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Editorial

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Inflammatory bowel diseases: where we are and where we should go

In Western countries, and especially in the Mediterranean area, inflammatory bowel diseases (IBD) show an increasing epidemiological trend as measured by incidence per year. Data is more evident for Crohn's disease than for ulcerative colitis, although some differences exist according to geographical area [1]. The causes of the increase are not clear, but dietary habits and environmental or socioeconomic factors seem to play an important role in the onset of IBD, especially Crohn's disease [2].

The costs of these chronic diseases are very high as patients require strict monitoring, a continuous use of pharmacological therapy, and often surgical treatment at a rate of 30% for ulcerative colitis and 70% for Crohn's disease [3].

As there are no symptoms, biochemical changes, pathological features or endoscopic findings that can be considered completely specific for Crohn's disease or for ulcerative colitis, it is not always easy to distinguish between these two forms. The symptoms can be confounded with irritable bowel syndrome, which, in the Western world, is the gastroenteric condition for which patients most frequently request a medical consultation; or with infectious diseases, be they protozoan, parasitic, or bacterial in nature. The frequency of the latter is also growing in the West due to immigration from other continents. Echography of the intestine is endowed with a very high sensitivity for detecting thickening of the wall of the terminal ileum, expression of edema and inflammation; and can differentiate in minutes at the patient bedside between an inflammatory disease and a functional disturbance. However, specificity is low and the method does not distinguish an IBD from an infectious disease.

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are widely used as a means for diagnosing Crohn's disease, but the sensitivity is in the range of 40%–60%. Their specificity is not absolute as ASCA may be present in patients with celiac disease or intestinal tuberculosis, suggesting that they may reflect a non-specific immune response in the course of various types of small bowel disease. Acute phase reactants such as ESR and protein C-reactive are generally elevated but they can be normal even in the stages where the disease is active or severe. Thus, to date, colonoscopy with terminal ileoscopia is the standard means for the diagnosis of IBD, after excluding infectious and inflammatory granulomatous diseases such as tuberculosis, *Yersinia* infection, schistosomiasis, lymphoma and Behçet's disease.

For these reasons, there is a constant push to find diagnostic and monitoring approaches which are low in cost and minimally invasive, but still able to support diagnosis, stratify and sub-classify IBD and predict therapy response.

The two articles of Basso [4] and Roggenbuck [5] in this issue of *Clinical Chemistry and Laboratory Medicine* (*CCLM)* provide important data to support this push, based on three fundamental aspects.

The first regards the use of fecal markers of inflammation: lactoferrin and calprotectin. These markers have been used for many years in clinical practice, both as a diagnostic confirmation and in monitoring IBD. The work of Basso and co-authors gives a detailed view of the clinical significance and diagnostic applications which highlights the possibility of measuring these markers with different methods and diverse types of instrumentation; the rapid immunochromatographic test (point-of-care tests; FC-POCT) for measuring calprotectin can be very useful in specialist clinics where it is decided whether or not to pursue colonoscopy in a suspected IBD case. In these cases, a semi-quantitative or even a qualitative test might have a relevant role in the diagnostic decisional process. The measuring of fecal markers with random access instruments or with ELISA methods, however, falls to the laboratory where quantitative results must be returned quickly, using analytical methods with a wide linear range for therapeutic decision-making.

The second aspect regards the significance and use of IBD serological markers. The contributions of Basso and Roggenbuck offer a complete panorama of markers that can be used in clinical laboratories, reporting the data from the most recent literature on clinical applications in the diagnostic arena for monitoring and subclassification of IBDs.

The work of Roggenbuck, in particular, presents interesting data on the significance and role of anti-glycoprotein 2 (GP2) antibodies. These antibodies recognize the GP2 molecule identified as a major autoantigenic target of the so-called pancreatic antibodies. GP2 is likewise expressed as an intestinal receptor located at the epithelial border of the intestine on the surface of M cells. In this context, GP2 appears to play an important role in keeping the balance of the intestinal immune system, controlling the equilibrium between the potentially pathogenic microbiota and the commensal microbiota. Especially intriguing is the hypothesis that the loss of control of the pancreatic/intestinal GP2 system might have a role in the pathogenesis of IBDs and, in particular, of Crohn's disease, and this aspect certainly should be explored in greater depth, especially because the anti-GP2 antibodies are the only true autoantibodies among all the antibodies identified in patients with IBD. In fact, ASCA, anti-laminaribioside (ALCA), anti-chitobioside (ACCA), anti-mannabioside (AMCA), anti-flagellin of *Clostridium subphylum* (CBir1), anti-*Pseudomonas fluorescens* (I2 IgA) and anti-outer-membrane porin (OmpC) are all antibodies directed against glucidic or proteic structures belonging to microbial agents present in the digestive tract, and therefore not against autoantigens.

The need to utilize all these markers in association clearly emerges from these two studies so as to provide the clinic with a complete antibody profile with a significant increase in diagnostic sensitivity. It has been noted for some time that the contemporaneous determination of ASCA and anti-neutrophil cytoplasmic antibodies (ANCA) is useful in differentiating Crohn's disease (ASCA-pos/ANCA-neg) from ulcerative colitis (ASCA-neg/ANCA-pos), in particular, in undifferentiated colitis. However, it is less noted that anti-GP2 antibodies of the IgA class are present even in a significant percentage of patients with celiac disease and this suggests that it could be useful to associate anti-transglutaminase antibodies during the diagnostic phase.

The association of multiple antibodies, especially in patients with Crohn's disease, is also relevant in the stratification of risk: patients with greater positivity for serological markers in general present a disease course that is more likely to have complications (fibrostenosis, fistolization, etc) and is more at risk of surgical intervention [6]. In light of these considerations, it would seem advantageous to have multiplex tests (microarray, blot) using predefined profiles [7], in which diverse markers are tested simultaneously with the objective of providing a complete report, useful either for diagnostic orientation (Crohn's disease, ulcerative colitis or celiac disease) or for determining risk stratification. Multiple pathology-oriented antibody profiles might, in addition, demonstrate their usefulness in predicting IBD onset in the pre-diagnostic phase, as already demonstrated for ASCA [8], and very recently for anti-CBir1 and anti-OmpC [9] that have been detectable in the sera of apparently healthy subjects, on average 3–4 years before the disease became manifest, thus introducing a window of opportunity for early intervention.

The third and last aspect concerns pharmacogenetics and, in general, the therapeutic monitoring of patients with IBD. Basso and co-authors conveniently underline the relevance of evaluating sera levels and polymorphism of thiopurine methyl transferase (TPMT) enzyme in patients undergoing treatment with azathioprine (AZA) and 6-mercaptopurine (6-MP) because, since TPMT facilitates the metabolism of AZA and 6-MP converting them into inactive metabolites, treatment is contraindicated in homozygous TPMT mutant allele carriers who have an extremely low TPMT enzyme activity and are therefore at a high risk of myelotoxicity. In addition, the use of immunosuppressive drugs must take place once tuberculosis, amebic dysentery, and the other great mimics of IBD have been excluded, the treatment of which with steroids or immunosuppressive drugs would be fatal for the patient.

Another relevant aspect is linked to the use for some years of anti-tumor necrosis factor (anti-TNF) agents in the treatment of IBDs, especially Crohn's disease. These biological drugs which are composed of monoclonal antibodies or their fragments are very costly, but their therapeutic efficacy has been clearly demonstrated. In any case, some patients treated with anti-TNF develop anti-drug antibodies that can be a cause of lowered response to treatment, or to adverse reactions at the moment of the infusion. Numerous studies have demonstrated the importance of testing for antibodies to biological drugs in that a positive test in the presence of an unsatisfactory response to therapy can suggest to the physician the need for alternative biological treatments or for returning to conventional drugs. This last aspect is also relevant in indicating the fundamental importance of laboratory diagnostics in profiling IBD and in follow-up treatment.

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