes in its biodiversity and composition compared to healthy controls. Patients with SSc are characterized

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by an increased abundance of *Fusobacterium, Desulfovibrio, Ruminococcus*, and *Lactobacillus*, and a decreased abundance of *Faecalibacterium*.[8–10] Furthermore, some of the SSc complications, such as interstitial lung disease (ILD) and esophageal dysmotility, are associated with more pronounced dysbiosis.[11,12] Currently, research on modifying microbiota in SSc is emerging. Some studies have shown that probiotics or fecal microbiota transplantation may improve SSc, particularly gastrointestinal symptoms of the disease.[13–16]

In systemic sclerosis, metabolic proficiencies of gut microbiota also differ from the control group;[9] thus, it is highly probable that patients are exposed to a different spectrum of bacterial substances. This exposure can be magnified by vascular abnormalities and intestinal involvement[17] which increase intestinal permeability.[18,19] The pathways by which microbiota can regulate human homeostasis have recently been intensively investigated. Among them, the impact of microbiota metabolites is the most direct and has been the best described. Some bacterial metabolites present in increased concentrations in the state of dysbiosis are considered detrimental to homeostasis. These metabolites include trimethylamine N-oxide (TMAO).[20,21] TMAO is known for its pro-inflammatory properties, which exacerbate several diseases, especially atherosclerosis, chronic kidney disease and type 2 diabetes.[22–24]

TMAO is a small molecule that is transformed by the liver from bacterial metabolite – trimethylamine (TMA).[22,25] The literature reports that the concentration of plasma TMAO is increased in conditions such as cardiovascular diseases,[26–28] and renal insufficiency,[29] also in rheumatic diseases such as psoriatic arthritis,[30] and rheumatoid arthritis.[31] Furthermore, a recent pre-clinical study has found that TMAO significantly increases the expression of extracellular matrix proteins associated with fibrosis and the major profibrotic cytokine TGF-β1, which are the key elements of SSc pathomechanism.[32]

The study aimed to determine concentrations of the bacteria-derived metabolite TMAO in plasma of SSc patients as well as assess potential correlation between plasma TMAO concentration and various clinical (age, disease subtype, concomitant lung, gastrointestinal, or cardiac involvement) and metabolic factors in SSc.

Materials and Methods

Subject

The study included 63 adult patients diagnosed with systemic sclerosis based on the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2013 classification criteria. Patients were recruited from the Department of Dermatology at the Medical University of Warsaw between February 2021 and January 2022.

Exclusion criteria included gastrointestinal infection during the last 3 months before the study, concomitant chronic gastrointestinal disorder (eg, Crohn's disease, celiac disease), any gastrointestinal surgical procedures or unexplained weight loss during the last 6 months prior to the study, dietary restrictions or intake of medications modulating gut microbiota (antibiotics, probiotics or prebiotics) within the previous 3 months, chronic liver and pancreatic disease, other autoimmune diseases, estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, pregnancy, and breastfeeding.

The control group consisted of 47 individuals who were matched for age, body mass index (BMI), and gender. Control group subjects fulfilled the same exclusion criteria.

Clinical Assessment

Detailed medical history was obtained from all participants. The extent of skin involvement was analyzed with a modified Rodnan skin score (mRSS). Microvascular abnormalities assessed in capilaroscopy were classified into early, active, or late scleroderma patterns according to Cutolo et al.[33] To evaluate the gastrointestinal manifestation of systemic sclerosis a barium swallow X-ray was performed. To determine lung involvement, high-resolution computed tomography (HRCT) and pulmonary function tests, including diffusing capacity for carbon monoxide (DLCO), were performed. Cardiac involvement was assessed in echocardiography and by measuring N-terminal pro-B-type natriuretic peptide (NT-proBNP). To quantify organ damage in the course of SSc, we used Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI).

Laboratory Assessment

Patients have the following tests performed: complete blood count, erythrocyte sedimentation rate glucose, lipid, kidney and liver profiles. Venous blood samples were taken for analysis following a 12-hour fast. Estimated glomerular filtration

rate (eGFR) was calculated using the CDK-EPI equation. Antinuclear antibodies were detected and identified with immunoblot assay.

Plasma TMAO concentration was measured using high-performance liquid chromatography (HPLC) coupled with triple-quadrupole mass spectrometry as previously described.[34,35]

Statistical Analysis

The statistical software STATISTICA 13.3 was used for all calculations (TIBCO, Palo Alto, CA, USA). The Shapiro–Wilk test was used to determine if the distribution of the data was normal. The mean with standard deviation were used to represent normally distributed data, while the median and interquartile range (IQR) were used to express non-normally distributed variables. A chi-squared test was used to compare categorical data that were presented as counts and percentages. For two independent samples, a Student's *t*-test after assessment of the equality of variances by Levene's test, or Mann–Whitney *U*-test was used to assess the continuous variables that were parametric and nonparametric, respectively. To evaluate potential linear correlations between two continuous variables, the Spearman rank test with correlation coefficient was applied. P-values <0.05 were considered to be statistically significant.

Ethics

The study was approved by the Regional Bioethical Committee at the Medical University of Warsaw (KB/136/2021). Written informed consent was obtained from all participants. The study has been carried out in accordance with the Declaration of Helsinki.

Results

Characteristics of the Study Group

The study included 63 SSc patients (57 women and 6 men) and 47 controls (42 women and 5 men) matched for age, sex and BMI. The mean age of patients and volunteers was 59.9 and 55.5 years, respectively. The SSc cohort consisted of 34 (54.0%) individuals with dcSSc and 29 (54.0%) individuals with lcSSc based on LeRoy's criteria.

Detailed characteristics of SSc patients including demographical, clinical, and serological features are presented in Table 1.

TMAO in Systemic Sclerosis

The median concentration of TMAO in the group of patients with systemic sclerosis was 283.0 ng/mL (IQR 188.5–367.5) and it was significantly higher compared to the control group (205.5 ng/mL, IQR 101.0–318.0, p = 0.006, Figure 1).

Furthermore, we found a significant moderate correlation between TMAO concentration and the age of patients (rho = 0.446; p = 0.0002; Figure 2).

When patients were categorized according to antinuclear antibodies, there were no significant differences between patients with positive anti-RNA polymerase III (298.5 ng/mL, IQR 230.5–368.0), antitopoisomerase I (262.5 ng/mL, IQR 169.0–340.0) and anticentromere antibodies (284.7 ng/mL, IQR 189.0–345.0).

TMAO and Skin Involvement

There was no significant difference between patients with lcSSc (280.7 ng/mL; IQR 183.5–330.7) and dcSSc (287.7 ng/mL; IQR 185.2–375.7). No significant correlation was found between TMAO concentration and skin involvement measured by mRSS.

TMAO level did not differ between SSc patients presenting early (284.7 ng/mL; IQR 206.2–632.0), active (253.5 ng/mL; IQR 178.0–315.0), and late (308.7 ng/mL; IQR 204.0–366.0) patterns in NVC.

Table 1 The Main Characteristics of SSc Patients and the Control Group

	SSc Patients (n = 63)	Control Group (n = 47)	p-value
Age, years	59.9±12.7	55.5±14.4	0.09
Sex Men, n (%) Women, n (%)	 6 (5.4%) 57 (51.8%)	 5 (4.5%) 42 (38.2%)	0.85
Body mass index, kg/m^2	24.1±3.4	24.8±3.2	0.11
Disease subset, n (%) Limited cutaneous SSc Diffuse cutaneous SSc	 29 (46%) 34 (54%)	 - -	 - -
Modified Rodnan skin score	5 [2–9]	-	-
Disease duration, years	8.5 [4–14]	-	-
Autoantibody positivity, n (%) Antinuclear autoantibodies (ANA) Anticentromere autoantibodies (ACA) Antitopoisomerase I autoantibodies (ATA) Anti-RNA polymerase III autoantibodies (ARA)	 62 (98.4%) 27 (42.9%) 29 (46.0%) 4 (6.3%)	 - - - -	 - - - -
Interstitial lung disease, n (%)	47 (74.6%)	-	-
Pulmonary hypertension, n (%)	2 (3.2%)		
Esophageal dysmotility, n (%)	37 (58.7%)	-	-
Current Immunosuppressive therapy, n (%) Methotrexate Mycophenolate mofetil	 13 (20.6%) 24 (28.0%)	 - -	 - -
Current vasoactive therapy, n (%) Alprostadil Calcium channel antagonist Phosphodiesterase 5 inhibitors Sulodexide	 42 (66.7%) 15 (23.8%) 26 (41.3%) 48 (76.1%)	 - - - -	 - - - -

TMAO and Interstitial Lung Disease

A comparison of TMAO concentration in the group of patients showed significantly higher TMAO levels in SSc patients with interstitial lung disease (Figure 3). The median concentration of TMAO in the subgroup with ILD was 302.0 ng/mL (IQR 212.0–385.5) compared to 204.0 ng/mL (IQR 135.5–292.0) in the subgroup without this complication ($p = 0.001$). Furthermore, DLCO, a marker of the restriction caused by ILD, showed a significant negative correlation with TMAO level (rho=−0.53; $p = 0.013$ (Figure 4).

TMAO and Gastrointestinal Manifestations of the Disease

In SSc patients with concomitant esophageal dysmotility (confirmed in barium swallow) TMAO concentration was significantly higher (289.75 ng/mL [IQR 213.75–387.5]) than in subgroup with normal esophageal passage (209.5 ng/mL [IQR 141.5–315.0] $p = 0.026$, Figure 5).

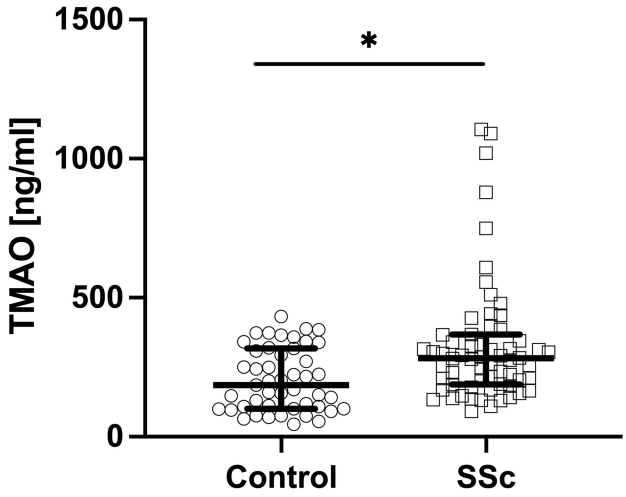

Figure 1 Plasma concentration of trimethylamine N-oxide (TMAO) in patients with systemic sclerosis compared to controls (* $p < 0.05$).

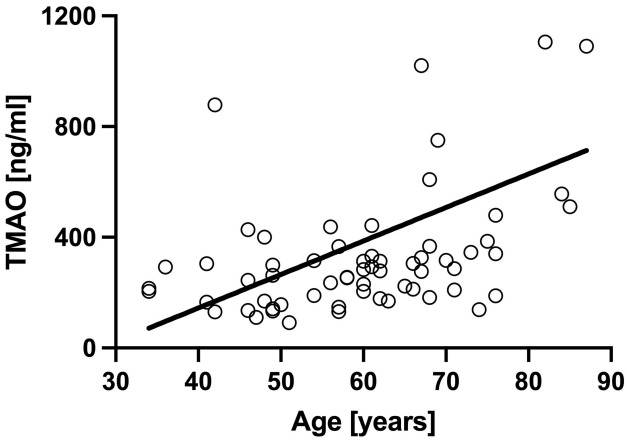

Figure 2 Correlation of TMAO with the age of patients with SSc (rho = 0.446; $p < 0.001$).

TMAO and Cardiac Involvement of SSc

Spearman correlation coefficient revealed a significant negative correlation between TMAO plasma concentration and left ventricle (LV) ejection fraction in echocardiography (rho=−0.39; p = 0.009, Figure 6), and a significant positive correlation between TMAO and NT-proBNP concentration (rho = 0.41; p = 0.0009, Figure 7).

TMAO and Organ Damage in SSc

There was a significant correlation (rho = 0.78; p = 0.0001) between TMAO concentration and the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI) as shown in Figure 8.

Discussion

This work provides evidence that intestinal microbiota metabolite TMAO is associated with some specific complications of systemic sclerosis. To the best of our knowledge, this is the first human study investigating microbiota metabolite TMAO in SSc. We found that this compound is present in a greater concentration in patients with SSc compared to the age-, sex-, and BMI-matched control subjects. The possible explanation for this could be that bacterial genera which are more abundant in the dysbiotic microbiota of SSc patients (ie, *Fusobacterium, Desulfovibrio, Ruminococcus*) have been observed to be linked with

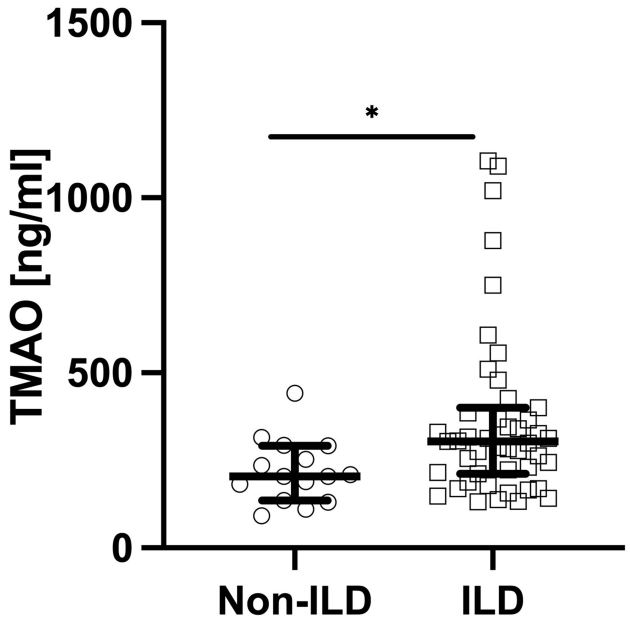

Figure 3 Plasma concentration of trimethylamine N-oxide (TMAO) in SSc patients with interstitial lung disease (ILD) and without this complication (* $p < 0.05$).

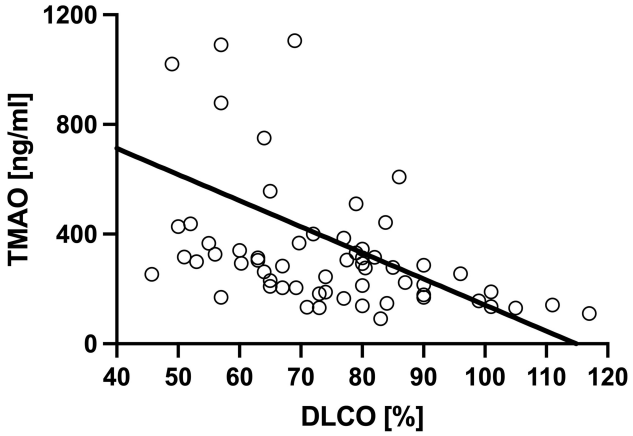

Figure 4 Correlation of trimethylamine N-oxide (TMAO) with diffusing capacity of lung for carbon monoxide (DLCO; rho = −0.53; $p < 0.05$).

greater plasma TMAO concentrations.[36] Moreover, dysbiosis with an abundance of these bacteria has been observed in conditions such as end-stage renal disease,[37] pulmonary arterial hypertension,[38] and cardiac failure.[39]

Additionally, SSc patients with concomitant interstitial lung disease and reflux disease were characterized by an increased level of TMAO compared to patients without mentioned comorbidities. Significant correlations between the TMAO level and markers of cardiac involvement were also revealed. The observed results are corresponding to the previous preclinical studies. Endothelial injury is an early feature of systemic sclerosis and plays a key role in pathogenesis.[40] Recent preclinical studies have shown that TMAO can potentially impact the pathogenesis of systemic sclerosis by inducing endothelial injury, promoting profibrotic cytokine signaling and collagen deposition, suppressing endothelium-dependent dilation of arteries, and inhibiting nitric oxide release in the epithelium.[32,41,42] It was observed that TMAO can induce the differentiation of fibroblasts into myofibroblasts and endothelial-mesenchymal transition.[32] The presence of myofibroblasts in involved organs is one of the histopathological hallmarks of systemic sclerosis.[33]

The available literature reveals that the incidence and increased severity of SSc-associated complications may be linked to an increased level of TMAO. An elevated level of this metabolite has been observed in pulmonary arterial hypertension

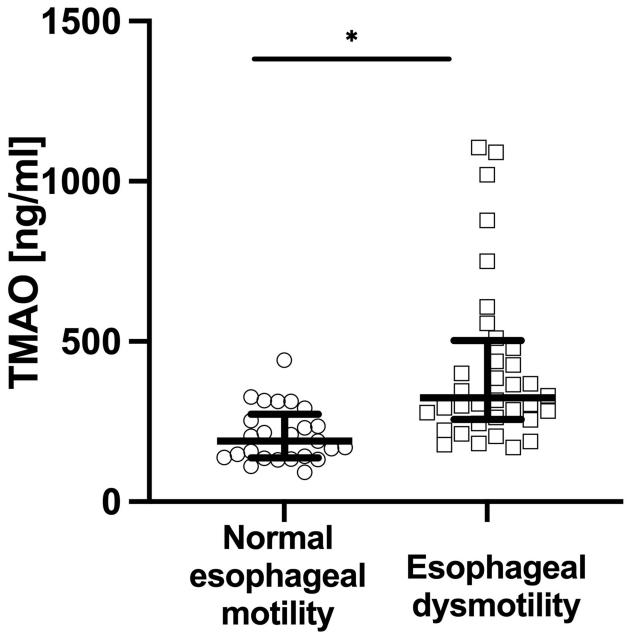

Figure 5 Plasma concentration of trimethylamine N-oxide (TMAO) in SSc patients subgroup with esophageal dysmotility and without this complication (* $p < 0.05$).

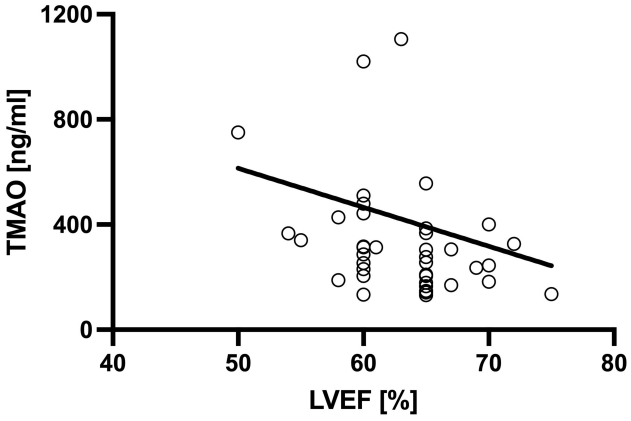

Figure 6 Correlation of trimethylamine N-oxide (TMAO) with left ventricular ejection fraction (LVEF; rho = −0.39; $p < 0.01$).

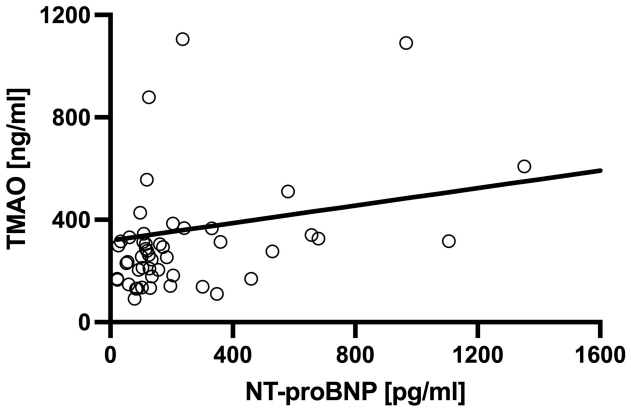

Figure 7 Correlation of trimethylamine N-oxide (TMAO) with N-terminal pro B-type natriuretic peptide (NT-proBNP; rho = 0.41; $p < 0.001$).

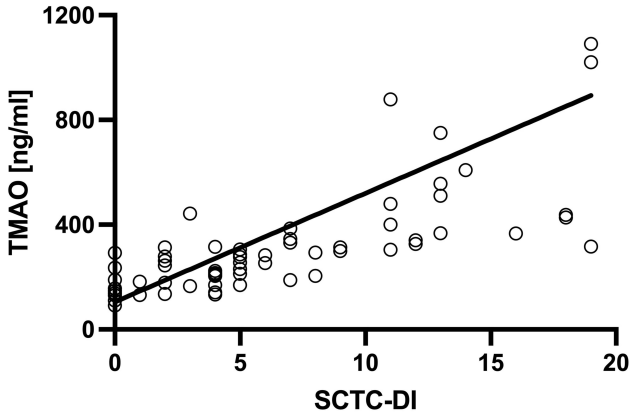

Figure 8 Correlation of trimethylamine N-oxide (TMAO) with the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI; rho = 0.78; $p < 0.001$).

(PAH)[43,44] which is a common comorbidity in systemic sclerosis and affects about 15% of patients.[1] Additionally, higher TMAO level was related to the worse course of PAH.[44,45] In heart involvement, microbiota metabolites could be also an important factor in prognosis. Literature reports that TMAO can aggravate heart fibrosis and therefore decrease ejection fraction and contractility of the myocardium.[46] More severe gastrointestinal symptoms, including esophageal reflux, were observed in patients with higher circulating TMAO.[47] Despite these encouraging findings, it is still unknown whether a rise in TMAO is a causative factor of the mentioned conditions or just an effect of the disease. To accurately evaluate the pathogenic effect of TMAO on the complications of systemic sclerosis more studies are needed, especially prospective and multi-centered, that would evaluate the long-term exposition to TMAO and its potential effects. However, several studies reported the advantages of an approach based on reducing the TMAO level. Lowering TMAO by 3,3-dimethyl-1-butanol (DMB), an inhibitor of the bacterial synthesis of TMA, improved hemodynamic parameters and pulmonary vascular remodeling in mouse and rat models of PAH.[44,45] In a mouse model of cardiac fibrosis lowering TMAO was associated with the decreased gene expression level of collagen I and TGF-β, and decreased fibrosis in a microscopic examination of cardiomyocytes.[48] According to the mentioned studies, patients with systemic sclerosis may benefit from adjuvant medication intended to reduce harmful bacteria metabolites and possibly experience reduced disease activity. A growing number of evidences suggest that TMAO concentration can be modulated by several bioactive compounds or dietary patterns. The low-fat diet, resveratrol, capsanthin and allicin decrease TMAO concentrations, whereas high-fat and high protein diet promote overproduction of TMAO. Diet can affect TMAO in several pathways, including the metabolism of its precursors, modulating intestinal permeability as well as shifting gut microbiota.[49,50]

Understanding the mechanisms of how dysbiotic microbiota and its metabolites may influence systemic sclerosis seems to be valuable in clinical practice. Our study suggests that TMAO may serve as a useful marker for identifying the severity of multi-organ involvement in SSc due to exhibiting a strong positive correlation with SCTC-DI. Therefore, measurement of TMAO may help to stratify patients into different disease severity categories, which could help to optimize the treatment of the disease.

Limitations of our study include small sample size, single-center type, and cross-sectional character. To assess the causative effects of prolonged exposure to increased plasma levels of bacterial metabolites on the development of complications of SSc prospective, multicenter studies are needed.

Conclusion

Our cross-sectional study showed that increased plasma TMAO level was associated with increased prevalence and severity of interstitial lung disease and heart involvement in systemic sclerosis. Also, a higher concentration of TMAO was observed in patients with concomitant gastroesophageal dysmotility. This is the first study evaluating plasma TMAO in systemic sclerosis. The mentioned findings indicate the possible usefulness of TMAO as a biomarker of incidence, and severity of complications of the disease. A more profound knowledge of the causal link between microbiota metabolites and SSc may point to pathophysiological pathways that may be addressed for targeted therapeutics to improve disease severity and complications.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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The Clinical Significance of Serum Biomarkers of the Intestinal Barrier in Systemic Sclerosis: A Cross-Sectional Study

Albert Stec [1,*], Magdalena Maciejewska [1], Michał Zaremba [1], Karolina Paralusz-Stec [1], Milena Michalska [2], Lidia Rudnicka [1] and Mariusz Sikora [3]

[1] Department of Dermatology, Medical University of Warsaw, Koszykowa 82A, 02-008 Warsaw, Poland
[2] Department of General, Vascular and Transplant Surgery, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland
[3] National Institute of Geriatrics, Rheumatology and Rehabilitation, Spartańska 1, 02-637 Warsaw, Poland; drmariuszsikora@gmail.com
* Correspondence: albertmstec@gmail.com

Abstract: Systemic sclerosis (SSc) is an immune-mediated connective tissue disease. Recent studies reported differences in the composition of intestinal microbiota (dysbiosis) in patients with SSc compared to nonsclerodermic subjects. Dysbiosis may disrupt the intestinal barrier, which leads to immunological activation via microbial antigen and metabolite translocation. The study aimed to assess the differences in intestinal permeability between SSc patients and controls and to examine the correlation between intestinal permeability and complications of SSc. The study comprised 50 patients with SSc and 30 matched subjects. Serum intestinal permeability markers: intestinal fatty acid binding protein, claudin-3, and lipopolysaccharides (LPS) were determined using an enzyme-linked immunosorbent assay. SSc patients had a significantly increased concentration of LPS compared to control subjects (232.30 [149.00–347.70] versus 161.00 [83.92–252.20] pg/mL, $p < 0.05$). The patients with shorter SSc duration ($\leq$6 years) had an increased concentration of LPS and claudin-3 compared to the subgroup with longer disease length: LPS (280.75 [167.30–403.40] versus 186.00 [98.12–275.90] pg/mL, $p < 0.05$), and claudin-3 (16.99 [12.41–39.59] versus 13.54 [10.29–15.47] ng/mL, $p < 0.05$). The patients with esophageal dysmotility had a decreased LPS level compared to those without this complication (188.05 [102.31–264.40] versus 283.95 [203.20–356.30] pg/mL, $p < 0.05$). Increased intestinal permeability in SSc may exacerbate the course of the disease and increase the risk of developing complications. Lower LPS levels in SSc might be a hallmark of esophageal dysmotility.

Keywords: systemic sclerosis; microbiota; gut–skin axis; dysbiosis; inflammation; immune-mediated inflammatory diseases; intestinal barrier; intestinal permeability

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1. Introduction

Systemic sclerosis (SSc) is an immune-mediated connective tissue disease with a chronic, progressive course that causes multiorgan failure and the patient's disability [1]. The disease is characterized by progressive fibrosis of skin and internal organs with concomitant impairment of microcirculation and persistent inflammation. The pathogenesis of systemic sclerosis is still poorly understood [1].

Accumulating evidence suggests that alterations in the composition of the gut microbiome, which is called dysbiosis, may play a role in the pathogenesis of systemic sclerosis [2–4] and other immune-mediated skin conditions such as psoriasis [5] and systemic lupus erythematosus [6]. Changes in the intestinal microbiota in the course of systemic sclerosis are characterized by an increased presence of bacteria of the genera *Fusobacterium*, *Desulfovibrio*, *Ruminococcus*, and *Lactobacillus* and a decreased presence of bacteria of the genus *Faecalibacterium* [2–4]. The concept of the gut-skin axis suggests a connection between changes in the intestinal microbiota and skin immunological responses [7]. However, the exact mechanism causing this crosstalk is unknown yet. One of the possible factors is the

disruption of the intestinal barrier, which causes increased intestinal permeability. It can result in the translocation of intestinal luminal content, e.g., allergens, bacterial endotoxins, metabolites, or even whole bacterial cell components, into the circulation, which further initiates or exacerbates systemic inflammation [8,9].

Techniques that have been used so far for determining gut barrier integrity, such as histological examination of intestinal biopsies or oligosaccharide absorption assays, are complicated, time-consuming, and difficult to apply in routine clinical practice [10]. Alternatives for the mentioned methods include novel biomarkers, which can be assessed from the blood in routine blood collection. The biomarkers are intestinal fatty acid binding protein (IFABP), claudin-3, and lipopolysaccharides (LPS). IFABP is a protein that exclusively presents in the cytoplasm of enterocytes in the small intestine. In normal conditions, passage of this marker into circulation is minimal, whereas damage to the intestinal epithelium causes a marked increase of IFABP levels in the blood [11]. In turn, claudin-3 is a component of tight junctions between the enterocytes, and their disruption can be observed as an increase in the blood level of this marker [12]. LPS is a marker of the translocation of bacteria through the intestinal epithelium [9,10]. An increased concentration of this element indicates an extensive bacterial translocation and, additionally, it is a potent proinflammatory factor that may exacerbate inflammatory responses [9,13]. When measured together, the mentioned markers can reliably provide information about the state of the intestinal barrier.

The study aimed to investigate potential differences in intestinal permeability between the patients with systemic sclerosis and the matched controls with the use of serum intestinal permeability markers. The impact of various clinical factors on the concentration of the mentioned markers was also assessed.

2. Materials and Methods

2.1. Study Participants

The study comprised 50 adult patients diagnosed with systemic sclerosis who fulfilled the classification criteria of the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) 2013. Those patients were recruited from the Department of Dermatology at the Medical University of Warsaw between January 2022 and June 2022.

To avoid potential bias the following exclusion criteria were used: acute or chronic gastrointestinal infection within the three months before the study, concomitant inflammatory bowel disease, any gastrointestinal surgery or unexplained weight loss within the six months before, intake of nonsteroidal anti-inflammatory drugs within the previous week, dietary restrictions, intake of antibiotics, probiotics or synbiotics within the previous 3 months, a history of malignancy, drug or alcohol abuse, chronic liver and pancreatic disease, estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, pregnancy, and breastfeeding.

The control group consisted of 30 individuals who were matched for age, gender, and body mass index (BMI). Subjects in the control group met the same exclusion criteria.

2.2. Clinical Assessment

Detailed medical history was obtained from all of the participants. The severity of the disease was assessed with Valentini Disease Activity Score. The stage of skin involvement was analyzed with the modified Rodnan skin score (mRSS). To evaluate the gastrointestinal manifestation of systemic sclerosis a normal barium swallow was performed. In addition, high-resolution computed tomography (HRCT) and body plethysmography were performed to determine lung involvement. Besides, the cardiac manifestation of systemic sclerosis was assessed in echocardiography as well as by measuring N-terminal pro b-type natriuretic peptide (NT-proBNP).

2.3. Laboratory Assessment

The patients had the following tests performed: complete blood count, erythrocyte sedimentation rate, creatinine, and estimated glomerular filtration rate (calculated using the CDK-EPI equation). The venous blood samples were taken after a 12-h fast.

Antinuclear antibodies were evaluated by indirect immunofluorescence pattern on HEp-2 cells and detected by immunoblot analysis.

The samples were tested by use of commercially available ELISA kits: I-FABP: R&D Systems, Inc., Minneapolis, MN, USA (assay range: 15.6 pg/mL–1000 pg/mL); LPS: CUSABIO, Wuhan, China (assay range: 6.25 pg/mL–400 pg/mL); and CLDN3: Wuhan Fine Biotech Co., Wuhan, China (assay range: 0.313 ng/mL–20 ng/mL) in order to quantify the serum concentration of permeability markers. The concentrations of target proteins in the test samples were estimated based on the previous studies, and a proper dilution factor was selected to make the diluted target proteins' concentration fall within the optimal detection range of the kit. The concentrations read from the standard curve were multiplied by the dilution factor. The samples for these analyses were collected according to the manufacturers' instructions: blood samples were collected from the peripheral vein after a 12-h fasting period and placed in the serum separator tubes. After 2 h of clotting, the samples were centrifuged for 20 min at 1000× *g*. Serum aliquots obtained by centrifugation were stored at −80 °C until further analysis. All of the measurements were performed in accordance with the manufacturers' instructions. Additionally, they were assessed in duplicate and the means were utilized to further analysis. For the ELISA analyses mentioned above, intra-assay coefficients of variation were below 8% and inter-assay coefficients of variation were below 11%.

2.4. Statistical Analysis

The statistical software STATISTICA 13.1 was used for all calculations (StatSoft, Krakow, Poland). The Shapiro-Wilk test was applied so as to determine if a continuous variable follows a normal distribution. The mean standard deviation (SD) was used to represent normally distributed data, whereas the median and interquartile range were utilized to describe non-normally distributed variables (IQR). A chi-square test (with the Yates correction for small groups [n < 10]) was used to compare categorical data that were presented as counts and percentages. The Student's *t*-test was chosen to assess the continuous variables with a parametric distribution, whereas the Mann-Whitney U test was selected in order to assess continuous variables with a nonparametric distribution. Besides, Spearman's rank correlation coefficient was applied to evaluate potential correlations between two continuous variables. The *p*-value of <0.05 was considered statistically significant.

2.5. Ethics

The study was approved by the Regional Bioethical Committee at the Medical University of Warsaw. Written informed consent was obtained from all of the participants. The study was carried out in accordance with the Declaration of Helsinki, and it also gained the approval of the local bioethical committee (approval code: KB136/2021; Bioethics Committee at the Medical University of Warsaw, Warsaw, Poland).

3. Results

3.1. Patients' Characteristics

The study included 50 patients with systemic sclerosis and 30 age-, sex-, and BMI-matched control individuals. According to the design of the study, there was no difference between the two participant groups in terms of age, sex distribution, or BMI. Table 1 provides an overview of the key demographic, clinical, laboratory, and serological characteristics of individuals with SSc and the control group.

Table 1. The characteristics of the control group and individuals with SSc.

	Systemic Sclerosis (n = 50)	Control (n = 30)	*p*-Value
General characteristics			
Age, years	57 [48–65]	54 [49–59]	0.22
Sex, women, n (%)	42 (84.00%)	25 (83.33%)	0.94
Body mass index, kg/m^2	23.56 [21.31–27.44]	25.29 [22.35–26.25]	0.67
Characteristics of systemic sclerosis			
Modified Rodnan skin score	4 [2–9]	-	-
Limited cutaneous systemic sclerosis, n (%)	27 (54%)	-	-
Diffuse cutaneous systemic sclerosis, n (%)	23 (46%)	-	-
Systemic sclerosis duration, years	6 [4–13]	-	-
Autoantibody positivity			
Anticentromere (ACA), n (%)	23 (46%)	-	-
Antitopoisomerase I (ATA), n (%)	19 (38%)	-	-
Anti-RNA polymerase III, n (%)	5 (10%)	-	-
Current treatment			
Methotrexate, n (%)	12 (24%)	-	-
Mycophenolate mofetil, n (%)	14 (28%)	-	-
Calcium channel blockers, n (%)	9 (18%)	-	-
Sildenafil, n (%)	25 (50%)	-	-
Sulodexide, n (%)	29 (58%)	-	-
Prostaglandins, n (%)	45 (90%)	-	-
Pentoxifylline, n (%)	4 (8%)	-	-

3.2. Markers of Intestinal Permeability in Systemic Sclerosis

In patients with systemic sclerosis, we found a significantly higher concentration of LPS compared to control subjects (232.30 pg/mL [149.00–347.70] versus 161.00 pg/mL [83.92–252.20], $p < 0.05$; Figure 1). The differences between SSc and control groups in other markers of intestinal permeability, i.e., IFABP and claudin-3, were not statistically significant: IFABP (1505.0 pg/mL [1108.0–1865.0] versus 1598.0 pg/mL [921.8–1835.0], $p = 0.45$); claudin-3 (14.44 ng/mL [11.85–94.53] versus 15.22 ng/mL [11.83–23.11], $p = 0.63$).

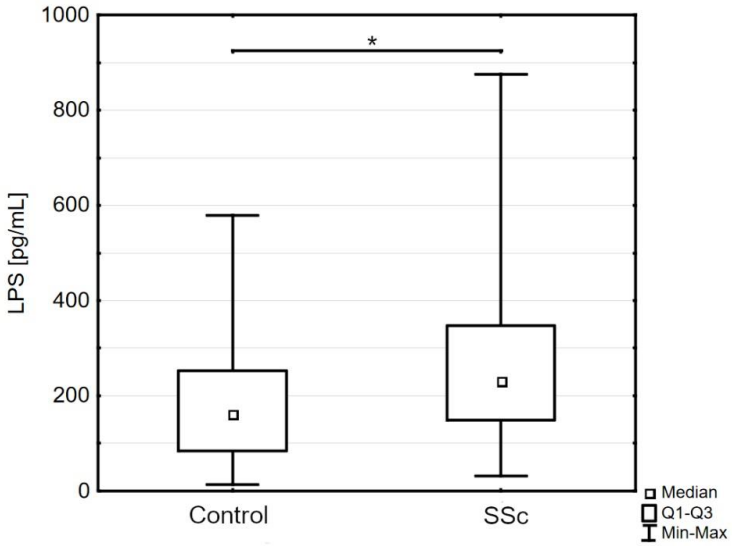

Figure 1. The serum concentration of lipopolysaccharides (LPS) in patients with systemic sclerosis compared to controls; SSc–systemic sclerosis; Q1—the first quartile; Q3—the third quartile; Min—the lowest value; Max—the highest value (* $p < 0.05$).

3.3. Markers of Intestinal Permeability in Subgroups of Disease Duration

Further analysis revealed a significant negative correlation between claudin-3 and disease duration (rho = −0.31, $p < 0.05$). Because of this fact, the patients' group has been split in two by the median disease duration. A comparison of levels of intestinal permeability markers between these two groups revealed a significantly higher concentration of LPS and claudin-3 in the group with a shorter duration of SSc than the group with a longer duration: LPS (280.75 pg/mL [167.30–403.40] versus 186.00 pg/mL [98.12–275.90], $p < 0.05$; Figure 2A); claudin-3 (16.99 ng/mL [12.41–39.59] versus 13.54 ng/mL [10.29–15.47], $p < 0.05$; Figure 2B). Detailed characteristics of both groups are provided in Table 2.

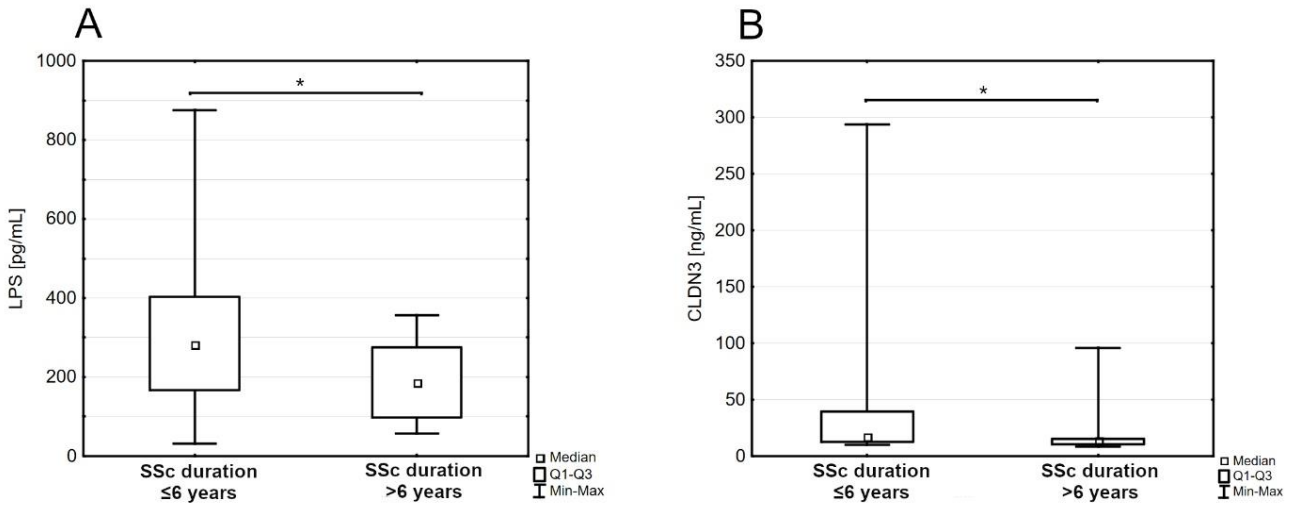

Figure 2. The serum concentration of lipopolysaccharides (LPS) (**A**) and claudin-3 (CLDN3) (**B**) in patients' subgroups with different durations of systemic sclerosis (SSc); Q1—the first quartile; Q3—the third quartile; Min—the lowest value; Max—the highest value (* $p < 0.05$).

Table 2. The characteristics of the patients with shorter (≤6 years) and longer (>6 years) durations of systemic sclerosis.

	Shorter Duration of the Disease (≤6 Years; n = 28)	Longer Duration of the Disease (>6 years; n = 22)	*p*-Value
General characteristics			
Age, years	55.9 ± 11.7	57.0 ± 12.3	0.70
Sex, women, n (%)	21 (75.0%)	21 (95.5%)	0.12
Body mass index, kg/m^2	23.1 [21.1–27.5]	23.8 [21.9–27.4]	0.70
Characteristics of systemic sclerosis			
Limited cutaneous systemic sclerosis, n (%)	16 (57.1%)	11 (50.0%)	0.62
Diffuse cutaneous systemic sclerosis, n (%)	12 (42.9%)	11 (50.0%)	0.62
Modified Rodnan skin score	4 [2–6]	4.5 [2–9]	0.41
Interstitial lung disease, n (%)	14 (50.0%)	19 (86.4%)	**0.02**
Diffusing capacity of the lungs for carbon monoxide (DLCO), %	71.14 ± 21.08	72.96 ± 13.62	0.62
Left ventricular ejection fraction (LVEF), %	65 [60–67]	65 [60–65]	0.99
Esophageal dysmotility, n (%)	13 (46.4%)	14 (63.6%)	0.55
Autoantibody positivity			
Anticentromere (ACA), n (%)	16 (57.1%)	7 (31.8%)	0.13
Antitopoisomerase I (ATA), n (%)	9 (32.1%)	10 (45.5%)	0.50
Anti-RNA polymerase III, n (%)	3 (10.7%)	2 (9.1%)	0.78

Table 2. *Cont.*

	Shorter Duration of the Disease (≤6 Years; n = 28)	Longer Duration of the Disease (>6 years; n = 22)	*p*-Value
Intestinal barrier parameters			
Intestinal fatty acid binding protein (IFABP), pg/mL	1375.0 [1060.0–1828.5]	1534.0 [1108.0–1885.0]	0.61
Claudin-3 (CLDN3), ng/mL	16.99 [12.41–39.59]	13.54 [10.29–15.47]	**0.02**
Lipopolysaccharides (LPS), pg/mL	280.75 [167.30–403.40]	186.00 [98.12–275.90]	**0.02**
Laboratory parameters			
Erythrocyte sedimentation rate, mm/h	13.0 [6.0–21.0]	8.5 [7.0–12.0]	0.41
Estimated glomerular filtration rate (eGFR), mL/min./1.73 m^2	87.41 ± 19.49	79.19 ± 18.28	0.15
N-terminal pro b-type natriuretic peptide (NT-proBNP), pg/mL	126 [74–230]	145 [64.5–206.5]	0.90

3.4. Lipopolysaccharides (LPS) in Specific Comorbidities of Systemic Sclerosis

The patients with esophageal dysmotility were characterized by a significantly decreased level of LPS compared to the patients without this complication (188.05 pg/mL [102.31–264.40] versus 283.95 pg/mL [203.20–356.30], $p < 0.05$; Figure 3).

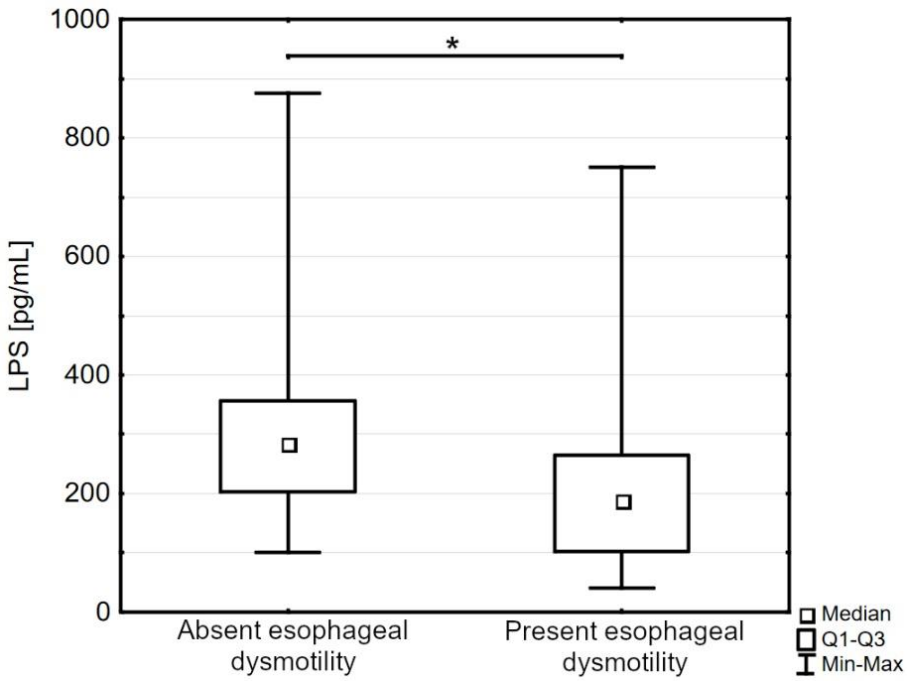

Figure 3. The serum concentration of lipopolysaccharides (LPS) in the patients with systemic sclerosis and absent esophageal dysmotility compared to the subgroup with present esophageal dysmotility; Q1—the first quartile; Q3—the third quartile; Min—the lowest value; Max—the highest value (* $p < 0.05$).

In the subgroup with a shorter duration of SSc and concomitant interstitial lung disease, we observed an increased level of LPS compared to the subgroup with a shorter duration of SSc and absent ILD (385.55 pg/mL [266.90–506.50] versus 217.75 pg/mL [157.25–280.75], $p < 0.05$; Figure 4).

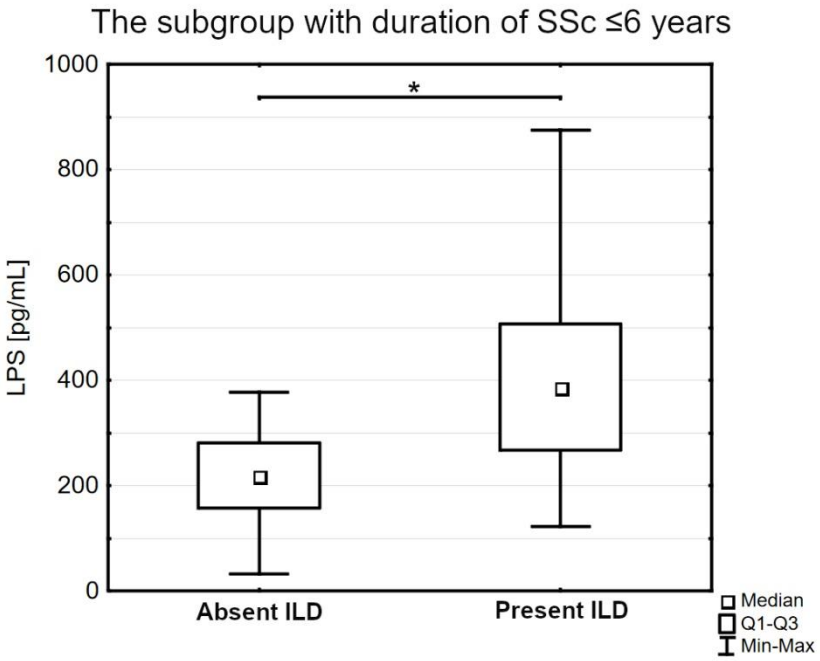

Figure 4. The serum concentration of lipopolysaccharides (LPS) in the patients' subgroup with a shorter duration of the disease with or without concomitant interstitial lung disease (ILD); Q1—the first Q3—the third quartile; Min—the lowest value; Max—the highest value (* $p < 0.05$).

We did not observe any relevant correlations between the markers themselves or between markers and left ventricle ejection fraction, NT-proBNP, DLCO, or characteristics of systemic sclerosis (i.e., disease activity index, modified Rodnan Skin score, disease subtype, antibody profile).

4. Discussion

Intestinal barrier integrity has recently been the focus of extensive studies about the interactions between the gastrointestinal tract and general homeostasis. The findings of our research are consistent with the previous studies and confirm that disruption of the intestinal barrier is a feature of the early stage of systemic sclerosis [14,15]. The assessment of intestinal permeability in the mentioned studies was based on sugar permeability tests and we observed similar results using serum protein markers. Furthermore, our findings seem to prove the hypothesis proposed in these studies regarding the translocation of pro-inflammatory antigens from the intestinal lumen induced by increased intestinal permeability [14,15].

The obtained findings could reflect the natural course of systemic sclerosis. Depending on the study, the highest disease activity and rate of progression occur between the 3rd and 5th years of the disease, and after this stage, the disease often slows down or even slightly reverses, as in the case of skin sclerosis [16,17]. Consistently, our study implies that in the early period of SSc, the intestinal barrier is damaged, which results in increased gut permeability and translocation of bacterial lipopolysaccharides. The observed results could be the consequence of abnormalities in the ultrastructure of the small intestinal mucosa found in progressive systemic sclerosis, mainly dilated intraepithelial spaces [18]. Since claudin-3 is an element of tight junctions, widened spaces between enterocytes suggest a disruption of these components, which could be the cause of an increased concentration of claudin-3 and LPS in patients with SSc [10]. The lack of differences in IFABP concentration between patients and controls may be due to the absence of pathology in enterocytes since IFABP is a protein that exclusively presents in the cytoplasm of these cells [10,18].

Lipopolysaccharides are well-recognized pro-inflammatory factors, and an increased serum LPS concentration in patients with early systemic sclerosis, which was observed

in our study, may have a detrimental impact on the further course of the disease. Recent publications have reported that TLR4, which is a receptor for LPS among others, can be substantially involved in the pathogenesis of SSc [19]. Skin and lung biopsies from SSc patients are characterized by an increased presence of TLR4 on fibroblasts compared to nonsclerodermic subjects [20]. Moreover, in vitro studies revealed that LPS can stimulate the expression of extracellular matrix genes, especially collagen, in skin fibroblasts and markedly increase their capacity to initiate a profibrotic response when challenged with TGF-β1, a cytokine known as a major profibrotic trigger in SSc [20,21]. Furthermore, LPS can induce the transdifferentiation of skin fibroblasts into myofibroblasts and promote profibrotic gene expression, which is particularly important in the case of systemic sclerosis, in which myofibroblasts are responsible for organ fibrosis [20,22,23]. LPS is known for its ability to induce lung inflammation and fibrosis, and an LPS-treated mouse is a model for acute lung injury [24]. However, LPS in this model is administered at supraphysiological concentrations [24]. The data on the effects of prolonged exposition to lower doses on lung physiology remain elusive. Despite this fact, LPS was found to exacerbate coexisting interstitial lung disease in the mouse model [25]. Moreover, in patients with systemic sclerosis and concomitant intestinal lung disease, compared to the control group, blood monocytes were found to secrete significantly higher amounts of IL-6 after exposure to LPS [26]. IL-6 levels are elevated in the sera of SSc patients and have been shown to be strongly associated with the severity of skin thickening and disease progression in interstitial lung disease [27,28]. In this context, an approach based on lowering the exposure to LPS could be a potential therapeutic option in systemic sclerosis.

Observed lower in the subgroup with a longer disease duration than in the subgroup with a shorter disease duration, the concentration of permeability markers may be an effect of coexisting disturbances in intestinal absorption due to fibrosis. Impaired absorption of saccharides, i.e., lactose and fructose, was found to be often present in patients with systemic sclerosis [29,30]. Furthermore, the present malabsorption of the mentioned saccharides was associated with increased gastrointestinal symptoms [29,30]. Malabsorption can be a result of multifactorial pathology of the affected intestinal wall, which includes fibrosis of the arterial tunica intima in the submucosal arteries of the small bowel, damage to the enteric nervous system, and marked fibrosis of the circular layer of the muscularis propria [31,32]. Available diagnostic methods for the involvement of the gastrointestinal tract are limited, complex, and expensive [31]. Since we observed a significantly lower LPS concentration in the patients with esophageal involvement compared to patients with normal esophageal motility, it could be hypothesized that the lower translocation of LPS in those patients may be due to decreased intestinal absorption of LPS. Further studies are needed to evaluate this relationship and its clinical significance.

As mentioned, substances from the intestinal lumen, such as LPS, can reach the circulation and exert proinflammatory effects, which can potentially accelerate disease progression. Many prognostic factors have been identified as a consequence of the complicated pathophysiology of SSc, and the presence and interplay between them might reflect the course of the disease [33]. It is possible that increased intestinal permeability is one of the causes exacerbating the natural course of systemic sclerosis, and a further decrease in permeability markers could be an effect of developing fibrosis of the gastrointestinal tract. However, due to the limitations of our study, including the relatively small sample size, single-center type, and cross-sectional character, the conclusions on the impact on disease progression are limited. To exactly assess the pathogenetic effects of a disrupted gut barrier on the course of SSc, prospective, multicenter studies are needed. The prospective assessment of intestinal permeability and exposition to LPS at different stages of the disease seem to be particularly valuable.

Understanding the mechanisms of how dysbiotic microbiota and a disrupted intestinal barrier may influence the course of systemic sclerosis seems valuable in clinical practice. Hypothetically, measurement of the intestinal barrier markers may help to stratify patients into different prognostic categories, which could help optimize treatment in the early stages

of the disease. An approach based on the modification of the gut microbiota could break the vicious circle driven by inflammation induced by increased translocation of bacterial elements. It could lead to mitigating the progression of the disease, especially in the early stages of the disease when the complications are not developed. Available techniques of modulation of microbiota include probiotics, prebiotics, and fecal microbiota transplantation. These methods have been intensively studied in a number of dermatological conditions and are known to improve the gut barrier [34–36]. An improvement of the intestinal barrier by the mentioned interventions leads to a decrease in intestinal permeability and can be an effect of elevated expression of proteins of tight junction complexes in intestinal epithelial cells, stimulation of the proliferation of intestinal epithelial cells, and an increased secretion of mucins, which protect enterocytes from stressors [34–36]. Such attempts were also made in systemic sclerosis and were associated mainly with the improvement of gastrointestinal symptoms [37–41]. One study reported the immunomodulatory effect of a probiotic mixture manifested as a significant decrease in the proportion of Th17 cells compared with placebo [38]. It is worth noting that the administration of probiotics as well as fecal microbiota transplantation exhibited an excellent safety profile, and the only noticed adverse events were mild and transient, i.e., diarrhea, bloating, and abdominal pain [37–41]. However, there is still little information available on the most appropriate probiotic strain composition and donor matching for fecal microbiota transplantation. Measurements of intestinal permeability markers in such trials could be a marker of intervention outcome.

5. Conclusions

Our cross-sectional study revealed that the patients with systemic sclerosis are characterized by increased LPS serum concentration compared to the control subjects. Furthermore, biomarkers of intestinal barrier permeability, LPS and claudin-3, are elevated in the patients with a shorter duration of the disease compared to the subgroup with a longer duration of the disease. Additionally, in the subgroup with a shorter duration of SSc, we observed increased LPS concentration in patients with concomitant interstitial lung disease compared to the patients with the absence of this complication. Concomitant esophageal dysmotility was associated with a decrease in LPS in the patients with SSc. The study highlighted the importance of disease duration in gut barrier research. On account of the potent proinflammatory and profibrotic properties of LPS, increased circulating LPS may be an exacerbating factor in the early stage of the disease, whereas the subsequent decrease of LPS may be due to the development of gastrointestinal disturbances. However, to exactly determine the impact of LPS in systemic sclerosis, prospective studies are needed to demonstrate the effect of prolonged exposition to LPS. The modulation of the gut barrier may represent a new therapeutic approach for systemic sclerosis.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the Medical University of Warsaw (approval code: KB136/2021, 6th September 2021, Bioethics Committee at the Medical University of Warsaw, Warsaw, Poland).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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7. Publikacje stanowiące pracę doktorską

7.1. Stec, A.; Sikora, M.; Maciejewska, M.; Paralusz-Stec, K.; Michalska, M.; Sikorska, E.; Rudnicka, L. Bacterial Metabolites: a Link between Gut Microbiota and Dermatological Diseases. Int. J. Mol. Sci. 2023, 24, 3494. https://doi.org/10.3390/ijms24043494

Celem powyższej pracy było podsumowanie dotychczasowych doniesień publikacyjnych na temat roli poszczególnych grup metabolitów mikrobioty jelitowej w wybranych chorobach dermatologicznych ze szczególnym uwzględnieniem jednostek o podłożu autoimmunizacyjnym. Omówiono również dotychczasową wiedzę dotyczącą wpływu mikrobioty jelitowej na mechanizmy immunologiczne mające istotny wpływ w patogenezie danych chorób. Dodatkowo podsumowano dostępne możliwości modyfikacji mikrobioty jelitowej i ich wpływ na przebieg chorób dermatologicznych obserwowany w badaniach klinicznych.

W ostatnich latach coraz większe znaczenie w patogenezie chorób dermatologicznych przypisuje się metabolitom bakteryjnym wytwarzanym przez mikrobiotę jelitową. Współczesne badania wykazały, że metabolity te wpływają na stan skóry, a ich zaburzenia mogą prowadzić do rozwoju chorób skóry, takich jak łuszczyca, trądzik, czy atopowe zapalenie skóry.

Jedną z kluczowych grup metabolitów mikrobioty jelitowej są krótkołańcuchowe kwasy tłuszczowe (SCFA), takie jak octan, propionian i maślan. SCFA są wytwarzane przez bakterie jelitowe w procesie fermentacji nieprzyswajalnych resztek pokarmowych. Działają one przeciwzapalnie i immunomodulująco, głównie poprzez zwiększenie populacji i aktywności limfocytów T regulatorowych oraz zmniejszenie ekspresji cytokin prozapalnych w makrofagach i limfocytach T. Ponadto krótkołańcuchowe kwasy tłuszczowe wpływają na procesy proliferacyjne i różnicowanie keratynocytów, a tym samym, wpływają pozytywnie na barierę skórną i w konsekwencji zmniejszają przeznaskórkową utratę wody (ang. transepidermal water loss; TEWL). Wymienione właściwości oraz obserwacje na modelach zwierzęcych wskazują, że zaburzenia w wytwarzaniu krótkołańcuchowych kwasów tłuszczowych mogą być istotnym elementem patogenezy atopowego zapalenia

skóry, alergii, łuszczycy oraz chorób tkanki łącznej takich jak toczeń rumieniowaty układowy i twardzina układowa.

Kolejną grupą metabolitów omówionych w publikacji są pochodne tryptofanu. Powstają one w wyniku przekształcenia tryptofanu przez bakterie jelitowe głównie na drodze szlaku indolowego. Działanie tej grupy metabolitów wiąże się z aktywacją receptora węglowodorów aromatycznych (aryl hydrocarbon receptor; AhR), który występuje na wielu komórkach, między innymi fibroblastach, keratynocytach, komórkach Langerhansa, melanocytach, sebocytach, komórkach tucznych i limfocytach. Udowodniono, że jeden z metabolitów, kwas indolo-3-pirogronowy, zwiększa odporność keratynocytów na promieniowanie UVB. Ponadto zaburzenia w stężeniu niektórych metabolitów tryptofanu obserwowano w atopowym zapaleniu skóry i toczniu rumieniowatym okładowym.

Ostatnią z omówionych grup metabolitów są monoaminy – N-tlenek trimetyloaminy (TMAO) i trimetyloamina (TMA). Powstają one między innymi z dostarczanych z pożywieniem choliny, L-karnityny i fosfatydylocholiny w wyniku przekształcenia przez mikrobiotę jelitową. Dostępne publikacje sugerują, że gatunki bakterii jelitowych związane z dysbiozą mają większy potencjał do tworzenia wymienionych metabolitów w porównaniu do eubiotycznej mikrobioty. Ponadto u pacjentów z łuszczycą zaobserwowano istotnie wyższe stężenie TMAO we krwi w porównaniu do grupy kontrolnej. Podobne wyniki obserwowano w łuszczycowym zapaleniu stawów, gdzie dodatkowo zaobserwowano istotną dodatnią korelację między stężeniem TMAO a nasileniem choroby, mierzone zarówno przez wskaźniki aktywności skórnej, jak i stawowej. W pracy opisano również potencjalny negatywny wpływ N-tlenku trimetyloaminyny na hidradenitis suppurativa, toczeń rumieniowaty układowy oraz twardzinę układową.

W dalszej części pracy podsumowano dostępne metody modyfikacji mikrobioty i wyniki prób klinicznych zastosowania ich w terapii chorób skóry. W skład opisanych metod wchodzi podaż probiotyków, prebiotyków, postbiotyków oraz zastosowanie przeszczepu mikrobioty jelitowej. Metody te cechuje duże bezpieczeństwo, a obserwowane skutki uboczne są łagodne i przemijające. Dostępne publikacje wskazują, że podejście oparte na modyfikacji mikrobioty jelitowej może przynieść najwięcej korzyści w atopowym zapaleniu skóry i w chorobach alergicznych. Badania na temat zastosowania wymienionych terapii

w chorobach autoimmunizacyjnych, choć mniej liczne, również wskazują na pozytywny efekt w jednostkach takich jak łuszczyca i toczeń rumieniowaty układowy.

W konkluzji zwrócono uwagę, że metabolity mikrobioty jelitowej mogą mieć istotny wpływ na procesy patogenetyczne w chorobach dermatologicznych. Metabolity indolu i krótkołańcuchowe kwasy tłuszczowe wydają się działać poprzez ich właściwości immunomodulacyjne i przeciwzapalne. Pochodne amin, zwłaszcza TMAO, mogą przyspieszać postęp chorób skóry i przyczyniać się do rozwoju powikłań ze względu na ich prozapalne działanie. Modyfikacje mikrobioty, mogące zmieniać stężenia metabolitów wytwarzanych przez mikrobiotę, są obiecującą opcją terapeutyczną dla chorób dermatologicznych oraz wykazują bardzo dobry profil bezpieczeństwa.

7.2. Stec A, Maciejewska M, Paralusz-Stec K, Michalska M, Giebułtowicz J, Rudnicka L, Sikora M.: The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis. Journal of Inflammation Research 2023;16:1895-1904. https://doi.org/10.2147/JIR.S409489

Celem badania było określenie stężenia bakteryjnego metabolitu TMAO w osoczu pacjentów z twardziną układową w porównaniu do grupy kontrolnej oraz ocenę potencjalnej korelacji pomiędzy stężeniem TMAO w osoczu a różnymi czynnikami klinicznymi, takimi jak wiek, podtyp choroby, współwystępujące zajęcie płuc, przewodu pokarmowego, serca.

Do badania włączono 63 pacjentów z twardziną układową oraz 47 ochotników dopasowanych pod względem wieku, płci i wskaźnika BMI. Stężenie TMAO w osoczu pacjentów zbadano z użyciem techniki chromatografii cieczowej sprzężonej ze spektrometrem mas (HPLC-MS). Otrzymane wyniki zostały opracowane przy pomocy programu Statistica 13.3. Dopasowanie rozkładu danych do rozkładu normalnego zostało ocenione za pomocą testu Shapiro-Wilka. Porównanie zmiennych jakościowych przeprowadzono wykorzystując test chi-kwadrat (z poprawką Yatesa dla grup $n < 10$). Test t-Studenta został wykorzystany do oceny zmiennych ciągłych o rozkładzie normalnym, natomiast test U Manna-Whitney'a do oceny zmiennych ciągłych o rozkładzie różnym od rozkładu normalnego. Współczynnik korelacji rang Spearmana został wyliczony, aby określić korelację między zmiennymi. Poziom istotności statystycznej przyjęto dla $p < 0,05$.

W badaniu stwierdzono, że pacjenci z twardziną układową mają istotnie statystycznie wyższe stężenie TMAO w osoczu w porównaniu do osób w grupie kontrolnej (283,0 ng/ml (IQR 188,5-367,5) vs. 205,5 ng/ml (IQR 101,0-318,0); $p < 0,01$). Dodatkowo wykazano, że pacjenci z twardziną ze współwystępującą śródmiąższową chorobą płuc (interstitial lung disease, ILD) charakteryzują się wyższym stężeniem TMAO w osoczu w porównaniu do pacjentów bez ILD (302,0 ng/ml (IQR 212,0-385,5) vs. 204,0 ng/ml (IQR 135,5-292,0); $p < 0,01$), natomiast marker restrykcji spowodowanej przez ILD, zdolność dyfuzyjna płuc dla tlenku węgla (diffusing capacity for carbon monoxide, DLCO), wykazywał istotną ujemną korelację ze stężeniem TMAO ($rho = -0,53$; $p = 0,013$). Stężenie TMAO było również wyższe

w grupie pacjentów ze współwystępującymi zaburzeniami motoryki przełyku (289,75 ng/ml (IQR 213,75-387,5) vs. 209,5 ng/ml (IQR 141,5-315,0); p = 0,026). Ponadto zaobserwowano istotną ujemną korelację pomiędzy stężeniem TMAO w osoczu a frakcją wyrzutową lewej komory serca (left ventricle ejection fraction; LVEF): rho = -0,39; p = 0,009 oraz istotną dodatnią korelację pomiędzy stężeniem TMAO a stężeniem N-końcowego fragmentu propeptydu natriuretycznego typu B (NT-proBNP): rho = 0,41; p = 0,0009. Poziom TMAO u pacjentów wykazywał też silną dodatnią korelację z wynikiem w skali Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI): rho = 0,78; p < 0,001.

Na podstawie powyższych wyników można wnioskować, że TMAO może stanowić wartościowy marker uszkodzenia narządowego w twardzinie układowej. Obserwowane zwiększenie stężenia TMAO w zajęciu płuc, przełyku i dodatnia korelacja stężenia metabolitu z markerami zajęcia serca świadczą o potencjalnej roli mikrobioty jelitowej i jej metabolitów w wystąpieniu swoistych objawów narządowych choroby. Potwierdzenie związku przyczynowego zwiększonej ekspozycji na TMAO z wystąpieniem objawów narządowych w przyszłych badaniach prospektywnych mógłby prowadzić do wykorzystania TMAO jako markera ogólnego postępu choroby, co dałoby możliwość lepszej stratyfikacji pacjentów i w konsekwencji optymalizacji terapii.

7.3. Stec, A.; Maciejewska, M.; Zaremba, M.; Paralusz-Stec, K.; Michalska, M.; Rudnicka, L.; Sikora, M. The Clinical Significance of Serum Biomarkers of the Intestinal Barrier in Systemic Sclerosis: a Cross-Sectional Study. J. Pers. Med. 2023, 13, 678. https://doi.org/10.3390/jpm13040678

Badanie miało na celu ocenę potencjalnych różnic w przepuszczalności jelit między pacjentami z twardziną układową i grupą kontrolną oraz zbadanie korelacji między przepuszczalnością jelit a objawami narządowymi twardziny układowej. W pracy oceniono stężenie wybranych markerów przepuszczalności jelitowej, jelitowego białka wiążącego kwasy tłuszczowe (I-FABP), klaudyny-3 i lipopolisacharydów bakteryjnych (LPS), w surowicy 50 pacjentów z twardziną układową i 30 ochotników dopasowanych pod względem wieku, płci i wskaźnika BMI.

Stężenia wybranych markerów w surowicy uczestników oceniono metodą testu immunoenzymatycznego (I-FABP: R&D Systems, Inc., Minneapolis, USA; LPS: CUSABIO, Wuhan, China; CLDN3: Wuhan Fine Biotech Co., Wuhan, China). Wyniki zostały opracowane przy użyciu oprogramowania Statistica 13.3. Zgodność rozkładu danych z rozkładem normalnym oceniono za pomocą testu Shapiro–Wilka. Porównywanie zmiennych jakościowych przeprowadzono za pomocą testu chi-kwadrat (z poprawką Yatesa dla małych grup [n < 10]). Do oceny zmiennych ciągłych o rozkładzie normalnym wybrano test t-Studenta, natomiast do oceny zmiennych ciągłych o rozkładzie różnym od rozkładu normalnego wykorzystano test U Manna-Whitneya. W celu określenia korelacji pomiędzy zmiennymi obliczono współczynnik korelacji rang Spearmana. Poziom istotności statystycznej przyjęto dla $p < 0,05$.

Analiza wykazała, że pacjenci z twardziną układową mieli istotnie wyższe stężenie LPS w surowicy w porównaniu z grupą kontrolną (232,30 pg/mL (IQR 149,00–347,70) vs. 161,00 pg/mL (IQR 83,92–252,20); $p < 0,05$). Ponadto pacjenci z krótszym czasem trwania twardziny układowej (≤6 lat) mieli zwiększone stężenie LPS (280,75 pg/mL (IQR 167,30–403,40) vs. 186,00 pg/mL (IQR 98,12–275,90); $p < 0,05$) i klaudyny-3 (16,99 ng/ml (IQR 12,41–39,59) vs. 13,54 ng/ml (IQR 10,29–15,47); $p < 0,05$) w porównaniu z podgrupą

z dłuższym czasem trwania choroby (powyżej 6 lat). W podgrupie pacjentów z krótszym czasem trwania choroby obserwowano istotnie wyższe stężenie LPS w surowicy pacjentów chorujących na śródmiąższową chorobę płuc w porównaniu do pacjentów bez zajęcia płuc (385,55 pg/mL (IQR 266,90–506,50) vs. 217,75 pg/mL (IQR 157,25–280,75); $p < 0,05$). Dodatkowo pacjenci z zaburzeniami motoryki przełyku charakteryzowali się niższym poziomem LPS w porównaniu z pacjentami z prawidłowym wynikiem badania kontrastowego przełyku (188,05 pg/ml (IQR 102,31–264,40) vs. 283,95 pg/ml (IQR 203,20–356,30); $p <0,05$).

Wyniki pracy wskazują, że bariera jelitowa w twardzinie układowej może być uszkodzona, szczególnie w początkowym okresie choroby. Przenikające do krwi substancje w tym LPS mogą być związane z wystąpieniem objawów choroby takich jak śródmiąższowa choroba płuc. Badanie wskazało również, że stan bariery jelitowej może się zmieniać w trakcie trwania choroby, co może być użyteczną informacją przy projektowaniu przyszłych badań nad barierą jelitową.

8. Wnioski

1. Stężenie metabolitu mikrobioty jelitowej – N-tlenku trimetyloaminy (ang. trimethylamine N-oxide; TMAO) w surowicy jest istotnie wyższe u pacjentów z twardziną układową w porównaniu do grupy kontrolnej. Występowanie niektórych objawów choroby, m. in. śródmiąższowej choroby płuc oraz zaburzeń motoryki przełyku, wiąże się ze szczególnie podwyższonym stężeniem TMAO.

2. Metabolity mikrobioty jelitowej mogą być łącznikiem między dysbiozą jelitową i zajęciem narządów w przebiegu twardziny układowej. Modulacja metabolitów pochodzących z bakterii jelitowych może stanowić nowe podejście terapeutyczne w leczeniu twardziny układowej.

3. Stężenie markera przepuszczalności jelitowej – lipopolisacharydów (ang. lipopolysaccharides; LPS) w surowicy jest istotnie wyższe u pacjentów z twardziną układową w porównaniu do grupy kontrolnej; podgrupa pacjentów o krótszym czasie trwania choroby (czas mniejszy lub równy 6 lat) charakteryzuje się istotnie statystycznie większym stężeniem markerów przepuszczalności jelitowej, LPS i klaudyny-3, w porównaniu do podgrupy o dłuższym czasie trwania choroby (powyżej 6 lat), co sugeruje występowanie zwiększonej przepuszczalności jelit na wczesnym etapie choroby.

4. Niższe stężenie markerów przepuszczalności jelitowej (LPS i Klaudyny-3) u pacjentów z dłuższym okresem trwania choroby (powyżej 6 lat) w porównaniu do chorych o krótszym przebiegu choroby (czas mniejszy lub równy 6 lat) może być spowodowane współwystępującymi zaburzeniami wchłaniania wynikającymi z zajęcia przewodu pokarmowego.

9. Bibliografia

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10. Opinia Komisji Bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

KB/ 136 /2021

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
w dniu 06 września 2021 r. po zapoznaniu się z wnioskiem:

Dr n. med. Mariusz Sikora
Katedra i Klinika Dermatologiczna
ul. Koszykowa 82a, 02-008 Warszawa

dotyczącym: wyrażenia opinii w sprawie badania pt.:"Rola zaburzeń bariery jelitowej i metabolitów bakteryjnych w twardzinie układowej-implikacje kliniczne i terapeutyczne."

- Badanie może być prowadzone wyłącznie w okresie obowiązywania polisy ubezpieczeniowej.

wyraża następującą
opinię

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .

Przewodnicząca Komisji Bioetycznej
Prof. dr hab. n. med. Magdalena Kuźma-Kozakiewicz

*niepotrzebne skreślić

11. Oświadczenia wszystkich współautorów publikacji

Publikacja nr 1:

Bacterial Metabolites: A Link between Gut Microbiota and Dermatological Diseases.

Stec, A.; Sikora, M.; Maciejewska, M.; Paralusz-Stec, K.; Michalska, M.; Sikorska, E.; Rudnicka, L. Bacterial Metabolites: A Link between Gut Microbiota and Dermatological Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 3494. https://doi.org/10.3390/ijms24043494

Lp.	Autor	Rodzaj wkładu merytorycznego	Procentowy wkład w powstanie publikacji	Data, podpis
1.	Albert Stec	• Stworzenie projektu badania • Opracowanie artykułu • Stworzenie rycin i tabeli	70%	25.04.2023 Albert Stec
2.	Mariusz Sikora	• Stworzenie projektu badania • Opracowanie artykułu	10%	
3.	Magdalena Maciejewska	• Rewizja artykułu	3%	25.04.2023
4.	Karolina Paralusz-Stec	• Opracowanie artykułu • Stworzenie rycin i tabeli	3%	24.04.2023 Karolina Paralusz-Stec
5.	Milena Michalska	• Rewizja artykułu • Stworzenie rycin i tabeli	2%	
6.	Ewa Sikorska	• Rewizja artykułu • Stworzenie rycin i tabeli	2%	
7.	Lidia Rudnicka	• Stworzenie projektu badania • Rewizja artykułu	10%	

Publikacja 2:

The gut microbial metabolite trimethylamine N-oxide is linked to specific complications of systemic sclerosis.

Stec A, Maciejewska M, Paralusz-Stec K, Michalska M, Giebułtowicz J, Rudnicka L, Sikora M.: The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis. Journal of Inflammation Research 2023;16:1895-1904. https://doi.org/10.2147/JIR.S409489

Lp.	Autor	Rodzaj wkładu merytorycznego	Procentowy wkład w powstanie publikacji	Data, podpis
1.	Albert Stec	• Stworzenie projektu badania • Gromadzenie danych • Analiza danych • Opracowanie artykułu	60%	28.04.2023 Albert Stec
2.	Magdalena Maciejewska	• Rewizja artykułu	2%	29.04.2023 Magdalena Maciejewska
3.	Karolina Paralusz-Stec	• Opracowanie artykułu • Gromadzenie danych	2%	29.04.2023 Karolina Paralusz-Stec
4.	Milena Michalska	• Rewizja artykułu	2%	Milena Michalska
5.	Joanna Giebułtowicz	• Rewizja artykułu • Gromadzenie danych • Analiza danych	5%	Joanna Giebułtowicz
6.	Lidia Rudnicka	• Stworzenie projektu badania • Rewizja artykułu	10%	
7.	Mariusz Sikora	• Stworzenie projektu badania • Opracowanie artykułu • Analiza danych	19%	

Publikacja 3:

Serum biomarkers of the intestinal barrier in systemic sclerosis: clinical significance

Stec A, Maciejewska M, Zaremba M, Paralusz-Stec K, Michalska M, Rudnicka L, Sikora M.: The Clinical Significance of Serum Biomarkers of the Intestinal Barrier in Systemic Sclerosis: A Cross-Sectional Study. Journal of Personalized Medicine 2023; 13(4):678. https://doi.org/10.3390/jpm13040678

Lp.	Autor	Rodzaj wkładu merytorycznego	Procentowy wkład w powstanie publikacji	Data, podpis
1.	Albert Stec	• Stworzenie projektu badania • Gromadzenie danych • Analiza danych • Opracowanie artykułu	64%	25.04.2023 Albert Stec
2.	Magdalena Maciejewska	• Rewizja artykułu • Gromadzenie danych	2%	25.04.2023
3.	Michał Zaremba	• Gromadzenie danych	5%	25.04.2023
4.	Karolina Paralusz-Stec	• Opracowanie artykułu • Gromadzenie danych	2%	24.04.2023
5.	Milena Michalska	• Rewizja artykułu	2%	
6.	Lidia Rudnicka	• Stworzenie projektu badania • Rewizja artykułu	10%	
7.	Mariusz Sikora	• Stworzenie projektu badania • Opracowanie artykułu • Analiza danych	15%	