# To progress understanding of disease triggers and modifiers in sarcoidosis, stratification is the key

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The vast majority of sarcoidosis literature starts with something similar to the following sentence "Sarcoidosis is a multi-system granulomatous disease of unknown aetiology predominantly affecting the lung". The unknown cause continues to inspire researchers to search for a possible trigger, especially if new and high-throughput methods are at their disposal. The histological hallmark of sarcoidosis is epithelioid cell granuloma. Formation of granuloma is a common reaction towards, for example, bacterial agents. Evidence for bacterial involvement in sarcoidosis consist of presence of bacterial peptides inside lung granuloma and other products derived from sarcoidosis patients, the ability of these products to produce a granulomatous reaction in human and in mice, and the success of anti-microbial therapy in patients [1, 2]. Although active infection has not been found, bacteria, and in particular mycobacteria, are thought to be a causative or disease modifying factor in sarcoidosis.

High-throughput molecular methods for identification of the complete bacterial microbiome in bronchoalveolar lavage (BAL) have recently been applied to sarcoidosis, but have not identified a clear disease-associated dysbiotic profile. GARZONI et al. [3] studied the microbiome in interstitial lung disease, and did not find disordered airway microbiota in sarcoidosis (n=7). A report by SCHER et al. [4] described the microbiome in immune-mediated disease and concluded that distal airway dysbiosis in untreated early rheumatoid arthrithis was similar to that seen in sarcoidosis (n=10). Recently a study by CLARKE et al. [5] analysed the microbiome content in lymph node biopsies, but also 16 BAL samples from sarcoidosis subjects and did not identify significantly different bacterial communities between BAL samples from sarcoidosis and controls subjects.

In this issue of the *European Respiratory Journal*, ZIMMERMANN et al. [6] present the largest study so far and describe the microbial composition in 71 patients with sarcoidosis, 10 healthy controls and 15 IPF patients. Mycobacterial DNA was only present in two sarcoidosis patients. Two taxa were more frequent in

sarcoidosis than in controls: *Atopobium* sp. and *Fusobacterium* sp., both potentially alive and active. However, interpretation of these results is difficult because both taxa have also been described to associate with other lung diseases, such as chronic obstructive pulmonary disease [7] and cystic fibrosis [8].

While researchers are just beginning to understand what constitutes a healthy lung microbiome, they simultaneously try to interpret findings in disease-associated microbiomes. *Fusobacterium*, for instance, is by some regarded as a true pathogen that should always be treated, while others describe it to be part of a healthy microbiome [9].

The lung microbiome largely overlaps with the oral microbiome, and is the result of microbial immigration, elimination and relative reproduction rates [10, 11]. Microbiome communities are complex and dynamic and vary throughout life under the influence of the season, geography, local environment, body site and host characteristics like genotype, ethnicity, age, sex, body mass index, diet, drugs and health status [9–12]. This wide variety of influences makes it difficult to develop studies that account for all these factors. It is therefore surprising that microbiome studies continuously keep comparing their findings in disease with those in health.

In inflammatory bowel disease, LEVI *et al.* [13] call for a more individualised approach that links microbial community structure and disease phenotypes. Comparison between phenotypes might be more valuable than differentiation between patients and healthy subjects. The value of the microbiome may lie in differentiating subtypes that share microbiome signatures indicative for prognosis or treatment. Such an approach requires stratification within patient cohorts.

Because the sample size in the ZIMMERMANN *et al.* [6] study was large, the authors stratified the patients by lung phenotype according to radiographic Scadding stages. In comparison with controls, ZIMMERMANN *et al.* [6] found that the increase in *Atopobium* sp. was associated with stage I (n=17) and II (n=39) and the increase in *Fusobacterium* sp. with stage II and III (n=6) of sarcoidosis. The observation that disease-associated pathogens may differ per stage is interesting. But, whether *Atopobium* is truly associated with hilar lymphadenopathy and *Fusobacterium* with parenchymal involvement in sarcoidosis requires further investigation, ideally in a study designed to sufficiently power strata for direct comparison amongst each other instead of with controls.

The value of stratification in sarcoidosis is well illustrated by looking at what, biologically, can be called specific phenotypes of sarcoidosis: Blau syndrome, chronic beryllium disease and Löfgren syndrome.

Blau syndrome is an inflammatory disorder characterised by the triad of granulomatous arthritis, uveitis and dermatitis [14]. It is a familial monogenic disease with autosomal dominant inheritance pattern thought to be caused by gain of function mutations in NOD2 that result in the continuous stimulation of nuclear factor κB [15].

Chronic beryllium disease is a granulomatous lung disease caused by exposition to beryllium. The disease is clinically indistinguishable from pulmonary sarcoidosis and nowadays extrapulmonary manifestations are regarded rare [16]. Carriership of human leukocyte antigen (HLA)-DPB1 E69 contributes significantly to the risk of disease with odds ratios for E69 homozygotes *versus* non-carriers of over 20 [17].

Löfgren syndrome is an acute form of sarcoidosis characterised by erythema nodosum, bilateral hilar lymphadenopathy (BHL), and bilateral ankle arthritis or periarticular inflammation. This syndrome is common among Caucasians and has a good prognosis [18]. The trigger is unknown but disease occurrence is seasonal and disease susceptibility and prognosis are highly dependent on carriership of HLA-DRB1*0301 genotype [19].

There are three commonalities between Blau syndrome, chronic beryllium disease and Löfgren syndrome: first, the necessity to cluster patients prior to identification of factors causal to disease; secondly, organ involvement is informative for the identification of disease phenotypes, particularly in combination with demographics and disease course; and thirdly, genetic association with each specific phenotype is very high.

Genetic penetrance, meaning what percentage of patients with the variant get the disease, is extremely high for the NOD2 mutations, but extremely low for the HLA-types associated with chronic beryllium disease and Löfgren syndrome. Low penetrance is a sign that, apart from the HLA-type, other factors, genetic or environmental, play a key role in development of disease. In chronic beryllium disease, the environmental agent was identified by occupational clustering, for others forms of sarcoidosis bacteria might be such an environmental key factor.

BTNL2 is a disease risk locus for sarcoidosis [20]. In the study by ZIMMERMANN *et al.* [6], stratification of patients by genotype of BTNL2 (rs2076530) showed that carriership of the risk allele A was significantly associated with a decrease in bacterial burden. BTNL2 is part of the HLA region and has

immunomodulating properties. BTNL2 variants have been associated with many immune mediated diseases, *e.g.* ulcerative colitis, Graves' disease, type 1 diabetes, asthma, systemic lupus erythematosus, and rheumatoid arthritis, whether or not secondary to HLA-DRB1 alleles [21].

Odds ratios for BTNL2 in sarcoidosis however, are low, much lower than many risks associated with HLA types in sarcoidosis phenotypes [22]. HLA class II molecules, like DPB1 and DRB1, are involved in the presentation of peptides derived from extracellular proteins. In search for combinations between disease triggers and host genetics, analysis of HLA class II variation would be a first choice. The ACCESS study combined detailed environmental histories with high resolution HLA class II typing and found that HLA DRB1*1101 and insecticide exposure at work was associated with extrapulmonary sarcoidosis, specifically cardiac sarcoidosis and hypercalcaemia and that HLA DRB1*1101 and exposure to molds and musty odours was associated with pulmonary only sarcoidosis [23]. This is the result of a large, laborious and well-designed study and one of the rare examples where clustering (by exposure), localisation (pulmonary *versus* extra-pulmonary) and genetics (HLA DRB1*1101 positive) come together. Still, translation of the results to the clinic remains difficult.

However, there is something more we can learn from Blau syndrome, chronic beryllium disease and Löfgren syndrome. Genetic expressivity, meaning the extent to which a genetic variant is expressed at the phenotypic level, is relatively uniform. Patients with Blau syndrome, chronic beryllium disease or Löfgren syndrome have clinically clearly defined forms of the disease. There is reason to assume that more, yet to be discovered, clearly defined forms of sarcoidosis may exist.

Defining a phenotypic uniform cohort within this heterogeneous pool of sarcoidosis patients might significantly aid research, even, or perhaps especially, when proportionally small groups of patients can be identified that would easily have been overlooked or that were underpowered in previously performed all-inclusive sarcoidosis studies. Authors suggesting sarcoidosis phenotyping systems have been trying to be as inclusive as possible (reviewed by PEREIRA *et al.* [24]), but from a scientific perspective the identification of a small, clinically homogeneous group of patients has a better chance of producing clinically applicable results in the future.

In pulmonary fibrosis, a recent research statement formulated criteria for the provisional category "interstitial pneumonia with autoimmune features" [25]. This sparked research on the subject which led, in a very short period of time, to better understanding of the natural history of the disease entity. Currently, there is an unmet scientific need for defining phenotypic clusters of sarcoidosis patients. Therefore, a call for a European Respiratory Society scientific task force to identify one or several phenotypes of sarcoidosis. Successful phenotyping could be key to clinically meaningful and translational results in sarcoidosis.

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