U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2022/2023

Sara Miguel Cardeal Dourado

O Papel do Microbioma nas Doenças Cardiovasculares: uma Revisão Sistemática

The microbiome role in Cardiovascular Diseases: A systematic review

março, 2023

Sara Miguel Cardeal Dourado

O Papel do Microbioma nas Doenças Cardiovasculares: uma revisão sistemática

The microbiome role in cardiovascular diseases: A systematic review

Mestrado Integrado em Medicina

Área: Doenças Cardiovasculares

Tipologia: Dissertação- Revisão sistemática

Trabalho efetuado sob a Orientação de:
Professora Doutora Isabel Miranda

E sob a Coorientação de:

Professor Doutor António Barros

Trabalho organizado de acordo com as normas da revista:

Revista Portuguesa de Cardiologia

março, 2023

Index

UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

Eu, Sara Miguel Cardeal Dourado, abaixo assinado, nº mecanográfico 201604604, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 18 / 01 / 2023

Assinatura conforme cartão de identificação: Sara Miguel Cardeal Dourado

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Sara Miguel Cardoal Dourado

NÚMERO DE ESTUDANTE | E-MAIL

201604604 | saradourado22@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Doenças Cardiovasculares

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

The microbiome role in Cardiovascular diseases: a systematic review.

ORIENTADOR

Isabel Alexandra Marques Miranda

COORIENTADOR (se aplicável)

António Sousa Barros

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 18/01/2023

Assinatura conforme cartão de identificação: Sara Miguel Cardoal Dourado

Agradeço à minha família, por tudo o que representa na minha vida e por acreditarem em mim.

Agradeço à minha orientadora e às pessoas da equipa do Departamento de Cirurgia e Fisiologia da FMUP por todo o apoio prestado.

The microbiome role in cardiovascular diseases: A systematic review

Sara Dourado [a], Diana Martins [a], Ana Albuquerque [a], António S. Barros [a, #], Isabel Miranda [a, #]

[a] Cardiovascular R&D Centre - UnIC@RISE, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto

[#] These authors contributed equally to this work

Word Count: 3996

Corresponding author:

Sara Miguel Cardeal Dourado

saradourado22@gmail.com

Cardiovascular R&D Centre – UnIC@RISE, Department of Surgery and Physiology
Faculty of Medicine of the University of Porto
Alameda Prof. Hernâni Monteiro
4200-319 Porto, Portugal

RESUMO

Introdução: As doenças cardiovasculares são a maior causa de morbilidade e de mortalidade mundialmente. Apesar de cada vez mais estudadas, a sua complexidade tem justificado a relevância de procurar novos mecanismos fisiopatológicos associados de forma a promover estratégias terapêuticas mais eficazes. Recentemente, o papel desempenhado pela microbiota intestinal nas vias inflamatórias e metabólicas tem sido explorado e considerado relevante na progressão das doenças cardiovasculares, embora em termos mecanísticos o conhecimento seja insípido.

Objetivo(s): O objetivo deste estudo é sistematizar e avaliar a relação entre o microbioma intestinal e as doenças cardiovasculares, em estudos baseados apenas na população Humana.

Métodos: A pesquisa bibliográfica foi feita através da MEDLINE e da Web of Science. De acordo com as orientações PRISMA, foram incluídos apenas estudos observacionais e experimentais, realizados em humanos, que avaliassem o microbioma intestinal em doentes com Fibrilhação Auricular (FA), Insuficiência Cardíaca (IC) e Acidente Cerebrovascular (AVC).

Resultados: Globalmente, e considerando a classe de metabolitos, verificam-se níveis elevados de TMAO (N-óxido de trimetilamina) nas patologias cardiovasculares quando doentes são comparados a controlos. Relativamente à microbiota intestinal, os filos predominantes foram as Actinobacteria, Bacteroidetes, Firmicutes e as Proteobacteria. Na FA, as amostras estavam enriquecidas com os géneros: *Bacteroides, Parabacteroides, Enterococcus, Dorea, Ruminococcus,* e *Streptococcus*. Na IC, comprovou-se um aumento de *Streptococcus* e *Veillonella*. Nos estudos relativos ao AVC, constatou-se um aumento da família *Enterobacteriaceae* e do seu género *Enterobacter*.

Conclusão: Apesar da falta de informação quantitativa dos metabolitos e da microbiota intestinal por parte dos trabalhos incluídos, este estudo suporta a existência de uma relação entre os mecanismos fisiopatológicos das doenças cardiovasculares e o microbioma intestinal. Este trabalho demonstra que os trabalhos, de forma geral, são bastante heterógenos, sem poder amostral, o que torna frágil a evidência entre a microbiota e algumas doenças cardiovasculares.

Palavras-chave: Doenças cardiovasculares; Metabolitos; TMAO; Fibrilhação auricular; Acidente vascular cerebral; Insuficiência cardíaca; Microbioma.

ABSTRACT

Background: Cardiovascular Diseases **(**CVD) are a set of heterogeneous diseases affecting the heart and blood vessels whose underlying cause of the development is most often atherosclerosis. The basic mechanisms of atherosclerosis involve a complex interaction of vasculature, the immune system, and lipid metabolism. The gut microbiome plays a role in these mechanisms, with most of the contributions related to microbial metabolites. Therefore, it is crucial to clarify the link between the gut microbiome and cardiovascular diseases in humans to find new possible therapeutic pathways for the foreseeable future.

Objectives: The purpose of this study is to systematize and evaluate the relationship between the gut microbiome and CVD, in human-based studies.

Methods: The bibliographic research was carried out at MEDLINE and Web of Science. Based on PRISMA Guidelines, were included human-based observational and experimental studies assessing gut microbiome and CVD, namely Atrial Fibrillation (AF), Heart Failure (HF) and stroke.

Results: Overall, when compared with controls, higher TMAO levels were associated with CV diseases' patients. Relatively to the gut microbiota, the predominant phyla were Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. In AF, patients' samples were enriched with the genera *Bacteroides, Parabacteroides, Enterococcus, Dorea, Ruminococcus,* and *Streptococcus*. In HF patients, there was an increase in the genera *Streptococcus* and *Veillonella*. Studies with stroke patients reported the enrichment of the family *Enterobacteriaceae* and its genus *Enterobacter*.

Conclusions: Despite the lack of quantitative data regarding metabolites and microbiota, this study supports a relationship between the pathophysiology of CVD and the gut microbiome. However, this work also demonstrates heterogenicity among studies, mostly without sample power, that affect the construction of a strong evidence between the gut microbiome and CVD.

Keywords: Cardiovascular diseases; Microbiota; Metabolites; TMAO; Atrial Fibrillation; Stroke; Cerebrovascular accident; Heart Failure; Microbiome.

Introduction

By definition, Cardiovascular Diseases (CVD) are illnesses that affect the heart and blood vessels of our body[1]. They can be divided accordingly by the region of the affected blood vessels. On the one hand, we have coronary heart disease (a disease of the blood vessels supplying the heart muscle); cerebrovascular disease (a disease of the blood vessels supplying the brain); peripheral arterial disease (a disease of blood vessels supplying the arms and legs); rheumatic heart disease (damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria); congenital heart disease (birth malformations that affect the heart); heart rhythm problems (such as atrial fibrillation), and deep vein thrombosis (blood clots in the leg veins, which can dislodge and travel to the heart and lungs)[1]. On the other hand, we have myocardial infarction and stroke (acute episodes mainly caused by a disturbance that prevents blood from flowing to the heart or brain, respectively). Stroke is an acute neurologic condition resulting in either ischemia (formation of blood clots) or hemorrhage (bleeding from a blood vessel). The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain, leading to arterial wall thickening (hardening) and elasticity loss[1]. This process is called atherosclerosis.

Certain risk factors like smoking, high blood pressure/hypertension, high cholesterol/dyslipidemia, unhealthy diet, lack of exercise, type 2 diabetes and obesity are heavily linked with the risk of CVDs[2].

In the modern age, cardiovascular diseases became the most relevant cause of morbidity and mortality worldwide. Approximately 17.9 million people died from CVD in 2019, representing 32% of all global deaths [3].

As a major concern of global health, researchers have tried to understand and prevent the complex and multifactorial pathophysiology of CVD. Studies have shown that microbiota is involved in the occurrence and development of CVD and its risk factors [4].

The human microbiota consists of trillions of symbiotic microbial cells, and the gastrointestinal tract is the most diverse body site with 500-1000 species, including bacteria, viruses, and fungi [5]. Bacteroidetes and Firmicutes are the two phyla more representative, constituting more than 90% of the total bacterial

community[6]. Indeed, they provide essential nutrients, produce vitamin K, help the digestion of cellulose, and stimulate angiogenesis and enteric nerve function[7].

The gut microbiota functions like an endocrine organ that produces bioactive metabolites, which can be absorbed into the circulation and serve as mediators of gut microbial effects on the host [8]. The production of these metabolites occurs from bacterial metabolism of dietary substrates, modification of host molecules or directly from bacteria[9]. Metabolites like short-chain fatty acids (SCFAs) and bile acids (BA) increased levels are associated with antiatherogenic properties[10,11]. In contrast, trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS), and uremic toxins are associated with pro-atherogenic properties [11].

SCFAs, such as acetic acid, propionic acid and butyric acid, have been proven critical in modulating the pathological atherosclerosis process, attenuating lipid profile and reactive oxygen species, and reducing monocyte adhesion, cholesterol aggregation, macrophage inflammation and foam cell formation. Moreover, SCFAs are associated with reduced blood pressure and less incidence of cardiovascular mortality. In HF, SCFAs play a role in cardiac function improvement, suppression of fibrosis and hypertrophy development by modulating inflammatory responses [12]. Increasing evidence demonstrates that SCFAs play a part in stroke modulation and post-stroke recovery, controlling blood-brain barrier structure, metabolism, inflammation, and gut microbiota dysbiosis [13]. Also, SCFAs display a protective effect on AF development by alleviating atrial remodelling [14].

The relationship between BA synthesis and gut microbiota is bidirectional. BA regulate gut bacteria overgrowth, protect against invasive organisms, and exert anti-inflammatory responses [15]. While the gut microbiota plays a role in the biotransformation into secondary BA, impacting BA composition affects lipid and glucose metabolism via activating different receptors (such as farnesoid X receptor, vitamin D receptor, Takeda G-protein-coupled receptor 5, sphingosine-1-phosphate, muscarinic receptors, and big potassium channels) [16].

Regarding TMAO, it plays a significant role in CVD (Supplementary Figure 1). Gut microbiota metabolizes choline, betaine, and carnitine nutrients from the diet, generating trimethylamine (TMA). Then, TMA is absorbed into the bloodstream and oxidized to TMAO in the liver. Acting as a proatherogenic factor, TMAO

promotes macrophage migration and foam cell formation and increases the expression of inflammatory cytokines (TNF-α and interleukin 6) [17].

About LPS, these bacterial glycolipids can enter the systemic circulation, inducing a pro-inflammatory state, and are associated with an enhanced risk of major adverse cardiovascular events [18].

Regarding uremic toxins, gut dysbiosis resulting from renal dysfunction in chronic kidney disease is associated with its secretion, promoting CVD [19].

Besides TMAO, mainly indoxyl sulfate and p-cresyl sulfate lead to endothelial inflammation and loss of endothelial integrity conducting to atherosclerosis [20].

In this line of thought, altered gut bacterial composition (dysbiosis) can be harmful[21]. Indeed, *Tabata et al., 2020* verified that the poorer the gut microbiota the higher is the metabolic and chronic inflammation leading to more diseases[22].

Environmental factors, dietary habits, genetics, antibiotics, and comorbidities are the leading causes of this microbial diversity and composition alterations[23].

Cardiovascular diseases, proceed with the same logic. The body passes through many physiological disparities[7] (neuro-hormonal activation, overload of volume and pressure, venous and pulmonary congestion, microcirculatory lesions, among others) that lead to a higher intestinal barrier permeability, contributing to a bacterial and endotoxin translocation from the intestines. This process will increase even more systemic inflammation through multiple cytokines like interleukin 1, interleukin 6, and tumor necrosis factor (TNF)[24].

Thus, this study aims to reveal the relationship between microbiome and CVD, in humans.

Methods

Materials and Methods

Study Design

This systematic review adhered to PRISMA (Preferred Reporting Items for Systematic Reviews) guidelines [25] (see Appendix).

Literature Search

A literature search was conducted on MEDLINE, and Web of Science, representing a broad search of cardiovascular diseases research. No date restrictions were applied on any of the databases. For the nominated databases, search keywords were developed and applied (Table 1).

Table 1. Keywords used in the Literary search conducted on MEDLINE and Web of science on the 22nd of November 2022.

#1	ALL (stroke OR cerebrovascular AND accident OR cerebrovascular AND disease OR heart AND failure OR cardiac AND failure OR atrial AND fibrillation)
#2	ALL (microbiome OR microbiota OR gut AND microbiota OR intestinal AND microbiota OR intestinal AND bacteria OR bacterial AND communities OR commensal AND bacteria OR dysbiosis OR mycobiome OR gastrointestinal AND microbiome OR gut AND flora)
#3	ALL (metabolome OR metabolite OR metabolism OR metabolic OR metagenome OR biomarker OR gut AND derived AND metabolites OR microbiota AND derived AND metabolites)

Eligibility Criteria

Studies on the following cardiovascular diseases were selected: stroke, atrial fibrillation and heart failure. Observational studies were considered if they involved human participants and evaluated plasma levels of TMAO as well as other metabolites such as bile acids, short-chain fatty acids, and other microbiota metabolites. Additionally, studies that provided data on the gut microbiome were included.

Meta-analyses, systematic reviews, reviews, editorials, animal or *in vitro* studies were excluded. Additionally, any study in which it was not possible to extract data was also excluded from further analyses.

Paper Screening

Titles and abstracts attained from the search strategy were reviewed by three independent researchers (SD, DM, AA). The workflow process of literature assessment used Rayyan software. Ensuing this, after full text analysis eligible studies were included. Any disagreement was resolved through discussion and all the included articles are presented in Supplementary Table 1.

Data Extraction

Significant data were extracted by one researcher (SD) onto two worksheets representing metabolites levels and taxa associated with CVD (Supplementary tables 3, 4 and 5). Additional information regarding all the extracted data is available in the supplementary material, namely from the included articles: first authors, year of publication, countries where the studies were performed, number of controls and patients, methods of taxa assessment and metabolites quantification. In addition, all the references are also noted in the supplementary material.

Synthesis methods

From the analysed studies, it wasn’t possible to obtain quantitative data of the microbial metabolites, with the exception of TMAO. Therefore, TMAO levels (μM) are represented in Table 3. Taxa associated with CVD was qualitatively categorized, either as increased or decreased relatively to the control samples. Meta-analyses were performed for Stroke’s TMAO values using both models of

common and random effect. Heterogeneity was quantified accordingly with I^2 test.

Quality assessment

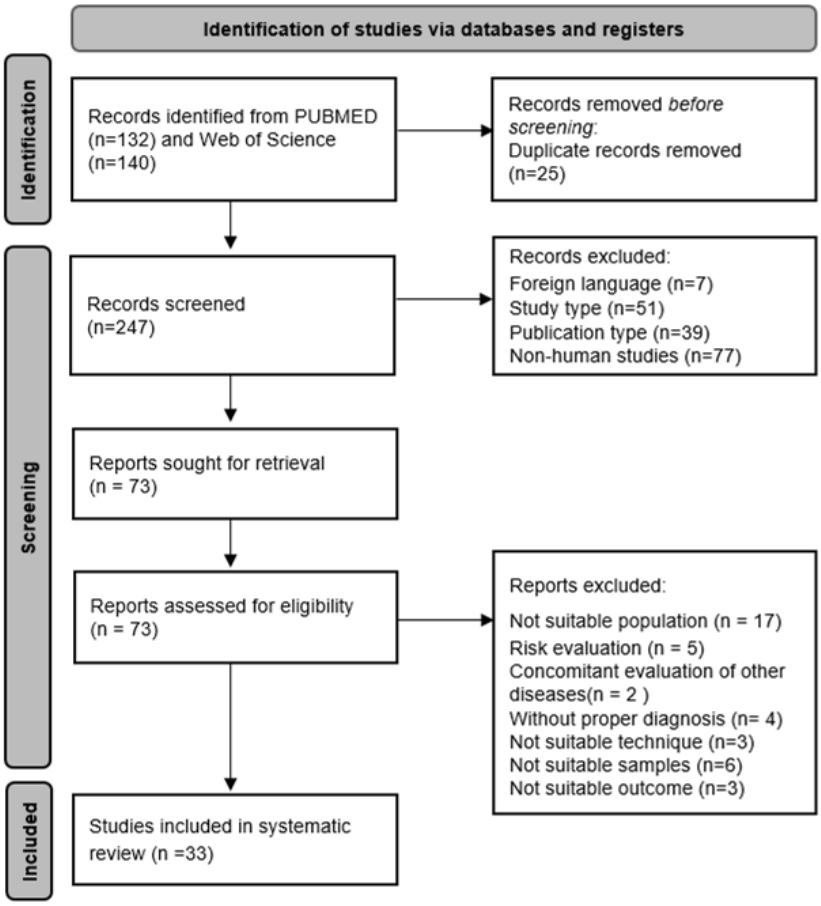
The quality and validity of the included studies were assessed using the Study Quality Assessment Tools (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools) developed by NHLBI (National Heart, Lung and Blood Institute). Evaluations were answered with “No”, “Yes”, or “Cannot determine/Not applicable/Not reported” and were scored according to the number of “No” responses. Therefore, studies were classified as good, fair or poor if the number of “No” responses were ≤2, between 3 and 4 or >4, respectively. In general terms, a good study has the least risk of bias, and results are considered to be valid. A fair study is susceptible to some bias. A poor rating indicates significant risk of bias. This assessment was performed by two researchers (SD and DM).

Results

Selection of gut microbiome-CVD studies

In the initial identification, 247 articles were screened, after the removal of duplicate records. After title and abstract screening, 73 studies were full text analysed. In the end, a total of 33 studies met the inclusion criteria advancing to data extraction (Figure 1).

Figure 1 - Flowchart representing the included and excluded articles during the different stages of the systematic review.



Characterization of gut microbiome-CVD studies

Supplementary table 1 summarizes the main characteristics of the 33 selected articles[22,26–57]. We included studies from 2011 to 2022, with most published in 2020 (n=10). The Asian continent accounts for 27 conducted studies, including 23 with Chinese cohorts. Europe conducted 5 studies and Australia accounts for

one study. Regarding study types, 30 are observational, and three are experimental.

This analysis included a total of 4195 cases and 3134 controls comprising three major cardiovascular diseases atrial fibrillation (n=7), heart failure (n=7), and stroke (n=19). Regarding study purposes, 25 carried out microbial identification. Within these, 16 carried out microbiota-derived metabolites identification. In addition, 8 articles exclusively performed metabolites analysis, with TMAO being the most evaluated target metabolite (n=7). The methods used for microbial characterization were 16S rRNA gene sequencing (n=18) and whole-genome shotgun sequencing (n=7), with stool as the biological sample. The metabolite analyses were performed using mass spectrometry coupled with different chromatography techniques (n=22). In 15 articles, blood plasma was the predominant biological sample used for metabolic analysis, while 7 used blood serum.

Quality Assessment of gut microbiome-CVD studies

For stroke studies, the quality and validity assessment suggested that 37% of studies were qualified as good and 63% of studies were categorized as fair. In HF studies, 29% of studies were considered of good quality and 71% of studies were given as fair. For AF studies, 71% of studies were qualified as good and 29% of studies were categorized as fair. None of the included studies incorporated a sample size calculation (Supplementary Table 2).

Taxa associated with CVD

Across all pathologies, the predominant gut microbial phyla were Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Within each pathology, some studies reported heterogeneous results (Table 2).

In AF, patients reported an enrichment in genera *Bacteroides* [34,44,47], *Parabacteroides* [22,47], *Enterococcus* [33,47], *Dorea* [22,33,34,37], *Ruminococcus* [22,33,37,44], and *Streptococcus* [22,33,44,47], whilst a depletion of *Butyricicoccus* [33,34]. At the species level, the studies reported increased levels of *Bacteroides vulgatus* [34,44]. However, the relative abundance regarding some genera, such as *Prevotella* [33,34,44,47] and its specie *Prevotella copri* [34,37,44], *Alistipes* [22,33,47], *Eubacterium* [33,34,37,47], *Blautia* [33,34,37,47], *Faecalibacterium* [33,34,37,44,47] and its

specie *Faecalibacterium prausnitzii* [33,34,37,44], were heterogeneous across studies. Studies including HF patients reported an increase in genera *Streptococcus* [30,48] and *Veillonella* [30,48]. Studies with stroke patients reported enrichment of the family *Enterobacteriaceae* [41,51,54] and its genus *Enterobacter* [26,35]. Moreover, the relative abundance of genera *Bifidobacterium* [27,41,42,57], *Phascolarctobacterium* [35,41], *Clostridium XIVa* [38,39], *Lactobacillus* [41,51,57], *Megasphaera* [26,41], *Desulfovibrio* [26,57], *Klebsiella*[51,57], *Streptococcus*[38,51], *Enterococcus* [41,55,57] were found to be increased in stroke patients. Data regarding genera *Bacteroides* [26,27,32,35,38,39,41,51,54,55], *Parabacteroides* [32,38,41,57], *Akkermansia*[27,35,41,50,54], *Prevotella* [26,27,32,35,38,41,57], *Roseburia* [32,35,38,51,54], *Faecalibacterium* [26,27,32,35,38,51,54], *Ruminococcus* [27,32,39,50,57], *Megamonas* [27,32,55], *Escherichia* [27,32,35,38,51,55], and *Shigella* [35,38,47,51] were heterogeneous across studies.

Table 2– Number of articles in which the main taxa composing the gut microbiota of CVD patients are increased  or decreased  relatively to the control samples.

			Atrial Fibrillation		Heart Failure		Stroke	
Phylum	**Family**	**Genus / Species**	↑	↓	↑	↓	↑	↓
Actinobacteria				1	1		5	
	Bifidobacteriaceae	*Bifidobacterium*	1		1		4	
		Bifidobacterium longum	1					
		Metascardovia					1	
		Parascardovia					1	
	Corynebacteriaceae	*Corynebacterium*	1					
	Eggerthellaceae	*Adlercreutzia*					1	
Actinomycetota	*Coriobacteriaceae*						1	
		Coriobacteriales					1	
		Eggerthella					1	
Bacteroidetes				2	1		5	1
	Saprospiraceae	*Sphingobacteriales*					1	
	Bacteroidaceae	*Bacteroides*	3		1		6	4
		Bacteroides vulgatus	2					
	Flavobacteriaceae	*norank_p_Flavobacteriaceae*					1	
	Odoribacteraceae	*Butyricimonas*	1					
		Odoribacter					2	
	Porphyromonadaceae						1	
	Prevotellaceae	*Paraprevotella*		1				
		Prevotella	2	2			4	3
		Prevotella copri	2	1				
	Rikenellaceae	*Alistipes*	2	1			1	
	Tannerellaceae	*Parabacteroides*	2				4	1
Cyanobacteria							1	
Euryarchaeota							1	
	Methanobrevibacteriaceae	*Methanobrevibacter*						
		Methanobrevibacter smithii	1					
		Methanobrevibacter curvatus	1					
Firmicutes			1	1	1		5	1
		Firmicutes bacterium CAG:95		1				
	Acidaminococcaceae	*Acidaminococcus*					1	
		Thermosinus	1					
		Phascolarctobacterium					2	
	Christensenellaceae	*Christensenellaceae_R-7_group*	1				1	
	Clostridiaceae	*Butyricicoccus*		2				
		Clostridium					1	
		Clostridium Bolteae	1					
		Clostridiuim sp. MSTE9					1	
		Clostridium viride					1	
		Clostridium sp. CAG:226					1	
		Clostridium sp. CAG:1024					1	
		SMB53				1		
	Clostridiales	*Flavonifractor*		1			1	
	Coprobacillaceae	*Coprobacillus*	1		1			
	Enterococcaceae	*Enterococcus*	2				3	
		Enterococcus faecium	1					
		Vagococcus					1	
	Eubacteriaceae	*Eubacterium*	2	1				
		Eubacterium rectale	1					

			Atrial Fibrillation		Heart Failure		Stroke	
Phylum	**Family**	**Genus / Species**	↑	↓	↑	↓	↑	↓
	Lachnospiraceae						1	1
		Agathobacter		1				
		Anaerostipes						1
		Anaerostipes caccae					1	
		Blautia	3	1			1	1
		Butyrivibrio		1				
		Clostridium XlVa					2	
		Coprococcus	1					1
		Dorea	4					
		Dorea longicatena	1					
		Lachnoclostridium	1					
		Lachnospira					1	
		Lachnospiraceae_UCG_001					1	
		Roseburia	1				3	2
		Oscillibacter		1			2	1
		Oscillibacter sp. CAG:241					1	
	Lactobacillaceae						2	
		Lactobacillus					3	
	Leuconostocaceae	*Weissella*	1					
	Ruminococcaceae						1	
		Faecalibacterium	3	2			3	4
		Faecalibacterium prausnitzii	2	2		1		
		norank_f_Ruminococcaceae					1	
		Ruminococcaceae_other					1	
		Ruminococcaceae_UCG_002					1	
		Ruminococcaceae_UCG_005					1	
		Ruminococcus	4			1	4	1
		Ruminococcus gnavus			1			
	Selenomonadaceae	*Megamonas*	1			1	2	1
	Staphylococcaceae	*Gemella*					1	
	Streptococcaceae						1	
		Streptococcus	4		2		2	
	Veillonellaceae							1
		Dialister	1				2	1
		Megasphaera					2	
		Veillonella	1		2		1	
	Sporomusaceae	*Anaeroarcus*	1					
Fusobacteriota			1					
	Fusobacteriaceae	*Fusobacterium*					1	
Planctomycetes							1	
Proteobacteria			1		1		6	
	Desulfovibrionaceae	*Desulfovibrio*					2	
	Enterobacteriaceae						3	
		Enterobacter		1			2	
		Escherichia					5	1
		Escherichia coli	1					
		Klebsiella	1				2	
		Raoultella	1					
		Shigella					3	1
	Holosporaceae	*Holospora*	1					

			Atrial Fibrillation		Heart Failure		Stroke	
Phylum	**Family**	**Genus / Species**	↑	↓	↑	↓	↑	↓
	Hyphomicrobiaceae	*Gemmiger*						1
	Methylobacteriaceae	*Methylobacterium-Methylorubrum*	1					
	Methylococcaceae	*Methylovulum*	1					
		Methylovulum miyakonense	1					
	Pasteurellaceae	*Haemophilus*	1					
	Sutterellaceae	*Sutterella*		1				
Spirochaetes							1	
Synergistetes	*Pyramidobacter*						1	
Tenericutes							1	
Thermodesulfobacteriota	*Desulfovibrionaceae*	*Bilophila*		1				
Lentisphaerota	*Victivallaceae*	*Victivallis*					1	
Candidatus Parcubacteria		*norank_p_Parcubacteria*					1	
Verrucomicrobia							1	
	Verrucomicrobiaceae	Akkermansia					4	1

Microbial metabolites associated with CVD

From the analysed studies, it wasn't possible to obtain quantitative data of the microbial metabolites, with the exception of TMAO. Therefore, TMAO levels (μM) are represented in Table 3.

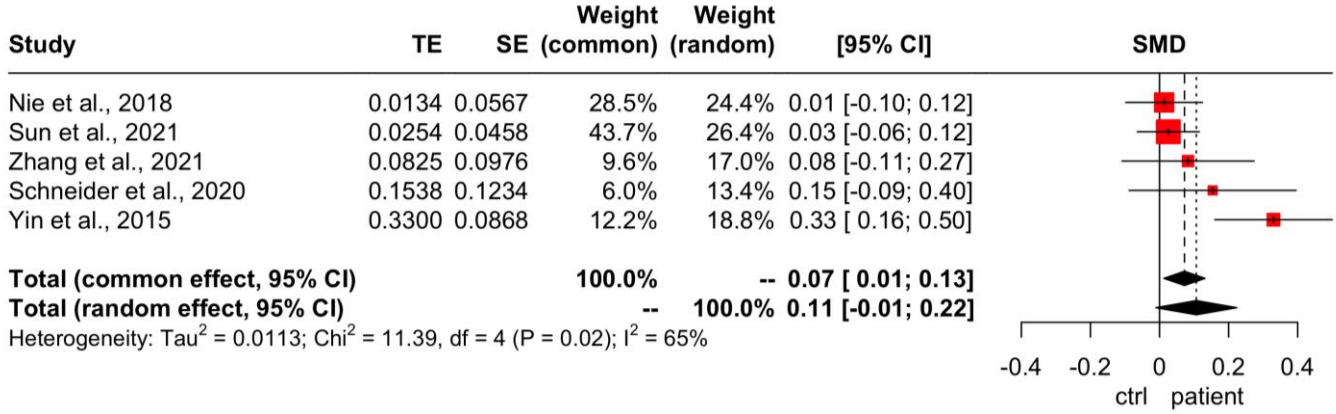
Most studies assessed the circulating levels of TMAO that generally reported an increase in CVD patients. However, regarding AF studies, Buttner *et al.* (2020) [36] showed decreased TMAO levels in diseased individuals. HF and stroke were the pathologies with the highest number of publications on TMAO levels. Concerning HF, three studies showed high patient TMAO levels [28,45,52]. Regarding stroke studies, seven revealed increased TMAO levels in the patients compared to healthy individuals [26,31,40,42,43,49,57], and one revealed the opposite [46].

When combining the results from each stroke study, a high level of heterogeneity was obtained (Figure 2). Indeed, only Yin *et al.* (2015) [26] favours the hypothesis of higher TMAO levels in patients when compared to controls.

Table 3 – TMAO levels across included studies.

			TMAO levels (μM)	
Articles	**References**	**TMAO**	**Patients**	**Controls**
Atrial fibrillation				
Buttner *et al.* (2020)	36	Decreased	3.7	4.2
Heart failure				
Troseid *et al.* (2015)	28	Increased	12.1	7.9
Hayashi *et al.* (2018)	45	Increased	Compensated 17.7 Decompensated HF 17.3	8.2
Emoto *et al.* (2021)	52	Increased	Low TMAO 6.4 High TMAO 21.0	Low TMAO 5.2 High TMAO 11.5
Stroke				
Yin *et al.* (2015)	26	Increased	2.7	1.91
Nie *et al.* (2018)	31	Increased	2.5	2.3
Schneider *et al.* (2020)	40	Increased	4.9	3.16
Zhu *et al.* (2020)	43	Increased	5.6	4.9
Haak *et al.* (2021)	46	Decreased	1.97	4.03
Xu *et al.* (2021)	57	Increased	Cardiometabolic stroke 4.22 Large artery atherosclerotic stroke 2.93	1.66
Sun *et al.* (2021)	49	Increased	2.85	2.33
Zhang *et al.* (2021)	42	Increased	6.1	4

Figure 2 – Forest Plot of TMAO levels across stroke studies.



Discussion

In this study, we systematically reviewed human studies evaluating gut microbiome alterations in CVD. CVD are a set of heterogeneous diseases affecting the heart and blood vessels whose underlying cause of development is most often atherosclerosis. The basic mechanisms of atherosclerosis involve a complex interaction of vasculature, the immune system, and lipid metabolism. The gut microbiome plays a crucial role in these mechanisms, with most of the contributions related to microbial metabolites. Therefore, foremost gut bacteria will be analysed and then their putative metabolites.

Relatively to the gut microbiota, the results revealed an overall increase of Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes.

Stroke cases, have been associated with an increase of opportunistic bacteria such as *Enterobacter, Megasphere, Oscillibacter* and *Desulfovibrio* and a diminution of commensal microbes such as *Bacteroides, Prevotella* and *Faecalibacterium* which are not in total agreement with our results[26]. Nevertheless, it is well known that *Enterobacteriaceae* produce LPS; release pro-inflammatory cytokines and accumulate amyloid-β, leading to neurodegeneration[38]. In addition, they also cause enteropathy, affect the mucosa's immunity and inhibit the growth of healthy gut microbes, like *Ruminococcus* and *Streptococcus*[54].

In Atrial Fibrillation, both *Ruminococcus sp.* and *Streptococcus* are increased which impacts the progression and are important traits of this pathology. In fact, they have been identified as proinflammatory agents, since they enhance the levels of gamma interferon, interleukin 17, and interleukin 22[44]. Moreover, *Lachnoclostridium, Parabacteroides* and *Dorea* are also increased, producing therefore higher levels of TMAO[22]. On the other hand, *Faecalibacterium* is mostly decreased resulting in low-grade chronic gut inflammation[38]. This occurs because it is a butyrate producing bacteria that preserves epithelial health and inhibits the production of pro-inflammatory cytokines. Reductions in *Alistipes*, *Oscillibacter* and *Bilophila* also occur in AF patients[33].

In HF cases, enhanced populations of pathogenic *Shigella*, *Salmonella* and *Campylobacter bacteria* as well as less abundant *Eubacterium rectale* and *Dorea longicatena* bacteria were isolated from fecal samples[58]. Chronic HF patients

demonstrated decreased *Faecalibacterium prausnitzii* and increased *Ruminococcus gnavus* populations[30].

Gut microbiota metabolizes choline, betaine, and carnitine nutrients from the diet, producing TMAO in the liver. TMAO plays a pivotal role in CVD. This systematic review included a total of 12 studies that examined the associations of TMAO with CVD pathologies. TMAO and its precursors choline and betaine are related with clinical, haemodynamic and neurohormonal disease severity, however only high TMAO levels are associated with severe long-term outcomes[28]. Indeed, TMAO may cause auricular electrophysiological instability, acute electrical remodelling and worsening of cardiac fibrosis in AF. Moreover, promotes end-organ lesions and adverse ventricular remodelling[59]. In stroke patients, there is a dose-dependent relation between TMAO and complications/mortality at 3 months, even after adjusting for traditional risk factors[60]. Indeed, TMAO incites mitochondria impairment which decreases ATP availability and exacerbates calcium buffer's disparity and H_2O_2 formation, both of whom play a crucial part in ischemic/reperfusion cerebral lesions[61].

Additionally, studies involving matched case-control research revealed high TMAO levels in CVD patients. This analysis supports that high circulating TMAO levels are associated with an increased risk of CVD as reviewed by Caroff *et al.* (2021) [62]. Nevertheless, from our analysed studies, we couldn't prove without a thought this relationship. In fact, only Yin *et al.* (2015)[26] favours the hypothesis of higher TMAO levels in stroke patients when compared to controls.

It wasn't possible to obtain quantitative data from other gut microbiota-derived metabolites, like SCFAs [63] , LPS [64], BA [65] and uremic toxins [66]. However, they have attracted attention due to their regulator role in CVD development. Although fewer observational studies exist on these metabolites, studies have measured the differences in gut microbiota composition between diseased and healthy individuals.

In this analysis, bacteria with the aptitude for SCFAs production in different CVD pathologies were identified. Zafar *et al.* (2021) [67] reported the genus *Bacteroides* as the primary producer of SCFAs in the human gut, mainly acetate and propionate. Genus *Clostridium* is the main drive to generate SCFAs from carbohydrate fermentation, as shown by Guo *et al.* (2020) [68]. Also, Bui *et al.* (2021) [69] described that the genus *Anaerostipes* converts dietary inositol into

propionate and acetate. Genus *Parabacteroides* has the physiological characteristics of secreting SCFAs [70] . Within the main SCFAs produced in the gut, the genera *Faecalibacterium*, *Roseburia* as reported by Faden *et al.* (2022) [71], are butyrate-producing bacteria, as well as *Lachnospiraceae* family and its genus *Blautia* as mentioned by Benítez-Páez *et al.* (2020) [72] evaluating the microbiota of obese children. The study of Scheiman *et al.* (2019) [73], which involved a cohort of elite athletes, found that the genus *Veillonella* participates in lactate metabolism into propionate. Genus *Dialister* participates in propionate production via the succinate pathway [74] .

As LPS producers, the genus *Ruminococcus* participates in the degradation and conversion of complex polysaccharides into various nutrients [75]. In Huang *et al.* (2022) [76] study involving patients with colorectal cancer, the genus *Megamonas* mediated LPS biosynthesis. Yoshida *et al.* (2020) [77] demonstrated that the genus *Bacteroides* contributes to faecal LPS levels.

Furthermore, the genera *Blautia* was identified as a secondary BA producer, as Vojinovic *et al.* (2019) [78] reported. Genus *Enterobacter* plays a significant role in BA dysmetabolism in an inflammatory bowel disease cohort, as reported by Baldelli *et al.* (2021) [79].

In addition, the genera *Escherichia* and *Desulfovibrio* are recognized as uremic toxins-producing bacteria, as identified by Popkov *et al.* (2022) [80].

Although there are studies to understand the microbiome's role and its interaction with the host, the cause-and-effect relationship between microbial composition and disease propensity is still unknown[81]. However, the microbiome is a significant contributor to several disease states and research efforts have proven its efficacy in therapeutic interventions. For instance, within the phyla Actinobacteria, *Bifidobacterium* is the genus more reported by the studies with greater abundance in stroke patients. *Bifidobacterium* is a lactic acid-producing bacteria used as a probiotic modulating immune response [82].The success of microbiome therapeutics is promising but suffers from a few challenges, namely microbial characterization, and disease-specific microbial marker identification [83].

Strengths and Limitations

This systematic review provided evidence of the associations between gut microbial communities and TMAO levels with CVD pathologies. Several

strength points stand out in this study: broad search using two databases (MEDLINE and Web of Science), different and accessible data presentation, and analyses of both microbiota and its metabolites. Moreover, the inclusion of only human-based articles focusing on cardiovascular diseases.

On the other hand, we considered several limitations. The main obstacle was obtaining relevant data regarding metabolites and microbiota due to the need for a control group for further comparisons and the lack of standardised data presentation and supplementary material with relevant data. Thus, we were unable to conduct a strong meta-analysis mainly because of the lack of results' harmonization reporting in the included articles. Furthermore, it is relevant to mention the variability of the presentation of data at the taxonomic level (Supplementary table 1). The high-throughput amplicon sequencing of the 16S rRNA gene was the most widely used technique. However, the main disadvantage of this technique is that it is insufficient for species-level resolution.

Therefore, we recommend some procedures to ease the association between different studies' information. First, data divulgation and transparency through supplementary spreadsheets or public consultation. Second, a more recent whole genome shotgun sequencing (WGS) would provide extensive information regarding microbial genetic material enabling to shed some light over microbial metabolism and consequently enhancing the knowledge on how microbiota interacts with the host. Third, the use of a standard microbial genome database since the use of several different ones in the studies may increase the studies' bias.

Conclusion

Despite limitations, we unearth putative relationships between the gut microbiome and CVD. However, this interconnection undoubtedly needs more clarification regarding its physiological pathways especially in future studies based on human populations. Therefore, it is necessary for more human-based studies, associated with large statistical-powered datasets and data transparency to verify proven pathways and explore possible therapeutic strategies for CVD.

References

1. WHO. [cited 2023 Mar 4]. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

2. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PWF, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. Circulation. 2006 Feb;113(6):791–8.

3. A RG, A MG, O JC. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019. J Am Coll. 2020;76(25):2982–3021.

4. Almeida C, Barata P, Fernandes R. The influence of gut microbiota in cardiovascular diseases—a brief review. Porto Biomed J. 2021;6(1):e106.

5. Xu F, Fu Y, Sun TY, Jiang Z, Miao Z, Shuai M, et al. The interplay between host genetics and the gut microbiome reveals common and distinct microbiome features for complex human diseases. Microbiome. 2020;8(1).

6. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The firmicutes/bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? Nutrients. 2020;12(5).

7. Zhang YJ, Li S, Gan RY, Zhou T, Xu DP, Li HB. Impacts of gut bacteria on human health and diseases. Int J Mol Sci. 2015;16(4):7493–519.

8. Gerard C, M SR, J KP, Catherine S. Minireview:Gut Microbiota:The Neglected Endocrine Organ. Mol Endocrinol. 2014;28(8):1221–38.

9. Sanchez-Gimenez R, Ahmed-Khodja W, Molina Y, Peiró OM, Bonet G, Carrasquer A, et al. Gut Microbiota-Derived Metabolites and Cardiovascular Disease Risk: A Systematic Review of Prospective Cohort Studies. Nutrients. 2022;14(13).

10. Ohira H, Tsutsui W, Fujioka Y. Are short chain fatty acids in gut microbiota defensive players for inflammation and atherosclerosis? J Atheroscler Thromb. 2017;24(7):660–72.

11. Zhang B, Wang X, Xia R, Li C. Gut microbiota in coronary artery disease: A friend or foe? Biosci Rep. 2020;40(5).

12. Pakhomov N, Baugh JA. The role of diet-derived short-chain fatty acids in regulating cardiac pressure overload. Am J Physiol - Heart Circ Physiol. 2021;320(2):H475–86.

13. Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurbier KR, et al. Short-chain fatty acids improve poststroke recovery via immunological mechanisms. J Neurosci. 2020;40(5):1162–73.

14. Zuo K, Fang C, Liu Z, Fu Y, Liu Y, Liu L, et al. Commensal microbe-derived SCFA alleviates atrial fibrillation via GPR43/NLRP3 signaling. Int J Biol Sci. 2022;18(10):4219–32.

15. Rodríguez-Morató J, Matthan NR. Nutrition and Gastrointestinal Microbiota, Microbial-Derived Secondary Bile Acids, and Cardiovascular Disease. Curr Atheroscler Rep. 2020;22(9).

16. Zhang R, Ma WQ, Fu MJ, Li J, Hu CH, Chen Y, et al. Overview of bile acid signaling in the cardiovascular system. World J Clin Cases. 2021;9(2):308–20.

17. Wang B, Qiu J, Lian J, Yang X, Zhou J. Gut Metabolite Trimethylamine-N-Oxide in Atherosclerosis: From Mechanism to Therapy. Front Cardiovasc Med. 2021;8.

18. Pastori D, Carnevale R, Nocella C, Novo M, Santulli M, Cammisotto V, et al. Gut-derived serum lipopolysaccharide is associated with enhanced risk of major adverse cardiovascular events in atrial fibrillation: Effect of adherence to mediterranean diet. J Am Heart Assoc. 2017;6(6).

19. Bryniarski MA, Hamarneh F, Yacoub R. The role of chronic kidney disease-associated dysbiosis in cardiovascular disease. Exp Biol Med. 2019;244(6):514–25.

20. Harlacher E, Wollenhaupt J, Baaten CCFMJ, Noels H. Impact of Uremic Toxins on Endothelial Dysfunction in Chronic Kidney Disease: A Systematic Review. Int J Mol Sci. 2022;23(1).

21. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36.

22. Tabata T, Yamashita T, Hosomi K, Park J, Hayashi T, Yoshida N, et al. Gut microbial composition in patients with atrial fibrillation: effects of diet and drugs. Heart Vessels. 2021;36(1):105–14.

23. Yong F, Oluf P. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021;19:55–71.

24. Zhang Y, Wang Y, Ke B, Du J. TMAO: how gut microbiota contributes to heart failure. Transl Res. 2021;228:109–25.

25. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Vol. 372, The BMJ. BMJ Publishing Group; 2021.

26. Yin J, Liao SX, He Y, Wang S, Xia GH, Liu FT, et al. Dysbiosis of gut microbiota with reduced trimethylamine-n-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. J Am Heart Assoc. 2015;4(11).

27. Ji W, Zhu Y, Kan P, Cai Y, Wang Z, Wu Z, et al. Analysis of intestinal microbial communities of cerebral infarction and Ischemia patients based on high throughput sequencing technology and glucose and lipid metabolism. Mol Med Rep. 2017;16(4):5413–7.

28. Trøseid M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, et al. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med. 2015;277(6):717–26.

29. Mayerhofer CCK, Ueland T, Broch K, Vincent RP, Cross GF, Dahl CP, et al. Increased Secondary/Primary Bile Acid Ratio in Chronic Heart Failure. J Card Fail. 2017;23(9):666–71.

30. Cui X, Ye L, Li J, Jin L, Wang W, Li S, et al. Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients. Sci Rep. 2018;8(1).

31. Nie J, Xie L, Zhao BX, Li Y, Qiu B, Zhu F, et al. Serum trimethylamine N-oxide concentration is positively associated with first stroke in hypertensive patients. Aha J. 2018;49(9):2021–8.

32. Wang W, Li X, Yao X, Cheng X, Zhu Y. The characteristics analysis of intestinal microecology on cerebral infarction patients and its correlation with apolipoprotein E. Med U S. 2018;97(41).

33. Zuo K, Li J, Li K, Hu C, Gao Y, Chen M, et al. Disordered gut microbiota and alterations in metabolic patterns are associated with atrial fibrillation. GigaScience. 2019;8(6).

34. Zuo K, Li J, Wang P, Liu Y, Liu Z, Yin X, et al. Duration of Persistent Atrial Fibrillation Is Associated with Alterations in Human Gut Microbiota and Metabolic Phenotypes. mSystems. 2019;4(6).

35. Li N, Weng X, Sun C, Wu X, Lu M, Si Y, et al. Change of intestinal microbiota in cerebral ischemic stroke patients. BMC Microbiol. 2019;19(1).

36. Büttner P, Okun JG, Hauke J, Holzwirth E, Obradovic D, Hindricks G, et al. Trimethylamine N-oxide in atrial fibrillation progression. IJC Heart Vasc. 2020;29.

37. Li J, Zuo K, Zhang J, Hu C, Wang P, Jiao J, et al. Shifts in gut microbiome and metabolome are associated with risk of recurrent atrial fibrillation. J Cell Mol Med. 2020;24(22):13356–69.

38. Ling Y, Gu Q, Zhang J, Gong T, Weng X, Liu J, et al. Structural Change of Gut Microbiota in Patients with Post-Stroke Comorbid Cognitive Impairment

and Depression and Its Correlation with Clinical Features. J Alzheimers Dis. 2020;77(4):1595–608.

39. Liu Y, Kong C, Gong L, Zhang X, Zhu Y, Wang H, et al. The Association of Post-Stroke Cognitive Impairment and Gut Microbiota and its Corresponding Metabolites. J Alzheimers Dis. 2020;73(4):1455–66.

40. Schneider C, Okun JG, Schwarz KV, Hauke J, Zorn M, Nürnberg C, et al. Trimethylamine-N-oxide is elevated in the acute phase after ischaemic stroke and decreases within the first days. Eur J Neurol. 2020;27(8):1596–603.

41. Xiang L, Lou Y, Liu L, Liu Y, Zhang W, Deng J, et al. Gut Microbiotic Features Aiding the Diagnosis of Acute Ischemic Stroke. Front Cell Infect Microbiol. 2020;10.

42. Zhang J, Wang L, Cai J, Lei A, Liu C, Lin R, et al. Gut microbial metabolite TMAO portends prognosis in acute ischemic stroke. J Neuroimmunol. 2021;354.

43. Zhu C, Li G, Lv Z, Li J, Wang X, Kang J, et al. Association of plasma trimethylamine-N-oxide levels with post-stroke cognitive impairment: a 1-year longitudinal study. Neurol Sci. 2020;41(1):57–63.

44. Zuo K, Yin X, Li K, Zhang J, Wang P, Jiao J, et al. Different Types of Atrial Fibrillation Share Patterns of Gut Microbiota Dysbiosis. mSphere. 2020;5(2):20–71.

45. Hayashi T, Yamashita T, Watanabe H, Kami K, Yoshida N, Tabata T, et al. Gut microbiome and plasma microbiome-related metabolites in patients with decompensated and compensated heart failure. Circ J. 2019;83(1):182–92.

46. Haak BW, Westendorp WF, van Engelen TSR, Brands X, Brouwer MC, Vermeij JD, et al. Disruptions of Anaerobic Gut Bacteria Are Associated with Stroke and Post-stroke Infection: a Prospective Case–Control Study. Transl Stroke Res. 2021;12(4):581–92.

47. Huang K, Wang Y, Bai Y, Luo Q, Lin X, Yang Q, et al. Gut Microbiota and Metabolites in Atrial Fibrillation Patients and Their Changes after Catheter Ablation. Microbiol Spectr. 2022;10(2).

48. Themistoklis K, Tomohito O. Non-Ischemic Heart Failure With Reduced Ejection Fraction Is Associated With Altered Intestinal Microbiota. Circ J. 2018;82:1640–50.

49. Sun T, Zhang Y, Yin J, Peng X, Zhou L, Huang S, et al. Association of gut microbiota-dependent metabolite trimethylamine N-oxide with first ischemic stroke. J Atheroscler Thromb. 2021;28(4):320–8.

50. Zhao L, Wang C, Peng S, Zhu X, Zhang Z, Zhao Y, et al. Pivotal interplays between fecal metabolome and gut microbiome reveal functional signatures in cerebral ischemic stroke. J Transl Med. 2022;20(1).

51. Zheng Q, Chen Y, Zhai Y, Meng L, Liu H, Tian H, et al. Gut Dysbiosis Is Associated With the Severity of Cryptogenic Stroke and Enhanced Systemic Inflammatory Response. Front Immunol. 2022;13.

52. Emoto T, Hayashi T, Tabata T, Yamashita T, Watanabe H, Takahashi T, et al. Metagenomic analysis of gut microbiota reveals its role in trimethylamine metabolism in heart failure. Int J Cardiol. 2021;338:138–42.

53. Beale AL, O'Donnell JA, Nakai ME, Nanayakkara S, Vizi D, Carter K, et al. The gut microbiome of heart failure with preserved ejection fraction. J Am Heart Assoc. 2021;10(13).

54. Tan C, Wu Q, Wang H, Gao X, Xu R, Cui Z, et al. Dysbiosis of Gut Microbiota and Short-Chain Fatty Acids in Acute Ischemic Stroke and the Subsequent Risk for Poor Functional Outcomes. J Parenter Enter Nutr. 2021;45(3):518–29.

55. Huang Y, Shen Z, He W. Identification of Gut Microbiome Signatures in Patients With Post-stroke Cognitive Impairment and Affective Disorder. Front Aging Neurosci. 2021;13.

56. W.-T. Z, J.-Y. NIU, C HE. Associations of OSAHS complicated by cerebral infarction with intestinal flora, inflammatory factors, homocysteine and adiponectin expression. 2020 p. 12993–9.

57. Xu DJ, Wang KC, Yuan LB, Li HF, Xu YY, Wei LY, et al. Compositional and functional alterations of gut microbiota in patients with stroke. Nutr Metab Cardiovasc Dis. 2021;31(12):3434–48.

58. Jin L, Shi X, Yang J, Zhao Y, Xue L, Xu L, et al. Gut microbes in cardiovascular diseases and their potential therapeutic applications. Protein Cell. 2021 May;12(5):346–59.

59. Zuo K, Liu X, Wang P, Jiao J, Han C, Liu Z, et al. Metagenomic data-mining reveals enrichment of trimethylamine-N-oxide synthesis in gut microbiome in atrial fibrillation patients. BMC Genomics. 2020 Jul;21(1).

60. Zhai Q, Wang X, Chen C, Tang Y, Wang Y, Tian J, et al. Prognostic Value of Plasma Trimethylamine N-Oxide Levels in Patients with Acute Ischemic Stroke. Cell Mol Neurobiol. 2019 Nov;39(8):1201–6.

61. Hou L, Zhang Y, Zheng D, Shi H, Zou C, Zhang H, et al. Increasing trimethylamine N-oxide levels as a predictor of early neurological deterioration in patients with acute ischemic stroke. Neurol Res. 2020 Feb;42(2):153–8.

62. Caroff C. Elevated levels of gut microbiota dependent trimethylamine N-oxide: An indicator of cardiovascular disease. Vol. 20, Human Microbiome Journal. Elsevier Ltd; 2021.

63. Nogal A, Valdes AM, Menni C. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. Gut Microbes. 2021;13(1):1–24.

64. Violi F, Cammisotto V, Bartimoccia S, Pignatelli P, Carnevale R, Nocella C. Gut-derived low-grade endotoxaemia, atherothrombosis and cardiovascular disease. Nature Reviews Cardiology. Nature Research; 2022.

65. Chong Nguyen C, Duboc D, Rainteau D, Sokol H, Humbert L, Seksik P, et al. Circulating bile acids concentration is predictive of coronary artery disease in human. Sci Rep. 2021;11(1).

66. Falconi CA, Junho CV da C, Fogaça-Ruiz F, Vernier ICS, da Cunha RS, Stinghen AEM, et al. Uremic Toxins: An Alarming Danger Concerning the Cardiovascular System. Vol. 12, Frontiers in Physiology. Frontiers Media S.A.; 2021.

67. Zafar H, Saier MH. Gut Bacteroides species in health and disease. Gut Microbes. 2021;13(1):1–20.

68. Guo P, Zhang K, Ma X, He P. Clostridium species as probiotics: Potentials and challenges. J Anim Sci Biotechnol. 2020;11(1).

69. Bui TPN, Mannerås-Holm L, Puschmann R, Wu H, Troise AD, Nijsse B, et al. Conversion of dietary inositol into propionate and acetate by commensal Anaerostipes associates with host health. Nat Commun. 2021;12(1).

70. Cui Y, Zhang L, Wang X, Yi Y, Shan Y, Liu B, et al. Roles of intestinal Parabacteroides in human health and diseases. FEMS Microbiol Lett. 2022;369(1).

71. Faden H. The role of Faecalibacterium, Roseburia, and Butyrate in Inflammatory Bowel Disease. Karger AG Basel. 2022;

72. Benítez-Páez A, Gómez del Pugar EM, López-Almela I, Moya-Pérez Á, Codoñer-Franch P, Sanz Y. Depletion of Blautia Species in the Microbiota of Obese Children Relates to Intestinal Inflammation and Metabolic Phenotype Worsening. mSystems. 2020;5(2).

73. Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, et al. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nat Med. 2019;25(7):1104–9.

74. Ganesan R, Jeong JJ, Kim DJ, Suk KT. Recent Trends of Microbiota-Based Microbial Metabolites Metabolism in Liver Disease. Vol. 9, Frontiers in Medicine. Frontiers Media S.A.; 2022.

75. La Reau AJ, Suen G. The Ruminococci: key symbionts of the gut ecosystem. J Microbiol. 2018;56(3):199–208.

76. Huang R, He K, Duan X, Xiao J, Wang H, Xiang G, et al. Changes of Intestinal Microflora in Colorectal Cancer Patients after Surgical Resection and Chemotherapy. Comput Math Methods Med. 2022;2022.

77. Yoshida N, Yamashita T, Kishino S, Watanabe H, Sasaki K, Sasaki D, et al. A possible beneficial effect of Bacteroides on faecal lipopolysaccharide activity and cardiovascular diseases. Sci Rep. 2020;10(1).

78. Vojinovic D, Radjabzadeh D, Kurilshikov A, Amin N, Wijmenga C, Franke L, et al. Relationship between gut microbiota and circulating metabolites in population-based cohorts. Nat Commun. 2019;10(1).

79. Baldelli V, Scaldaferri F, Putignani L, Del Chierico F. The role of enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. Microorganisms. 2021;9(4).

80. Gut E, Soulage O, Popkov VA, Zharikova AA, Demchenko EA, Andrianova NV, et al. Citation: Gut Microbiota as a Source of Uremic Toxins. Toxins Int J Mol Sci. 2022;2022:483.

81. Gulliver EL, Young RB, Chonwerawong M, D'Adamo GL, Thomason T, Widdop JT, et al. Review article: the future of microbiome-based therapeutics. Aliment Pharmacol Ther. 2022;56(2):192–208.

82. O'Neill I, Schofield Z, Hall LJ. Exploring the role of the microbiota member Bifidobacterium in modulating immune-linked diseases. Emerg Top Life Sci. 2017;1(4):333–49.

83. Yadav M, Chauhan NS. Microbiome therapeutics: Exploring the present scenario and challenges. Vol. 10, Gastroenterology Report. Oxford University Press; 2022.

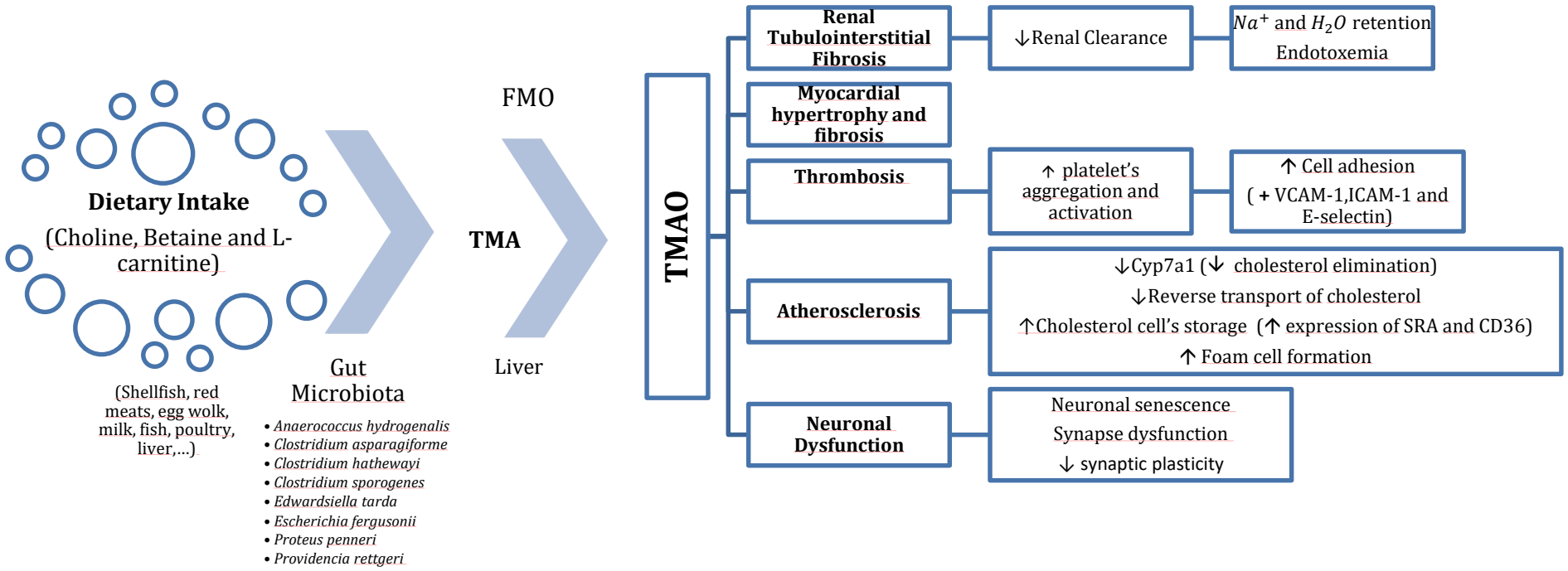
84. Liu Y, Dai M. Trimethylamine N-Oxide Generated by the Gut Microbiota Is Associated with Vascular Inflammation: New Insights into Atherosclerosis. Mediators Inflamm. 2020;2020.

85. Zheng L, Zheng J, Xie Y, Li Z, Guo X, Sun G, et al. Serum gut microbe-dependent trimethylamine N-oxide improves the prediction of future cardiovascular disease in a community-based general population. Atherosclerosis. 2019;280:126–31.

86. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. mBio. 2015 Mar;6(2).

Supplementary Material

Supplementary Figure 1 - Mechanisms around TMAO's production and its main effects on the human body[24,84–86] (Legend: TMA- trimethylamine; TMAO- trimethylamine N-oxide; FMO- flavin-containing monooxygenases, Cyp7a1- bile acid synthase; CD36- class B scavenger receptor; SRA- scavenger receptor A; VCAM-1- vascular cell adhesion molecule; ICAM-1- intercellular adhesion molecule).



Supplementary Table 1 - Articles included in this study and its characteristics (Legend: ELISA- Enzyme-linked immunosorbent assay; GC/MS-Gas chromatography–mass spectrometry; LC/MS-Liquid chromatography - mass spectrometer; LPS-Lipopolysaccharides; na- Not assigned; SCFA- Short-chain fatty acids; TMAO- Trimethylamine N-oxide; CE/TOF/MS- Capillary ELletrophoresis Time of Flight Mass Spectrometer; EI/MS- Electron ionization–mass spectrometry).

Year	Authors	References	Study Type	Country	Pathology	N. Controls	N. Patients	Microorganisms	Microorganisms Classification	Sample	Method	Sequencing Region	Metabolites	Metabolites Data	Sample	Method	Validation
2015	Yin et al.	26	Observational	China	Stroke	231	322	yes	Genus	Stool	16S rRNA gene sequencing	V4	yes	TMAO	Plasma	LC/MS	include
2017	Ji et al.	27	Observational	China	Stroke	na	10	yes	Genus	Stool	16S rRNA gene sequencing	V4	no	na	na	na	include
2015	Trøseid et al.	28	Observational	Norway	Heart Failure	33	155	no	na	na	na	na	yes	TMAO and precursors	Plasma	LC/MS	include
2017	Mayerhofer et al.	29	Observational	Norway	Heart Failure	20	142	no	na	na	na	na	yes	Bile acids	Plasma	LC/MS	include
2018	Cui et al.	30	Observational	China	Heart Failure	41	53	yes	Genus	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, plasma	LC/MS	include
2018	Nie et al.	31	Observational	China	Stroke	622	622	no	na	na	na	na	yes	TMAO and precursors	Serum	LC/MS	include
2018	Wang et al.	32	Observational	China	Stroke	10	10	yes	Species	Stool	16S rRNA gene sequencing	V4	yes	Apolipoprotein E	Serum	ELISA kit	include
2019	Kun Zuo et al.	33	Observational	China	Atrial Fibrillation	50	50	yes	Species	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, serum	LC/MS	include
2019	Kun Zuo et al.	34	Observational	China	Atrial Fibrillation	20	20	yes	Genus	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, serum	LC/MS	include
2019	Li et al.	35	Observational	China	Stroke	30	30	yes	Genus	Stool	16S rRNA gene sequencing	V1-V2	no	na	na	na	include
2020	Buttner et al.	36	Observational	Germany	Atrial Fibrillation	20	45	no	na	na	na	na	yes	TMAO	Plasma	EI/MS	include
2020	Li et al.	37	Observational	China	Atrial Fibrillation	40	50	yes	Species	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, serum	LC/MS	include
2020	Ling et al.	38	Observational	China	Stroke	25	41	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	no	na	na	na	include
2020	Liu et al.	39	Experimental	China	Stroke	35	30	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	yes	SCFA	Stool	GC/MS	include
2020	Schneider et al.	40	Observational	Germany	Stroke	100	196	no	na	na	na	na	yes	TMAO	Plasma	LC/MS	include
2020	Xiang et al.	41	Observational	China	Stroke	16	20	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	no	na	na	na	include
2020	Zhang et al.	56	Observational	China	Stroke	30	28	yes	Genus	Stool	16S rRNA gene sequencing	V4	no	na	na	na	include
2020	Zhu et al.	43	Observational	China	Stroke	170	86	no	na	na	na	na	yes	TMAO	Plasma	LC/MS	include
2020	Zuo et al.	44	Observational	China	Atrial Fibrillation	50	50	yes	Genus	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, serum	LC/MS	include
2018	Hayashi et al.	45	Experimental	Japan	Heart Failure	11	22	yes	Genus	Stool	16S rRNA gene sequencing	nd	yes	TMAO, uremic toxins	Plasma	CE/TOF/MS	include
2021	Haak et al.	46	Observational	Netherlands	Stroke	51	349	yes	Genus	Stool swab	16S rRNA gene sequencing	V3-V4	yes	TMAO, SCFA	Plasma	LC/MS	include
2021	Huang et al.	47	Observational	China	Stroke	19	76	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	no	na	na	na	include
2021	Tabata et al.	22	Observational	Japan	Atrial Fibrillation	66	34	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	no	na	na	na	include
2021	Tan et al.	54	Observational	China	Stroke	92	140	yes	Genus	Stool	16S rRNA gene sequencing	V4	yes	SCFA, LPS	Stool, serum	GC/MS; ELISA kit	include
2021	Xu et al.	57	Observational	China	Stroke	59	108	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	yes	TMAO	Plasma	LC/MS	include
2021	Zhang et al.	42	Observational	China	Stroke	150	351	no	na	na	na	na	yes	TMAO	Plasma	LC/MS	include
2022	Huang et al.	55	Observational	China	Atrial Fibrillation	30	36	yes	Species	Stool	16S rRNA gene sequencing	V3-V4	yes	Nonspecific	Stool	LC/MS	include
2018	Katsimichas et al.	48	Observational	Japan	Heart Failure	19	28	yes	Genus	Stool	16S rRNA gene sequencing	V1-V2	no	na	na	na	include
2021	Sun et al.	49	Observational	China	Stroke	953	953	no	na	na	na	na	yes	TMAO	Plasma	LC/MS	include
2022	Zhao et al.	50	Observational	China	Stroke	30	60	yes	Genus	Stool	Shotgun metagenomic sequencing	na	yes	Nonspecific	Stool, urine, plasma	GC/MS	include
2022	Zheng et al.	51	Observational	China	Stroke	33	30	yes	Genus	Stool	16S rRNA gene sequencing	V3-V4	yes	LPS	Plasma	ELISA kit	include
2021	Emoto et al.	52	Experimental	Japan	Heart Failure	11	22	yes	Genus	Stool	Shotgun metagenomic sequencing	na	yes	TMAO	Plasma	CE/TOF/MS	include
2021	Beale et al.	53	Observational	Australia	Heart Failure	67	26	yes	Genus	Stool	16S rRNA gene sequencing	V4-V5	no	na	na	na	include

Supplementary Table 2 – Quality Assessment of gut microbiome-CVD studies (Legend: NA-Not applicable).

Pathology	Articles	References	1. Was the research question or objective in this paper clearly stated?	2. Was the study population clearly specified and defined?	3.Was the participation rate of eligible persons at least 50%?	4.Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	5.Was a sample size justification, power description, or variance and effect estimates provided?	6.For the aNAlyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	7.Was the timeframe sufficient so that one could reasoNAbly expect to see an association between exposure and outcome if it existed?	8.For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome?	9.Were the exposure measures defined, valid, reliable, and implemented consistently across all study participants?	10.Was the exposure(s) assessed more than once over time?	11.Were the outcome measures defined, valid, reliable, and implemented consistently across all study participants?	12.Were the outcome assessors blinded to the exposure status of participants?	13.Was loss to follow-up after baseline 20% or less?	14.Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure and outcome?	Number of "No" responses	Quality Assessment
Stroke	Yin et al., 2015	26	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	1	Good
Stroke	Ji et al., 2017	27	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	No	Yes	NA	NA	No	3	Fair
Heart Failure	Trøseid et al.,2015	28	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	No	Yes	3	Fair
Heart Failure	Mayerhofer et al., 2017	29	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	No	Yes	3	Fair
Heart Failure	Cui et al., 2018	30	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	No	3	Fair
Stroke	Nie et al., 2018	31	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	Yes	NA	Yes	2	Good
Stroke	Wang et al., 2018	32	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	No	3	Fair
Atrial Fibrillation	Kun Zuo et al., 2019	33	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	2	Good
Atrial Fibrillation	Kun Zuo et al., 2019	34	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	2	Good
Stroke	Li et al., 2019	35	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Atrial Fibrillation	Buttner et al., 2020	36	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	Yes	Yes	NA	No	No	3	Fair
Atrial Fibrillation	Li et al., 2020	37	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	Yes	1	Good
Stroke	Ling et al., 2020	38	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Liu et al., 2020	39	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	Yes	No	3	Fair
Stroke	Schneider et al., 2020	40	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	Yes	No	Yes	No	Yes	3	Fair
Stroke	Xiang et al., 2020	41	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Zhang et al., 2020	56	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Zhu et al., 2020	43	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	Yes	2	Good
Atrial Fibrillation	Zuo et al., 2020	44	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	2	Good
Heart Failure	Hayashi et al., 2018	45	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	2	Good
Stroke	Haak et al., 2021	46	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Huang et al., 2021	47	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Atrial Fibrillation	Tabata et al., 2021	22	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Tan et al., 2021	54	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	Yes	2	Good
Stroke	Xu et al., 2021	57	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	2	Good
Stroke	Zhang et al., 2021	42	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	Yes	Yes	2	Good
Atrial Fibrillation	Huang et al., 2022	55	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	Yes	Yes	NA	NA	No	2	Good
Heart Failure	Katsimichas et al., 2018	48	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	Yes	2	Good
Stroke	Sun et al., 2021	49	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	Yes	2	Good
Stroke	Zhao et al., 2022	50	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	No	3	Fair
Stroke	Zheng et al., 2022	51	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	NA	NA	No	3	Fair
Heart Failure	Emoto et al., 2021	52	Yes	Yes	Yes	Yes	No	Yes	NA	Yes	Yes	No	Yes	NA	NA	No	3	Fair
Heart Failure	Beale et al., 2021	53	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	NA	No	3	Fair

Supplementary Table 3 – Articles' references in which the main taxa composing the gut microbiota of stroke patients are increased  or decreased  relatively to the control samples.

				Stroke			
Phylum	**Family**	**Genus**	**Species**	↑	↓	↑	↓
Actinobacteria				5		57, 27, 38, 41, 39	
	Bifidobacteriaceae	*Bifidobacterium*		4		57, 27, 41, 56	
		Metascardovia		1		56	
		Parascardovia		1		56	
	Eggerthellaceae	*Adlercreutzia*		1		56	
Actinomycetota	*Coriobacteriaceae*			1		41	
		Coriobacteriales		1		56	
Bacteroidetes				5	1	27, 35, 38, 41, 39	57
	Saprospiraceae	*Sphingobacteriales*		1		56	
	Bacteroidaceae	*Bacteroides*		6	4	32, 35, 38, 41, 39, 51	26, 27, 54, 47
	Flavobacteriaceae	*norank_p_Flavobacteriaceae*		1		35	
	Odoribacteraceae	*Odoribacter*		2		35, 50	
	Porphyromonadaceae			1		54	
		Prevotella		4	3	32, 35, 38, 41	26, 57, 27
	Rikenellaceae	*Alistipes*		1		38	
	Tannerellaceae	*Parabacteroides*		4	1	57, 32, 38, 41	27
Cyanobacteria				1		27	
Euryarchaeota				1		27	
Firmicutes				5	1	57, 27, 35, 38, 39	41
	Acidaminococcaceae	*Acidaminococcus*		1		41	
		Phascolarctobacterium		2		35, 41	
	Christensenellaceae	*Christensenellaceae_R-7_group*		1		35	
		Clostridium		1		50	
			Clostridiuim sp. MSTE9	1		50	
			Clostridium viride	1		50	
			Clostridium sp. CAG:226	1		50	
			Clostridium sp. CAG:1024	1		50	
	Clostridiales	*Flavonifractor*		1		39	
	Enterococcaceae	*Enterococcus*		3		57, 41, 47	
		Vagococcus		1		56	
	Lachnospiraceae			1	1	41	54
		Anaerostipes			1		54
			Anaerostipes caccae	1		56	
		Blautia		1	1	41	54
		Clostridium XlVa		2		38, 39	
		Coprococcus			1		39
		Lachnospira		1		41	
		Lachnospiraceae_UCG_001		1		35	
		Roseburia		3	2	32, 35, 38	54, 51
		Oscillibacter		2	1	26, 50	39
			Oscillibacter sp. CAG:241	1		50	
	Lactobacillaceae			2		54, 51	
		Lactobacillus		3		57, 41, 51	
	Ruminococcaceae			1		41	
		Faecalibacterium		3	4	32, 35, 38	26, 27, 54, 51
		norank_f_Ruminococcaceae		1		35	
		Ruminococcaceae_other		1		38	
		Ruminococcaceae_UCG_002		1		35	
		Ruminococcaceae_UCG_005		1		35	
		Ruminococcus		4	1	57, 27, 32, 50	39
	Selenomonadaceae	*Megamonas*		2	1	27, 32	47
	Staphylococcaceae	*Gemella*		1		39	
	Streptococcaceae			1		51	
		Streptococcus		2		38, 51	
	Veillonellaceae				1		51
		Dialister		2	1	27, 32	51
		Megasphaera		2		26, 41	
		Veillonella		1		41	
Fusobacteriota							
	Fusobacteriaceae	*Fusobacterium*		1		39	
Planctomycetes				1		27	
Proteobacteria				6		57, 27, 35, 38, 41, 39	
	Desulfovibrionaceae	*Desulfovibrio*		2		26, 57	
	Enterobacteriaceae			3		41, 54, 51	
		Enterobacter		2		26, 35	
		Escherichia		5	1	27, 32, 35, 38, 51	47
		Klebsiella		2		57, 51	
		Shigella		3	1	35, 38, 51	47
	Hyphomicrobiaceae	*Gemmiger*			1		39
Spirochaetes				1		27	
Synergistetes	Pyramidobacter			1		35	
Lentisphaerota	Victivallaceae	*Victivallis*		1		35	
Candidatus Parcubacteria		*norank_p_Parcubacteria*		1		35	
Tenericutes				1		27	
Verrucomicrobia				1		27	
	Verrucomicrobiaceae	Akkermansia		4	1	35, 41, 54, 50	27

Supplementary Table 4 – Articles' references in which the main taxa composing the gut microbiota of HF patients are increased 1 2 or decreased 1 relatively to the control samples.

				Heart Failure			
Phylum	**Family**	**Genus**	**Species**	↑	↓	↑	↓
Actinobacteria				1		45	
	Bifidobacteriaceae	*Bifidobacterium*		1		45	
Bacteroidetes				1		48	
	Bacteroidaceae	*Bacteroides*		1		48	
Firmicutes				1		48	
	Clostridiaceae	*SMB53*			1		48
	Coprobacillaceae	*Coprobacillus*		1		30	
	Ruminococcaceae	*Faecalibacterium*					
			Faecalibacterium prausnitzii		1		30
		Ruminococcus			1		53
			Ruminococcus gnavus	1		30	
	Selenomonadaceae	*Megamonas*			1		45
	Streptococcaceae	*Streptococcus*		2		48, 30	
	Veillonellaceae	*Veillonella*		2		48, 30	
Proteobacteria				1		48	

Supplementary Table 5 – Articles' references in which the main taxa composing the gut microbiota of AF patients are increased 1 2 3 4 or decreased 1 2 relatively to the control samples.

				Atrial Fibrillation			
Phylum	**Family**	**Genus**	**Species**	↑	↓	↑	↓
Actinobacteria					1		55
	Bifidobacteriaceae	*Bifidobacterium*		1		33	
			Bifibacterium longum	1		33	
	Corynebacteriaceae	*Corynebacterium*		1		34	
	Coriobacteriaceae		*Collinsella aerofaciens*	1		33	
Bacteroidetes					2		55, 44
	Bacteroidaceae	*Bacteroides*		3		55, 34, 44	
			Bacteroides vulgatus	2		34, 44	
	Odoribacteraceae	*Butyricimonas*		1		22	
	Prevotellaceae	*Paraprevotella*			1		34
		Prevotella		2	2	34, 44	55, 33
			Prevotella copri	2	1	34, 44	37
			Prevotella copri CAG:164		1		37
	Rikenellaceae	*Alistipes*		2	1	55, 22	33
	Tannerellaceae	*Parabacteroides*		2		55, 22	
Euryarchaeota							
	Methanobacteriaceae	*Methanobrevibacter*					
			Methanobrevibacter smithii	1		37	
			Methanobrevibacter curvatus	1		37	
Firmicutes				1	1	44	55
		Firmicutes bacterium CAG:95			1		33
	Acidaminococcaceae	*Thermosinus*		1		34	
	Christensenellaceae	*Christensenellaceae_R-7_group*		1		55	
	Clostridiaceae	*Butyricicoccus*			2		34, 33
		Clostridium	*Clostridium Bolteae*	1		34	
	Clostridiales	*Flavonifractor*			1		33
	Coprobacillaceae	*Coprobacillus*		1		33	
	Enterococcaceae	*Enterococcus*		2		55, 33	
			Enterococcus faecium	1		34	
	Eubacteriaceae	*Eubacterium*		3	1	34, 33, 37	55
			Eubacterium rectale	1		33	
	Lachnospiraceae						
		Agathobacter			1		55
		Blautia		3	1	34, 33, 37	55
		Butyrivibrio			1		55
		Coprococcus		1		34	
		Dorea		4		22, 34, 33, 37	
			Dorea longicatena	1		37	
		Lachnoclostridium		1		22	
		Roseburia		1		33	
		Oscillibacter			1		33
	Leuconostocaceae	*Weissella*		1		55	
		Faecalibacterium		3	2	55, 34, 44	33, 37
			Faecalibacterium prausnitzii	2	2	34, 44	33, 37
		Ruminococcus		4		22, 33, 44, 37	
	Selenomonadaceae	*Megamonas*		1		55	
	Streptococcaceae	*Streptococcus*		4		55, 22, 33, 44	
	Veillonellaceae	*Dialister*		1		37	
		Veillonella		1		33	
	Sporomusaceae	*Anaeroarcus*		1		34	
Fusobacteriota				1		55	
Proteobacteria				1		55	
	Enterobacteriaceae	*Enterobacter*			1		22
		Escherichia	*Escherichia coli*	1		33	
		Klebsiella		1		55	
		Raoultella		1		55	
	Holosporaceae	*Holospora*		1		44	
	Methylobacteriaceae	*Methylobacterium-Methylorubrum*		1		55	
	Methylococcaceae	*Methylovulum*		1		44	
			Methylovulum miyakonense	1		44	
	Pasteurellaceae	*Haemophilus*		1		55	
	Sutterellaceae	*Sutterella*			1		33
Thermodesulfobacteriota	*Desulfovibrionaceae*	*Bilophila*			1		33

Appendix

PRISMA 2020 Statement- Checklist that should be followed in a systematic review.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 2
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 10
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Pages 11 to 13
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 13
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 15
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 14
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 14
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 15
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 15
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 15
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 15
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 16 and Supplementary Table 2
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 15
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 15
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 15
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 15
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 15
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Page 15
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not applicable
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 16 and Supplementary

Section and Topic	Item #	Checklist item	Location where item is reported
			Table 2
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Pages 15 and 16
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 17
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 17
Study characteristics	17	Cite each included study and present its characteristics.	Pages 17 and 18
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 18 and Supplementary Table 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Pages 17 to 24
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Pages 17 to 24
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Pages 23 and 24
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not applicable
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not applicable
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 18 and Supplementary Table 2
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Page 24
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pages 25 to 27
	23b	Discuss any limitations of the evidence included in the review.	Pages 27 and 28
	23c	Discuss any limitations of the review processes used.	Pages 27 and 28
	23d	Discuss implications of the results for practice, policy, and future research.	Page 27
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not applicable
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Not applicable
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not applicable
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Not applicable
Competing interests	26	Declare any competing interests of review authors.	Not applicable
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not applicable

SPC's Guidelines for the Articles' Submission and Publication

The Portuguese Journal of Cardiology, the official journal of the Portuguese Society of Cardiology, was founded in 1982 with the aim of keeping Portuguese cardiologists informed through the publication of scientific articles on areas such as arrhythmology and electrophysiology, cardiovascular surgery, intensive care, coronary artery disease, cardiovascular imaging, hypertension, heart failure and cardiovascular prevention.The Journal is a monthly publication with high standards of quality in terms of scientific content and production. Since 1999 it has been published in English as well as Portuguese, which has widened its readership abroad.

Please, take into account that as of January 2021, *Revista Portuguesa de Cardiologia* will require new article submissions to be written in English language.

The Journal accepts the following categories of articles:

Research (Original Investigation and Meta-Analysis), Review and Education (Narrative Reviews, Systematic Reviews -without meta-analysis, Guidelines, Case Reports, Images in Cardiology and Snapshots), Opinion (Current Perspective), Correspondence (Editorial Comment, Letters to the Editor, Research Letter and Observation)

Types of article

Manuscripts submitted for publication should be prepared in accordance with the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" of the International Committee of Medical Journal Editors (ICMJE). This document is available at http://www.icmje.org/recommendations/.

Summary table of *Revista Portuguesa de Cardiologia* types of article characteristics.

Article type	Abstract	Keywords	Main text structure	Max. words	Tables/figures	References
Original Article	Max. 250 words; structured (Introduction and Objectives, Methods, Results and Conclusion(s))	3-10	Introduction; Objectives; Methods; Results; Discussion; Conclusion(s); Learning points/Take home messages; Acknowledgments, if any; References; Central figure, figure legends	4000 + 100 (learning points)	Total up to 10	up to 50
Review Article	Max. 250 words; unstructured	3-10	Introduction; thematic sections at the discretion of the authors; Conclusion(s); Acknowledgements, if any; References; and figure legends, if any	5000	Total up to 15	up to 75
Systematic Review with or without Meta-analysis	Max. 300 words; Structured	3-10	Introduction, Methods, Results, Discussion and Conclusion(s); Acknowledgements, if any; References; and figure legends, if any (PRISMA)	4000	Total up to 6	Up to 100
Case Report	Max. 250 words; unstructured	3-6	Introduction; Case report; Discussion; Conclusion(s) (optional); Learning points/Take home messages; References; and figure legends, if any (CARE)	1000 + 100 (learning points)	Up to 4	Up to 15
Images in Cardiology	None	None	Unstructured	250	Up to 2 figures; no tables	Up to 5
Editorial Comment	None	None	Unstructured	1000	1 table, 1 figure + photo of the author	Up to 20
Letter to the Editor	None	None	Unstructured	600	Up to 2 figures; no tables	Up to 5
Guidelines	Max. 350 words; unstructured	3-10	Introduction; thematic sections at the discretion of the authors; Conclusion(s); Acknowledgements, if any; References; and figure legends, if any	4000	Total up to 6	Up to 100
Current Perspectives	None	3-6	Unstructured	1200	Up to 2	Up to 10
Observational or Research Letter	None	3-6	Unstructured	600	Up to 2	Up to 6
Study Protocols	Max. 300 words; Structured	3-10	PRISMA-P or SPIRIT	4000	Up to 3	Up to 30

Review Articles and Systematic Reviews

Review Articles should have a maximum of 5000 words, with a total of up to 15 tables and/or figures, and should be structured as follows: Abstract (maximum 250 words; unstructured); 3-10 keywords; Introduction; thematic sections at the discretion of the authors; Conclusion(s); Acknowledgements, if any; References (up to 100); and figure legends, if any.

Systematic Reviews should be structured as Introduction, Methods, Results, Discussion and Conclusion(s). The subject should be clearly defined. The objective of a systematic review should be to produce an evidence-based conclusion. The Methods should give a clear indication of the literature search strategy, data extraction, grading of evidence and analysis.

Systematic Reviews should not normally exceed 4000 words, with a total of up to 6 tables and/or figures and up to 100 references.

Authors are strongly recommended to consult the PRISMA statement (http://www.prisma-statement.org/), which is intended to help improve the reporting of systematic reviews and meta-analyses. We encourage authors to develop a systematic review protocol (e.g. following PRISMA-P) and register with PROSPERO.

Guidelines

It is recommended to consult the AGREE II instrument for which items should be reported that highlighted particular quality aspects of guideline development. In general, published statements intended to guide clinical care (e.g., guidelines, practice parameters, recommendations, consensus statements and position papers) should describe the clinical problem to be addressed, the mechanism by which the statement was generated, a review of the evidence for the statement (if available), and the statement on practice itself.

To minimize confusion and to enhance transparency, such statements should begin with the following questions, followed by brief comments addressing each question:

- What other guideline statements are available on this topic?
- Why was this guideline developed?
- How does this statement differ from existing guidelines?
- Why does this statement differ from existing guidelines?

The statement should have an unstructured abstract of up to 350 words, 3 to 10 keywords and can include up to 4000 words, a total of up to 6 tables and/or figures and up to 100 references.

Contact details for submission

You can send your manuscript at https://www.editorialmanager.com/repc

Language

This journal is published in Portuguese and in English language.
The title (and abstract and key words if applicable) must be submitted in both English and Portuguese.

Articles submitted to the Journal should be clearly written in Portuguese (from Portugal) and/or English of a good standard. Text may be edited to maintain linguistic quality and to conform with standard American English.

ADVANCE NOTICE FOR AUTHORS

Please, take into account that as of January 2021, *Revista Portuguesa de Cardiologia* will require new article submissions to be written in English language.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

¿ E-mail address

¿ Full postal address

All necessary files have been uploaded:

Manuscript:

¿ Include keywords

¿ All figures (include relevant captions)

¿ All tables (including titles, description, footnotes)

¿ Ensure all figure and table citations in the text match the files provided

¿ Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

Further considerations

¿ Manuscript has been 'spell checked' and 'grammar checked'

¿ All references mentioned in the Reference List are cited in the text, and vice versa

¿ Permission has been obtained for use of copyrighted material from other sources (including the Internet)

¿ A competing interests statement is provided, even if the authors have no competing interests to declare

¿ Journal policies detailed in this guide have been reviewed

¿ Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' section of our ethics policy for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Authorship

Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. One or more authors should take responsibility for the integrity of the work as a whole, from inception to published article. According to the guidelines of the International Committee of Medical Journal Editors (ICMJE), authorship credit should be based on the following 4 criteria:

1. substantial contributions to conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; and
2. drafting of the work or revising it critically for important intellectual content; and
3. final approval of the version to be published; and
4. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Changes to authorship. Role of the corresponding author

A single corresponding author (or coauthor designee in the event that the corresponding author is unavailable) will serve on behalf of all coauthors as the primary correspondent with the editorial office during the submission and review process. If the manuscript is accepted, the corresponding author will review an edited manuscript and proof, make

decisions regarding release of information in the manuscript to the news media or federal agencies, handle all postpublication communications and inquiries, and will be identified as the corresponding author in the published article. The corresponding author also is responsible for ensuring that the Acknowledgment section of the manuscript is complete and that the conflict of interest disclosures reported of the manuscript are accurate, up-to-date, and consistent with the information provided in each author's potential conflicts of interest section in the Authorship Form.

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright
Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Open Access

Please visit our Open Access page from the Journal Homepage for more information.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good American English. Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article

Please submit your article via https://www.editorialmanager.com/repc

Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

Peer review

This journal operates a rigorous single blind peer review process, in which manuscripts are sent to external reviewers selected from an extensive database. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Peer reviewers will respond to the Editor within 30 days recommending acceptance, revision or rejection. The Editor will decide within 10 days whether to accept the manuscript without modification, to send the reviewers' comments to the authors for modification, or to reject it. When modifications are proposed, the authors have 30 days (which can be extended on request) to submit a revised version of the manuscript, incorporating the comments of the reviewers and the Editor. Any amendments should be highlighted in a different colour. The Editor will decide within 10 days whether to accept the new version, reject it, or send it for further review by one or more reviewers.

Letters to the Editor and Editorials will be reviewed by the Editorial Board, but external peer review may also be requested.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared

in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'. Use generic names of drugs (first letter: lowercase) whenever possible. Registered trade names (first letter: uppercase) should be marked with the superscript registration symbol ® or ™ when they are first mentioned.

The Journal recommends the guidelines for publication of the EQUATOR network (http://www.equator-network.org), including the CONSORT statement and its extensions for randomized trials (http://www.consort-statement.org/), STROBE for observational (cohort, case-control and cross-sectional) studies (http://www.strobe-statement.org/), STARD for diagnostic accuracy studies (http://www.stard-statement.org/), PRISMA for systematic reviews and meta-analyses (http://www.prisma-statement.org/), SQUIRE for quality improvement studies (http://www.squire-statement.org/) and CARE for case reports (http://www.care-statement.org/). Reporting of the statistical aspects of studies should be in accordance with the Statistical Analyses and Methods in the Published Literature (SAMPL) guidelines (http://www.equator-network.org/reporting-guidelines/sampl/).

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference.

If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Cover letter and Essential title page information

Submission of an article must include a cover letter with the following information:

1. a brief description of the article's significance and/or interest;
2. a declaration of originality, specifying that none of the paper's contents have been published or are under consideration elsewhere;
3. a declaration that all authors have read and approved the manuscript;
4. a full disclosure of any potential conflict of interest for any of the authors;
5. and which manuscript type is being submitted for publication.

Title page must contain the following information:

• *Title*. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. Preferably not exceed 12 words. It may also include a subtitle of up to 4 words. All nouns, adjectives and verbs in the title and subtitle must begin with a capital letter.

• *Author names and affiliations*. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's

name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• *Corresponding author*. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
• *Present/permanent address*. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.
• *Word count* of the manuscript text.

Structured abstract

A structured abstract, by means of appropriate headings, should provide the context or background for the research and should state its purpose, basic procedures (selection of study subjects or laboratory animals, observational and analytical methods), main findings (giving specific effect sizes and their statistical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations.

Abstracts for all article types should not contain any references. Abbreviations should be avoided or kept to a minimum.

The headings will consist of: Introduction and Objectives, Methods, Results and Conclusion(s))

Keywords

Immediately after the abstract, provide the keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. Keywords should ideally be selected from the list of MeSH terms available at www.nlm.nih.gov/mesh/. These keywords will be used for indexing purposes.

Abbreviations, Product Names and Gene Names

Do not use abbreviations in the title or abstract and limit their use in the text. Expand all abbreviations at first mention in the text.

Ensure consistency of abbreviations throughout the article.

Do not use abbreviations in the title or abstract and limit their use in the text. Expand all abbreviations at first mention in the text.

Ensure consistency of abbreviations throughout the article.

Names of Drugs, Devices, and Other Products

Use nonproprietary names of drugs, devices, and other products and services, unless the specific trade name of a drug is essential to the discussion. In such cases, use the trade name once and the generic or descriptive name thereafter. Do not include trademark symbols.

Gene Names, Symbols, and Accession Numbers

Authors describing genes or related structures in a manuscript should include the names and official symbols provided by the US National Center for Biotechnology Information (NCBI) or the HUGO Gene Nomenclature Committee. Before submission of a research manuscript reporting on large genomic data sets (eg, protein or DNA sequences), the data sets should be deposited in a publicly available database, such as NCBI's GenBank, and a complete accession number (and version number if appropriate) must be provided in the Methods section or Acknowledgment of the manuscript.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.)

Units of Measure

Laboratory values are expressed using conventional units of measure, with relevant Système International (SI) conversion factors expressed secondarily (in parentheses) only at first mention. Articles that contain numerous conversion factors may list them together in a paragraph at the end of the Methods section. In tables and figures, a conversion factor to SI should be presented in the footnote or legend. The metric system is preferred for the expression of length, area, mass, and volume. For more details, see the Units of Measure conversion table on the website for the AMA Manual of Style.

Artwork

Tables and Figures. Image manipulation

Restrict tables and figures to those needed to explain and support the argument of the article and to report all outcomes identified in the Methods section. Number each table and figure and provide a descriptive title for each. Every table and figure should have an in-text citation. Verify that data are consistently reported across text, tables, figures, and supplementary material.

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.

• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then, at no additional charge, these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print. Further information on the preparation of electronic artwork.

Figures

Number all figures (graphs, charts, photographs, and illustrations) in the order of their citation in the text. The number of figures should be limited. Avoid complex composite or multipart figures unless justified.
See Categories of Articles for limits on the number of figures and/or tables according to article type.

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used written out in full and in alphabetical order. Different panels in a figure should be identified by capital letters (Figure 1A, Figure 2C, Figure 3A and B, etc. in the text and (A), (B), (C-E), etc. in the captions).

Text graphics
Text graphics may be embedded in the text at the appropriate position. See further under Electronic artwork.

Tables
Include a brief title for each table (a descriptive phrase, preferably no longer than 10 to 15 words) and number all tables in the order of their citation in the text. Refer to Categories of Articles for limits on the number of tables.

Do not embed tables as images in the manuscript file or upload tables in image formats, and do not upload tables as separate files. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end.

Place any table notes below the table body. Please avoid using vertical rules and shading in table cells.

All abbreviations used in the table must be written out in full, in alphabetical order, in the table legend immediately below the table. Footnotes may be used if necessary, indicated by superscript lower-case letters ([a], [b], [c] etc.) in the table and in the legend. Asterisks (*, **, *** etc.) may be used to indicate p values only. If a table contains a reference cited in the text, it should be cited with the name of the first author and "et al." followed by the reference number without space (e.g. Millard et al.[9]).

References

Citation in text. Reproduced material.

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Personal Communications and Unpublished Data

A signed statement of permission should be included from each individual identified as a source of information in a personal communication or as a source for unpublished data, and the date of communication and whether the communication was written or oral should be specified. Personal communications should not be included in the list of references but added to the text parenthetically.

Reproduced material

Please verify that all information and materials in the manuscript are original. The journal generally does not republish text, tables, figures, or other material from other publishers, except in rare circumstances. If you believe that you must include content that is owned by a third party, please let us know and provide information about all material that has been previously published and, when applicable, include author(s), title of article, title of journal or book or other publication, and complete citation, doi, and/or URL.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: Ribeiro J. Left ventricular noncompaction and Fabry disease: An unlikely association. Rev Port Cardiol. 2019. https://doi.org/10.1016/j.repc.2019.12.001. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. This identifier will not appear in your published article.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style

Text: Indicate references by superscript numbers in the text. The actual authors can be referred to, but the reference number(s) must always be given.
List: Number the references in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
17. Reis L, Paiva L, Costa M, Silva J, Teixeira R, Botelho A, et al. Registry of left atrial appendage closure and initial experience with intracardiac echocardiography. Rev Port Cardiol. 2018;37:763-72. https://doi.org/10.1016/j.repc.2018.03.009.

Reference to a journal publication with an article number:
2. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. *Heliyon*. 2018;19:e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:
30. Cohn PF. Silent myocardial ischemia and infarction. 3rd ed. New York: Mansel Dekker; 1993.

Reference to a chapter in an edited book:
23. Nabel EG, Nabel GJ. Gene therapy for cardiovascular disease. In: Haber E, editor. Molecular cardiovascular medicine. New York: Scientific American;1995.p.79-96.
Reference to a website:
12. Portuguese Registry on Acute Coronary Syndromes (ProACS). Available at: http://www.clinicaltrials.gov/identifier NCT01642329 [accessed 26 October 2013].
Reference to a dataset:
[dataset] 5. Oguro M, Imahiro S, Saito S, Nakashizuka T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. https://doi.org/10.17632/xwj98nb39r.1.

Note shortened form for last page number. e.g., 51–9, and that for more than 3 authors the first 6 should be listed followed by 'et al.' For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997;277:927–34)(see also Samples of Formatted References).

Journal abbreviations source
Journal names should be abbreviated according to the PubMed list.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure

that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

RESEARCH DATA

This journal encourages you to share data that supports your research publication in an appropriate data repository, and enables you to interlink the data with your published articles. If you are sharing data, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation.

Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Mendeley Data

This journal supports *Mendeley Data*, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. Before submitting your article, you can deposit the relevant datasets to Mendeley Data. Please include the DOI of the deposited dataset(s) in your main manuscript file. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.