

# The Microbiome–Imaging Axis: Can Radiology Detect Microbial Influences on Disease?

**Naeem Hamza\*, Majbour Lana, Shtat Shahad Hazar Ismael, Wardeh Mahdi, MHD Bashir Almonajjed, & Alexandre Wirman**

General Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj Napoca, Romania

**\*Corresponding author:** Naeem Hamza, General Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj Napoca, Romania.

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## Abstract

The microbiome–imaging axis, or radio microbiomics, is an emerging field that combines medical imaging with gut microbiome analysis to map how the gut communicates with distant organs, particularly the brain. While traditional research often focuses on simple correlations, this framework uses structural and functional imaging to visualize the actual physical impact of gut dysbiosis on host tissue. This review explores how microbial metabolites, such as short-chain fatty acids (SCFAs) and bile acids, act as molecular messengers that trigger changes in brain connectivity, cortical thickness, and liver fat deposition. We examine the clinical utility of these findings as non-invasive biomarkers for Alzheimer’s disease, Multiple Sclerosis, and NAFLD. Additionally, we discuss the development of pathogen-specific PET tracers that allow doctors to see active infections directly, rather than just the body’s inflammatory response.

**Keywords:** Radio Microbiomics, Microbiota-gut-brain axis (GBA), Multi-omics integration, Gut-liver axis, Functional Connectivity (FC), Diffusion Tensor Imaging (DTI), Voxel-based morphometry (VBM), Bacteria-specific PET tracers, Microbial metabolites, Bile acids (BAs), Short-chain fatty acids (SCFAs), Lipopolysaccharides (LPS).

## Introduction

The microbiome–imaging axis, often referred to as radio microbiomics, represents an emerging interdisciplinary framework that integrates advanced medical imaging with gut microbiome analysis to visualize and quantify interactions between the gastrointestinal tract and distant organ systems, particularly the brain [1-6]. This framework builds on accumulating evidence that the gut microbiota communicates with the central nervous system through metabolic, immune, and neural pathways, and that these interactions can be captured using structural, functional, and metabolic imaging techniques. By combining microbiome profiling with radiological data, radio microbiomics provides a systems-level approach to characterizing the biological mechanisms underlying the gut–brain axis (GBA) and the contribution of gut dysbiosis to disease pathogenesis [1-6].

The core strength of the microbiome–imaging axis lies in its capacity to integrate quantitative imaging parameters—including brain morphology, connectivity, and metabolic activity—with microbial compositional and functional data to map the down-

stream effects of gut-derived signals on the central nervous system. While early microbiome research primarily identified associations between specific microbial taxa and neurological disorders, this framework extends beyond correlation by linking microbial alterations to observable structural and functional brain changes. Radio microbiomics specifically leverages radiomics-derived imaging features alongside high-throughput microbiome datasets to identify imaging biomarkers and potential mechanistic pathways within the GBA. Importantly, this approach supports a bidirectional model, capturing not only how microbial metabolites and immune mediators influence CNS architecture, but also how brain activity feeds back to regulate gut physiology [1-6].

Understanding how gut microbial alterations are reflected in medical imaging is critical for uncovering disease mechanisms, identifying biomarkers, and improving clinical decision-making. Whereas microbiome studies typically provide compositional or functional snapshots, imaging enables visualization of the physiological consequences of dysbiosis, including altered

white matter integrity, cortical thinning, and disrupted functional connectivity. These imaging correlates help distinguish pathogenic microbial effects from adaptive or compensatory changes. Moreover, imaging-based signatures associated with dysbiosis show promise as non-invasive biomarkers for early diagnosis, risk stratification, and prognostication in conditions such as Alzheimer's disease, schizophrenia, and Crohn's disease. Integration of imaging with microbiome and metabolomic data further supports precision medicine approaches by improving patient stratification and therapeutic targeting. For instance, combined MRI–microbiome models have been used to estimate biological age in schizophrenia, enhancing assessment of cognitive decline, and to improve prediction of cumulative bowel damage in Crohn's disease. Imaging also provides an objective means of monitoring responses to microbiome-targeted interventions, such as probiotics, prebiotics, and dietary modification, through longitudinal assessment of brain structure and function [1-8].

Multiple imaging modalities contribute complementary insights into microbiome-related disease processes. Magnetic Resonance Imaging (MRI) is the most extensively utilized modality. Structural MRI and voxel-based morphometry have demonstrated associations between specific microbial taxa and alterations in hippocampal volume, cortical thickness, and gray matter morphology in disorders including Alzheimer's disease and irritable bowel syndrome [1-4].

Diffusion Tensor Imaging (DTI); has revealed correlations between taxa such as Eggerthellaceae and white matter tract integrity, particularly in pathways relevant to memory and language, and has identified microstructural abnormalities in germ-free animal models. Functional MRI (fMRI), both resting-state and task-based, has linked gut microbiota composition to altered connectivity in networks governing emotion, cognition, and autonomic regulation, including evidence of probiotic-induced modulation of the default mode network. Magnetic Resonance Spectroscopy (MRS) enables *in vivo* quantification of brain metabolites and has identified abnormal choline peaks in the anterior cingulate cortex of individuals at ultra-high risk for psychosis, consistent with membrane dysfunction potentially related to dysbiosis [1-7].

Beyond neuroimaging, Magnetic Resonance Enterography (MRE) enables macroscopic assessment of intestinal inflammation and structural damage in Crohn's disease, and its integration with microbiome signatures improves prediction of disease severity and progression [8]. Emerging ultra-high-field (UHF) MRI offers unprecedented spatial resolution for visualizing small brainstem and spinal structures implicated in vagal and spinal components of the GBA [2]. Positron Emission Tomography (PET) further complements MRI by providing metabolic and molecular specificity. FDG-PET and amyloid-targeted tracers have demonstrated associations between microbiome alterations and cerebral glucose metabolism, amyloid deposition, and neuroinflammation in Alzheimer's disease [1-4]. PET imaging of microglial activation offers insight into inflammatory processes potentially driven by microbial metabolites such as short-chain fatty acids [7]. Although modalities such as CT and ultrasound play supporting roles—particularly in hybrid approaches such as PET-CT—advanced MRI techniques remain central due to their superior soft tissue characterization and compatibility

with multi-omic integration [8, 9]. Overall, the microbiome–imaging axis represents a transformative approach for visualizing the systemic consequences of gut dysbiosis. By integrating radiological phenotyping with microbial and metabolic data, this framework enhances mechanistic understanding, supports the development of non-invasive biomarkers, and lays the foundation for personalized therapeutic strategies across neurological and gastrointestinal disorders.

Building on these imaging-based insights into gut–organ communication, the following section focuses on the gut–liver axis, where microbiome-driven metabolic and inflammatory pathways can be directly quantified using advanced hepatic imaging techniques.

### **Microbiome and Metabolic/Liver Diseases**

The gut–liver axis is a central regulator of metabolic homeostasis, reflecting the bidirectional interaction between the gut microbiota and hepatic physiology. Owing to its anatomical and functional connection to the intestine via the portal circulation, the liver is continuously exposed to gut-derived metabolites, microbial products, and inflammatory mediators, rendering it particularly susceptible to alterations in microbial composition and activity [2, 3]. Accumulating evidence indicates that gut dysbiosis plays a critical role in the initiation and progression of non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome by modulating hepatic lipid accumulation, inflammation, and fibrogenesis through multiple interconnected biological pathways.

### **Influence of Gut Microbiota on Liver Fat, Fibrosis, and Inflammation**

Several mechanisms link microbial imbalance to liver pathology. Increased intestinal permeability, a hallmark of dysbiosis, facilitates translocation of bacteria and microbial products such as lipopolysaccharide (LPS) into the portal circulation [2-4]. LPS activates Toll-like receptor 4 (TLR4) on hepatic Kupffer cells, inducing pro-inflammatory cytokine release—including TNF- $\alpha$  and IL-6—which promotes hepatic inflammation and triggers stellate cell activation, a key driver of fibrogenesis [1-5]. Dysregulation of bile acid metabolism further contributes to metabolic dysfunction. Microbiota-mediated modification of bile acids alters signaling through the FXR and TGR5 pathways, disrupting glucose and lipid homeostasis [1-7]. Secondary bile acids, such as deoxycholic acid, may also impair intestinal barrier integrity and exert hepatotoxic effects [5].

Additional microbially mediated pathways exacerbate NAFLD pathogenesis. Certain bacterial species, including *Klebsiella pneumoniae* and *Escherichia* spp., produce endogenous ethanol, increasing oxidative stress and intestinal permeability and thereby amplifying hepatic injury [2-5]. Microbial conversion of dietary choline into trimethylamine (TMA) reduces choline availability for very-low-density lipoprotein (VLDL) synthesis, impairing hepatic lipid export and promoting steatosis [1,5]. Alterations in tryptophan metabolism also contribute to disease progression. A shift from the protective indole pathway toward the pro-inflammatory kynurene pathway—driven by increased indoleamine 2,3-dioxygenase (IDO) activity—has been associated with hepatic inflammation and fibrosis [4]. Reduced levels of indole-3-propionic acid (IPA), a microbial metabolite that

supports gut barrier integrity, have likewise been linked to more advanced fibrotic disease [4].

### Imaging Methods Used to Evaluate Microbiome-Related Liver Changes

Non-invasive imaging modalities play a critical role in quantifying hepatic steatosis, inflammation, and fibrosis, particularly in research contexts where liver biopsy is impractical or unethical [8]. MRI–Proton Density Fat Fraction (MRI–PDFF) is a highly precise and reproducible technique for quantifying hepatic fat content and is widely adopted as a non-invasive biomarker, with a threshold of  $\geq 5\%$  commonly used to define NAFLD [6–8]. Magnetic Resonance Elastography (MRE) provides accurate assessment of liver stiffness as a surrogate marker of fibrosis and is considered the most sensitive non-invasive method for detecting advanced fibrosis, with values  $\geq 3.63$  kPa indicating clinically significant disease [6–8].

Ultrasound-based elastography techniques, including vibration-controlled transient elastography (VCTE; FibroScan), offer accessible alternatives for estimating liver stiffness; however, their diagnostic accuracy may be reduced in individuals with obesity and they are less reliable for staging disease severity [3–8]. Conventional ultrasonography remains widely used for detecting hepatic steatosis but has limited sensitivity for mild fat infiltration and cannot reliably distinguish simple steatosis from non-alcoholic steatohepatitis (NASH) [3–5]. Collectively, these imaging approaches provide non-invasive platforms for linking structural and functional liver changes with microbiome-derived metabolic and inflammatory signatures.

### Microbial Taxa and Metabolites Correlating with Imaging Findings

Recent studies integrating microbiome profiling with MRI–PDFF and MRE have identified characteristic microbial and metabolomic patterns associated with hepatic steatosis and fibrosis. Advanced fibrosis, as defined by MRE, is consistently associated with increased abundance of Gram-negative taxa, including Proteobacteria, Enterobacteriaceae, and *Escherichia coli*, alongside depletion of beneficial Firmicutes such as *Eubacterium rectale* and *Ruminococcus obeum* [1]. In NAFLD-related cirrhosis, microbial signatures shift further toward enrichment of *Streptococcus*, *Megasphaera*, and *Gallibacterium*, accompanied by marked reductions in *Faecalibacterium prausnitzii* [8]. Metabolomic analyses have identified 3-(4-hydroxyphenyl)lactate as a metabolite jointly associated with hepatic fibrosis and steatosis, correlating strongly with the abundance of *Bacteroides caccae*, *Clostridium* spp., and *Escherichia coli* [6].

Microbial correlates of hepatic steatosis, assessed using MRI–PDFF or ultrasound, include elevated abundance of the family *Veillonellaceae*, which has been linked to increased NAFLD risk [7]. Conversely, taxa such as *Rikenellaceae*, *Barnesiellaceae*, and *Bifidobacterium adolescentis* are associated with reduced disease likelihood [7]. Taurocholic acid, a bile acid derivative, positively correlates with NAFLD risk and higher microbiome-based risk scores [7]. Consistent with fibrosis-associated findings, elevated levels of 3-(4-hydroxyphenyl)lactate are also observed in individuals with MRI-defined NAFLD, reinforcing its role as a shared microbial metabolite associated with both hepatic fat accumulation and fibrotic remodeling [6]. Despite

these consistent associations, the predominantly cross-sectional design of existing studies limits causal inference, underscoring the need for longitudinal, multi-omic imaging studies to clarify temporal relationships.

### Microbiome and Brain Imaging: The Gut–Brain Axis

The gut–brain axis (GBA) operates as a bidirectional communication network through which the gut microbiota interacts with the central nervous system via neural, endocrine, immune, and metabolic pathways [9]. Dysbiosis—alterations in microbial composition—can affect brain plasticity, structural organization, and physiological activity by modulating neurotransmitter production, influencing the hypothalamic–pituitary–adrenal axis, activating inflammatory cascades, and changing microbial metabolite availability [9, 10]. Advances in neuroimaging have enabled the detection of these microbiome-driven effects on functional networks, cortical morphology, white-matter architecture, and neurometabolite signatures.

### Influence of Gut Microbiota on Brain Structure, Connectivity, and Metabolism

Evidence indicates that gut microbiota are critical modulators of intrinsic functional brain networks. Functional connectivity (FC) analyses reveal that microbial composition affects large-scale systems such as the default mode network (DMN), salience network (SN), and frontoparietal network (FPN). Genera including *Prevotella* and *Bacteroides* show strong associations with connectivity strength within these networks [11]. Microbial diversity correlates with global network topology, with higher diversity linked to small-world network properties that support cognitive functions like working memory [16]. Experimental studies in germ-free mice demonstrate widespread hyperconnectivity and poorly modularized networks, highlighting the importance of microbial colonization for normal synaptic pruning and network maturation [14]. Additionally, gut microbes influence structural–functional coupling in regions such as the fusiform gyrus and hippocampus, affecting cognitive control and attentional processes [16].

Microbiome-related changes also extend to brain structure and microstructure. Structural MRI studies indicate that microbial enterotypes, such as *Bacteroides* or *Prevotella* dominance, are associated with differences in cortical thickness and gray matter volume. Individuals with a *Bacteroides* enterotype often show reduced prefrontal cortical thickness compared with those dominated by *Ruminococcaceae* or *Prevotella* [10]. Diffusion tensor imaging (DTI) links families such as *Selenomonadaceae* and *Veillonellaceae* with white-matter integrity in the frontal cortex and cerebellum [11]. Germ-free mouse models complement these findings, showing immature microglia, altered dendritic spine density, and impaired structural organization in the absence of microbiota [14].

Inflammatory and metabolic pathways form another critical connection between gut microbial communities and neural function. In schizophrenia, peripheral cytokines (IL-2, IL-6, TNF- $\alpha$ ) mediate relationships between specific bacterial taxa, such as *Succinivibrio*, and altered anterior cingulate cortex activity [9]. Short-chain fatty acids (SCFAs), mainly produced by commensal bacteria, maintain blood–brain barrier integrity and reduce neuroinflammation [10]. Reduced SCFA-producing genera are

common in depression and schizophrenia and are linked to abnormal neural responses [9]. Microbial genera including *Bacteroides* and *Parabacteroides* also regulate glutamate–GABA pathways, connecting dysbiosis to altered metabolic activity in cerebellar and limbic circuits [10].

**Neuroimaging Techniques to Study the Gut–Brain Relationship**  
Radiomicromics, which integrates microbiome data with neuroimaging, has transformed the study of gut–brain interactions [10]. Resting-state fMRI (rs-fMRI) remains the primary tool for mapping FC alterations related to microbial variability, with dysbiosis linked to disrupted synchrony in DMN, SN, and limbic networks [11, 12]. Task-based fMRI shows complementary effects; probiotic supplementation can reduce amygdala reactivity to emotional stimuli and enhance executive control circuits during working-memory tasks [12, 13].

DTI reveals associations between microbial taxa and white-matter integrity in frontal lobes, cerebellum, and corpus callosum [11]. Structural MRI measures cortical thickness and gray-matter volume, showing microbiome-related differences in the hippocampus and prefrontal cortex [10]. Magnetic resonance spectroscopy (MRS) provides metabolic insights by quantifying neurometabolites such as GABA, glutamate, and N-acetylaspartate, corresponding to microbiome composition or probiotic interventions [9].

Machine learning models combining microbial sequencing with neuroimaging biomarkers improve disease classification. Support vector machines and deep learning approaches achieve high accuracy (AUC > 0.90) in distinguishing clinical populations from controls based on microbial abundance and neural features [9, 10].

#### Diseases Studied in the Gut–Brain–Imaging Context

Major depressive disorder (MDD) shows reduced SCFA-producing bacteria (*Faecalibacterium*, *Coprococcus*) and increased pro-inflammatory taxa (*Enterobacteriaceae*, *Eggerthella*), correlating with abnormal hippocampal and DMN connectivity [10]. IBS demonstrates structural alterations in the prefrontal cortex and hypothalamus, with disrupted SN connectivity [18]. Schizophrenia presents a strong inflammatory microbiota–brain axis, where elevated cytokines associated with *Succinivibrio* and *Proteus* correlate with reduced regional homogeneity and altered brain volume [9]. ASD is linked to microbial *Clostridium* overgrowth, associated with reduced fractional anisotropy in the corpus callosum [10]. Bipolar disorder and hepatic encephalopathy further illustrate the influence of microbial modulation on neural function and connectivity [5–21].

#### Imaging Microbial Infections Directly

Radiology and nuclear medicine are increasingly essential for detecting infectious processes; however, conventional imaging lacks sensitivity and specificity. CT and MRI are widely used to localize infections and determine tissue involvement [22], but they rely on structural changes like edema, necrosis, or fluid collections, which appear only at later stages [22, 23]. Early infection often goes undetected, and anatomical imaging cannot reliably differentiate active bacterial infection from sterile inflammation or malignancy [22, 23]. Conventional nuclear medicine methods using [18F]FDG or radiolabeled leukocytes

detect inflammatory activity rather than pathogens, generating false positives in sterile inflammatory lesions or tumors [22, 23]. To overcome these limitations, microbe-targeted radiopharmaceuticals have been developed to image pathogens directly. These agents exploit prokaryote- or fungal-specific pathways, such as siderophore-mediated iron acquisition, specialized sugar metabolism, and folate synthesis [22, 23]. Radiolabeled siderophores like [68Ga]Ga-DFO-B selectively accumulate in infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* [22]. Para-aminobenzoic acid (PABA) analogs target bacterial folate synthesis, allowing specific detection without uptake in noninfected host tissue [24].

Among promising tracers is 2-deoxy-2-[18F] fluoro-D-sorbitol ([18F]FDS), which selectively enters Enterobacteriales via a sorbitol-specific pathway absent in mammalian cells [24, 25]. [18F] FDS accumulates in infected tissues but not in *Staphylococcus aureus*, host tissues, or cancer cells, and can distinguish fungal species (*C. albicans* vs. *C. glabrata*) [24, 25]. In contrast, [18F] FDG accumulates non-specifically in metabolically active tissues, including sterile inflammation and tumors [25].

Microbial PET tracers are evaluated for diverse infections. In invasive aspergillosis, [18F]FDS distinguishes fungal infiltrates from bacterial pneumonia or sterile inflammation in immunocompromised patients [25]. Musculoskeletal infections use D-methyl-[11C]methionine and [68Ga]Ga-NOTA-UBI29-41 to differentiate septic from aseptic implant loosening [26]. Pulmonary and cardiovascular infections, including tuberculosis and endocarditis, are being studied using various pathogen-specific tracers [26]. MRI complements PET by providing structural information and elucidating microbiome–host interactions across organ systems [23, 24].

#### Molecular Pathways Linking Microbiome to Imaging Changes

The gut microbiome (GM) and its metabolites exert profound effects on host health and central nervous system (CNS) function. Research across neurodegenerative, psychiatric, and inflammatory diseases consistently highlights that the microbiota-gut-brain axis (GBA) represents a robust bidirectional communication system, necessitating advanced methodologies to elucidate underlying mechanisms [23].

#### Microbial Metabolites and Host Pathways

The mechanistic link between gut flora dysbiosis and host pathology is mediated by small-molecule metabolites that modulate immune, metabolic, and neural systems.

#### Short-Chain Fatty Acids (SCFA) and Neuroinflammation

SCFAs, primarily acetate (AA), propionate (PA), and butyrate (BA), are essential microbial products frequently depleted in disease states [28]. Lower plasma PA/AA and BA/AA ratios are strongly associated with increased T2 lesion load and higher disability scores (EDSS) in patients with multiple sclerosis (MS) [29]. These depleted SCFA ratios negatively correlate with pro-inflammatory cytokine-producing immune cells (GM-CSF+, TNF- $\alpha$ +, IFN- $\gamma$ + T and B cells), suggesting that SCFA imbalances promote environments that exacerbate neurodegenerative processes [29]. Similarly, in Alzheimer's disease (AD) and amnestic Mild Cognitive Impairment (aMCI), SCFA con-

centrations decline progressively, coinciding with a reduction in SCFA-producing Firmicutes taxa such as Clostridia and Blautia [28-30].

### Bile Acids, Lipids, and Toxic Byproducts

Other mediating metabolites include bile acids (BAs) and host lipids. Altered BA profiles correlate with neuroimaging biomarkers in AD; for instance, lower cholic acid (CA) levels are associated with decreased hippocampal volume and reduced FDG-PET brain glucose metabolism [28]. Lipid metabolism dysfunction in AD, reflected by declines in serum sphingomyelin (SM) and ether-containing phosphatidylcholines (PC), affects cellular lipid rafts—platforms influencing A $\beta$  accumulation and tau oligomer production, linking metabolic status to structural integrity [28].

Pro-inflammatory microbial products such as lipopolysaccharide (LPS), derived from Gram-negative bacteria (e.g., *Bacteroides*), translocate across impaired barriers, linking systemic inflammation to brain regions with elevated amyloid load (frontal, anterior cingulate, precuneus cortex) as visualized by PET imaging [23-31].

### Microbial Metabolic Deficiency and Organ Function

In the gastrointestinal tract, SCFA shortage due to reduced bacterial load (e.g., via broad-spectrum antibiotics) forces colonocytes to switch energy metabolism to glycolysis, resulting in measurable increases in colonic 18F-FDG uptake (SUV<sub>max</sub>/mean) on FDG-PET-CT [32]. This demonstrates a unique functional imaging application to monitor host-microbiota interactions [32].

**Quantitative Neuroimaging: Mapping the Microbiome's Impact**  
Quantitative neuroimaging is essential for translating GBA research into spatial and temporal visualization of microbial-induced brain effects [23].

### Functional MRI (fMRI) and Connectivity Changes

Resting-state fMRI (rsfMRI) measures functional connectivity (FC) and BOLD signal alterations due to GM changes [23]. In aMCI patients, regions with decreased intrinsic brain activity, particularly the cerebellar vermis IV-V (0.01–0.08 Hz), negatively correlate with Bacteroidetes abundance [30]. Functional disruptions in cerebellar regions, traditionally linked to motor control and cognition, parallel decreased cognitive scores. Probiotics or fermented milk products modulate brain activity in emotion- and sensation-related networks, such as the DMN and salience network, decreasing BOLD signals in viscero-sensory cortices [23-30].

### Structural and Microstructural Imaging (VBM and DTI)

Voxel-based morphometry (VBM) identifies structural changes; studies link GM composition to increased sensory region volumes and decreased insular and prefrontal cortices in IBS patients [27]. Germ-free (GF) mice models further demonstrate commensal bacteria are necessary for normal neural morphologic development, showing regional expansion of olfactory bulbs and prefrontal cortex [23-31].

Diffusion tensor imaging (DTI) provides fractional anisotropy (FA) and mean diffusivity (MD) measures of white matter in-

tegrity. Fecal matter transplantation (FMT) from ADHD patients into GF mice reduces FA and increases MD in hippocampus and fornix, indicating GM directly impacts neural microstructure [23-31]. Increased Actinobacteria abundance correlates with higher FA in amygdala and thalamus in obese men, underscoring DTI specificity beyond VBM [23].

### Multi-Omics Integration and Biomarker Discovery

Integration of microbiome, metabolome, and functional gene data identifies reproducible, disease-specific signatures, enhancing diagnostic accuracy.

### Integrative Analysis in IBD

Cross-cohort integrative analysis (CCIA) of IBD used nine metagenomic and four metabolomic cohorts, identifying 31 species, 25 KO genes, and 13 metabolites that consistently differentiated IBD from healthy controls [33]. Integration of multi-omics signatures improved AUROC to 0.98, outperforming single-omics models [33]. KEGG orthology (KO) analysis highlighted upregulated two-component systems and downregulated propionate metabolism, with *crp* gene expression correlating with fecal calprotectin [33]. Multi-omics correlation maps revealed impaired microbial biotransformation (e.g., *rocF* downregulation leading to urea accumulation) and enriched aminoacyl-tRNA biosynthesis, suggesting immune regulatory roles [33].

### Shotgun Metagenomics in Hematopoietic Cell Transplantation (HCT)

In HCT patients under chemotherapy and broad-spectrum antibiotics, shotgun metagenomic sequencing enabled high-resolution functional analysis of resistomes and virulence factors [31]. Metagenome-assembled genomes (MAGs) tracked bacterial population dynamics, including shifts in dominant *Enterococcus faecium* strains, validated with orthogonal PCR [31]. These analyses highlight clinical relevance for detecting microbial threats in vulnerable populations [31].

### Future Directions

Neuroimaging and multi-omics integration complement each other in characterizing GBA functional consequences [23-32]. While metagenomics and metabolomics reveal microbial components and molecular messengers, quantitative neuroimaging (fMRI, DTI, PET) provides measurable evidence of temporal and spatial effects on CNS and GI tissues [23-32]. Longitudinal studies and controlled preclinical models (GF and gnotobiotic animals) are critical for confirming causality [23]. The combined use of imaging and multi-omics data holds substantial potential for developing non-invasive, high-accuracy biomarkers for diagnosis, prognosis, and therapy monitoring in complex diseases such as AD, MS, and IBD [23-33].

### Radio microbiomics and Multi-Omics Integration

#### Defining Radio microbiomics and Neuroimaging-Omics

Radio microbiomics integrates quantitative brain imaging with gut microbiome data, enabling investigation of complex bidirectional communication systems like the GBA, particularly relevant in AD pathogenesis [28]. Neuroimaging-omics or multi-omics integration combines radiomic features with biological data (microbiomics, genomics, metabolomics) to identify multi-dimensional signatures critical for understanding interaction mechanisms and discovering biomarkers or therapeutic

targets [28-34]. Data layers include microbiome composition (via 16S rDNA or metagenomic sequencing) [28]; imaging-derived radiomics (multi-modal MRI, 18F-FDG-PET) [28-35]; and metabolomics profiling (e.g., SCFAs, BAs) as intermediate signals bridging gut microbiota and brain [28].

### AI and Deep Learning Approaches for Integration

Artificial intelligence (AI), especially deep learning (DL), is crucial for integrating high-dimensional, heterogeneous radiomics and multi-omics data [34]. CNNs process raw 2D/3D images, extracting features while maintaining spatial context [34]. Generative models (VAEs, GANs) handle incomplete data, generate synthetic samples, and infer missing modalities [34]. Transformers combined with GANs can relate MRI features to SNP data to predict cognitive decline [34].

Sequential models (RNNs) handle longitudinal imaging data in diseases like AD, and combined RNN-VAE frameworks capture both temporal and cross-modal dimensions [34]. Integration strategies—early, intermediate, late fusion—enable learning nonlinear inter-modality relationships and shared latent spaces [34].

### Challenges in Combining High-Dimensional Imaging and Microbiome Data

Integrating imaging and omics data presents challenges due to heterogeneity, scale differences, and missingness [35]. High feature dimensionality leads to overfitting and unreliable analyses. Spatial and temporal discrepancies occur because imaging is longitudinal while molecular profiling may not be systematic [35, 36]. Differences in technical platforms, measurement scales, and feature counts complicate integration [34, 35]. Missing modalities reduce usable sample size, limiting machine learning performance [36].

Lack of standardized nomenclature linking radiomic data with biological omics further hinders reproducibility and global correlation [35]. Addressing these challenges requires innovative multi-layer computational systems to ensure structured relationships and consistency across data types [35].

### Methodological Challenges and Study Quality

The central methodological challenge in microbiome-imaging research is that current imaging modalities do not visualize microorganisms directly; instead, they detect microbial metabolic activity or downstream effects on host tissues (37). This indirect detection paradigm reflects the physical limitations of existing imaging technologies, particularly their insufficient spatial resolution to resolve individual microbes *in vivo* (37). While intentional, this constraint introduces interpretative challenges when distinguishing microbial-derived signals from host background effects, especially in complex biological environments. These challenges are further compounded by the intrinsic complexity, inter-individual variability, and temporal instability of the human microbiome [37].

A substantial body of literature demonstrates that systematic biases may be introduced at nearly every stage of microbiome research, from sample acquisition to downstream bioinformatic analysis [38]. When such biases intersect with imaging-derived endpoints, they may propagate or amplify error, underscoring

the need for rigorous methodological control and cautious interpretation [38].

### Limitations and Biases in Microbiome-Imaging Studies

#### Low-Microbial-Biomass Samples and Contamination

Low-microbial-biomass (LMB) samples represent one of the most significant constraints in microbiome-imaging studies, particularly when derived from tissues traditionally considered sterile, such as blood, lung, placenta, or solid organs (39). In these settings, microbial DNA signals are often comparable to background contamination originating from laboratory reagents (“kitomes”), environmental exposure, equipment, or personnel [39]. This limitation is critical for imaging validation, as spurious microbial signals may result in false spatial or functional associations. Earlier reports describing a placental microbiome were later shown to be indistinguishable from contamination controls, highlighting the consequences of inadequate contamination control in LMB studies [39].

#### Biases in Standard Microbiome Analysis

Even prior to integration with imaging, sequencing-based microbiome analyses are subject to substantial technical bias [38].

#### Sample Collection and Storage

Sample collection methods impose biological constraints; mucosal biopsies capture adherent microbial communities, whereas stool or rectal swabs primarily represent luminal populations [38]. Storage conditions further influence microbial composition, as delayed freezing or room-temperature storage allows selective expansion of aerotolerant taxa such as Enterobacteriaceae, distorting community structure [38]. Chemical preservatives such as RNAlater may also bias diversity metrics and relative abundance estimates [38].

#### DNA Extraction and PCR

DNA extraction introduces significant bias due to differential lysis efficiency among bacterial taxa, particularly between Gram-positive and Gram-negative organisms [38]. The choice of extraction kit alone can alter inferred microbial composition (38). In addition, PCR-based approaches amplify DNA from both viable and non-viable cells, complicating interpretation when imaging aims to reflect active microbial metabolism [38].

#### Sequencing and Bioinformatics

Primer selection for 16S rRNA gene sequencing represents a major source of bias, as no universal primer set amplifies all taxa equally [38]. In metagenomic workflows, library preparation protocols can introduce GC-content bias, as demonstrated with certain commercial kits [38]. Downstream analytical decisions—including OTU clustering versus denoising algorithms (e.g., DADA2, Deblur) and reference database selection (e.g., SILVA, Greengenes)—can yield substantially different taxonomic profiles from identical datasets [38]. These methodological choices directly influence how imaging-derived signals are contextualized and interpreted.

#### Limitations of Specific Imaging Modalities

Each imaging modality operates within distinct physical and biological regimes that define its applicability [37]. Optical techniques, including fluorescence and bioluminescence imaging, are limited by shallow tissue penetration and oxygen dependence,

restricting their use largely to preclinical models and excluding obligate anaerobes that dominate the gut microbiota [40]. Metabolic labeling approaches, whether fluorescence-based or radionuclide-based, are constrained by signal dilution as labeled bacteria divide, limiting their utility for long-term colonization studies [41, 42].

MRI-based tracking using iron oxide nanoparticle labeling similarly suffers from signal dilution and lacks discrimination between live and dead bacteria, complicating functional interpretation [43]. Ultrasound-based acoustic reporter gene technologies represent a promising but still nascent approach; current limitations include genetic stability of reporter constructs and restricted applicability across diverse microbial taxa, particularly Gram-positive species [44]. PET imaging, while highly sensitive, is limited by spatial resolution, cost, radiation exposure, and tracer-specific pharmacokinetics, including non-target organ retention [37].

### Influence of Confounding Factors

Pharmacologic and host-related variables represent major confounders in microbiome-imaging studies [37, 38].

### Antibiotics

Antibiotic exposure is particularly influential, as it can profoundly alter microbial composition and function. Wang et al. demonstrated that broad-spectrum antibiotics eliminated the antitumor efficacy of anti-PD-1 immunotherapy by disrupting the gut microbiota [42]. Conversely, antibiotic treatment is now deliberately used as an experimental tool to confirm bacterial specificity of imaging signals or to monitor antimicrobial efficacy [40].

### Other Confounders

Additional variables, including diet, age, host genetics, and immune status, further modulate microbial activity and imaging readouts (37, 38). Animal models therefore remain essential for isolating microbial effects under controlled conditions, although this reliance introduces translational limitations when extrapolating findings to heterogeneous human populations [37].

### Recommended Standards for Study Quality

To mitigate these challenges, rigorous contamination control is essential, particularly for LMB samples [39]. Comprehensive negative controls, including extraction blank controls and no-template amplification controls, should be routinely incorporated to characterize background signal [39]. Quantitative validation methods such as qPCR should be used to confirm that microbial DNA levels in biological samples exceed those of control blanks [39]. Statistical decontamination tools, including Decontam, may then be applied to identify and remove contaminant sequences [39].

Standardization of protocols across studies remains critical for reducing inter-study variability (38). This includes consistency in sample collection, storage conditions, DNA extraction methods, and sequencing workflows [38]. For emerging imaging modalities, built-in validation controls are particularly important; for example, acoustic reporter gene signals can be selectively erased to confirm specificity and improve reproducibility [44].

### Clinical Applications and Future Perspectives

The microbiome-imaging axis is driven by its potential to move beyond correlative associations and provide spatially resolved, functional insight into host-microbe interactions [37]. By integrating imaging with microbiome profiling, this approach offers a pathway toward clinically actionable interpretation of microbial activity.

### Improving Diagnosis, Prognosis, and Treatment Monitoring

A primary clinical application lies in distinguishing active bacterial infection from sterile inflammation, a limitation of conventional imaging techniques [45]. Bacteria-specific PET tracers targeting metabolic pathways absent in host cells, such as folate and peptidoglycan synthesis, represent a rational solution to this diagnostic challenge [45]. This approach is particularly promising for infections in anatomically inaccessible or sterile sites, including vertebral osteomyelitis, septic arthritis, diabetic foot infections, and pneumonia [45].

Functional imaging of microbial activity also enables early assessment of treatment response, often preceding anatomical changes detectable by CT or MRI [45]. Parker et al. demonstrated the ability to distinguish antibiotic-sensitive from resistant *E. coli* strains *in vivo* using D-[<sup>3-11</sup>C]alanine PET imaging, confirming therapeutic efficacy in real time [45]. Complementary metagenomic analyses may further guide therapy by identifying antimicrobial resistance genes and informing targeted antibiotic selection [39].

As microbiome-based therapeutics such as fecal microbiota transplantation and engineered probiotics gain clinical traction, imaging tools capable of tracking delivery, engraftment, and persistence will become increasingly important [40-42].

### Target Diseases for Clinical Application

**Cancer:** Imaging microbiome modulation of immunotherapy (e.g., anti-PD-1) and tumor microbiota interactions in colorectal and breast cancer [42]. **Infectious Diseases:** Targeted imaging for difficult-to-diagnose infections such as pneumonia, vertebral discitis-osteomyelitis, and septic arthritis [45].

**Inflammatory and Autoimmune Disorders:** Conditions like IBD, where microbial dysbiosis plays a role, are potential targets [38]. **Neurological and Metabolic Disorders:** Microbiome involvement in neuropsychiatric and metabolic diseases can be investigated via functional imaging of gut-brain interactions [37, 38].

### Technological and Ethical Challenges

Despite rapid progress, several barriers to clinical translation remain. Many PET tracers exhibit taxonomic bias or background host uptake, while metabolic labeling strategies are inherently limited by signal dilution, preventing long-term tracking of colonization [41, 42]. Optical imaging techniques remain constrained by tissue penetration, although emerging fluorophores in the near-infrared window offer potential improvements [37-40]. Reporter gene approaches face challenges related to microbial genetic engineering, particularly for obligate anaerobes that dominate the gut microbiota [37-41]. Methods requiring bacterial pre-labeling, such as MRI-based approaches, remain largely restricted to animal models [43]. Ultimately, widespread clinical

adoption will depend on robust validation, standardization, and ethical oversight to prevent misinterpretation and potential patient harm [38, 39].

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