DECODING MICROBIOME DYSBIOSIS THROUGH METAGENOMIC ALPHA DIVERSITY. IMPLICATIONS FOR SARCOIDOSIS AETIOLOGY

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ABSTRACT

Background: Sarcoidosis is a chronic inflammatory disease that can affect multiple organs. The aetiology of sarcoidosis is not fully understood, but there is increasing evidence that the microbiome may play a role. The blood microbiome is a collection of microorganisms that live in the bloodstream. It is a complex and dynamic community that is influenced by a variety of factors, including the host's lifestyle and pathology. Recent studies have shown that people with sarcoidosis have alterations in their blood microbiome. These alterations include changes in the diversity, richness, and evenness of the microbial community. The abundance measures by which the blood microbiome diversity may detect instances of dysbiosis related to sarcoidosis aetiology. It should be clearly distinguished from microbiome changes related to unspecific inflammation or sepsis. However, the available evidence suggests that the microbiome may be a promising target for therapeutic interventions.

Aim: The primary goal of this review was to assess and compare the existing metrics of microbiome composition and diversity as established by metagenomic analyses. Additionally, we aim to elucidate the potential causal relationship between these measures, the phenomenon of blood microbiome dysbiosis and the pathogenesis of sarcoidosis.

Conclusion: In the present review, we investigated alpha diversity measures as characteristics of microbiome communities, examining their potential as indicators of dysbiosis, and the probable mechanisms of microbiome participation. A descriptive qualitative comparison was conducted between lung microbiome data of sarcoidosis patients and blood microbiome data of healthy adults. This comparison elucidates common taxa between the two microbiomes and identifies taxa potentially involved in sarcoidosis.

Key words: sarcoidosis, dysbiosis, blood microbiome, alpha diversity

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INTRODUCTION

Sarcoidosis is characterized by the formation of small inflammation areas (granulomas) in various organs, most commonly the lungs and lymph nodes [1]. Current theories on sarcoidosis aetiology suggest a complex interaction between genetic susceptibility, immune response, and exposure to specific environmental, occupational, or infectious agents, but the precise pathogenesis remains unclear [2,3]. Corticosteroids are commonly used as first-line therapy, but a significant proportion of patients may require additional treatment due to refractory disease or adverse effects, pointing out the necessity for novel therapeutic strategies [4].

The blood microbiome is now being recognized as potentially affected by various systemic and inflammatory diseases, as microbial components and metabolites were identified in the blood and can directly interact with the immune system [5]. There is a growing body of evidence suggesting that alterations in microbiome, or dysbiosis, could play a role in sarcoidosis [6,7]. Dysbiosis may influence sarcoidosis development through several mechanisms, including immune dysregulation, metabolic shifts, or increased permeability of mucosal barriers that allow the translocation of bacteria or bacterial products into

the bloodstream [8–10]. These alterations in the microbiome could contribute to the granulomatous inflammation observed in sarcoidosis, suggesting a possible link between microbial dysbiosis and the pathogenesis of the disease. Future studies investigating the blood microbiome in patients with sarcoidosis could provide valuable insights into the disease's aetiology and offer novel therapeutic targets [11].

The composition of blood microbiome is mainly assessed through metagenomic sequencing, a method that allows a comprehensive survey of the microbial community within a given sample [12]. This high-throughput technique provides a detailed picture of the diversity of microbial community [13]. Metagenomics can identify both known and novel microorganisms, including bacteria, viruses, fungi, archaea, and eukaryotic unicellular and multicellular parasites, that would otherwise be missed with traditional culture techniques [14]. This technique has significantly advanced our understanding of blood microbiome, and its role in health and disease [15].

The primary goal of the present review was to assess and compare the existing metrics of microbiome composition and diversity as established by metagenomic analyses. Additionally, we aim to elucidate the potential causal relationship these measures hold with the phenomenon of blood microbiome dysbiosis and the pathogenesis of sarcoidosis.

MEASURES OF MICROBIOME ABUNDANCE

Metrics for analysing microbiome's composition and diversity using metagenomic data primarily come from ecological studies. These metrics can be generally classified into alpha diversity and beta diversity measures [16]. The primary focus of this review is alpha diversity, as various measures of alpha diversity provide increasingly detailed insights into the structure of microbial communities. This information is vital for evaluating the health of a community, and is crucial for identifying signs of microbiome dysbiosis. In contrast, beta diversity, in all its forms, serves as a straightforward measure that essentially quantifies the numerical distance between two communities — the higher the beta diversity, the greater the distance — requiring no further interpretation [16].

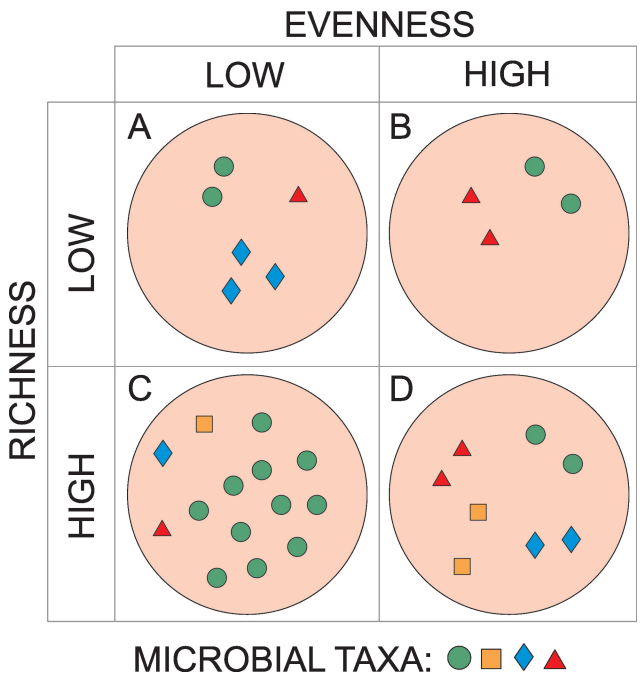

Figure 1. Schematic representation of four distinctive states of microbiome alpha diversity assessed by the measures of evenness and richness; A) low evenness vs. low richness; B) low richness vs. high evenness; C) low evenness vs. high richness; D) high evenness vs. high richness.

Alpha diversity is a key measure used to describe the complexity of microbial communities within a particular sample. It represents both the richness (number of different species) and evenness (distribution of individual species) of the community (**Figure. 1**) [16]. Different alpha diversity indices can provide various insights into the composition of the microbiome and potential dysbiosis. (1) Richness: The total number of unique species in a sample provides a basic measure of diversity. **Richness** is the simplest measure and only takes into account the number of different species, without considering their relative abundance. As such, it might overlook important shifts in the distribution of individual species. If there is a substantial reduction in the richness of the blood microbiome it may suggest a loss of beneficial microorganisms or overgrowth of a few species, characteristic of dysbiosis. (2) **Evenness** typically ranges within an interval of 0 to 1; 0 indicates complete unevenness (with one taxon dominating the entire community), while 1 signifies

perfect evenness (all taxa are equally abundant within the community). Such a community maintains a balanced distribution of species abundances, with no single taxon overshadowing others. This scenario represents a healthy and stable microbiome, fostering a more resilient community. A low evenness value suggests that the microbiome is dominated by one or a few species, with others present in much lower abundances. Consequently, the community might be more susceptible to disturbances; in other words, low evenness could be indicative of microbiome dysbiosis. (3) Shannon Index and Simpson Index: These are more complex indices that consider both **richness** and **evenness**. **The Shannon Index and Simpson Index** combine both richness and evenness into a single measure. The Shannon Index places more weight on richness, while the Simpson Index places more weight on evenness (dominance). Therefore, they complement each other and offer a more comprehensive overview of diversity. For instance, a microbiome sample might have high richness but low evenness due to the overrepresentation of a particular species. This could lead to a low Shannon Index value but a high Simpson Index value. Understanding these nuances can help detect subtle changes in microbiome composition, such as those that may occur during dysbiosis [17,18]. A decrease in the Shannon index or an increase in the Simpson index can both indicate a decline in diversity and potential dysbiosis. For example, decreased alpha diversity reflecting reduced richness and/or evenness (measured by the Shannon index) has been associated with inflammatory bowel disease, indicating a less diverse and potentially dysbiotic microbiome [19]. An increase in the Simpson index indicating the dominance of certain species might be associated with conditions like periodontitis [20]. By assessing alpha diversity, researchers can get a sense of the overall health of the microbiome. Changes in alpha diversity can indicate shifts towards dysbiosis, and studying these changes over time could help to elucidate the onset and progression of diseases linked to microbiome alterations.

While some overlap exists between the various alpha diversity metrics, they primarily serve as complementary tools that capture different aspects of microbial diversity. Indeed, the use of these various metrics in tandem allows researchers to capture different aspects of microbiome complexity. For instance, observing high richness but low evenness might suggest that while a large number of species are present, the community is being dominated by a few species, potentially indicating dysbiosis. Similarly, a low richness but high evenness could suggest a more balanced community, but with fewer species present, indicating a possible loss of beneficial microorganisms [21]. Metrics like the Shannon and Simpson indices which integrate both richness and evenness, could help identify more nuanced shifts in the microbiome. Therefore, the combined application of these metrics offers a multi-faceted view of the microbial community, capturing its richness, balance, and overall diversity. This comprehensive approach is crucial for a thorough understanding role of microbiome in health and disease, and the potential implications of microbiome dysbiosis.

MICROBIOME DYNAMICS

Regardless of the mechanisms by which the microbiome affects health, it is crucial to develop reliable methods to describe and differentiate alterations in the blood microbiome. Microbiome alterations could be graded as random fluctuations, microbiome dysbiosis and infection or sepsis.

(1) **Random fluctuations** occur even in healthy individuals. The composition of microbiome may naturally fluctuate due to factors such as diet, sleep, stress, and other environmental factors [22]. Therefore, discerning between these random fluctuations and disease-related dysbiosis is a critical challenge. Longitudinal studies that track individual microbiomes over time can help to set a baseline for these natural fluctuations [23]. Random fluctuations in microbiome composition are natural variations that can occur due to factors such as daily diet, short-term illnesses, minor changes in the environment, or even the circadian rhythm. These fluctuations typically do not lead to significant shifts in the overall structure of the microbial community, and the latter tends to return to its original state (baseline) once the influence of the transient factors ends. Alpha diversity metrics can reflect these fluctuations as random and transient changes over time. Similarly, the relative abundances of different species may

fluctuate slightly due to random variations, while the overall evenness of the community should remain stable unless a certain species starts to consistently dominate or become marginalized. The combined measures of richness and evenness can be sensitive to random fluctuations. However, if the community is resilient, these indices should return to baseline levels once the temporary influencing factor is removed. Thus microbiome random fluctuations are highly unlikely to contribute to sarcoidosis aetiology.

(2) **Microbiome dysbiosis,** or chronic conditions often involves sustained and significant shifts in the microbiome composition. Differentiating these shifts from random fluctuations requires a detailed understanding of the diversity and abundance of microbial species, often achieved through metagenomic sequencing [13]. Microbiome dysbiosis refers to a state where the natural balance of the microbial community is disrupted, often in association with a disease or a pathological condition. Alpha diversity metrics can help detect and quantify such disruptions. In a state of dysbiosis, the richness of the microbiome, or the total number of different species might decrease significantly. This is because certain species may outcompete others or some may not survive the altered conditions. A decrease in richness may indicate that beneficial species have been lost or that pathogenic species have overgrown. During dysbiosis, the evenness of the microbiome can also be affected as some species become overrepresented while others become underrepresented. This means that even though many species may still be present, their distribution is uneven, often favouring pathogenic or opportunistic species. As previously mentioned, Shannon and Simpson's indices combine richness and evenness into a single measure, and changes in these indices can indicate dysbiosis. A decrease in the Shannon index or an increase in the Simpson index suggests a decrease in diversity and an indication for dysbiosis. Therefore, alpha diversity metrics can help detect shifts in the microbiome associated with dysbiosis, providing valuable insights into the microbiome's role in health and disease.

(3) **Infection or sepsis**. Sepsis represents a clear disturbance of blood microbiome usually linked to the proliferation of a particular pathogen. Rapid diagnostic tools like PCR or next-generation sequencing can help identify pathogens directly from blood samples [24]. Sepsis is a severe, systemic response to infection that can lead to organ failure and death. The dysbiosis that accompanies sepsis represents a significant disruption of the normal microbial community structure with potentially life-threatening consequences. Alpha diversity metrics can provide insights into these microbial changes. In sepsis, a decrease in species richness can occur due to the overwhelming presence of a particular pathogen, leading to the reduction or elimination of other microbial species. This can also be the result of broad-spectrum antibiotic treatment commonly used in sepsis management, which can indiscriminately kill both harmful and beneficial microorganisms [25,26]. Similarly, the evenness of the microbiome is likely to decrease in sepsis as the pathogen causing the infection dominates the microbial community, or as antibiotics alter the relative abundance of various species. A significant decrease in the Shannon index or an increase in the Simpson index could indicate a state of dysbiosis associated with sepsis. There is increasing evidence that microbiome analysis, including the use of alpha diversity metrics, may provide valuable insights for sepsis diagnosis and prognosis. For instance, a study by Yin and colleagues [27] found that lower diversity (assessed using the Shannon index) of gut microbiome was associated with a higher six-month mortality rate in patients with sepsis. However, while these metrics can provide a snapshot of the microbial community at a given point in time, they do not capture the dynamic changes of microbiome over time.

ASSOCIATION OF MICROBIOME DYSBIOSIS AND HOST PATHOGENESIS

(1) **Developing pathology.** Changes in blood microbiome may be a result, rather than a cause of disease development. From this perspective, the disease process causes systemic changes, including immunological or metabolic shifts that subsequently lead to dysbiosis. Thus dysbiosis is an effect of the disease rather than its initiator [28]. For example, changes in gut microbiome composition have been observed in numerous diseases such as obesity and diabetes, and it was suggested that those changes may be a reflection rather than the cause of the

Table 1. Members of the extended microbiome included used in the metagenomic analysis of BAL of sarcoidosis patients

Taxon/Species	Phylum	Alpha Diversity Measure	Richness Measure
Streptococcus	Firmicutes	Shannon	Sequence count [6]
Corynebacterium	Actinobacteria	Shannon	Sequence count [6]
Neisseria	Proteobacteria	Shannon	Sequence count [6]
Atopobium	Actinobacteria	Shannon	Bacterial burden [7]
Fusobacterium	Fusobacteria	Shannon	Bacterial burden [7]
Mycobacterium	Actinobacteria	Shannon, Simpson, Inverse Simpson	Sequence count [33]
Cutibacterium	Actinobacteria	–	Sequence count vs. total number of sequences [34]

altered metabolic state [29].

(2) **Unlocking pathology**. Conversely, there is substantial evidence suggesting that dysbiosis can contribute to the development and progression of a disease. From this perspective microbial imbalance is a trigger event that unlocks pathological processes. For example, alterations in the gut microbiome can disrupt the gut barrier, leading to translocation of bacteria and bacterial products into the bloodstream. This in turn can trigger systemic inflammation, a common feature of various diseases [30]. Similarly, changes in blood microbiome could contribute to disease by triggering an inappropriate immune response or causing direct tissue damage [5]. Dysbiosis in the subgingival microbial plaque is the reason for the development of periodontitis. The specific mechanisms for development of periodontitis are not sufficiently well understood, but microbiome dysbiosis, as a cause of immune dysregulation, has its place in the general picture. In this case, the relationships between the oral, intestinal, and blood microbiome are not sufficiently well studied. *Porphyromonas gingivalis* is the causative agent of chronic periodontitis and has been identified in the brain of patients with Alzheimer's disease [31]. Toxic proteases from the bacterium called gingipains have also been identified in the brains of Alzheimer patients and their levels correlate with tau and ubiquitin proteins in pathology [31]. Microbial translocation to the blood is evident, but whether the oral or the intestinal microflora is the primary source of pathogen, is not clear [32].

ASSOCIATION OF PULMONARY MICROBIOME DYSBIOSIS AND THE ETIOLOGY OF SARCOIDOSIS

Currentlyin sarcoidosis research, bronchoalveolar lavage (BAL) is the primary sample type used for comparing microbiome composition in patients with sarcoidosis and control subjects [6,7,33]. **Table 1**. shows microbial genera with specifically increased their alpha abundance in BAL samples of sarcoidosis patients. Overall, such samples were characterized by high richness and low evenness values (**Figure. 1 C**).

BLOOD MICROBIOME AND THE ETIOLOGY OF SARCOIDOSIS

Blood microbiome is a complex community of bacteria, fungi, viruses, and other microorganisms. The composition of this microbiome is influenced by various factors, including diet, age, gender, and overall health status. While the exact composition can vary from person to person, certain common microbial species have been identified. To our knowledge, two studies have specifically addressed the characterization of blood microbiota in healthy adults:a study conducted by Paise et al., in 2016 [35], and another by Panaiotov et al., in 2021 [9]. Both investigations reported similar findings regarding

taxon compositions and proportions at both the phylum and genus levels. The dominant bacterial classes identified were Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria, while the prevalent genera included *Staphylococcus, Micrococcus, Corynebacterium, Acinetobacter, Streptococcus, Fusobacterium, Pelomonas, and Rothia*. A scrutiny of the prevalent genera in sarcoidosis BAL reveals a comparable taxon composition [6, 7, 33, 34]. Notably, the presence of potentially pathogenic microbial species such as *Mycobacterium* and *Neisseria spp.*, as well as commensals associated with sarcoidosis like *Atopobium* [7] and *Cutibacterium*, have been documented.

In light of the high similarity in taxon composition and the presence of commensals, several implications can be drawn. Firstly, the data suggest a potential interaction between lung and blood microbiomes, indicating a complex interplay between the microbial communities inhabiting these sites. Secondly, the findings raise the possibility that microbiome dysbiosis could significantly contribute to the aetiology of sarcoidosis, highlighting the need for further investigations into the role of microbiome dysbiosis in the aetiology of this condition. Lastly, the presence of Mycobacterium spp. hints at the potential contribution of latent infections to the disease process, suggesting a nuanced role of these microbial species in sarcoidosis, which deserves deeper exploration.

Based on the above data, one can propose a hypothetical scenario associating sarcoidosis aetiology with the blood microbiome, based on our current understanding of microbiome dysbiosis. In sarcoidosis, the immune response is thought to be triggered by an unknown antigenic stimulus, which could potentially be linked to the bloodstream or dysbiosis in the blood microbiome. Less compelling alternatives, such as the absence of certain taxa or those with reduced abundance, do not align so well in this context. This is because the contribution of microbiome to health conditions is more likely linked to the presence and proliferation of certain microbes, rather than their absence or reduced presence. In the context of sarcoidosis, it could be hypothesized that genera typically associated with pathogenic traits, such as *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Pseudomonas*, might be overrepresented. Conversely, genera that are typically associated with a healthy microbiome, such as *Cutibacterium*, *Prevotella*, *Veillonella*, and *Fusobacterium*, might be transformed by microbiome interactions into opportunistic pathogens and also increase in their abundance. However, it is important to emphasize that this is a hypothetical scenario and actual research may show different results. The relationship between blood microbiome and sarcoidosis, and the potential role of specific microbial genera, need to be confirmed through empirical studies.

CONCLUSIONS

Microbiome dysbiosis in the blood is the main scope of this review because of its hypothesized influence on chronic inflammatory conditions and sarcoidosis in particular. Microbiome dysbiosis represents a significant shift in the relative abundance and diversity of different microbial species that populate the body's ecosystems, and these changes can be particularly evident in the blood microbiome. For instance, in cardiovascular diseases, there has been growing evidence of alterations in the blood microbiome composition, with a relative abundance of specific bacteria such as Proteobacteria and decreased diversity observed in patients with atherosclerosis [36]. Similarly, in autoimmune conditions such as rheumatoid arthritis, dysbiosis of the blood microbiome has been identified, with an increase of rare or pathogenic species and an overall decrease in diversity as compared to healthy controls [37]. Chronic kidney disease has also been linked to blood microbiome dysbiosis, with increased levels of circulating bacterial DNA and a predominance of certain bacterial genera, such as *Staphylococcus* and *Pseudomonas,* in the bloodstream [38]. Given the significant associations between blood microbiome dysbiosis and various chronic conditions, the deciphering of these microbial changes could potentially lead to the identification of novel diagnostic markers and therapeutic targets. Still, comprehensive metagenomic sequencing studies are required to better understand these complex relationships and their implications for human health [13]. In particular, measures of microbiome

abundance could play a crucial role in evaluating the aetiology of sarcoidosis. Not only might they help identify specific microbial taxa associated with the disease, but they could also shed light on the dynamic interplay between the host and its microbiome. These insights could, in turn, enhance our understanding of how sarcoidosis develops and progresses, resulting inmore effective strategies for prevention and treatment.

CONFLICT OF INTERESTS:

The authors declare no conflict of interest.

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