SYMPOSIUM

VIII WORKSHOP ON AUTOIMMUNITY

ANCA-ASSOCIATED VASCULITIS

BIOMARKERS OF INTEREST IN INFLAMMATORY BOWEL DISEASE: DIAGNOSIS AND MONITORING

Grifols Academy,
Parets del Vallès, Barcelona, Spain.
October 2nd 2019

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VIII WORKSHOP ON AUTOIMMUNITY

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Introduction

Autoimmune diseases derive from abnormal immune responses against normal self-tissues, organs, or cells. In recent times, the incidence and prevalence of autoimmune diseases have increased markedly, representing a significant global health problem. Autoimmune diseases are often chronic and can be life-threating. Recent advances have greatly increased our knowledge and understanding of the pathogenesis of autoimmune diseases, and it is widely accepted that environment, genes, and immunity contribute to the development of these diseases. These advances may therefore aid in the development of effective immunotherapeutic strategies.

On 2 October 2019, Grifols Academy held its VIII Workshop on Autoimmunity to share some of the latest developments in the area of autoimmunity. This publication provides a summary of two hot topics in autoimmunity which were presented by key opinion leaders: ANCA-associated vasculitis and biomarkers in inflammatory bowel disease.

VIII WORKSHOP
ON AUTOIMMUNITY

ANCA-associated vasculitis

ALEJANDRO OLIVÉ

**SERVICIO DE REUMATOLOGÍA
HOSPITAL UNIVERSITARIO GERMANS TRÍAS
I PUJOL, BADALONA, BARCELONA, SPAIN**

1. INTRODUCTION

Vasculopathy is a disease of the blood vessels which can be caused by conditions such as atherosclerosis, Thomboangitis obliterans (Buerger disease) and vasculitis. Vasculitis (or arteritis) is the term used to describe inflammation of the blood vessels, sometimes accompanied by fibrinoid necrosis. In vasculitis, there is a narrowing of, or blockage in, the arteries supplying blood to the tissues, leading to ischemia of the organs and the development of aneurysms.

Vasculitis is a heterogeneous group of rare but severe diseases. However, in recent years there has been an increase in the number of cases, likely due to increased knowledge and awareness among specialists and primary care physicians, the use of anti-neutrophil cytoplasmic antibodies (ANCAs) for diagnosis, and high-resolution consultations.

In recent decades, different proposals have also been developed to classify vasculitis. The most recent of these is the 2012 revised International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides (Table 1) [1]. The classification of vasculitis considers vessel size, clinical manifestations, and the presence of ANCAs.

Several different clinical manifestations can lead physicians to suspect that a patient has vasculitis:

- **Mononeuritis multiplex** (asynchronous and asymmetric inflammation of various nerves) **with fever** might indicate the presence of vasculitis
- **Livedo reticularis**, with or without ulcers, may suggest vasculitis
- Entities that are typical of the elderly population (i.e. stroke, abdominal wall necrosis, orchitis, intermittent claudication) but occur during adult age
- **Calf pain**: spontaneous or during direct manual pressure
- Palpable purpura – can be the first manifestation of ANCA-associated vasculitis. It is recommended to perform a skin biopsy (deep and of a recent lesion). Immunohistochemistry should also be performed
- Laboratory tests should include: hemogram, biochemistry, urine sediment, complement analysis, rheumatoid factor, and ANCAs.

The determination of ANCAs plays a critical role in the classification, pathogenesis and diagnosis of vasculitis. ANCAs are a group of autoantibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes and monocytes. Indirect immunofluorescence (IIF) on ethanol-fixed neutrophils is used for the detection/screening of various ANCAs. The enzyme-linked immunosorbent assay (ELISA) is used to detect antibodies to individual antigens. However, these techniques are not well standardized and there is wide interlaboratory variability. Rapid tests for the qualitative detection of ANCAs are also available commercially and may be useful for early detection and treatment in some

Table 1. Classification of vasculitis according to the 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides

Large vessel vasculitis	Takayasu arteritis Giant cell arteritis
Medium vessel vasculitis	Polyarteritis nodosa Kawasaki disease
Small vessel vasculitis	Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis • Microscopic polyangiitis • Granulomatosis with polyangiitis (Wegener) • Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) Immune complex • Anti–glomerular basement membrane disease • Cryoglobulinemic vasculitis • IgA vasculitis (Henoch-Schönlein) • Hypocomplementemic urticarial vasculitis (anti-C1q vasculitis)
Variable vessel vasculitis	Behçet's disease Cogan's syndrome
Single-organ vasculitis	Cutaneous leukocytoclastic angiitis Cutaneous arteritis Primary central nervous system vasculitis Isolated aortitis Others
Vasculitis associated with systemic disease	Lupus vasculitis Rheumatoid vasculitis Sarcoid vasculitis Others
Vasculitis associated with probable etiology	Hepatitis C virus–associated cryoglobulinemic vasculitis Hepatitis B virus–associated vasculitis Syphilis-associated aortitis Drug-associated immune complex vasculitis Drug-associated ANCA-associated vasculitis Cancer-associated vasculitis Others

Adapted from [1].

patients [2]. On the other hand, it has been reported that some drugs, such as levamisole, hydralazine, carbimazole, propylthiouracil, penicillamine, or minocycline can induce the formation of ANCAs; this should be considered when differential diagnosis is performed.

2. ANCA-ASSOCIATED VASCULITIS

The frequency of ANCA-associated vasculitis is low, and the etiology is unknown. Notably, some clinical characteristics are common, including fever and constitutional syndrome, mononeuritis multiplex, as

well as cutaneous (purpura, livedo reticularis, ulcers), pulmonary and renal manifestations. In addition, regardless of etiology, the treatment of ANCA-associated vasculitis is similar and standardized.

2.1. Granulomatosis with polyangiitis (Wegener's granulomatosis)

- A long-term systemic disorder that involves the formation of granulomas and inflammation of small- and medium-size blood vessels (vasculitis). Although it can affect many organs, the upper respiratory tract, lungs and kidneys are most commonly affected.
- Symptoms typically include nose bleeds, stuffy nose, crustiness of nasal secretions, and inflammation of the uveal layer of the eye. In addition, in the kidney, rapidly progressive glomerulonephritis may occur. In the lungs, pulmonary nodules, infiltrates, and cavitary lesions have been observed. Arthritis (pain or swelling) has also been reported. In the skin, subcutaneous nodules (granulomas) on the elbow and purpura have also been described.
- Although the cause is unknown, an inhaled exogenous factor that leads to an immune reaction has been associated with Wegener's granulomatosis.
- The number of new cases annually of Wegener's granulomatosis is estimated to be 3–6 per million individuals.
- Biopsy often reveals granulomatous inflammation involving the respiratory tract, and vasculitis of small- to medium-sized vessels.

2.2. Microscopic polyangiitis

- It was first described in 1948 as a glomerulonephritis in polyarteritis nodosa, and during subsequent years it was named microscopic polyarteritis nodosa.
- It is characterized as systemic, pauci-immune, necrotizing, small-vessel vasculitis (medium- and small-sized arteries, capillaries, venules and arterioles) without clinical or pathological evidence of necrotizing granulomatous inflammation.
- Epidemiological data are scarce.
- The median age of onset is 57 years and it is more common in males.
- Biopsy often reveals a typical vasculitis of the capillaries, arterioles and small-sized arteries, without granulomatous inflammation. Immunohistochemistry is negative (no immune complexes).
- Symptoms include fever and constitutional syndrome with arthralgias and myalgias, cutaneous manifestations, dyspnea, cough and hemoptysis (pulmonary hemorrhage), renal insufficiency (rapidly progressive glomerulonephritis), mononeuritis multiplex, bowel angina, and testicle pain.
- Laboratory parameters include increased erythrocyte sedimentation rate, elevated C-reactive protein, normochromic normocytic anemia, increased urea and creatinine levels, urinary sedimentation with hematuria and positive perinuclear ANCAs (p-ANCAs) with myeloperoxidase specificity.
- Differential diagnosis: pulmonary hemorrhage or rapidly progressive renal insufficiency.

2.3. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome)

- This form was first described in 1951 by Drs. Jacob Churg and Lotte Strauss.

- It is a rare condition, occurring in approximately 1/3 cases per million individuals.
- Although it can occur at any age, the median age is 48 years, and it occurs at a ratio of 2:1 in males/females.
- Systemic vasculitis is characterized by eosinophilic granulomatosis with polyangiitis, asthma and extravascular granulomas.
- American College of Rheumatology (ACR) criteria include asthma, eosinophilia >10% on differential white blood cell count, mononeuropathy (including multiplex) or polyneuropathy, migratory or transient pulmonary opacities detected radiographically, paranasal sinus abnormality, and biopsy containing blood vessels showing an accumulation of eosinophils in extravascular areas [3].
- It is more common in patients with a history of atopy, allergic rhinitis, nasal polyps, and sinusitis.
- Eosinophilic granulomatosis with polyangiitis consists of three stages (not all patients develop all three stages, or in the same order or with the same severity):
  - Initial: allergic stage (almost all patients experience asthma and/or allergic rhinitis)
  - Medium: eosinophilic stage (abnormally high level of eosinophils in the blood and tissues)
  - Final: vasculitic stage (inflammation of the blood vessels, and the consequent reduction of blood flow to various organs and tissues).
- Symptoms typically include a history of asthma that develops constitutional syndrome with fever and myalgias. It may also include mononeuritis multiplex, pulmonary infiltrates, purpura in the lower extremities, abdominal pain (angina secondary to vasculitis). Remarkably, the most serious complication of the vasculitic stage is heart complications.
- Laboratory: increased acute-phase reactants and IgE levels, leukocytosis, absolute eosinophilia, eosinophilia in the bone marrow aspiration. Positive p-ANCA with myeloperoxidase specificity (70%).
- Diagnosis should be suspected in cases of multisystemic involvement, asthma, eosinophilia, and pulmonary infiltrates. The diagnosis should be confirmed by biopsy (nerve, lung, kidney or skin).

3. TREATMENT OF ANCA-ASSOCIATED VASCULITIS

According to EULAR recommendations [4], patients with newly-diagnosed ANCA-associated vasculitis, with organ or life-threatening disease, should receive induction treatment with cyclophosphamide or rituximab together with intravenous glucocorticoids. In cases of rapidly progressive renal failure or pulmonary hemorrhage, plasma exchange should be considered. However, in patients with non-organ threatening disease, methotrexate or mycophenolate mofetil with glucocorticoids may be considered. If the patient is in remission, it is recommended to continue azathioprine or methotrexate or rituximab and taper glucocorticoids. After 2 years of remission, azathioprine or methotrexate may be tapered and rituximab can be stopped [4].

In patients with ANCA-associated vasculitis and severe pulmonary hemorrhage or severe renal insufficiency, plasmapheresis is required. If this is not the case, the proposed therapeutic approach includes an induction phase and a maintenance phase (Figure 1).

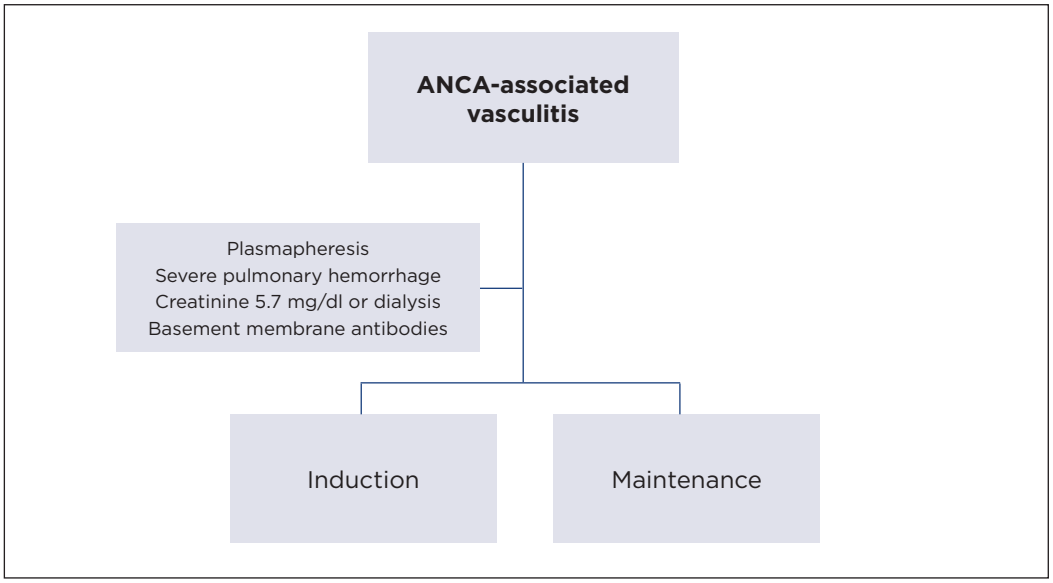

Figure 1. ANCA-associated vasculitis therapeutic approach..

During the induction phase, patients are treated with intravenous (IV) glucocorticoids (500–1000 mg) for 3 days and rituximab or cyclophosphamide (Figure 2). A clinical trial which compared rituximab (375 mg/m^2/week for 4 weeks) with cyclophosphamide (2 mg/kg of body weight per day) for remission induction in patients with ANCA-associated vasculitis, showed that 64% of patients in the rituximab group and 53% in the control group reached the primary endpoint (disease remission without the use of prednisone at 6 months; P<0.001 for noninferiority). In addition, the rituximab-based regimen was more effective than the cyclophosphamide-based regimen for inducing remission of relapsing disease (67% vs 42%; P=0.01). Rates of adverse events were similar between both groups [5]. In another clinical trial, performed in patients with ANCA-associated renal vasculitis, rituximab was not superior to IV cyclophosphamide [6]. However, overall, it has been reported that the tolerability of rituximab is better than that of cyclophosphamide, including the absence of gonadal toxicity.

Cyclophosphamide can be administrated either orally (United States; 1.5–2 mg/kg/day) or IV (Europe; every 2–3 weeks, with a lower risk of leucopenia and infections). Rates of complete remission at 6 months are high [7]. Resistance to cyclophosphamide is rare. In many cases, resistance to cyclophosphamide is associated with inadequate doses. In cases of resistance, infection should be ruled out. Notably, there are two conditions with low responses to cyclophosphamide: subglottic stenosis secondary to Wegener's granulomatosis, and orbital pseudotumor. In these cases, treatment with glucocorticoids and rituximab may be useful. For patients with Wegener's granulomatosis, treatment with rituximab may be of particular interest in young women to avoid sterility issues, in those who have received previous induction therapy with cyclophosphamide and in those with ANCA PR3.

With regard to maintenance treatment (Figure 3), the majority of evidence has been obtained with

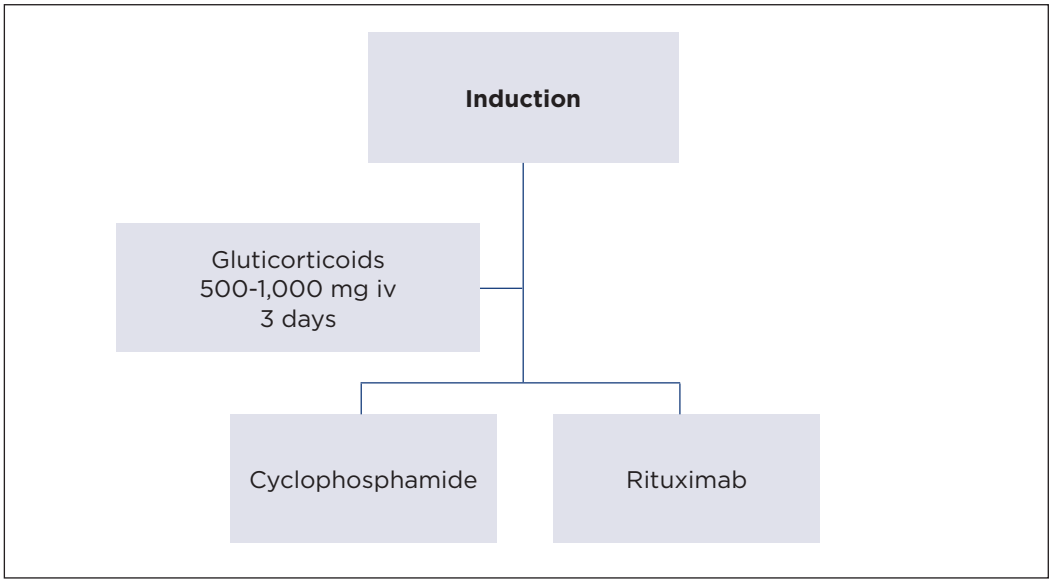

Figure 2. Induction phase.

rituximab and azathioprine and, to a lesser extent, with mycophenolate mofetil and methotrexate.

After 3–6 months of induction cyclophosphamide therapy in the CYCAZAREM study, in patients with ANCA-associated vasculitis involving the kidneys or another vital organ, maintenance treatment with azathioprine had similar recurrence rates and adverse events as long-term follow-up with cyclophosphamide [7]. Data with methotrexate are scarce; there are some limited data which were obtained only after induction with cyclophosphamide (not with rituximab), but recurrence rates are high. Mycophenolate mofetil is less effective than azathioprine, and no comparisons with methotrexate are currently available. In a clinical trial that compared rituximab (375 mg/m^2 administered once weekly for 4 weeks) followed by placebo with cyclophosphamide administered for 3 to 6 months followed by azathioprine for 12 to 15 months, 64% of patients treated with rituximab had complete remission by 6 months (vs 53% in the control group). At 12 and 18 months, these numbers were 48% and 39%, respectively, with rituximab, and 39% and 33%, respectively, in the comparison group. Adverse events occurred similarly in both groups [8]. In another clinical trial, patients with newly-diagnosed or relapsing granulomatosis with polyangiitis, microscopic polyangiitis, or renal-limited ANCA-associated vasculitis who were in complete remission after a cyclophosphamide-glucocorticoid regimen received either rituximab 500 mg on days 0 and 14 and at months 6, 12, and 18 after study entry, or daily azathioprine until month 22. At month 28, major relapse had occurred in 29% of patients treated with azathioprine and in 5% of patients treated with rituximab (HR 6.61; 95% CI 1.56–27.96; P=0.002), with similar rates of adverse events [9]. In summary, rituximab is effective as maintenance therapy among patients with ANCA-associated vasculitis. Key EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis are summarized in Table 2 [4].

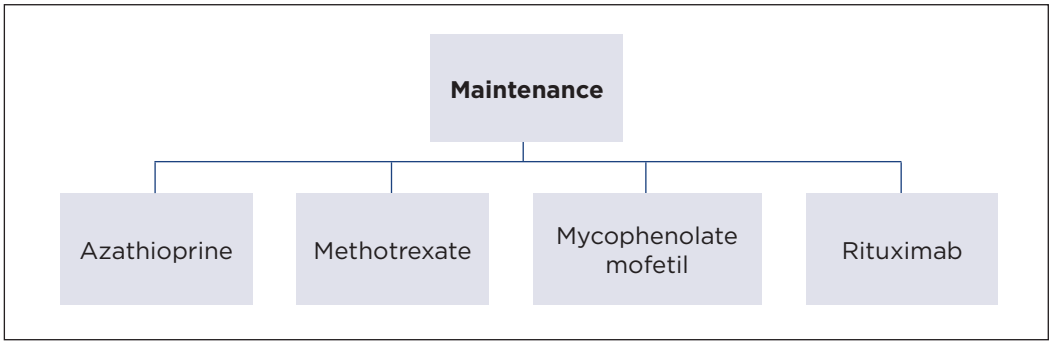

Figure 3. Maintenance treatment.

Table 2. Recommendations for the management of ANCA-associated vasculitis (EULAR/ERA-EDTA)

It is recommended that these patients are managed at (or in collaboration with) centers of expertise
Biopsies are recommended to assist in establishing a new diagnosis and for further evaluation for patients suspected of having relapsing vasculitis
For remission-induction of new-onset organ-threatening or life-threatening ANCA-associated vasculitis, a combination of glucocorticoids and either cyclophosphamide or rituximab is recommended
For a major relapse of organ-threatening or life-threatening disease in ANCA-associated vasculitis, a combination of glucocorticoids and either cyclophosphamide or rituximab is recommended
Plasma exchange should be considered for patients with ANCA-associated vasculitis and serum creatinine ≥5.7 mg/dL and can be considered for patients with diffuse alveolar hemorrhage
For remission-maintenance of ANCA-associated vasculitis, treatment with a combination of low-dose glucocorticoids and either azathioprine, rituximab, methotrexate or mycophenolate mofetil, at least 24 months following induction of sustained remission, is recommended
Periodic assessment of cardiovascular risk is recommended

Adapted from [4].

A Five-Factor Score for systemic necrotizing vasculitides (polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome) has been proposed to evaluate prognosis at diagnosis (Table 3).

If the score is 0, patients can be treated only with glucocorticoids and if the score is >1, patients should be treated with cyclophosphamide or rituximab plus glucocorticoids [10]. However, although some conditions (i.e. mononeuritis multiplex) can be severe, the Five-Factor Score may be low, and early treatment (intravenous glucocorticoids and likely rituximab) is required.

Table 3. Factors included in the Five-Factor Score for systemic necrotizing vasculitides

Age >65 years
Heart failure
Renal insufficiency (serum creatinine 1.7 mg/dL)
Gastrointestinal involvement
No otorhinolaryngology involvement

4. SUMMARY POINTS

Polyangiitis with granulomas

- The use of cyclophosphamide should be limited to 3–6 months to avoid side effects
- Only use drugs that have been proven to be beneficial in clinical trials and not just in case series (i.e. etanercept had more side effects in clinical trials)
- It is better to maintain patients on low-doses of glucocorticoids rather than withdraw them
- Resistance to cyclophosphamide is rare. If it occurs, suspect co-infection
- ANCAs are very valuable in the diagnosis, but not during follow-up
- Patients with ANCA-associated vasculitis have a higher risk of cardiovascular disease and cancer, a poorer quality of life and a higher loss of work.
- Hematuria does not always indicate activity (i.e. bladder cancer, renal lithiasis, etc.).
- A chest computed tomography (CT) scan may show lesions in patients with normal chest X-ray.
- Gingival hyperplasia is typical, but rare.
- In case of very aggressive lesions in the roof of the mouth, nasal cavity, or eyeball, consider lymphoma, midline granuloma, cocaine, mucormycosis (e.g. diabetics).

Polyangiitis with eosinophilic granulomatosis

- Symptoms may include: adenopathies, increased parotid gland, arthritis, and nodules (differential diagnosis with rheumatoid arthritis)
- ANCA positivity is low (<50%)
- ANCA-associated vasculitis causes more mononeuritis multiplex and cardiac disease
- Patients with positive ANCA have more renal and central nervous system disease than patients with negative ANCA
- The role of leukotrienes is controversial
- Clinical control: eosinophils, acute-phase reactant and organ damage (differential diagnosis with hypereosinophilic syndrome)
- Always consider renal damage
- Clinical problem: patients with controlled vasculitis but active asthma

In summary, for induction therapy, a combination of glucocorticoids and either cyclophosphamide or rituximab is recommended and, for maintenance therapy, azathioprine or rituximab. The prognosis of ANCA-associated vasculitis has improved in recent years, mortality rates are currently low, and most deaths are not associated with vasculitis (i.e. cardiovascular disease, renal failure, status asthmaticus, etc.).

New treatments are currently being investigated. For example, in patients with eosinophilic

granulomatosis with polyangiitis, mepolizumab resulted in significantly more weeks in remission and a higher proportion of participants were in remission than placebo recipients; however, only 44% of patients achieved protocol-defined remission [11]. In addition, there has been great interest recently in therapeutically targeting B cell cytokines, such as B cell-activating factor (BAFF). Dual B cell-targeted immunotherapy, combining B cell depletion and BAFF blockade, could be of great interest [12].

5. CONCLUSIONS

ANCA-associated vasculitis is uncommon. It is classified according to the 2012 Chapel Hill Consensus criteria. Treatment is based on induction, with glucocorticoids and cyclophosphamide or rituximab, and maintenance, with azathioprine, methotrexate or rituximab.

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Biomarkers of interest in inflammatory bowel disease: diagnosis and monitoring

JUAN MANUEL ACEDO

**UNIDAD DE PATOLOGÍA CLÍNICA/
ANÁLISIS CLÍNICOS,
HOSPITAL UNIV. FUNDACIÓN ALCORCÓN,
MADRID, SPAIN**

1. INTRODUCTION

It has been estimated that approximately 15% of patients consult a physician in the primary care setting due to digestive disorders. Symptoms are frequently diffuse and may include abdominal pain, abdominal distension, diarrhea, constipation, nausea, vomiting, and reflux. It is very difficult for physicians to perform an adequate diagnosis because many conditions share symptoms, such as lactose intolerance, celiac disease, inflammatory bowel disease (IBD), food allergy, tumors, infections, or primary immunodeficiencies. Differential diagnosis should be based on clinical suspicion (medical history, nutrition habits, physical examination), with the aid of endoscopic, histological and laboratory tests [1].

2. INFLAMMATORY BOWEL DISEASE

2.1. Definition

IBD can be defined as a chronic condition, with no trend to spontaneous cure, periods of high and low symptomatic activity, with no specific treatment and prognosis based on disease chronicity [1].

The etiopathogenesis and pathophysiology of IBD are partially understood, and are associated with an excessive immune response, in which immune system cells synthesize and release many molecular effectors and there is a loss of the intestinal microbiota [2].

The principal types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). UC is a chronic condition that results in inflammation and ulcers of the intestinal mucosa of the colon and rectum, with an intermittent course (relapsing-remission). CD is a transmural inflammation that may cause stenosis, fistulas or abscesses. It affects the small intestine and large intestine, as well as the mouth, esophagus, stomach and anus. In contrast to UC, CD lesions are not continuous, with patchy areas of inflammation. Finally, nonspecific IBD is characterized by chronic inflammation of the digestive tract which cannot be classified as UC or CD [1].

2.2. Epidemiology

It has been estimated that approximately 2 million people in Europe and 500,000 in United States have IBD. Although the incidence is stable in developed countries, it is increasing in developing countries, likely due to industrial development. The prevalence in Europe is 1 in 300 inhabitants. In adults, IBD occurs similarly in males and females; however, in the pediatric population, IBD is slightly more common in males. In Spain, there are 5 to 12 cases of UC per 100,000 inhabitants, and 4 to 10 cases of CD per 100,000 inhabitants. The incidence of IBD is more common in the north than in the south of Spain [3].

2.3. Physiopathology

IBD is a complex condition, resulting from the interaction of different environmental and genetic factors which leads to particularly excessive immunological responses and intestinal inflammation [2].

In IBD, regulation of the cellular metabolism is altered, including mechanisms of autophagy. The external agent recognition system (i.e. Toll-like receptors, NOD2) is modified by genetic causes. In addition, with regard to innate immunity, patients with IBD express more receptors and release more cytokines (proinflammatory activation). Different immune effector systems are also implicated (cytokines, TH1, TH2, TH7 and regulatory T cells) [2].

Genetic predisposition is the most important factor. Concordance between monozygotic twins has been reported: 15–20% for UC, 20–50% for CD. The first gene linked to IBD (in 2001) was NOD2 (CARD 15). Since then, more than 200 single nucleotide polymorphisms have been associated with susceptibility to IBD [2].

Epigenetic mechanisms are also important as they modulate disease progression and are associated with the interaction between genes and environment. Thus, 5-mC RNA methylation affects the severity, duration and extent of UC, and increases the risk of dysplasia and neoplasia. Histone H4 acetylation affects the regulation of the innate immunity from the microbiota in CD. The role of micro-RNA is also important as there is a differential expression in the intestinal mucosa in CD according to the activity or the location.

There is a clear relationship between the microbiota and IBD. The relationship between the bowel immune system and the microbiota is key. Thus, antibiotics, alterations in the microbiota and changes in some specific bacteria, modulate the severity/progression of IBD. Patients with IBD have a 30–50% reduced biodiversity of commensal bacteria, such as Firmicutes and Bacteroidetes and increased numbers of harmful bacteria, such as *Fusobacterium varium* or *Mycobacterium avium*. Some disease-directed treatments (i.e. probiotics, prebiotics or fecal microbiota transplantation) could be beneficial in this context.

With regard to immunology, the innate immune system is altered in IBD: there is a loss of integrity of the intestinal epithelium, neutrophils infiltrate through the intestinal epithelium (crypt abscess), the number and activity of innate lymphoid cells (natural killer T cells and mucosal associated invariant T cells) are increased, as well as macrophages and dendritic cells. In the adaptive immune response, the activation of T lymphocytes plays a key role in the pathogenesis of IBD. In patients with CD, there is an increase of IFN-γ and IL-12 (Th1 pattern) whereas, in patients with UC, there is an excessive synthesis of interleukin (IL)-4, IL-5 and IL-13 (Th2 pattern). In addition, in both conditions, the IL-23/Th17 axis is increased. Moreover, there is a loss of tolerance in the IL-10 pathway, leading to an activation and proliferation of T cells. The humoral immune response is also activated in IBD.

Environmental factors have a direct role in the development of IBD. There are some protective factors, such as breastfeeding (which favors immunotolerance to food and bacterial antigens, as well as the transfer of maternal antibodies which modify the microbiota) or appendectomy (in UC). With regard to smoking, non-smokers have an increased risk of UC (vs smokers), but ex-smokers also have an increased risk. On the other hand, smokers have a double risk of developing CD compared to non-smokers. Hormonal therapy (either hormone replacement or hormonal contraceptive therapies) increases the risk of IBD. An increased sociosanitary level and living in an urban area also raise the risk of developing IBD. By contrast, sharing the same

room during childhood and having a high number of brothers, decrease the risk. In addition, coinfection with *Campylobacter*, *Mycobacterium avium* or adherent-invasive *Escherichia coli*, as well as treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or isotretinoin, increase the risk of IBD. By contrast, co-infection with *Helicobacter pylori* or warm summers are protective factors [2].

2.4. Signs and symptoms

Despite the fact that UC and CD are different conditions, they often have common symptoms [4]:

- Gastrointestinal symptoms: increased frequency of bowel movements and a decrease in stool consistency, abdominal pain, rectal bleeding, alternation of bowel rhythm (constipation and diarrhea). Nocturnal diarrhea is rare in functional disorders.
- Extra-gastrointestinal manifestations: systemic symptoms, such as fever or weight loss, particularly in children. Clinical manifestations in the skin, eyes, joints.

Differential diagnosis should be performed with infectious enterocolitis, and gastroenteritis, but in this case, fever is higher and is not preceded by alterations in bowel rhythm over the medium to long term [4].

The main differences between UC and CD are shown in Table 1 [1,4,5] and the Montreal classification is presented in Table 2 [6]. In UC, age <16 years is associated with a more aggressive evolution. After 10 years from the time of diagnosis, 50% of patients maintain clinical remission, 40% have intermittent decompensations and 10% have continuous chronic activity. In CD, after 20 years of follow-up, 56% of patients develop fistulae and 30% stenosis.

2.5. Biomarkers

IBD is an idiopathic inflammation of the gastrointestinal tract. Diagnosis is based on a combination of different data (clinical, biological, radiologic, endoscopic and histologic). Although endoscopic and histologic assessment are key in the diagnosis and management of IBD, they are invasive procedures and may not be well tolerated. In this context, there is a great interest in the search for new biomarkers which can differentiate between organic and functional disorders, act as a marker of disease activity and evolution, and exhibit changes in response to treatment [7].

Biomarkers can be obtained from serum (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) or feces (calprotectin or lactoferrin). Biomarkers can be acute-phase reactants, derived from the synthesis of proteins after the activation of immune cells of the intestinal mucosa, or can be antibodies associated with the activation of humoral immunity.

2.5.1. Serum markers

C-reactive protein

CRP is an acute-phase protein of hepatic origin that increases following IL-6 or tumor necrosis factor-alpha (TNF-α) secretion. The terminal half-life of CRP is 19 hours. The reference value is <1 mg/L; in severe conditions, it can increase to >250 mg/L. CRP is a simple and economical marker that can differentiate between organic and functional disorders. Because there is a closer relationship between IL-6 and the transmural involvement and also due to the direct synthesis from the inflamed mesentery, CD is associated with higher CRP increases than UC. In CD, the sensitivity and specificity of CRP

Table 1. Differences between ulcerative colitis and Crohn's disease

	Ulcerative colitis	Crohn's disease
Symptoms and signs		
Diarrhea	Common	Common
Defecation	Often mucus-like and with blood	Often porridge-like, sometimes steatorrhea
Abdominal pain	Uncommon (related with defecation)	Common (related with food ingestion)
Tenesmus	More common	Less common
Fever	Indicates severe disease	Common
Weight loss	More seldom	Often
Blood in the stool	Common	Uncommon
Fatigue	Common	Common
Complications		
Anemia	Common	Common
Fistulae	Seldom	Common
Stenosis	Seldom	Common
Extra gastrointestinal manifestations	10% of patients (joints, skin, eyes, and liver)	20–40% of patients (skin, eyes)
Distribution		
Distribution of disease	Continuous area of inflammation	Patchy areas of inflammation
Terminal ileum involvement	Seldom	Common
Colon involvement	Always	Usually
Rectum involvement	Usually	Seldom
Anus involvement	Seldom	Common
Endoscopy	Continuous ulcer	Deep geographic and serpiginous ulcers
Depth of inflammation	Shallow, mucosal	Deep into tissues (may be transmural)

Based on data from [1,4,5].

is high (>80%). However, approximately 10% of patients with clinical activity have normal CRP values, particularly when the disease is limited to the terminal ileum or has a stenosing pattern. The risk of recurrence is higher in patients with elevated CRP levels (prognostic marker). In contrast, the role of CRP is more limited in UC (high levels of CRP are only seen in active and extensive UC). The relationship between disease activity and the proportion of patients with increased CRP levels is shown in Table 3 [8]. The correlation is better in CD than in UC, but in moderate to severe patients there is a good correlation between CPR and both conditions.

Table 2. Montreal classification of ulcerative colitis (extent and severity) and Crohn's disease

Ulcerative colitis		Crohn's disease	
Extent	**E1** Ulcerative proctitis (limited to the rectum) **E2** Left sided (limited to a proportion of the colorectum distal to the splenic flexure) **E3** Extensive (extends proximal to the splenic flexure)	**Age at diagnosis**	**A1** below 16 years **A2** between 17 and 40 years **A3** above 40 years
Severity	**S0** Clinical remission (asymptomatic) **S1** Mild (≤4 stools/day, absence of any systemic illness, and normal inflammatory markers) **S2** Moderate (>4 stools per day but with minimal signs of systemic toxicity) **S3** Severe (≥6 bloody stools daily, pulse rate of at least 90 beats per minute, temperature of at least 37.5°C, hemoglobin <10.5 g/100 mL, and erythrocyte sedimentation rate ≥30 mm/h)	**Location**	**L1** ileal **L2** colonic **L3** ileocolonic **L4** isolated upper disease*
		Behavior	**B1** non-stricturing, non-penetrating **B2** stricturing **B3** penetrating p perianal disease modifier**

*L4 is a modifier that can be added to L1–L3 when concomitant upper gastrointestinal disease is present.
**"p" is added to B1–B3 when concomitant perianal disease is present.
Based on data from [6].

In summary, CRP is a valuable serum biomarker as there is a good correlation with the Crohn's Disease Activity Index (CDAI) and inflammation markers (IL-6). In addition, it is a good marker to assess the beneficial effect of therapy on intestinal inflammation. Patients with CRP levels 5–10 mg/L have a high response to infliximab (and other biological agents). However, there is not a clear cut-off point for IBD. In addition, there is an overlap between CRP levels and activity rates; the predictive value is low, and it can be modified by external causes (i.e. nutritional status, hepatic function, body mass index, or genetic polymorphisms).

Erythrocyte sedimentation rate (ESR)

ESR is the rate at which red blood cells (RBCs) in anticoagulated whole blood descend in a standardized tube over a period of 1 hour. ESR is a non-specific measure of inflammation and is increased with acute-phase reactants. It has a long terminal half-life and levels decrease very slowly after disease

Table 3. Disease activity and proportion of patients with increased CRP levels

	Ulcerative colitis	Crohn's disease
Quiescent	14%	54%
Mild	42%	70%
Moderate	64%	75%
Severe	83%	100%

Adapted from [8].

resolution. As a result, it is not a good marker for disease follow-up. In addition, ESR is limited by many external factors (i.e. number or size of RBCs, age, smoking, some drugs). It is a simple and economical determination.

However, ESR is not very helpful for the diagnosis of IBD. ESR levels are very similar between UC and CD. In addition, levels may be normal in stenosing CD and in distal UC, and levels may vary according to disease location. However, although there is a good correlation between ESR levels and the CDAI, results are controversial as a predictor and it is not useful for treatment monitoring.

Alpha-1-acid glycoprotein or orosomucoid

Alpha-1-acid glycoprotein is an acute phase plasma alpha-globulin glycoprotein. It is limited to detect clinical changes in patients with severe IBD. There is a good correlation with the CDAI, and with UC with protein loss.

Other biomarkers: leukocytes, platelets, albumin

2.5.2. Immune markers

IBD is associated with activation of humoral immunity. In this context, the determination of p-ANCA or Anti-Saccharomyces Cerevisiae Antibodies (ASCA) is of interest. ANCAs are a group of autoantibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes and monocytes. They are more common in UC. In CD they are detected in 2–25% of patients, mainly in left sided CD. Assessment of ANCA can be performed by IIF and ELISA. ASCAs are immune proteins, mainly of the IgG or IgA type, which are more common in CD (40–80%) than in UC (2–15%). Consequently, the determination of both markers could be helpful in differentiating between both entities in patients with IBD. Thus, in the case of ANCA-positive and ASCA-negative, UC is more likely but, in the case of ASCA-positive and ANCA-negative, CD is the more likely diagnosis.

2.5.3. Fecal markers

Fecal markers are proteins or leukocytes associate with degradation products. In addition, they are stable, easily preserved, and resistant to enzymatic degradation. Fecal markers have high specificity for the diagnosis of IBD. Moreover, they have good correlation with mucosal lesions, the activity intensity and the excretion of ^{111}-indium-labelled leucocytes (gold standard).

Fecal calprotectin

Calprotectin, a calcium- and zinc-binding protein, is a dimer of the calcium-binding proteins S100A8 and S100A. The complex accounts for up to 60% of the soluble protein content of the neutrophil cytosol. Calprotectin has many biological functions, including those associated with genetic, structural and immunological interactions. In addition, it has bacteriostatic and fungistatic properties. The presence of calprotectin in feces indicates an organic disorder and is directly related with the migration of neutrophils to the gastrointestinal tract. Thus, the amount of calprotectin reflects the number of neutrophils participating in the inflammatory process. Calprotectin is stable and resistant to heat and proteolysis. There may be variations with some factors such as treatment with NSAIDs, proton-pump inhibitors, age, diet or physical activity. Fecal calprotectin is measured using immunochemical techniques

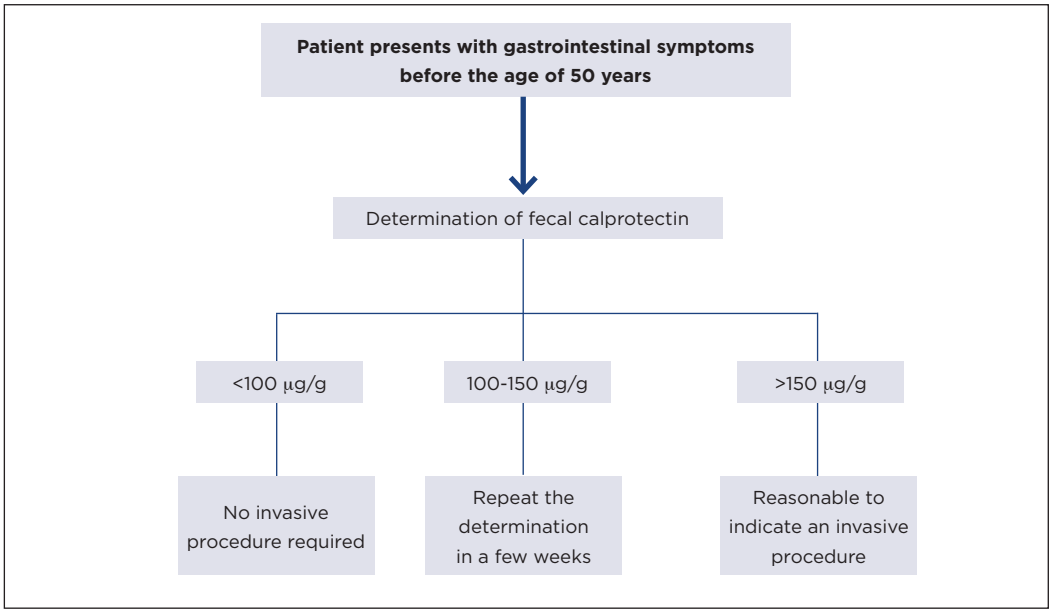

Figure 1. Reference values for fecal calprotectin.

such as ELISA or immunochromatographic assays; it is simple and economical to measure. The reference value is <50 µg/g. However, some studies have established that, for fecal calprotectin <100 µg/g, no invasive procedure is recommended; in the case of 100–150 µg/g, it is recommended to repeat the determination in a few weeks and, for levels >150 µg/g, it is reasonable to indicate an invasive procedure (Figure 1).

Fecal calprotectin has a high sensitivity and specificity (>90%) for differentiating between organic and functional disorders. However, specificity is lower in organic disorders, as levels are increased in conditions such as colorectal neoplasia or gastrointestinal infections. Fecal calprotectin has a high negative predictive value and a good correlation with IBD activity (although poorer in the case of small intestine involvement). Endoscopic correlation is lower than with histology. Fecal calprotectin has a high capacity to predict the risk of clinical recurrence (sensitivity 90%; specificity 83%). Combined with CRP, fecal calprotectin predicts the risk of relapse after treatment withdrawal. A value of 250 µg/g predicts large ulcers. In contrast, a value of <250 µg/g predicts mucosal healing. Fecal calprotectin levels start to increase 4 or 6 months before relapse. Consequently, it is recommended to perform fecal calprotectin assessments every 3 or 4 months. The normalization of calprotectin levels predicts endoscopic healing. Although a reduction of calprotectin levels is more difficult to achieve than for CRP levels, a reduction implies better mucosal healing [9].

Fecal lactoferrin

Lactoferrin is a glycoprotein expressed by activated neutrophils. Currently, only limited experience exists for the use of fecal lactoferrin as a marker. Although fecal lactoferrin levels are increased in IBD, they are

also elevated in gastrointestinal infections of either viral or bacterial origin. For the diagnosis of IBD, fecal lactoferrin has a sensitivity of 82% and a specificity of 93%, and values are similar for active disease and remission.

2.5.4. New biomarkers

A number of potential new biomarkers (i.e. genetic polymorphisms, electronic nose, L-arginine, short-chain fatty acids, anti-I2, anti-OmpC, anti-OmpW, anti-CBir1) are currently being investigated for the management of patients with IBD.

In summary, biomarkers are simple, economical, non-invasive and complementary instruments that provide additional information to the standard clinical approach, and endoscopic, radiologic and histologic exams. They are very helpful for differentiating between organic and functional disorders. The most important biomarkers are CRP (particularly for CD) and fecal calprotectin. Biomarkers have good correlation with disease activity, are good predictors of recurrence and they are useful for assessing treatment responses.

2.6. Treatment

The treatment of IBD is complex and heterogeneous. The selection of therapy is based on disease severity, the clinical characteristics of the patient, prior treatment (response and side effects). The therapeutic approach is challenging and should be individualized [2].

Treatment options for IBD include corticosteroids, aminosalicylates (i.e. mesalamine), thiopurines, methotrexate or calcineurin inhibitors (i.e. ciclosporin or tacrolimus). However, among biological agents, monoclonal antibodies are key for the management of patients with IBD [2,10].

Infliximab is a chimeric IgG1 monoclonal antibody against TNF-α, comprised of 75% human and 25% murine sequences. It has a high specificity for, and affinity to, TNF-α, leading to inhibition of the transcription of proinflammatory cytokines and adhesion molecules that favors the migration of leukocytes. Other anti-TNF-α antibodies include adalimumab, golimumab and certolizumab. There are also other monoclonal antibodies with different therapeutic targets, such as cell adhesion molecules (natalizumab, vedolizumab, etrolizumab), IL-12 and IL-23 antagonists (ustekinumab), Janus kinase (JAK) inhibitors (tofacitinib, filgotinib, upadacitinib) and sphingosine-1-phosphate (S1P) receptor agonists (ozanimod, etrasimod).

It is important to monitor the response to treatment, because 10–40% of patients do not respond to therapy after induction (no primary responders) and 30–40% of patients lose the response during maintenance (no secondary responders). This is due to pharmacodynamic (the main inflammation pathway does not depend on TNF-α) and pharmacokinetic (insufficient drug levels because of immunogenicity or increased clearance) issues [11]. There is a direct relationship between trough plasma levels of these drugs, anti-drug antibodies (ADAs) and clinical efficacy. Consequently, it is not only important to maintain drug levels within the therapeutic range, but also to decrease immunogenicity to the drug.

In IBD, it has been observed that, when treatment optimization is based solely on clinical response, this leads to low concentrations and the presence or formation of ADAs. In contrast, monitoring the therapeutic dose and antibodies is a very

Table 4. Monitoring and optimization of the dose of biological agents

Classically	• Infliximab prior to the first dose of maintenance (week 14) • Adalimumab prior to the first dose of maintenance • Etanercept prior to the fourth dose
During induction phase	• High probability of antibodies at the beginning of treatment • Low levels of infliximab in week 4, increase the risk of developing high levels of antibodies • Monitor levels in week 10 • Estimate levels in week 14
During maintenance phase	• Monitor to optimize the induction treatment • Main strategies: 1) Intensify the dose 2) Change anti-TNF (switch) 3) Change therapeutic target (swap)

useful tool to optimize anti-TNF-α therapy (i.e. infliximab >5 μg/mL; adalimumab >7.5 μg/mL) (Table 4) [2,10].

Therefore, therapeutic drug monitoring offers some advantages, including an early prediction of response, evolution and treatment failure. A double test can be performed to obtain drug levels and ADAs, in order to optimize the efficacy and safety, leading to individualized treatment, improving the cost and effectiveness of biological therapies, and reducing poor treatment adherence.

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Edited by: **Grifols, S.A.**
Parc Empresarial Can Sant Joan
Av. de la Generalitat, 152-158
08174 Sant Cugat del Vallès
Barcelona - SPAIN

Contact details and information:
medaffairs.diagnostic@grifols.com

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