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Editorial

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Mycobacterium Avium Subspecies *Paratuberculosis* Infection and Biological Treatment of IBD: Cause or Consequence?

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Comment on:
Isotype-Specific Antibody Responses to *Mycobacterium Avium* Subspecies *Paratuberculosis* Antigens are Associated with the use of Biological Therapy in Inflammatory Bowel Disease

Mycobacterium avium subspecies *paratuberculosis* [MAP] is an obligate intracellular mycobacterium that causes Johne's disease [JD], a disease characterized by chronic granulomatous inflammation in the gut of ruminants and other mammalian species, including humans.[1] The similarity between JD and Crohn's disease [CD], manifested through similar clinical symptoms such as diarrhoea and weight loss, similar transmural diffuse granulomatous inflammation in pathological reports [Figure 1] and overlapping epidemiological aspects [rising incidence, long incubation period and familial occurrence pattern], has raised the hypothesis of a possible aetiological connection between MAP and CD. This hypothesis is supported by the fact that various studies have reported a higher frequency of MAP in CD patients vs ulcerative colitis [UC] patients and healthy controls. However, small study sizes and inconsistent methodologies used in the detection and isolation of MAP have raised doubts regarding the causal relationship between this bacterium and CD.[2,3]

In this issue of the *Journal of Crohn's and Colitis*, van der Sloot *et al.* performed extensive serological assays mapping the humoral response to MAP in a large cohort of patients with inflammatory bowel disease [IBD]. In particular, 21 indirect ELISA assays were designed to detect seven immunoglobulin [Ig] isotypes [IgA, IgE, IgM, IgG1–4] specific for three different MAP antigens [MAP0210c, MAP2942c and MAP2609] and strict technical standards were applied to select only those assays that guaranteed high-quality isotype-specific serological responses. Of these, only four ELISAs were reliable enough to draw meaningful conclusions: two of the IgA ELISAs and two of the assays detecting anti-MAP IgM levels. Although the comparison was done with a small group of 50 healthy individuals, van der Sloot *et al.* confirmed that IBD patients [n = 812] had higher levels of anti-MAP antibodies compared to controls. The relationship between antibody levels and numerous patient characteristics and clinical data was investigated. The most important correlation, found for three of the four analysed antibodies, was the association between elevated anti-MAP humoral response and the use of biological therapies, while no relationship was found with the need for surgery [Figure 2].

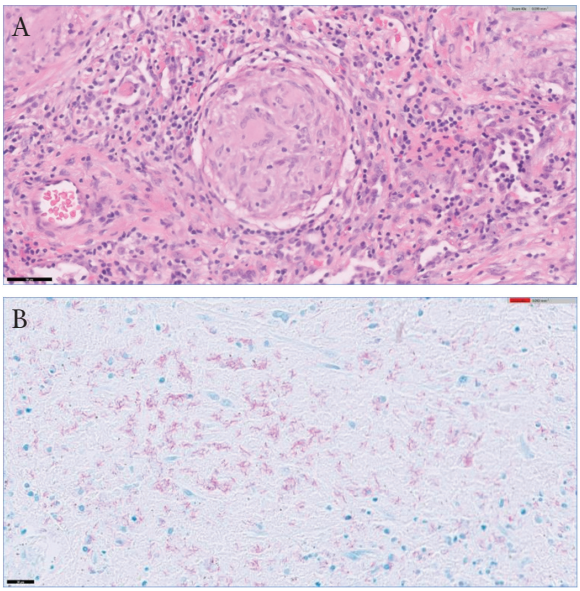

Figure 1. [A] *Mycobacterium*-associated granuloma. [B] Ziehl–Neelsen staining for mycobacterium detection in another sample from the same patient as in A [different location].

Their study is unique in its patient numbers and its critical appraisal of the methods used to detect anti-MAP antibodies. Indeed, the technical challenges in MAP detection are well known to all laboratories that have had to deal with this elusive microorganism. The golden standard for detection of MAP is based on isolation of the organism through culture methods. However, MAP cultivation from tissue samples was proven to be largely unsuccessful because of its very specific nutritional requirements and very slow growth. Culture of MAP in liquid or agar-based media requires weeks to months of laboratory incubation. This has led scientists to the utilization of molecular and serological methods as alternatives for

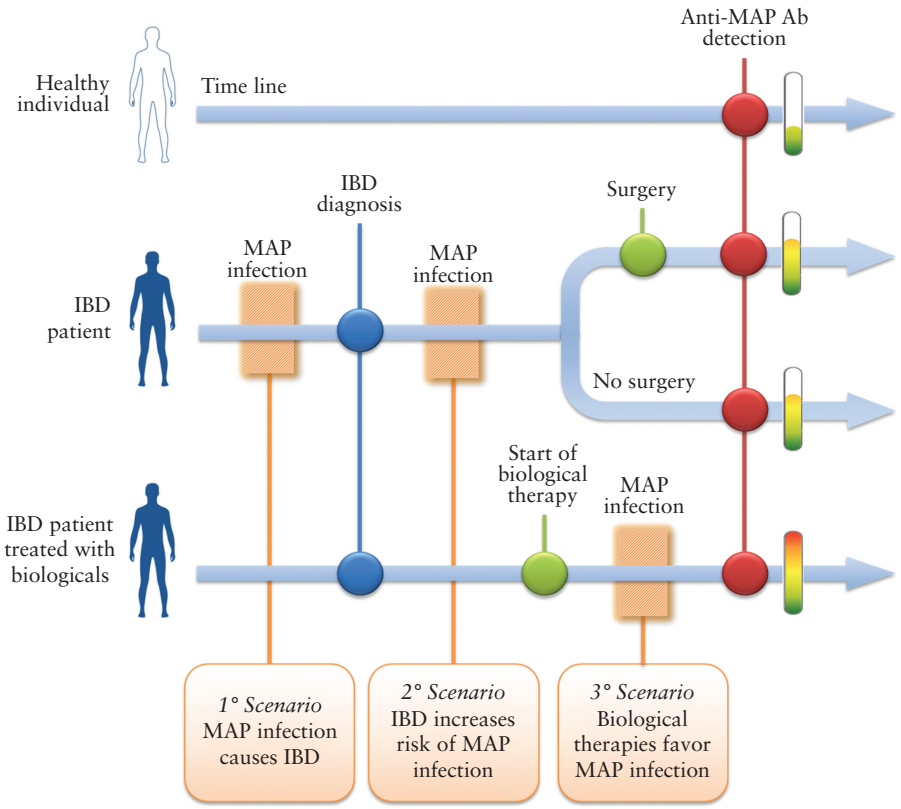

Figure 2. The detected anti-MAP antibody levels can be explained by three possible scenarios. First scenario: MAP infection occurs prior to the development of IBD and can therefore be seen as a possible aetiological cause of CD and UC. Second scenario: higher levels of anti-MAP antibodies in IBD patients compared to healthy individuals may also be explained by a greater risk for IBD patients to contract MAP infection. In both cases the need for surgery does not correlate with antibodies levels. Third scenario: patients undergoing biological therapies may be at increased risk of developing MAP infection, resulting in higher anti-MAP antibodies. IBD: inflammatory bowel disease, MAP: *Mycobacterium avium* subsp. *paratuberculosis*, Ab: antibody

MAP infection diagnosis.[4] Many studies based on PCR techniques have reported a higher percentage of positivity for MAP DNA in tissue from CD patients compared to non-IBD patients. At the same time, other studies have failed to detect MAP DNA in CD and concluded that there is no correlation between MAP and CD.[3] One of the reasons for the observed discrepancies between these studies may result from the fact that the methods for the detection of MAP are often in-house methodologies and have not been standardized for use on human samples. In low-abundance samples such as biopsies, nested PCR may be more sensitive than single PCR. Moreover, it is important to point out that most PCR studies targeted the MAP DNA insertion element IS900. IS900 has long been thought to be specific for MAP, but IS900 elements have also been found in environmental mycobacteria, raising doubts about the assay specificity.[2,5] For these and other reasons, standard PCR based on a single amplification is not ideal for detection of MAP in CD.

MAP-specific antibodies have previously been detected in CD patients. A meta-analysis of serology studies in CD patients found a higher prevalence of MAP-specific antibodies compared to controls.[2] However, also in this case the assays are not standardized and differ between the various studies, often without showing whether reliability and reproducibility tests have been performed.[6] Moreover, in many of the studies, evaluation of humoral responses through ELISA tests was executed through the use of protein G conjugates to measure a general IgG response. Thus, one of main the merits of the current study is the detailed reliability analysis of the ELISA immunoassays used for the detection of the anti-MAP antibodies. While unfortunately IgG data could not be reported, it is of interest to note that serum levels of IgA, an isotype commonly associated with mucosal surfaces, was found to be increased in serum from anti-tumour necrosis factor [TNF]α-treated patients. Similarly, it is intriguing that IgM levels, which would be expected to decrease upon isotype switching to IgG, are highly detectable in patients. As suggested by the authors, it is conceivable that isotype switching is absent, or that MAP infection is recent or recurrent. As such, determining the timing of MAP infection relative to serum sampling infection would be of added value. No positivity for IS900 was shown in a small subset of PCR-tested samples, although it remains unclear whether these individuals showed IgM serum positivity. Thus, having a feedback through the use of other MAP detection methodologies could provide greater clarity on the timing and specificity of the identified antibody responses. Coating of peptides to ELISA plates in carbonate buffer, although commonly performed, may not be sensitive enough to detect individual IgG responses. Other strategies [e.g. antigen peptides linked to biotin spotted on neutravidin-coated plates[7]] may be envisaged. Alternatively, fluorescence *in situ* hybridization of MAP has been shown to be a promising tool for detection of current MAP infection in intestinal biopsies,[8] and Ziehl-Neelsen staining, although detecting multiple mycobacteria, may provide an indication of MAP infection [Figure 1B].

Despite the technical challenges underlying the detection of MAP, the study by van der Sloot *et al.* suggests that disease severity *per se* is not related to MAP infection, because while MAP levels were correlated to anti-TNFα treatment of patients, there was no correlation between MAP antibodies and surgery. One alternative interpretation is that the use of anti-TNFα increases the patient's risk of MAP infection [Figure 2]. Indeed, anti-TNFα is known to be able to reactivate latent tuberculosis infection,[9] and predispose to bacterial and fungal infections.[10] With the clinical presentation of CD and MAP being so similar, the possibility of MAP infection as an alternative reason for loss of response to anti-TNFα treatment should perhaps be investigated more systematically in clinical diagnosis protocols. Finding accurate ways to detect current MAP infections is therefore paramount, and stringency protocols such as used by van der Sloot *et al.* provide a first step towards such implementation.

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Conflict of Interest

The authors have no conflicts of interest to disclose. The current manuscript, including related data and figures, has not been previously published and is not under consideration elsewhere.

Author Contributions

E.P., G.M.F.: study of the literature and drafting of the manuscript. M.P.P.: critical revisions and final approval of the version to be submitted.

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