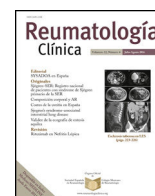




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Original Article

Does human microbiota interact with immunosuppressive treatment of systemic autoimmune rheumatological diseases? A systematic review



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ABSTRACT

Objective: To collect and analyse studies evaluating the interaction between the human microbiota (HM) and immunosuppressive (IS) treatments for systemic autoimmune rheumatological diseases (ARDs), and their impact on the disease.

Methods: A systematic review was performed based on an electronic search strategy in Medline, Embase, and Cochrane Library (inception-02/2024). We included papers studying the interaction of HM and IS treatments in adult patients with ARDs in which parameters of diversity and taxonomic composition were measured. We excluded spondyloarthritis for which more extensive knowledge is available. Studies of any language were allowed, prioritising clinical trials but also including observational longitudinal prospective and retrospective, and case-control studies.

Results: Of 2570 papers identified, 20 were included (15 from rheumatoid arthritis, 3 from systemic lupus erythematosus, 1 from primary Sjögren's syndrome and 1 from systemic sclerosis), overall, with a moderate risk of bias. The paucity of studies and niche specificity limited the study to the gut microbiota. A trend towards decreased diversity and compositional changes in gut microbiota and partial restitution in patients responding to IS treatment was identified. The heterogeneity observed in the design and outcome measures of the studies precluded a metaanalysis; however, the results point to a possible relationship between HM alterations and response to IS treatments in ARDs.

Conclusions: Available studies suggest a potential association between the HM and the response to IS therapies in ARDs. However, the overall moderate quality of evidence and substantial methodological heterogeneity limit the strength of combined conclusions. Standardization of microbiota-related studies is needed to enable data integration and support more robust inferences.

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¿Interacciona la microbiota humana con el tratamiento inmunosupresor de las enfermedades reumatológicas autoinmunes sistémicas? Revisión sistemática

RESUMEN

Objetivo: Recopilar y analizar los estudios en que se evalúa la interacción entre la microbiota humana (MH) y los tratamientos inmunosupresores (IS) de las enfermedades reumatológicas autoinmunes sistémicas (ERAS), y su repercusión en la enfermedad.

Métodos: Se realizó una revisión sistemática basada en una estrategia de búsqueda electrónica en Medline, Embase y Cochrane Library (inicio-02/2024). Se incluyeron aquellos trabajos que estudiaban la interacción de la MH y el tratamiento IS en ERAS en pacientes adultos en que se midieran parámetros de diversidad y composición taxonómica. Se excluyeron las espondiloartritis por disponerse de un conocimiento más extenso. Se permitieron estudios de cualquier idioma, priorizándose los ensayos clínicos, pero incluyendo también estudios observacionales longitudinales prospectivos y retrospectivos, y casos y controles.

Palabras clave:

Enfermedades del tejido conectivo
Artritis reumatoide
Lupus eritematoso sistémico
Síndrome de Sjögren
Esclerosis sistémica
Microbiota
Disbiosis
Resultado del tratamiento
Medicina de precisión

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Recurrencia
 Valor predictivo de las pruebas
 Terapia farmacológica
 Terapéutica

Resultados: De 2570 trabajos identificados, se incluyeron 20 (15 de artritis reumatoide, 3 de lupus eritematoso sistémico, 1 de síndrome de Sjögren primario y 1 de esclerosis sistémica), en general con un riesgo de sesgo moderado. La escasez de estudios y la especificidad de nicho limitó el estudio a la microbiota intestinal. Se identificó una tendencia a la disminución de diversidad y cambios en la composición en la microbiota y una restitución parcial en los pacientes respondedores a tratamiento IS. La heterogeneidad observada en el diseño y las medidas de resultado de los estudios impidió realizar un metaanálisis; no obstante, los resultados apuntan a una posible relación entre las alteraciones de la MH y la respuesta a los tratamientos IS en las ERAS.

Conclusiones: Los estudios disponibles sugieren una posible asociación entre la MH y la respuesta a terapias IS en ERAS. Sin embargo, la calidad global moderada de la evidencia y la alta heterogeneidad metodológica limitan la solidez de conclusiones combinadas. Es necesario la estandarización de los estudios sobre la MH para poder combinar resultados y establecer conclusiones con mayor confianza.

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Introduction

Autoimmune rheumatic diseases are complex chronic and systemic diseases with varying prevalence, depending on the specific disease type, and with a higher incidence in women.¹ Their pathogenic mechanism is not fully understood, but they are considered to be the result of an imbalance in the immune system's regulatory mechanisms, involving genetic and environmental factors such as lifestyle, nutrition, medications, and infections.¹

The human body is a symbiotic ecosystem inhabited by trillions of microorganisms that coexist in niches or microhabitats, forming the human microbiota (HM). This microbiota varies in composition and abundance, both between and within individuals, and plays a crucial role in various biological functions.²

At the intestinal level, homeostasis is maintained through a mutualistic relationship between the gut microbiota (GM) and the host immune system.³ When this relationship is altered, intestinal dysbiosis can occur, characterised by a reduction in microbial diversity and an overgrowth of opportunistic pathogens. An imbalance in intestinal homeostasis can trigger an immune response against the host through the loss of immunological tolerance, which has been associated with the development of autoimmune diseases.⁴

GM is involved in the processing of exogenous substances at the digestive level, such as drugs. This interaction may occur in which the drug alters the composition and function of GM, and GM, in turn, directly or indirectly modifies the chemical structure of the drug and, therefore, its pharmacokinetics and pharmacodynamics.⁵

Pharmacomicrobiomics is the field that studies how variations in GM affect drug action. Studies have shown that GM and its enzymatic products can influence drug bioavailability, efficacy, and toxicity through direct and indirect mechanisms.⁶ This discipline could be key to precision medicine, especially in the treatment of systemic autoimmune rheumatic diseases (SARDs), by predicting therapeutic response and optimising treatment by modulating the microbiota.⁷

The chronic course of SARDs requires long-term immunosuppressive therapy (IS), usually administered orally with gastrointestinal absorption and subsequent renal or hepatic elimination. The interaction between IS therapy and GM during drug absorption could offer new possibilities for more efficient and safer patient management, minimising side effects and improving clinical response.⁷

Studies that have specifically analysed the interaction of the different HM niches in SARDs with their IS treatments are scarce. The available studies show changes in GM composition due to interaction with IS therapy, but a lack of understanding of the molecular and cellular mechanisms that mediate this interaction persists.⁸

This systematic review analyses the current evidence and is aimed at identifying consistent and reproducible findings that may guide future research and therapeutic strategies.

Methods

A systematic review was conducted using the Cochrane Collaboration guidelines.⁹ The study protocol was not pre-registered with PROSPERO as the study selection process had already been initiated. However, our findings are reported in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement¹⁰ and are available upon request to ensure transparency.

Search strategy and selection process

Based on the PICoT (Population Intervention Comparator Outcome Time or Type of Study) framework, a search strategy was generated with MeSH terms and free terms, detailed in [Appendix A](#). The MeSH terms included subheadings related to: drug therapy, drug effects, therapy, therapeutic use, and physiopathology.

One of the study investigators (NF) proposed a strategy, which was supervised by another investigator (LC). Based on this, a systematic literature search was conducted in the Medline (via PubMed), Embase, and Cochrane Library databases with no start date and up to February 18, 2024 ([Appendix A](#)). The only restriction applied was to limit the search to human studies. Articles written in a language other than English were included and translated.

The inclusion criteria were: studies that analysed the composition of HM in biological samples (1) from patients with SARDs, with the exception of spondyloarthritis because this information has already been extensively reviewed,¹¹ (2) and compared it based on the IS treatment used or the response to it. (3) No limits were set regarding disease stage, geographic location, or publication date of the studies. The titles and abstracts of the identified works were reviewed. In the case of oral communications, an extension search was conducted after publication, retaining the most recent. The exclusion criteria were as follows: reviews, guidelines, or editorials; animal studies, in vitro or ex vivo; studies on SARDs in children and adolescents; and studies that only included patients with spondyloarthritis.

The results obtained were transferred to the Rayyan[®] software tool. After eliminating duplicate studies, two researchers (PS, NF) independently screened the titles and abstracts, eliminating those that did not meet the inclusion criteria and that presented any exclusion criteria. For studies that raised concerns, the full text was extracted to further assess their inclusion. Studies with dis-

Table 1
Microbiota prognostic factors studied in response to immunosuppressive treatment. Definition and measures used in the identified studies.

Variable	Definition	Measures
Alfa-diversity	Species diversity present in a single environment or sample in terms of richness and structure/uniformity	Shannon index Simpson index Chao index Fisher index Pielou uniformity Faith Phylogenetic Diversity
Beta-diversity	Variability in species composition between different samples. Used to understand how microbial communities differ from one environment to another	Bray-Curtis distance or difference UniFrac distance Operational Taxonomic Units (OTUs) Hierarchical Clustering PCoA (Principal Components Analysis)
Abundance	Amount of a specific microorganism in relation to the total number of microorganisms in a sample	Total number of individuals Proportion of a specific microorganism compared to others
Taxonomic composition	The diversity and identity of the microorganisms present in a sample	Operational taxonomic units (OTUs)

agreement regarding their selection were reviewed jointly with the assistance of a third researcher (LC).

Following this, the full texts of all selected studies were compiled and manually assessed by one of the researchers (NF) to confirm their final inclusion or exclusion, and any concerns were confirmed with the other researchers. The reasons for exclusion were noted at this stage.

Data collection and synthesis

Data extraction was performed by one reviewer (NF) using the ELICIT[®] tool, a research assistant powered by artificial intelligence.

The data collected in Excel[®] tables included 1) publication information: title; first author; year of publication; format; language; citation, and DOI (digital object identifier); 2) general characteristics of the study: objective and design; type of autoimmune disease studied; classification criteria and IS treatment administered to the patient; type of biological sample collected for the study; patient selection criteria; study duration; sample size; number of patients; mean age; gender, and demographic data; 3) study methods and results: sample analysis and study techniques and results; association of outcome measures with microbiota study variables (richness, structure, and composition) of a niche at different times and in different individuals in relation to IS treatment; association measures used and their corresponding p-value, together with other relevant findings from the studies documented in free text format. Microbiota-related prognostic factors were categorised into alpha-diversity; beta-diversity; abundance, and taxonomic composition parameters¹² (Table 1).

Regarding the study characteristics and results for which information was not available, after checking Appendix A for additional data, they were left blank, except for the geographic location, which was assumed to be the first author's work centre.

Following this, a qualitative analysis and assessment of study heterogeneity was performed based on study design; methodology and analysis; disease and subgroups; measures and outcomes, and study quality.

The methodological quality of the included studies was assessed by one reviewer (NF), with any uncertainties resolved by another (LC) using the Newcastle-Ottawa Score (NOS). To maintain uniformity in the assessment of the studies' risk of bias, the NOS was applied to all studies, including one randomised controlled trial (RCT).

Due to the limited number of comparable studies by disease, methodological design, and outcome measures, publication bias was not assessed.

Results

The flowchart for study selection is shown in Fig. 1. The search strategy applied to the Medline, Embase, and Cochrane databases yielded 322, 2238, and 10 studies, respectively; A total of 2570 studies were included. An initial screening process eliminated 202 duplicate studies, and a further 2328 studies were eliminated by title and abstract. The remaining studies were examined in detail, and 20 studies were excluded (Fig. 1).

The characteristics of the included studies are shown in Table 2. Of the 20 studies finally selected, 15 were in article format and 5 were oral communications. The most studied rheumatological disease was rheumatoid arthritis (RA), with 15 studies, followed by systemic lupus erythematosus (SLE), with 3 studies. We also identified one study on systemic sclerosis (SSc) and another on primary Sjögren's syndrome (pSS). The total number of participants included by disease was 998 in RA, 117 in SLE, 133 in pSS, and 23 in SSc. The patients recruited in each study ranged from 22 patients in a controlled RCT of RA to 165 patients in a prospective observational study of the same disease. The mean age of patients ranged from 33 to 70 years, with the lowest mean age being concentrated in the SLE studies. Females were predominant in all studies, 100% in SLE and over 50% in the other diseases. Forty percent of the studies were conducted in Asia, 35% in the Americas, and the remaining 25% in Europe.

Eleven studies were conducted using a prospective observational design, allowing comparison of the microbiota of the same patient under different therapeutic conditions. One was retrospective; and seven were cross-sectional, comparing the microbiota between patient subgroups. The remaining seven observational studies were cross-sectional, comparing microbiota characteristics between patient subgroups. Most studies recruited a cohort of healthy controls (65%) or a validation cohort (15%), matched by age and sex to the cases. The assessment of the studies' risk of bias using the NOS ranged from 4 to 8 points, indicating high risk of bias in some cases and moderate and low risk of bias in most cases (Appendix A).

The IS therapies analysed in relation to the microbiota were highly varied, including conventional disease-modifying antirheumatic drugs (cDMARDs), biologics (bDMARDs), and targeted therapies (tDMARDs), as well as corticosteroids (CS), cyclophosphamide (CPM), mycophenolate mofetil (MMF), cyclosporine A (CyA), and dapsone. The most frequently studied IS treatment group was cDMARDs (85%), with methotrexate (MTX) being the most prevalent (55%). Additionally, two Chinese RA studies studied the action of specific components available in traditional Chinese medicine (TCM) practices: glycosides from Tripterygium

Table 2
Table of evidence. Characteristics of the included studies.

Author, year	Country	SARD type	SARD duration (years)	Study type	Length (weeks)	N	Age ^b	Woman (%) ^c	IS treatment	Outcome measurement
Ubeda, 2016 ^a	U.S.A.	AR	< 1	Prospective	12–192	33			MTX	
Isaac, 2019 ^a	U.S.A.	AR	< 1	Prospective	16	27			MTX	
Ormseth, 2020	U.S.A.	AR		Prospective	24	70	54	69	MTX with/without CS	Inflammatory markers (NSJ, NTJ, DAS28, RSR, IL-6, TNF-α)
								81	ADA with/without MTX/CS	Microbial-specific sRNA sequences derived from tRNA and rRNA, [sRNA] total microbial, tDR1, tDR3
Artacho, 2021	U.S.A.	RA	< 1	Prospective	16	26	42	73	TCZ with/without MTX/CS	DAS28
							44	76	MTX	
Gupta, 2021	U.S.A.	RA		Retrospective	24–48	32	65	66	CS, cDMARD, bDMARD	CDAI, MCII
Zaragoza-García, 2023	Mexico	RA	< 1	Cross-sectional	0	23	45	100	CS, MTX, CQ	Metabolic pathways DAS28, HAQ-DI Serum levels TNF-α, IL-10, IL-17A e IFABP2
Zhang, 2015	China	RA		Prospective	≥ 4	115			MTX and/or TCM	NSJ, NTJ, DAS28, APR, FR, ACCP
Mei, 2021	China	RA		Controlled RCT	24	22	48	86	MTX, TCM, LEF	DAS28-CRP
Koh, 2023	South Korea	RA	> 1	Prospective	24	94	57	93	CS, cDMARD (MTX, LEF, SSZ, HCQ), bDMARD	DAS28, ACCP
Qiao, 2023	China	RA		Cross-sectional	0	145	55	71	MTX, NSAIDs	Enterotypes
							54			APR, FR, ACCP, anti-MVC, lymphocyte number, CD4+ T, cytokine levels (IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, IFN-γ)
							52			
							67			
Gremese, 2019 ^a	Italy	RA		Cross-sectional	0	44	55	80	cDMARD, bDMARD	DAS28
Dos-Santos, 2021 ^a	Spain	RA		Prospective	8	47	55	85	CS, cDMARD, bDMARD, tDMARD	Periodontal disease, DAS28,
							43			ACCP, FR, APR
Marazzato, 2022	Italy	RA	< 1	Prospective	12	25	61	55	MTX, CS	Th17/Treg circulation and cytokines SCFA concentration
Morales-Águila, 2022 ^a	Spain	RA		Cross-sectional	0	110	56	80	bDMARD, tDMARD (± cDMARD)	DAS28, severity variables (erosions, ACCP level, HAQ)
Danckert, 2024	United Kingdom	RA	< 1	Prospective	12	95	54	73	MTX, HCQ, SSZ	DAS28, CDAI, MCII
De Araújo Navas, 2012	Brazil	SLE		Cross-sectional	0	40	33	100	CS, CQ, AZA, MMF, MTX, CyA, Dapsone, CPM	SLEDAI, lymphocyte and leukocyte count, serum creatinine level
Li, 2019	China	SLE		Cross-sectional	0	40	37	100	CS, HCQ, CPM, bDMARD	DMFT, stimulated salivary flow SLEDAI, complement C3, APR and anti-dsDNA levels
							46			
Guo, 2020	China	SLE		Prospective	≥ 8	37	34	100	CS	SLEDAI, serum immune cytokines (IL-1β, IL-6, IL-10, IL-35, TWEAK, IL-2R, IL-17, TNF-α, IL-8, IL-21, IFN-γ e IL-22)
							41			
							34			
							25			
Tan, 2023	Singapore	SSc		Cross-sectional	0	23	54	100	MMF, CPM, AZA, MTX	
Wang, 2022	China	pSS		Prospective	12–48	133	49	89	HCQ	ESSDAI, APR, xerostomia, xerophthalmia, laboratory pathological indicators
							48	88		

ACCP: anti-cyclic citrullinated peptide antibodies; ADA: adalimumab; Anti-MVC: anti-mutant citrulline vimentin antibody; APR: acute phase reactants, includes CRP, C-reactive protein and ESR, erythrocyte sedimentation rate; AZA: azathioprine; CDAI: Clinical Disease Activity Index; CPM: cyclophosphamide; CQ: chloroquine; CS: corticosteroids; CyA: cyclosporine; DAS28: Disease Activity Score; DMFT: Decayed, Missing, Filled Teeth; bDMARD: biologic disease-modifying antirheumatic drug; cDMARD: conventional disease-modifying antirheumatic drug; tDMARD: disease-modifying antirheumatic drug targeted therapy; IS: immunosuppressant; ESSDAI: EULAR Sjögren's syndrome (SS) disease activity index; HAQ: Health Assessment Questionnaire; HCQ: hydroxychloroquine; IFABP2: intestinal fatty-acid binding protein 2; IFN-γ: interferon gamma; IL: interleukin; LEF: leflunomide; MCII: minimal clinically important improvement; MMF: mycophenolate mofetil; MTX: methotrexate; N: number of enrolled patients; NSAIDs: nonsteroidal anti-inflammatory drugs; NTJ: number of tender joints; NSJ: number of swollen joints; pSS: primary Sjögren's syndrome; RCT: randomized controlled trial; rRNA: ribosomal RNA; RA: rheumatoid arthritis; SARD: systemic autoimmune rheumatological disease; SCFA: short-chain fatty acids; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity; SLICC: Systemic Lupus International Collaborating Clinics; sRNA: small RNA; SSc: systemic sclerosis; SSZ: sulfasalazine; TCM: traditional Chinese medicine; TCZ: tocilizumab; tDR: tRNA Derived Fragment; tRNA: transfer RNA; TWEAK: Tumour necrosis factor (TNF)-like weak inducer of apoptosis.

Bold: Prospective study or RCT with faecal samples (excluding oral communications).

^a Oral communication.

^b Mean age of the total patient group or, if not available, of the intervention groups. Rounded up.

^c % women of the total patient group or, if not available, of the intervention groups.

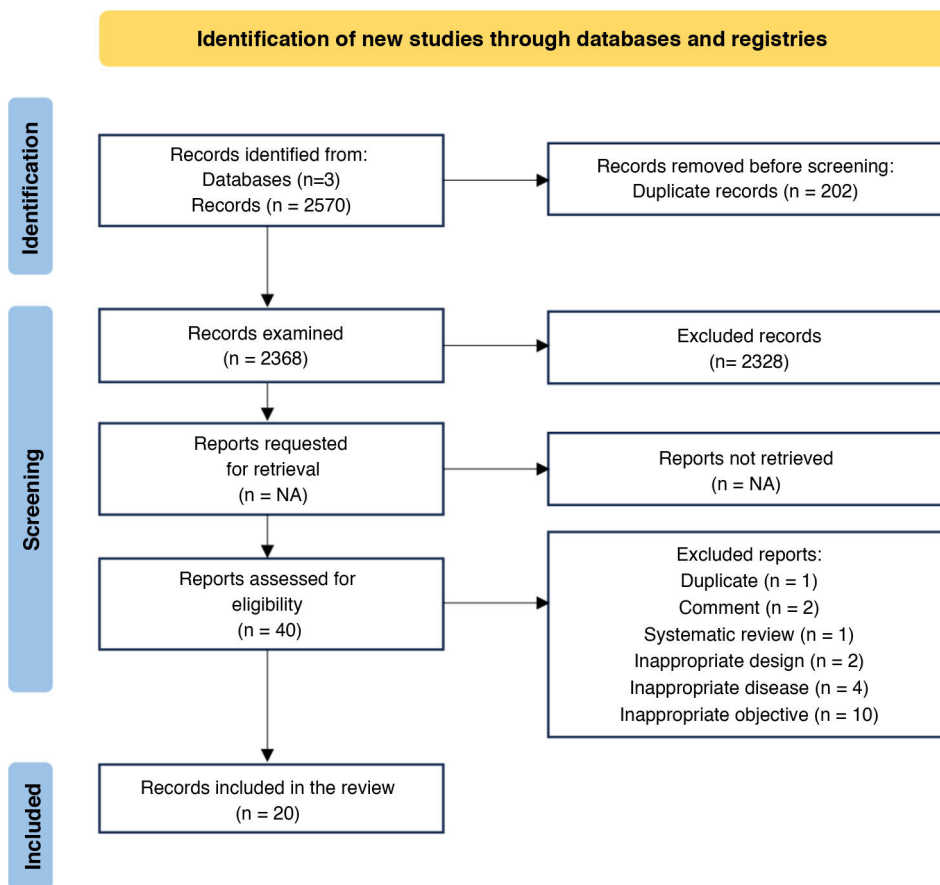


Figure 1. Flowchart of reviewed studies.

wilfordii (thunder god vine)¹³ and the Huayu-Qiangshen-Tongbi formula.¹⁴

Eighty-five percent of the studies analysed biological samples of faeces (17), of which three studies also analysed samples from other locations (saliva and dental plaque, saliva, and mouthwash and vaginal swab); and the remaining 15% (3) analysed exclusively non-faecal samples of mouth swab, mouthwash, and plasma¹⁵ (Table 3).

Sample processing was performed using genetic material sequencing in 17 studies, 16S rRNA variable region V3 and/or V4 sequencing in 10 studies, massive metagenomics in 8 studies, and sRNA sequencing in one study.¹⁵ One study used plate culture for the isolation and subsequent identification of microorganisms. The remaining studies did not provide any information on this subject. The subsequent DNA processing, extraction, sequencing, and bioinformatics analysis were performed using commercially available equipment.

Table 4 lists all the variables analysed in the studies and relevant to this review. Below, we review the results by disease.

Rheumatoid arthritis

Of the 15 included studies, 13 collected faecal samples; 2 of these also included saliva and dental plaque samples. The remaining 2 studies used plasma and oral swab samples. The limited representation of samples from the oral cavity and other types of samples limits their comparative analysis. Consequently, subsequent analysis will focus on faecal samples from the 13 studies.

Most of these studies recruited patients with recent-onset and/or DMARD-naïve RA.^{17–21} Eight studies were observational, prospective (7) and retrospective (1), with one RCT providing more

robust data. The remaining three studies were cross-sectional. All studies analysed the interaction of GM with cDMARDs, and specifically MTX in six of these studies.

Seven of the 13 studies directly exclusively analysed the interaction of IS therapy with GM. Two studies measured changes in GM based on changes in clinical activity in patients under treatment, allowing for inferences about the relationship between GM and IS therapy, and four studies employed direct and indirect outcome measures.

The most commonly used measure of association to measure alpha diversity was the Shannon index, followed by the Simpson index and the Chao index. Of the 9 studies with available alpha-diversity data, 89% presented statistically significant data, 5 directly and 3 indirectly.

Most studies use the Bray-Curtis Distance to measure beta diversity, with some studies using the UniFrac Distance and OTU (operational taxonomic unit). Of the 9 studies with available beta diversity data, 78% presented statistically significant results, 6 directly and one indirectly.

All studies analysed abundance changes using different measures, with the results being statistically significant in the 9 studies that defined it: 7 directly and one indirectly. Several studies agree on the existence of a baseline GM profile that could predict treatment response. In patients responding to treatment, Prevotella, Bacteroidetes, and Clostridiales species show a tendency to decrease in several studies. One study analysed the Firmicutes/Bacteroidetes ratio, and another compared two populations of RA patients based on the predominance of Prevotella or Bacteroidetes.

In the studies by Marazzato et al.¹⁷ and Úbeda et al.,¹⁸ patients who initiated treatment with MTX with or without CS reported

Table 3
Sample analysis and processing methods.

Author, year	Sample type	Study method	Extraction	Sequencing	Computational Pipeline	Taxonomic database	Taxonomic analysis method
Ubeda, 2016	Stool	16S rRNA V4	MoBio	Illumina MiSeq®	QIIME®		Two-tailed nonparametric Wilcoxon test
Isaac, 2019	Stool	16S rRNA Metagenomics					
Ormseth, 2020	Plasma	sRNA	Total RNA Purification Kits® (Norgen)	Illumina HiSeq®	TIGER®	SILVA®	Wilcoxon test
Artacho, 2021	Stool	16S rRNA V4	MoBio Powersoil®	Illumina MiSeq® NextSeq	VSEARCH®	SILVA®	Pearson chi-squared test Kaiju v1.7.0®
Gupta, 2021	Stool	Metagenomics	Qiagen® Power Faecal Kit	Illumina HiSeq®	HUMAnN2®	MetaPhlan2	MRLM
Zaragoza-García, 2023	Stool	Metagenomics	QIAamp® DNA stool Minikit			v2.7.8®	
Zhang, 2015	Stool, saliva, dental plaque	Metagenomics		Illumina®		IMG v400®	Wilcoxon test
Mei, 2021	Stool	Metagenomics	NucleoSpin® Soil kit	Illumina HiSeq®	HUMAnN2®	MetaPhlan2 v2.0®	Wilcoxon test
Koh, 2023	Stool	16S rRNA V3-V4	QIAamp® PowerFecal Pro DNA Kit	Illumina MiSeq®	QIIME2®	IMG® SILVA®	LEfSe
Qiao, 2023	Stool	16S rRNA V3-V4			Calypso® PICRUST2®	Ribosomal Database Project® (RDP)	LEfSe
Gremese, 2019	Stool	16S rRNA V3-V4		Illumina MiSeq®	QIIME (v1.9.1)®		STAMP® LEfSe
Dos-Santos, 2021	Buccal swab				VSEARCH (v1.1)®		
Marazzato, 2022	Stool	16S rRNA V3-V4	QIAGEN® kit	Illumina MiSeq®		Greengenes rDNA v13.10®	Mann-Whitney U test Wilcoxon test
Morales-Águila, 2022	Stool			Ion Torrent S5®	QIIME2®		
Danckert, 2024	Stool, saliva	Metagenomics		Illumina NovaSeq®	HUMAnN3®	Kraken2®/Bracken®	
De Araújo Navas, 2012	Mouthwash	Plate culture	Sample seeding, incubation and colony counting API 20 C AUX®, API 20 E® M API Staph®				
Li, 2019	Stool	16S rRNA V3-V4	LONGSEE STOOL DNA KIT®	Illumina MiSeq®	QIIME v1.9.1®	Greengenes®	LEfSe
Guo, 2020	Stool	16S rRNA V4	DNA extraction kit (#DP328®)	Illumina TruSeq® HiSeq®	PICRUST® QIIME2 v2018.2® PICRUST®		LEfSe
Tan, 2023	Stool	Metagenomics	QIAamp® Fast Stool Mini Kit	Illumina TruSeq® HiSeq®			Kaiju v. 1.7.3 «nr_2019-11-22»® LEfSe
Wang, 2022	Stool, mouthwash, vaginal swab	16S rRNA V3-V4	Qiagen® Gel Extraction Kit	Illumina TruSeq® HiSeq®	QIIME 2®	SILVA®	

16S rRNA: ribosomal ribonucleic acid; IMG: integrated microbial genomes; LEfSe: linear discriminant analysis effect size; MS: mass spectrometry; sRNA: small RNA. Bold: Prospective study or RCT with faecal samples (excluding oral communications).

a decrease in GM diversity and composition 3 mo after starting the IS treatment¹⁷ and small changes in GM diversity. They concluded that GM characteristics predict clinical response to MTX, including overgrowth of unclassified Coriobacteriaceae and related Coprococcus species.¹⁸

The results of Isaac et al.¹⁹ also reveal lower diversity, both alpha and beta, in patients who respond to MTX treatment.

In the 2024 study by Danckert et al.,²⁰ stool samples were taken at three time points: baseline, 6 weeks after, and 12 weeks after the start of cDMARDs. Changes in the patient’s microbiota were found,

Table 4
Parameters analysed in the studies.

Author, year	Alfa-Diversity	Beta-Diversity	IS therapy	Measurements	Others
<i>Ubeda, 2016</i>	Abundance	Unweighted UniFrac d	MTX	2 or more	
<i>Isaac, 2019</i>	Diversity Relative abundance	Bray-Curtis d	MTX	2	Ability in recent-onset RA to metabolize oral MTX and predict response Ex vivo incubation, NMRS, LC-MS
<i>Ormseth, 2020</i>	Composition (sRNA in plasma)		(MTX, ADA + /- MTX, TCZ + /- MTX) + /- CS MTX	2	
Artacho, 2021	OTUs observed Shannon I Relative abundance	Bray-Curtis d		4	Mechanisms of GM influencing response to MTX Residual MTX levels Ex vivo incubation NMRS y LC-MS
<i>Gupta, 2021</i>	Fisher I Abundance/Composition (cDMARD yes/no)	Bray-Curtis d	CS, CDMARD, BDMARD	2	Machine learning model to predict LMII based on baseline GM, clinical data, and dMSographic data H2O Python v3.26.0.3 [®] , Scikit-learn Python v0.24.1 [®]
<i>Zaragoza-García, 2023</i>	Abundance		MTX, CS	1	Correlation between activity and GM: Relative abundance – Ratio: Spearman correlation
Zhang, 2015	Relative abundance		MTX, TCM, MTX ± TCM	2	Determining whether dysbiosis occurs in OM Concordance and divergence between GM and OM
Mei, 2021	Shannon I Relative abundance	Bray-Curtis d (LFM: basal-t1 Vs basal-t6)	MTX + TCM/LFM	4	
Koh, 2023	Chao I (SSZ) Shannon I (SSZ) Simpson I Relative abundance	OTUs (SSZ)	CDMARD ± BDMARD BDMARD ± CDMARD SSZ, MTX, LFM, HCQ aTNF/ABA/TCZ aTNF/ABA MTX	2	Method to measure response prediction capacity ROC analysis (AUC)
<i>Qiao, 2023</i>	Chao I 1 Shannon I Simpson I Relative abundance/Composición	Bray-Curtis d		1	Enterotype-based index to predict response to MTX
<i>Gremese, 2019</i>	Diversity Relative abundance		CDMARD, BDMARD	1	
<i>Dos-Santos, 2021*</i>	Relative abundance		aTNF	1	Potential predictors of the presence of multiple species
Marazzato, 2022	OTUs observed Shannon I Simpson I Relative abundance	Adjusted Bray-Curtis d Bray-Curtis d Unweighted UniFrac d	MTX	2	
<i>Morales-Águila, 2022</i>	Abundance/Composition		BDMARD, FAMEtd	1	
Danckert, 2024	Chao I 1 Shannon I Simpson I Abundance	Adjusted Bray-Curtis d	CDMARD naïve/CDMARD en tratamiento	3	Predictive model for MCII Scattered partial least squares discriminant analysis (AUC, sPLS-DA)
<i>De Araújo Navas, 2012*</i>	Relative abundance		IS (CS, CQ, AZA, MMF, MTX, CyA, Dapsona, CPM)	1	
<i>Li, 2019</i>	Species observed Shannon I Relative abundance	Unweighted UniFrac d Weighted Unifrac	CS, HCQ, CPM, BDMARD	1	Correlation of GM dysbiosis and corresponding metabolic pathways Kruskal-Wallis test, Kruskal-Wallis test, KEGG (Heatmap)
Guo, 2020	OTUs observed Shannon I Faith's phylogenetic diversity Pielou's evenness Relative abundance/composition	Unweighted UniFrac d	CS	2	Dysregulation of serum immune cytokine levels: associated genera, specific genera correlations NTJos Predictive model to evaluate treatment efficacy based on a biomarkerr ROC (AUC) analysis
<i>Tan, 2023</i>	Shannon-Weber I Relative abundance/Composition	Bray-Curtis d	IS (MMF, CPM, AZA, MTX)	1	Functional profile (metabolic pathways) KEGG (Heatmap)
Wang, 2022	Alfa-diversity (Treatment no/yes 1) Relative abundance	Bray-Curtis d	HCQ	3	Relationship between microbiota in different body areas

Green: statistically significant; Red: not statistically significant; Black: no data; Yellow: non-faecal sample.

ABA: abatacept; AUC: area under the curve; ADA: adalimumab; ANOSIM: Analysis of Similarities; RA: rheumatoid arthritis; aTNF: anti-tumor necrosis factor; AZA: azathioprine; CPM: cyclophosphamide; LC-MS: liquid chromatography-mass spectrometry; CQ: chloroquine; CS: corticosteroids; CyA: cyclosporine A; E-NMRS: nuclear magnetic resonance spectroscopy; bDMARD: biologic disease-modifying antirheumatic drug; cDMARD: conventional disease-modifying antirheumatic drug; tDMARD: targeted disease-modifying antirheumatic drug; HCQ: hydroxychloroquine; I: index; IS: immunosuppressant; KEGG: Kyoto Encyclopaedia of Genes and Genome; LDA: linear discriminant analysis; LEfSe: linear discriminant analysis effect size; LFM: leflunomide; GM: gut microbiota; MCII: minimal clinically important improvement; MMF: mycophenolate mofetil; MLRM: multiple linear regression models; TCM: traditional Chinese medicine; MTX: methotrexate; OM: oral microbiota; OTU: Operational Taxonomic Unit; PCoA: principal components analysis; SSZ: sulfasalazine; TCZ: tocilizumab.

Bold: Prospective study or RCT with faecal samples (excluding oral communications).

showing clinical improvement after the start of cDMARDs and a reduction in the relative abundance of microorganisms predominant in the microbiota of RA patients: *Prevotella* spp. at 6 weeks and *Streptococcus* spp. at 12 weeks. These findings suggest that treatment with DMARDs restores the immune system to a eubiotic state and, therefore, that the biodiversity characteristics of the immune system could be used to predict the likelihood of response to DMARDs.

Artacho et al.²¹ evaluated the clinical response to MTX at 16 weeks of treatment, demonstrating significant differences in the diversity and composition of the baseline immune system between responders and non-responders. Their findings suggest that the immune system may be involved in MTX metabolism, modulating its bioavailability and therapeutic efficacy. Based on these results, the authors proposed the development of a model based on the prediction of non-response to MTX based on GM diversity parameters.

The work published in 2023 by Koh et al.²² classified patients based on their disease activity into moderate and high according to the Disease Activity Score 28 (DAS28), and the treatment they received, cDMARDs or bDMARDs, with a DAS28 reassessment at 6 mo. Differences in GM diversity were studied based on the type of IS treatment (monotherapy or combination therapy). No differences in GM diversity were identified based on the IS administered, except for sulfasalazine (SSZ), which significantly reduces GM richness and uniformity. When evaluating the taxonomic composition based on the response to cDMARDs, responding patients showed an increase in *Fusicatenibacter*, *Subdoligranulum*, and uncultured *Clostridia* genus 014; and a decrease in *Faecalitalea*. The combination of *Fusicatenibacter* and *Subdoligranulum* distinguishes responders from non-responders to a second line of treatment.

The retrospective study by Gupta et al.²³ analyses therapy with cDMARDs, bDMARDs, and SC, in which GM diversity is associated with disease activity, and microbial composition with cDMARD treatment.

The only registered RCT, by Mei et al.,¹⁴ compares the effects of treatment with MTX + TCM (Huayu-Qiangshen-Tongbi formula) versus MTX + leflunomide (LFM) in GM. Both treatments reduce various clinical indices of disease activity (number of tender joints, number of swollen joints, and morning stiffness) at 3 mo in the case of TCM and at 6 mo in the case of LFM. However, microbial diversity does not differ significantly over time, except for beta diversity, which increases in patients treated with LFM when compared at 1 mo and 6 mo after starting treatment. Regarding the relative abundance of species, there were no significant changes over time in patients treated with LFM, while there were in the TCM group. However, *Prevotella* spp., the predominant microorganism in RA patients, did not show significant differences between the two groups.

Zhang et al.¹³ compared the relative abundance of various niches of the oral microbiota and GM. Dysbiosis of both microbiotas was observed in RA patients when compared to healthy controls, but it partially recovered upon initiation of IS treatment. Furthermore, there were concordant changes associated with RA between the two microbiotas, suggesting an overlap in the abundance and function of species in different body locations. Treatment with TCM, specifically glycosides of the *Tripterygium wilfordii* component, reduced the number of MLGs (metagenomic linked groups) enriched in the gut, *H. filiformis* and *Bacteroides* spp., to a greater extent than treatment with MTX or MTX + TCM.

Systemic lupus erythematosus

Regarding the studies conducted in patients with SLE, two analysed faecal samples and one analysed mouthwash. Two of the studies had a cross-sectional design, and the third was prospec-

tive. The GM studies determined alpha and beta diversity, as well as relative abundance, using the same measures for alpha diversity (Shannon index) and beta diversity (unweighted UniFrac distance and its respective PCoA expression measure).^{24,25} The results of Guo et al.²⁴ showed greater alpha diversity in patients without corticosteroid treatment, while Li et al.²⁵ found no relationship between IS treatment and microbial diversity, partly explained by the small number of patients recruited without IS treatment.

The work of Guo et al.²⁴ highlights the variation in the *Bacteroidetes*/*Firmicutes* ratio depending on treatment with/without CS, with the *Firmicutes* population being restored when CS was administered. These results suggest that the *Firmicutes*/*Bacteroidetes* ratio could be used as a diagnostic and treatment response biomarker. In the taxonomic composition analysis by Gou et al., some differentially abundant taxa were recognised in each of the groups. In SLE patients treated with CS, there was an increase in *Bifidobacterium* and *Streptococcus*, and a decrease in *Enterobacteriales*; and in the SLE group not treated with CS, there was an increase in *Bacteroidetes* and *Bilophila*.

In the study by De Araújo et al.,¹⁶ mouthwash samples were processed using plate culture, which may have led to an underestimation of microbiota diversity and abundance, as well as limiting comparisons with other studies. Their results did not reveal significant differences based on IS treatment.

Systemic sclerosis

The cross-sectional observational study by Tan et al.²⁶ in patients with SSc did not generate significant results, but a tendency toward decreased alpha diversity was inferred in patients receiving IS treatment, moving away from the profile of untreated patients and closer to that of healthy controls.

Primary Sjögren's syndrome

Wang et al.²⁷ recruited patients with pSS and collected stool, saliva, and vaginal swab samples. An increase in microbiota richness was recorded in all three niches at 3 and 6 mo after starting treatment, but only at the intestinal level was this increase sustained at 6 and 12 mo. The overall microbiota composition at all three sites showed significant changes since the start of treatment, which remained unchanged in the GM and oral areas, but was still different from that of healthy controls. Certain phyla showed significant changes in relative abundance; *Fusobacteria* stands out, initially increasing and then decreasing.

Discussion

This study analysed whether there is an interaction between HM and IS treatment in SARDs patients through a systematic review. The analysis of 20 studies suggests a complex interaction between HM and IS therapy in SARDs patients, the understanding and management of which could optimise clinical response and advance toward more personalised medicine.

The main findings were: 1) there are few studies that focus on evaluating the interaction between HM and SARDs patients; 2) there is considerable heterogeneity among the studies conducted in terms of design, profile of patients recruited and treated, and sample and data collection and processing; 3) most studies were conducted in patients with RA, given its higher prevalence compared to other SARD patients; 4) virtually all studies analysed both alpha and beta diversity to obtain a comprehensive view of HM function in these diseases. However, the variety of measurement methods applied complicated their comparison; 5) few studies analysed microhabitats other than the intestinal microhabitat, so this review is limited to comparing GM; 6) a tendency toward

decreased GM diversity is observed in patients receiving IS treatment with good clinical response; 7) although several of the RA studies had a similar design, the lack of consistency between the measures used hampered their comparison and limited the possibility of combining them for meta-analysis.

There are several limitations to this study: 1) the review was not registered in PROSPERO, but to ensure transparency of the process, the protocol and methodological details are available upon request; 2) data extraction was performed by a single reviewer, assisted by artificial intelligence tools, which could have led to errors or omissions and should be considered when interpreting the results; 3) abstracts of oral communications were included, which tend to offer less methodological detail and are subject to less rigorous review, but their inclusion was justified by the scarcity of available studies and the relevance of the recent data they could provide; 4) marked methodological heterogeneity was observed among studies, ranging from cohort characterisation and sample collection and processing procedures to the diversity indices used and the taxonomic analysis strategies applied; 5) although most studies assessed alpha and beta diversity of the microbiota, heterogeneity in methodological approaches and the predominantly GM-focused approach limited the possibility of direct comparisons between studies, as well as with results obtained in other microbiological niches; 6) clinical-therapeutic variability, despite the predominance of studies in RA patients treated with MTX, together with the concomitant use of several IS, made it difficult to attribute changes in GM to a specific drug; 7) The small sample size of individual studies, their moderate overall quality, and considerable heterogeneity among them limit the robustness and generalisability of combined conclusions, as well as the development of a meta-analysis.

Furthermore, the diseases and treatments studied varied (15 studies in RA versus only a few in other diseases, and a diverse range of immunosuppressive therapies), and most studies analysed GM exclusively (faecal samples), while very few examined other body sites. The authors found marked heterogeneity among studies in multiple dimensions, from the way patients were profiled and samples were collected/processed, to the diversity indices and taxonomic analysis methods used.

The interaction between GM and IS therapy has been addressed primarily through a bacterial lens, with little attention paid to fungal and viral factors. Consequently, the included studies do not provide relevant findings in this regard. Furthermore, none of the studies evaluated the relationship between microbial functional pathways and IS therapy, which limits the possibility of interpreting the results with biological plausibility.

In conclusion, the individual studies provide relevant evidence that changes in HM could be related to the response to IS treatments in SARDs, although the moderate overall quality and considerable heterogeneity in the studies' approach to this association limit the robustness and generalisability of combined conclusions. Standardisation of HM studies is necessary to combine results and draw conclusions with greater confidence.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.reumae.2025.501938>.

Declaration of competing interest

The authors have no conflict of interests to declare.

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