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Present and future in the diagnosis and treatment of autoimmune diseases

Santiago, Chile, May 31, 2018

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CONTENTS

IV SYMPOSIUM ON AUTOIMMUNITY
Present and future in the diagnosis
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Utility of alternative markers in the diagnosis of antiphospholipid syndrome

MARÍA JOSÉ CUADRADO

SENIOR CONSULTANT, HEAD OF THE DEPARTMENT OF RHEUMATOLOGY, CLÍNICA UNIVERSITARIA DE NAVARRA-MADRID, SPAIN

Antiphospholipid syndrome (APS) is the most common type of acquired thrombophilia and the only one that is characterized by the appearance of venous and arterial thromboses, unlike genetic thrombophilia, which usually progresses with venous thrombosis only. APS is responsible for complications during pregnancy, leading to early miscarriage (first trimester), fetal death (second and third trimesters), preeclampsia, and intrauterine growth restriction. The antiphospholipid antibodies (aPL) taken into consideration for diagnosis include anticardiolipin antibody (aCL) and anti-B2-glycoprotein-1 antibody (aB2GP1). Also important for diagnosis is lupus anticoagulant (LA), which is not an antibody, but an entity associated with the prolonged clotting time that indirectly reflects the presence of aPL, specifically dilute Russell viper venom time (dRVVT) and activated partial thromboplastin time (aPTT). Other frequent clinical manifestations of APS are microangiopathy, or small vessel thrombosis, the most relevant of which are renal thrombotic microangiopathy, cardiomyopathy, and, at the cutaneous level, ulcers, necrosis, and livedo reticularis.

Frequent manifestations of APS that are not thrombotic in origin include thrombocytopenia, which is usually moderate (80,000-100,000 platelets/μL; normal range, 15,000-450,000 platelets/μL). Only 25% of patients have <50,000 platelets/μL and only 5% experience bleeding (<10,000 platelets/μL). Other frequent complications of APS include hemolytic anemia, heart valve thickening due to deposition of antibodies, and neurological complications such as transverse myelitis (**Table 1**).

As mentioned above, the criteria for classifying APS include positive aPL titer (aCL and aB2GP1) and presence of LA. The standard clotting times are dRVVT and aPTT. However, patients who receive anticoagulation with acenocoumarol or the new anticoagulants should be assessed using Taipan snake venom time (TSVT).

Table 1. Common clinical manifestations in patients with antiphospholipid syndrome associated and not associated with thrombosis.

Associated with thrombosis	Not associated with thrombosis
Cutaneous microangiopathy — Ulcers — Necrosis — Livedo reticularis	Thrombocytopenia Hemolytic anemia Arterial hypertension
Cardiac microangiopathy	Heart valve involvement Epileptic seizures
Renal microangiopathy	Chorea Transverse myelitis

TREATMENT OF THROMBOSIS

Treatment of patients with APS is classified as follows:

- Primary prophylaxis: for prevention in patients who have not yet manifested thrombosis, eg, patients with lupus or women with obstetric complications who have given birth.
- Secondary prophylaxis: to prevent new episodes of thrombosis or recurrent thrombosis.
- Treatment of obstetric disease.

Patients who do not meet the classification criteria owing to low or intermittent-positive aPL titers are not generally treated. In contrast, patients with persistently moderate or high titers with venous thrombosis should receive anticoagulant treatment until their international normalized ratio (INR) reaches 2.0-3.0 after treatment with vitamin K antagonists such as acenocoumarol and warfarin. Direct oral anticoagulants (antithrombin or anti–factor X) are also currently used to treat the first venous thrombosis, although they have not been shown to be equally efficacious as vitamin K antagonists in the prevention of thrombosis. In patients with APS and arterial thrombosis, the objective of treatment is to achieve an INR of 3.0-4.0. A systematic review showed that these patients experienced little bleeding, with a total of 18 deaths from thrombosis and only 1 from bleeding[1]. While hematologists prefer the INR not to be so high, arterial thromboses tend to recur when the INR is lower.

SERONEGATIVE ANTIPHOSPHOLIPID SYNDROME

Seronegative APS involves suggestive symptoms (recurrent thrombosis, obstetric disease, or both), with clinical manifestations that are not part of the diagnostic criteria (**Table 2**), but with negative aPL titers (aCL and aB2GP1) and negative LA titers. In these patients, it is important to identify other possible causes of disease and, above all, rule out neoplasm.

Table 2. Diagnostic criteria for seronegative antiphospholipid syndrome.

Recurrent thrombosis and/or morbidity during pregnancy
Absence of other identifiable diseases
Presence of manifestations of APS not included in the diagnosis of APS
Negative titers for aPL and LA and positive titers for aPL that are not part of the diagnostic criteria

aPL, antiphospholipid antibody; LA, lupus anticoagulant.

The classification criteria are used in research studies to ensure that samples are homogeneous, although a patient can be diagnosed with APS without fulfilling the criteria. A patient with low antibody titers and APS-associated obstetric morbidity cannot be included in a study because she does not fulfill the criteria, although she must be treated. Other aPL are not classification criteria because there is no evidence for their association with symptoms. These include antiphosphatidylserine (aPhS), antiprothrombin (aPT), antiphosphatidylserine/prothrombin complex (aPhS/PT), aB2GP1 IgA, and, more recently, aB2GP1 domain 1 (aB2GP1-D1) (**Table 3**). More studies are being developed to determine whether these antibodies are associated with symptoms.

ANTIPHOSPHATIDYLSERINE ANTIBODIES

Two studies have been published. The first included 212 patients with thrombotic and/or obstetric problems

Table 3. Antiphospholipid antibodies found in seronegative antiphospholipid syndrome.

Antiphosphatidylserine (aPhS)
Antiprothrombin (aPT)
Antiphosphatidylserine/prothrombin complex (aPhS/PT)
Anti-beta-2-glycoprotein 1 isotype IgA (aB2GP1 IgA)
Anti-beta-2-glycoprotein 1 domain 1 (aB2GP1-D1)

and compared various parameters of aPhS with other aPL. Specificity was 87% for aPhS. Positive titers for aPhS were detected in 70% of patients, in whom APS was confirmed. Many were also positive for aCL and/or anti-B2GP1[2]. The second study included patients with obstetric APS who were positive for aCL, anti-B2GP1, and LA or who fulfilled other parameters that were not part of the classification criteria. aPhS were found in 73% of patients who also had positive titers for standardized aPL, compared with 88% in patients with negative titers for standardized aPL[3].

ANTIPROTHROMBIN AND ANTI-PHOSPHATIDYLSERINE/PROTHROMBIN COMPLEX ANTIBODIES

aPhS and aPhS/PT antibodies are detected using enzyme-linked immunosorbent assay (ELISA). A study that used 2 coated ELISA plates (aPhS in one, aPsH/PT in the other) showed that these were 2 well-differentiated antibody populations and those that have been most associated with positive LA titers[4]. A systematic review of 38 studies on aPhS and 10 studies on aPhS/PT with more than 7,000 patients concluded that both antibodies were associated with a significantly increased risk of arterial or venous thrombosis; this risk was almost twice as high in patients with aPhS and up to 5-fold greater in patients with aPhS/PT complex antibodies[5]. Another study involving 7 countries analyzed aPhS/PT complex antibodies in 247 patients with APS. An association was observed with respect to the IgG isotype, although this association was less pronounced with the IgM isotype. Furthermore, 51% of patients with APS (defined by aCL and anti-B2GP1) also carried aPhS/PT, as did 9% of patients with seronegative APS. Therefore, the sensitivity was not very high (51%), although the specificity was 91%[6], indicating that aPhS/PT should be reassessed as a criterion.

IGA ISOTYPE ANTI-BETA-2 GLYCOPROTEIN 1 ANTIBODIES

The association between the IgG and IgM isotypes of aB2GP1 and thrombosis is already well established, although evidence for IgA is not so plentiful. A study that compared 40 patients with established APS and 40 patients with seronegative APS using 9 specific ELISAs to determine the IgG, IgM, and IgA isotypes of aCL, aB2GP1, and aB2GP1-DI antibodies found that 62.5% of patients with established APS had a positive result for IgA. The most relevant finding was that 10% of the patients were also seronegative[7]. Another study showed aB2GP1 IgA to be a risk factor for thrombosis after a 5-year follow-up. A total of 45 patients (9.7%) developed clinical manifestations of APS (38 patients [15.6%] in the aB2GP1-positive group vs 7 patients [3.2%] in the group with negative titers for all 3 antibody types); the difference was statistically significant ($p<0.001$). The incidence of thrombosis (3.1%) was similar to that observed in patients with APS defined based on classical antibodies. In aB2GP1 IgA–positive patients, the most common event was arterial thrombosis, which was one of the risk factors observed, together with age and male sex[8]. Therefore, it is important to take aB2GP1 IgA into account in patients with negative APS findings based on classical antibodies.

ANTI-BETA-2 GLYCOPROTEIN 1 DOMAIN 1 ANTIBODIES

B2GP1 comprises 5 domains that present a conformational change in patients with APS. It becomes antigenic when domain 1 (D1) is exposed. aB2GP1-D1 antibodies bind to the domain and are closely associated with thrombosis. All patients with aB2GP1-D1 antibodies are positive for B2GP1. aB2GP1-D1 antibodies have been identified, and specific assays have been applied to detect their IgA, IgG, and IgM isotypes[9]. A monoclonal antibody against D1 is currently being developed to block the onset of thrombosis.

A study of 157 patients, of whom 51 had APS, evaluated the aPhS/PT complex and aB2GP1-D1 and found that these antibodies made it possible to diagnose 90% of patients with APS. The remaining 10% were diagnosed using classical antibodies[10]. This is important, because LA is negative in anticoagulated patients, except when TSVT is used (see above), although this is not so frequent. A diagnosis cannot be made if aCL and aB2GP1 titers are also negative.

OTHER ANTIPHOSPHOLIPID ANTIBODIES

One study evaluated 11 nonclassical antibodies in patients with seronegative APS and used these to diagnose 36.8% of seronegative patients[11]. The antibodies included antiphosphatidylethanolamine (aPE), anticardiolipin/vimentin (aCL/Vim), aPhS/PT, and aPhS. However, to date, the antibodies showing most evidence for an association with thrombosis are aPhS/PT and aB2GP1-D1.

GLOBAL SCALE FOR EVALUATION OF THE THROMBOTIC RISK OF APS

The Global Antiphospholipid Syndrome Score (GAPSS) was developed to measure the probability of thrombosis in a patient based on the aPL titer. A triple-positive result (aB2GP1, aCL, and LA) implies a poorer prognosis than a positive finding for only 1 of the antibodies. The GAPSS also takes into account cardiovascular risk factors (hypertension, smoking, diabetes, hypercholesterolemia). The score is calculated based on the presence of positive findings for several parameters. For example, it gives 5 points to a positive aCL result, 4 to aB2GP1, 3 to aPhS/PT, 4 to LA, 3 to hyperlipidemia, and 1 to hypertension[12]; the higher the score, the greater the risk of thrombosis, obstetric conditions, and even recurrence of thrombosis. Patients with a GAPSS greater than 16 are 6 times more likely to experience thrombosis (HR, 6.17; 95%CI, 1.70-22.40). In addition, the IgG isotype of the aPhS/PT complex is significantly associated with a risk of thrombosis (HR, 2.95; 95%CI, 1.02-8.51)[12].

RECOMMENDATIONS AND CONCLUSIONS

- The IgA isotype of aCL and aB2GP1 and the IgG and IgM isotypes of the aPhS/PT complex should be evaluated in patients with symptoms of APS whose first classical antibody screen yielded negative results.
- The aPhS/PT complex should be included in the new criteria for classification of APS.
- aPE and aPT should be assessed, even though their value is lower.
- In the case of patients anticoagulated with vitamin K antagonists, LA should be determined using TSVT instead of dRVVT and aPTT. However, since TSVT is available in few laboratories, aPhS/PT can be used.
- In patients with seronegative APS, it is recommended to use antibodies not included in the classification criteria for APS.

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Present and future in the diagnosis
and treatment of autoimmune diseases

Vitamin D and autoimmune diseases

PATRICIA ABUMOHOR

CLINICAL IMMUNOLOGIST AND RHEUMATOLOGIST, DEPARTMENT OF INTERNAL MEDICINE, CLÍNICA LAS CONDES, REGIÓN METROPOLITANA, CHILE

VITAMIN D METABOLISM

Appropriate levels of vitamin D are associated with general wellbeing and absence of disease. Vitamin D deficiency, which is a widespread problem in the general population and in pregnant women[1], has been associated with a greater prevalence and incidence of autoimmune diseases and allergic diseases. Vitamin D comes from 2 sources: foods (vitamin D2 or ergocalciferol) and sunlight (vitamin D3 or colecalciferol). Sunlight provides most of the vitamin D that we need. This provitamin is oxidized by liver enzymes (CYP2R1) to the 25-hydroxy-vitamin D form (25OHD, or calciferol), which undergoes additional oxidation to the active form 1,25-dihydroxy-vitamin D ($1,25(OH)_2D_3$, or calcitriol) in the kidneys. This active form participates in calcium metabolism, thus favoring intestinal absorption for deposition in bone and absorption by the kidneys and suppressing parathyroid hormone[2]. Normal functioning prevents the onset of osteoporosis or rickets. Vitamin D status is assessed by measuring 25OHD in peripheral blood using various techniques, because it has a half-life of 2-3 weeks, unlike the active metabolite, which has a half-life of a few hours. The most widely agreed upon optimal blood concentration for bone maintenance is >30 ng/mL. Insufficiency is defined as <30 ng/mL and >10 ng/mL. Deficiency is defined as < 10 mg/mL[3]. Vitamin D receptors (VDRs) are found in the musculoskeletal tissue, the intestine, and kidneys and are widely distributed in other tissues (brain, muscle, heart, endothelium, breast, prostate, colon, skin) and in the immune system[4].

The influence of vitamin D on different types of tissue has been evaluated in various studies. Thus, we can observe "nonclassical" action of vitamin D, for example, suppression of cell growth, regulation of apoptosis, and control of activity in cells from tissues that carry VDRs, such as the reproductive tract (uterus, ovaries, placenta, testicles, and prostate), pancreas, pituitary gland, thyroid gland, adrenal cortex, smooth and skeletal muscle, heart, skin, brain, and liver, and even in the immune system. In particular, the immune system is affected not only by the active metabolite $1,25(OH)_2D_3$, but also by the 25OHD form[5], which is capable of metabolizing to the active form.

VDRs can affect 3% of the genome, since elements that respond to vitamin D are found at specific points along their sequence. The gene for VDRs is found at chromosome cr12q13.11. Polymorphisms, that is, small sequence variants (FokI, BsmI, TaqI, and ApaI) have been reported, and these can translate into lesser biological effects of vitamin D, even if blood levels are adequate[6].

ROLE OF VITAMIN D IN THE IMMUNE SYSTEM

VDRs and hydroxylase enzymes (CYP27A1, CYP27B1) in the immune system can respond and synthesize the active form of vitamin D. Levels of $1,25(OH)_2D_3$ may be high in immune tissue. The effect of vitamin D can manifest in the cell that synthesizes it (autocrine effect) or in neighboring cells (paracrine effect).

Vitamin D has an effect on both innate and adaptive immunity (**Figure 1**):

- Innate immunity:
  - VDRs are found in monocytes and macrophages, as are enzymes that are hydroxylated to 25OHD and $1,25(OH)_2D_3$. If they receive vitamin D, their proliferation and bactericidal capacity are enhanced

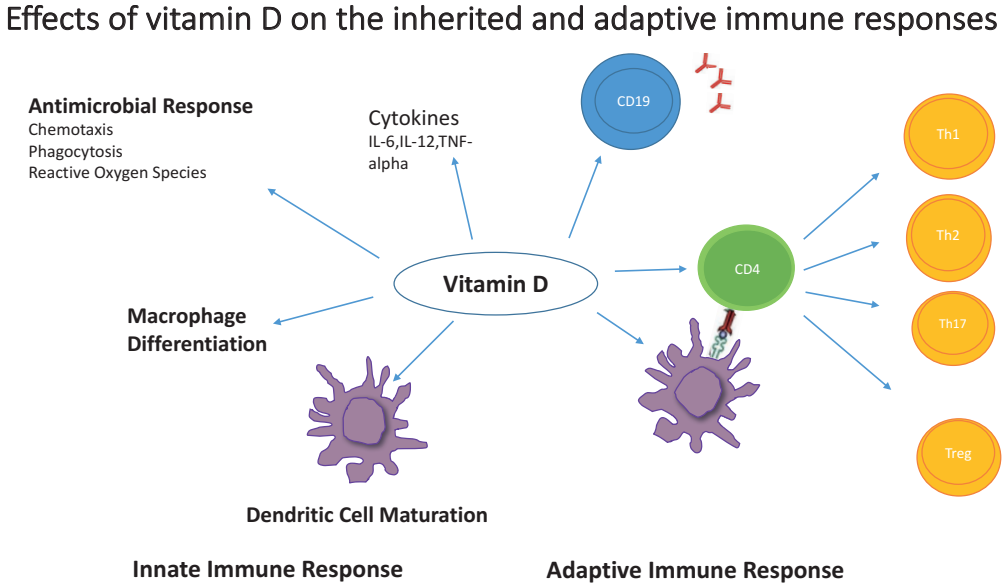

Figure 1. Effects of vitamin D on the inherited and adaptive immune responses.

owing to the increase in levels of enzymes that act in the host's defense. This function may be affected in patients with low vitamin D levels.

- Adaptive immunity:
  - Dendritic cells capture the antigen and initiate the response to suppress it. Vitamin D acts on the dendritic cells, diminishing their maturation and the expression of the surface markers necessary for an appropriate antigen response. They are converted into more immature cells that favor tolerance, thus placating the adapted immune response.
  - The T_H1 and T_H17 immune responses are diminished. In B lymphocytes, their proliferation and ability to produce antibodies are diminished.

Therefore, vitamin D enhances the inherited immune response and downregulates the adaptive immune response and the antibody rate[8]. That is, vitamin D increases the anti-infectious capacity of macrophages, but also diminishes stimulation of dendritic cells, increases regulation of T_H2, and reduces the T_H1 response, which is the most aggressive immune response[9].

HYPOVITAMINOSIS D: CAUSES AND CONSEQUENCES

Vitamin D status can be affected by external factors such as sunscreens, latitudes or winter periods with low exposure to sunlight, and skin pigmentation, all of which prevent absorption of the sun's rays and of the conversion of provitamin D into vitamin D[1]. Various drugs and supplements are associated with

vitamin D abnormalities, for example, corticosteroids. Physiological and pathological situations that are accompanied by vitamin D deficiency include liver failure (diminished hydroxylase enzyme), kidney failure (loss of protein that is bound by the active metabolite of vitamin D or reduction in hydroxylase enzyme levels), obesity (fatty tissue traps vitamin D), or malabsorption caused by various factors[1].

Possible consequences of the lack of vitamin D include a higher frequency of infection, depression, asthma, arterial hypertension, coronary disease, adult diabetes, metabolic syndrome, autoimmune disease (type 1 diabetes mellitus, multiple sclerosis, Crohn disease, rheumatoid arthritis, and lupus), other diseases (arthrosis, osteoporosis), and cancer (owing to its ability to affect immune regulation)[1].

EVIDENCE ON VITAMIN D AND AUTOIMMUNE DISEASE

In vitro studies

In vitro studies have demonstrated the antiproliferative, antibacterial, anti-inflammatory, and immunomodulatory activity of vitamin D.

In vivo studies: experimental studies

A protective effect of vitamin D, by which expression of the disease is diminished, has been demonstrated in various experimental models of autoimmune disease:

- Inhibition of diabetes has been recorded in nonobese diabetic mice (experimental model of type 1 diabetes).
- Experimental autoimmune encephalitis, multiple sclerosis model, collagen-induced arthritis, arthritis model. Also in murine lupus, where vitamin D reduces the antibody rate.
- Autoimmune thyroiditis.
- Inflammatory bowel disease.

Administration of vitamin D and/or appropriate levels thereof at the time of antigen presentation is thought to create an environment of tolerance resulting from changes in antigen-presenting cells.

The current hypotheses are as follows:

1) Vitamin D sufficiency could reduce the risk of autoimmune disease (eg, type 1 diabetes mellitus, multiple sclerosis, thyroid disease, rheumatoid arthritis, or systemic lupus erythematosus [SLE]).
2) Vitamin D deficiency is an environmental risk factor that can affect the prevalence and severity of autoimmune disease.
3) Vitamin D supplements may prove useful for preventing specific autoimmune diseases or reducing their severity[10].

CLINICAL TRIALS

Several published studies have evaluated the effect of vitamin D in various diseases.

Prospective population studies

- Data from a Danish study of 12,555 patients with a mean follow-up of 10 years show that during that time there were 525 cases of autoimmune disease in which the highest levels of vitamin D were associated with a lower risk of

developing an autoimmune disease. The risk was significant, especially in the case of thyroid disease[11].

Studies in rheumatoid arthritis

- Cross-sectional studies and meta-analyses:
  - Meta-analysis of 3,489 patients with rheumatoid arthritis in whom the authors measured vitamin D levels and compared them with controls. An inverse association was observed between vitamin D levels and disease activity measured using DAS28, that is, the greater the disease activity, the lower the level of vitamin D. This association was observed in lower latitudes, owing to decreased sun exposure, and is more significant in developing countries[12].

- Vitamin D status:
  - Cross-sectional multicenter European study in 13 countries that evaluated vitamin D status in 625 patients with rheumatoid arthritis and 276 healthy controls. The results showed that vitamin D deficiency and insufficiency were greater in patients with rheumatoid arthritis than in the healthy controls (66% vs 53%, p=0.01), although high rates of deficiency and insufficiency were also found in these countries. Furthermore, a negative correlation was found between vitamin D levels and disease activity measured using DAS28 (p<0.0001), the disability index measured using the Rheumatoid Arthritis Impact of Disease (RAID) score (p=0.04), and quality of life measured using the Health Assessment Questionnaire (p=0.02)[13].

- Association with disease activity and severity:
  - A study of 1,143 patients with rheumatoid arthritis revealed lower vitamin D levels in patients than in controls (55.2% vs 33.2%; OR, 2.460; 95%CI, 1.135-5.330; p=0.023) and an inverse correlation between vitamin D levels and disease activity (CC=–0.278; 95%CI, –0.393 to –0.153). The authors concluded that vitamin D levels are associated with a risk of rheumatoid arthritis and activity thereof[14].

- Effect of vitamin D on the immune response:
  - Jeffery et al[15] provided a detailed report of the effects of vitamin D and their relevance in rheumatoid arthritis (mediation of T_H1 and T_H17 populations and inhibition of the cytokines involved in bone damage and erosion).

Studies on systemic lupus erythematosus

- Mechanism of action of vitamin D:
  - Vitamin D insufficiency has been reported in most patients with SLE (39%-96%) and severe deficiency in up to 30%. Appropriate levels of vitamin D in patients with SLE leads to inhibition of NF-kB and, consequently, reduced levels of interferon gamma and production of interleukin 12, as well as inhibition of proliferation of the B lymphocytes that produce the antibodies. The authors also consider that vitamin D could have beneficial effects in SLE, by improving some of the clinical manifestations (given its inflammatory and immunomodulatory ability), as well as by modifying the endothelial repair mechanism, improving

musculoskeletal capacity, and regulating the cell cycle[16].

- Causes and consequences of vitamin D deficiency:
  - Causes: Patients with SLE and kidney failure with loss of protein and hydroxylase are advised to reduce their exposure to sunlight and to use sunscreen. In addition, several medications alter vitamin D metabolism (calcineurin inhibitors, corticosteroids, antiepileptic drugs, and antimalarial drugs)[16].
  - Consequences: muscle weakness, kidney disease, cardiovascular disease, and arteriosclerosis[16].

- Association with disease activity: cohort studies and meta-analyses:
  - A recent review of the association between vitamin D insufficiency and lupus activity revealed that some studies demonstrated an association whereas others did not[17]. Studies are very heterogeneous, and there are no controlled studies. Several interfering factors may explain this observation.

Studies on vitamin D supplementation

- Franco et al[18] found no association was found between the administration of vitamin D supplements and modification in the activity of arthritis, although fewer recurrences of flare-ups were recorded. A reduction in the frequency of anti-DNA antibodies was also recorded in patients with SLE.
- A review of the literature on studies that had analyzed the immunoregulatory effects of vitamin D supplements in the development of SLE showed that each study had used a different dose of vitamin D. Therefore, comparison was hampered by the heterogenous nature of the study population. Some studies showed a reduction in disease activity, some showed an improvement in quality of life and fatigue, and others showed reduced antibody levels[7].

RECOMMENDATIONS AND CONCLUSIONS

- Evidence of the effect of vitamin D deficiency in various autoimmune diseases has been reported from in vitro studies, experimental models, and observational or cohort studies, although no controlled studies have been performed.
- Vitamin D deficiency is highly prevalent in patients with autoimmune disease and in healthy persons, although not everyone goes on to develop autoimmune disease, since this depends on susceptibility.
- Vitamin D has an immunomodulatory effect.
- Vitamin D deficiency is a risk factor in persons who are prone to autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, thyroid disease, rheumatoid arthritis, and SLE.
- Vitamin D deficiency can also affect disease severity and clinical manifestations.
- No categorical clinical evidence has been published to date on the benefit of vitamin D supplement, except in type 1 diabetes during the first year of life.
- Vitamin D can be administered in various regimens and doses.
- Numerous variables are involved in the metabolism of vitamin D.
- More controlled prospective studies are required.

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IV SYMPOSIUM ON AUTOIMMUNITY
Present and future in the diagnosis
and treatment of autoimmune diseases

Keys to the diagnosis of type 1 diabetes: main preclinical markers and evaluation of risk

FRANCISCO PÉREZ

NUTRITIONAL GENOMICS LABORATORY, DEPARTMENT OF NUTRITION, FACULTY OF MEDICINE, UNIVERSITY OF CHILE, REGIÓN METROPOLITANA, CHILE

In 1999, the American Diabetes Association (ADA) reclassified type 1 diabetes into types 1A and 1B, with 1A being classic autoimmune diabetes, ie, that in which the patient's cell response or humoral response can be quantified. Type 1B diabetes, which is also known as idiopathic or fulminant diabetes, progresses with total insulin deficiency caused by self-destruction of the pancreatic beta cells and no markers of autoimmunity.

The year 1986 saw the initiation of the Diamond Project, which collected epidemiological data on type 1 diabetes by country and enabled standardized recording of cases in patients aged 0 to 14 years, thus making it possible to compare data between countries[1]. The distribution of the incidence of type 1 diabetes by country published in 1999 showed incidence in Finland to be highest (>40 cases per 100,000 inhabitants) and that in Chile to be one of the lowest (<5 cases per 100,000 inhabitants)[2]. Specific articles following the Diamond methodology provided updated data for 2016 from various countries showing a growing trend in the variation of the incidence of the disease. These changes in the epidemiological pattern indicate that type 1 diabetes is not a genetic disease and that it is modified by environmental pressure, although susceptibility markers have been identified.

A 2011 review on the pathophysiology of type 1 diabetes addressed the natural course of the disease, which involves genetic susceptibility and environmental triggers, as occurs with celiac disease, in which flattening of the enterocyte after contact with gluten leads to symptoms of inflammation and an autoimmune response. In the case of diabetes, factors such as bacteria, diet, and various substances lead to a reduction in pancreatic beta cells in susceptible patients. The pancreas contains alpha cells, beta cells, and delta cells, each with a different function. However, the immune system only attacks beta cells. These cells then begin to regenerate (the so-called honeymoon period), although they are eventually destroyed by the immune system[3]. At this point, it is better for the honeymoon period to disappear as soon as possible owing to the risk of hypoglycemia that could be triggered in a patient receiving external insulin. In parallel to the cell response that destroys beta cells, antibodies begin to be produced as a result of exposure to antigens caused by the destruction of beta cells and the subsequent humoral response.

When autoimmunity does not develop, these autoantibodies can cause other types of diabetes, for example, maturity onset diabetes of the young (MODY), which has various isoforms and is metabolically stable, or Wolfram syndrome diabetes. In patients with type 1 autoimmune diabetes, we can detect antibodies such as antitransglutaminase antibodies and antithyroid antibodies (**Figure 1**). Autoimmune diseases usually progress with other manifestations associated with autoimmunity. For example, in Chile the incidence of celiac disease is 1%, which increases to 8% in patients with type 1 diabetes.

AUTOANTIBODIES IN DIABETES

Islet cell antibodies (ICA) were first described in 1970 and continue to be the gold standard, although they are difficult to titrate because they require a pancreas biopsy. In the 1980s, it became possible to detect other antibodies using radioimmunoassay (RIA), and today, we are using antibodies analyzed by enzyme-linked immunosorbent assay (ELISA) (**Table 1**).

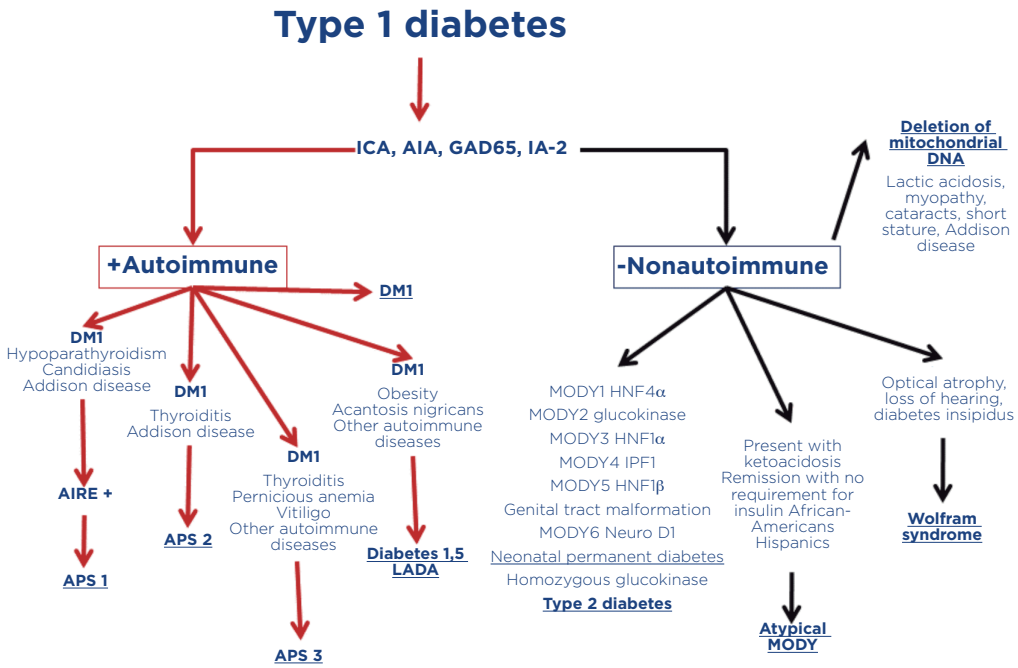

Figure 1. Types of type 1 diabetes and autoimmunity. DNA, deoxyribonucleic acid; APS 1, type 1 autoimmune polyglandular syndrome; APS 2, type 2 autoimmune polyglandular syndrome; APS 3, type 3 autoimmune polyglandular syndrome; DM1, type 1 diabetes mellitus; HNF1a, hepatocyte nuclear factor 1 alpha; HNF1b, hepatocyte nuclear factor 1 beta; HNF4a, hepatocyte nuclear factor 4 alpha; IA-2, tyrosine phosphatase–like proteins; AIA, anti-insulin antibodies; IPF1, insulin-promoting factor 1; LADA, latent autoimmune diabetes in adults; MODY, maturity onset diabetes of the young.

Table 1. History of autoantibodies in diabetes.

Decade	Autoantibody
1970	Against islet cells (ICA) (gold standard) Against islet cell surface (ICSA)
1980	Anti-insulin antibodies (AIA) 64-kDA (64 KAs) Insulin receptor Carboxypeptidase H Heat shock proteins
1990	64-kDA (64 KAs) = glutamic acid decarboxylase (GADA) Against islets 51-kDa aromatic-L-amino acid decarboxylase, 30-kDa chymotrypsinogen, topoisomerase II DNA, glima 38, GLUT2, glycolipids, GM2-1 ganglioside, IA-2, IA-2b, ICA69, proinsulin, and 52-kDa RIN (rat insulin)
2000	CD38, ZnT8

IA-2, tyrosine phosphatase–like proteins

These antibodies can be used for the following:

- To help define the nature of diabetes (fulminant or autoimmune).
- To give an idea of damage to the pancreas.
- To predict disease, given that they are markers of disease activity, even during prodromal stages, and thus provide valuable information for relatives of patients with diabetes.
- To measure sensitivity and specificity for prediction and the positive predictive values.

Sensitivity is the quotient of the number of positive subjects with disease and the number of subjects with disease. Specificity is the quotient of the number of negative subjects without disease and the number of subjects without disease. Finally, the positive predictive value is the quotient of the number of positive subjects who have the disease and the number of positive subjects. If the risk of disease is high, the predictive value is greater. Using several antibodies increases the sensitivity of prediction, and there is a reciprocal relationship between sensitivity and specificity.

ISLET CELL ANTIBODIES (ICA)

- ICA are detected by indirect immunofluorescence and remain the gold standard.
- Their sensitivity in children with type 1 diabetes is 70%-80%, although this is lower in adults.
- Their specificity is 96%-98%.
- Their predictive value in the general population is 0.15%-0.2%.
- They are used mainly in research protocols but are not useful in patients.

ANTI-INSULIN ANTIBODIES (AIA)

- AIA are detected mainly using ELISA
- AIA bind to insulin in individuals who do not receive insulin treatment.
- They make it possible to detect pancreatic beta cell damage when the molecule is very immature.
- Sensitivity is low at the onset of type 1 diabetes.
- Specificity is almost 99%, and their predictive value is extremely high when used in combination with ICA or other antibodies.

GLUTAMIC ACID DECARBOXYLASE ANTIBODIES (GADA)

- The 2 isoforms are GAD65 and GAD67
- They are not specific to pancreatic beta cells and are also found in the glial cells of the brain.
- They are determined using RIA or ELISA and have good sensitivity and specificity.
- The GAD67 isoform is used mainly in laboratory protocols.
- The GAD65 isoform is the more frequently used of the two and has a sensitivity of 70%-75% and a specificity of 98%-99%.
- GAD65 is not affected by age, as is the case with other antibodies.
- The predictive value of GADA is greater than that of ICA or AIA. However, the highest predictive value is achieved with all 3 combined.

TYROSINE PHOSPHATASE–LIKE PROTEIN ANTIBODIES (IA-2)

- IA-2 are determined mainly using ELISA
- The manifest later that other antibodies.
- They are found in 70%-80% of children with type 1 diabetes.
- Studies show that they develop later than GADA and that they can serve as short-term markers of risk.
- As with AIA, IA-2 are more common in children than in adults.

Table 2. Antigens

Decade	GAD65	IA-2	Insulin
Molecular weight (Da)	65,000	106,000	6,000
Chromosome	10p11	2q35	11p15
Type of cell where expressed	Neuroendocrine Pancreatic islet cells	Neuroendocrine Pancreatic islet cells	Pancreatic islet beta cells
Intracellular location	Small vesicles (neuron-like)	Secretory vesicles	Secretory vesicles
Function	Convert glutamic acid to GABA, an inhibitory neurotransmitter	Enzymatically inactivates members of the TPP family	Ligand of the insulin receptor, regulation of blood glucose

GABA, gamma aminobutyric acid; IA-2, tyrosine phosphatase–like protein; TPP, tyrosine phosphatase proteins.

A study comparing measurement of GADA by RIA and ELISA concluded that both techniques were useful for the diagnosis of type 1 diabetes[4].

Antigens have different molecular weights and different intracellular locations (**Table 2**).

The sensitivity and specificity of type 1 diabetes autoantibodies measured by RIA and ELISA is greater when several are combined (**Table 3**). These autoantibodies are useful, except in new-onset type 1 diabetes, owing to the honeymoon effect. They are probably more useful in the patient's relatives.

Some classic studies showed that combining detection tests/assays increased the sensitivity and specificity compared with when each was performed separately[5]. The usefulness of these antibodies as predictors of

Table 3. Sensitivity and specificity of type 1 diabetes autoantibodies by RIA and ELISA.

Autoantibody	Sensitivity (%)	Specificity (%)
ICA	70-90	98
AIA	40-70	99
GAD65	70-80	99
IA-2	50-70	99
GAD65 + ICA	99	—
GAD65 + AIA	97	—
GAD65 + IA-2	97	—
IA-2 + ICA	93	—
ICA + AIA	92	—
IA-2 + AIA	73	—

IA-2, tyrosine phosphatase–like protein antibody; AIA, anti-insulin antibody; ICA, islet cell antibody.

type 1 diabetes increases with analysis over time, taking into account both the general population and the at-risk population[6]. The Diabetes Autoimmunity Study in the Young (DAISY) evaluated the predictive value of autoantibody positivity in progression of type 1 diabetes in a population of 21,713 persons. The authors found 162 positive cases for these autoantibodies in at-risk families and 50 positive cases in the general cohort. Of these, only 24 went on to develop diabetes[7]. Therefore, it was demonstrated that predisposing genetics or an isolated antibody does not necessarily lead the patient to develop the disease. Furthermore, over time, the titers of these antibodies tend to become negative owing to the fact that the beta cell is no longer a target for the immune system[8].

Other more recently discussed antibodies include the following:

CD38 antibodies

- CD38 antibodies have been found in 4%-20% of patients with type 1 diabetes and in 8%-19% of patients with type 2 diabetes.
- CD38 is a cell surface receptor and is considered a physiological mediator of insulin secretion.

Anti-ZnT8 antibodies

- The first findings were in db/db rats with a zinc-deficient diet that exacerbated their fasting hyperglycemia.
- Anti-ZnT8 antibodies are not associated with genetics, thus giving them greater validity.
- Zinc is mobilized within the insulin granule, ZnT8 is exposed, and the immune system activated to generate the anti-ZnT8 antibody[9].
- There is an association between ZnT8 and age at onset, although this becomes negative as the patient gets older[10].

The usefulness of these antibodies depends on the genetic risk and on the probability of an immune response (**Figure 2**). In general, and from an

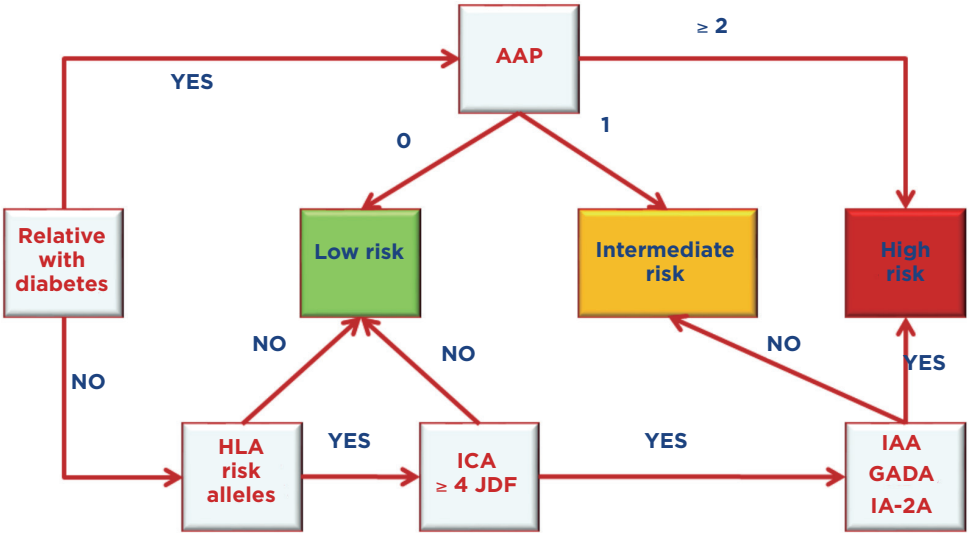

Figure 2. Algorithm for use of autoantibodies. GADA, glutamic acid decarboxylase; AIA, anti-insulin antibodies; ICA, island cell antibodies.

experimental perspective, the presence of risk alleles with a very positive ICA titer usually correlates with a positive pattern for AIA, GAD65, and IA-2, thus making it possible to predict disease in at-risk individuals.

In line with the previous chapter, a study in Finland, Sweden, and Norway showed that administering vitamin D supplements (5,000 units per day of 25 hydroxy-calcidiol) to at-risk families led to a decrease in the frequency of onset of diabetes. The study has been ongoing for 18 years, and results are published regularly.

RECOMMENDATIONS AND CONCLUSIONS

- Although the incidence of type 1 diabetes in Chile can be considered moderate, its cost must be taken into account before recommending screening.
- ELISA can be performed in the laboratory to determine the profile of GAD65, IA-2, and ZnT8.
- In the case of patients, this information might only be appropriate for those who are in the honeymoon phase. However, it is useful in relatives such as siblings of patients with onset of type 1 diabetes.
- In some countries, such as Finland, vitamin D is administered to persons with positive antibody titers in order to prevent type 1 diabetes.
- The risk of false positives and negatives can be reduced by combining antibodies, which can be selected from various panels.
- High-risk persons should undergo screening.

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IV SYMPOSIUM ON AUTOIMMUNITY
Present and future in the diagnosis
and treatment of autoimmune diseases

Rheumatoid arthritis: from diagnosis to personalized treatment

ANA MARÍA ORTEGA

MEDICAL DEPARTMENT, GRIFOLS, BARCELONA, SPAIN

RHEUMATOID ARTHRITIS: DEFINITION, EPIDEMIOLOGY, AND ETIOLOGY

Rheumatoid arthritis (RA) is a chronic, progressive, autoimmune, and disabling disease that is characterized by pain and inflammation of the joints. It usually affects the hands, wrists, elbows, knees, and feet. Patients report that it drastically affects their lives, which must be reconstructed from the time of diagnosis. Therefore, it is important to make an early diagnosis. The disease affects the hands and feet in 90% of cases. It progresses in the form flares with peaks of activity and remission periods. It is caused by the attack of immune cells on the synovial membrane, leading to inflammation and release of cytokines and proteolytic enzymes. Consequently, the patient feels pain, the joint becomes deformed, thus making it difficult to move. If the disease is not treated, it can lead to destruction of the cartilage and bone and even to irreversible malformations. Extra-articular symptoms may also appear and affect other parts of the body such as the skin, eyes, mouth, heart, and lungs.

RA affects 0.3%-1% of the population; therefore, between 20 and 70 million people throughout the world are thought to have the disease[1]. In Chile, the incidence is estimated to be 0.46%. According to the 2002 census, between 27,000 and 90,000 persons are affected[2]. The frequency of RA varies by sex and age, and the disease is 3 times more common in women, as is the case with most autoimmune diseases. Onset is usually between age 40 and 60 years, although the disease can also affect children and adolescents.

RA is a complex disease in which both genetic and nongenetic factors interact and combine to trigger onset. More than 60% of cases are caused by genetic factors, which predispose the patient to the disease. Therefore, having affected relatives increases the risk of developing the disease. Bacterial and viral infections can also favor onset of RA[3]. Lastly, exposure to toxic substances and smoking favors development of the disease in susceptible persons[4].

DIAGNOSIS OF RA

It is very important to make the diagnosis and administer treatment early, since the lesions affecting the joints may become irreversible. Therefore, it is necessary to have specific markers of inflammation and disease activity that can predict radiological progression (which will in turn determine the type and aggressiveness of the therapy to be administered), as well as markers for early monitoring to ensure that therapy is successful. The sooner treatment is started with disease-modifying antirheumatic drugs (DMARDs), the better the results will be.

Diagnosis is based on a review of the clinical history to confirm whether the patient has already experienced symmetrical involvement of multiple joints, inflammatory signs, morning stiffness, low-grade fever, or other nonspecific symptoms. A physical examination of the joints should then be performed

to evaluate the presence of inflammation, reddening, and heat. Lastly, the clinical history and the physical examination should be complemented with imaging and laboratory tests.

Imaging tests:
- Initial radiograph of hands, feet, and thorax
- Ultrasound
- Nuclear magnetic resonance

Laboratory/serology testing:
- Erythrocyte sedimentation rate (ESR)
- C-reactive protein (CRP)
- Rheumatoid factor (RF)
- Anticyclic citrullinated peptide antibodies/anticitrullinated protein antibodies (anti-CCP or ACPA)

In 2010, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) published classification criteria for RA based on a score index that evaluated whether a joint was affected by synovitis, the number of joints presenting it and degree of involvement, the size of the joints, serology (RF and ACPA), acute phase reactants (CRP and ESR), and time since onset of symptoms (more or less than 6 weeks)[5]. This new classification system was the first to include ACPA, thus enabling better identification of patients and earlier intervention. A score ≥6 indicates a positive diagnosis.

Therefore, the blood analysis should cover the following:

Inflammation parameters:
- ESR
- CRP

Values for these parameters increase quickly, although they are nonspecific and may be increased in the case of infection.

Presence of antibodies
- RF: antibodies against the fragment crystallizable region (Fc) of immunoglobulin G (IgG)
- ACPA

While the tests are useful for establishing a diagnosis, no single test can establish or rule out a diagnosis of RA[6].

RF is produced in response to IgG that has undergone conformational changes. The most common type is immunoglobulin M (IgM) against IgG. Its sensitivity for the diagnosis of RA is 65%-85%, and high concentrations are associated with more severe forms of joint disease. Its specificity is low owing to the fact that, although 80% of patients with RA present high concentrations in blood, levels can also be detected in other inflammatory or infectious disorders, and even in healthy persons[7].

Determination of ACPA facilitates early diagnosis, since these antibodies may be present 15 years before the appearance of the first symptom[6]. The sensitivity of ACPA is greater than that of RF for the diagnosis of RA (95%), as is its specificity (95%). Human filaggrin is the most widely used protein for determination of ACPA, and, since it was included in the classification criteria, several studies have been performed to determine differences between ACPA-positive and ACPA-negative patients. ACPA-positive patients experience greater disease activity, as well as more cardiovascular complications, greater rates of joint destruction, and greater mortality rates. In contrast, ACPA-negative patients have fewer cardiovascular

complications and lower rates of joint destruction and mortality[6]. Three studies were published in 2017. In one, Sirotti et al[8] concluded that ACPA-positive patients had a progressive and destructive form of RA at baseline. This criterion was a stronger independent predictor of radiological damage. The authors also recorded more inflammation and more extra-articular manifestations, as well as high rates of disability, cardiovascular disease, and premature death. Furthermore, the distinction between ACPA-positive and -negative patients could also help to select appropriate therapy, given that efficacy has been shown to be superior for biologics such as rituximab (anti-CD20), abatacept (T lymphocyte activation inhibitor), and other drugs such as methotrexate in ACPA-positive patients. Similarly, the study by Alivernini et al[9] showed that ACPA are specific for RA—even though not all patients have positive results for this biomarker—ACPA-positive patients have a high risk of developing RA, positive ACPA titers are associated with a severe erosive phenotype of RA with higher mortality, and ACPA status is associated with a favorable response to biologic drugs. Lastly, Lamerato et al[10] also concluded that ACPA-positive patients with RA had higher grades of inflammation and more marked disease activity, which translates into higher rates of joint erosion, increased medical care, and more specific treatment of RA.

According to the criteria of ACR and EULAR, determination of RF and ACPA is mandatory for the diagnosis of RA. However, given that they are not suitable for evaluating the success of treatment, other markers are necessary. As previously mentioned, RA involves inflammation of the synovial membrane, triggering of an invasion by immune cells, and release of cytokines and proteolytic enzymes, including metalloproteinase-3 (MMP-3). Without treatment, this process leads to erosion of the bone and cartilage of the affected joint (**Figure 1**).

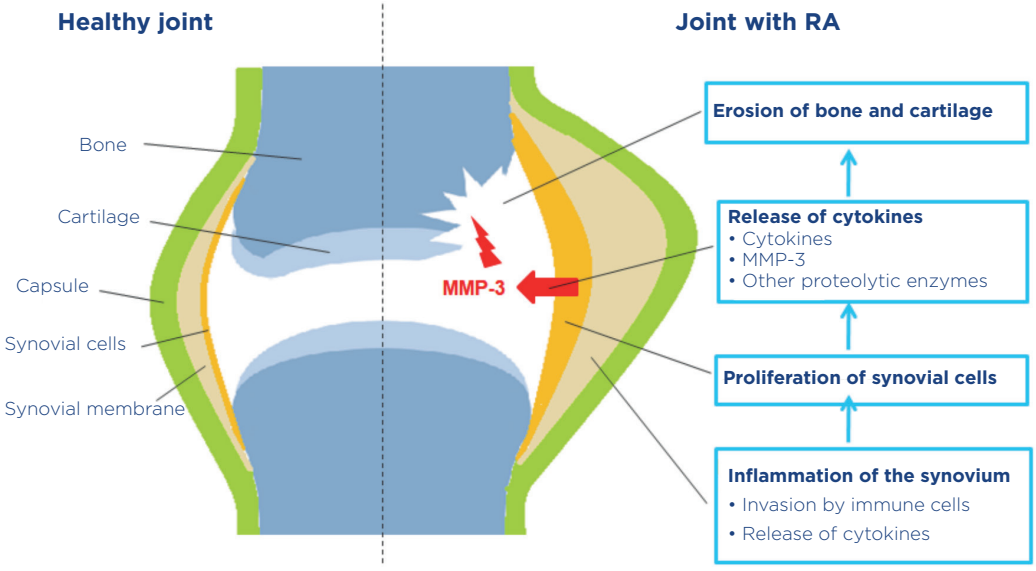

Figure 1. Inflammation, proliferation, and release of cytokines in rheumatoid arthritis (RA). Reproduced with the permission from AESKU. Diagnostics GmbH & Co. KG.
MMP-3, metalloproteinase-3.

The level of MMP-3 in synovial fluid is associated with the concentration of MMP-3 in serum, thus reflecting the inflammation process in the affected joints. Therefore, MMP-3 is a specific marker of disease activity, a prognostic marker of radiological progression, and a marker of the success of treatment, since its levels decrease after efficient therapy. Moreover, MMP-3 helps to identify RA patients who might benefit from early aggressive therapy. Galil et al[11] concluded that the baseline serum level of MMP-3 is a potent marker of disease activity and that it acts as an early predictor of progressive joint damage in RA. Determining MMP-3 values in serum reveals the risk of bone erosion and enables us to decide how aggressive initial therapy should be (**Figure 2**). After initiation of treatment, the MMP-3 level is re-evaluated to verify whether therapy has been successful.

TREATMENT OF RHEUMATOID ARTHRITIS

Currently available treatments do not cure the disease, although they do considerably relieve symptoms such as pain and minimize joint damage, thus improving physical function and helping to maintain quality of life. The objective is to achieve clinical remission or maintain the disease at a low level of activity. Drugs are the basic pillar of treatment, although patients are also recommended to develop good habits such as weight loss to reduce stress on joints. Surgery is only used when the pain is unbearable and the patient's mobility is considerably affected.

Currently available pharmacological options include the following:

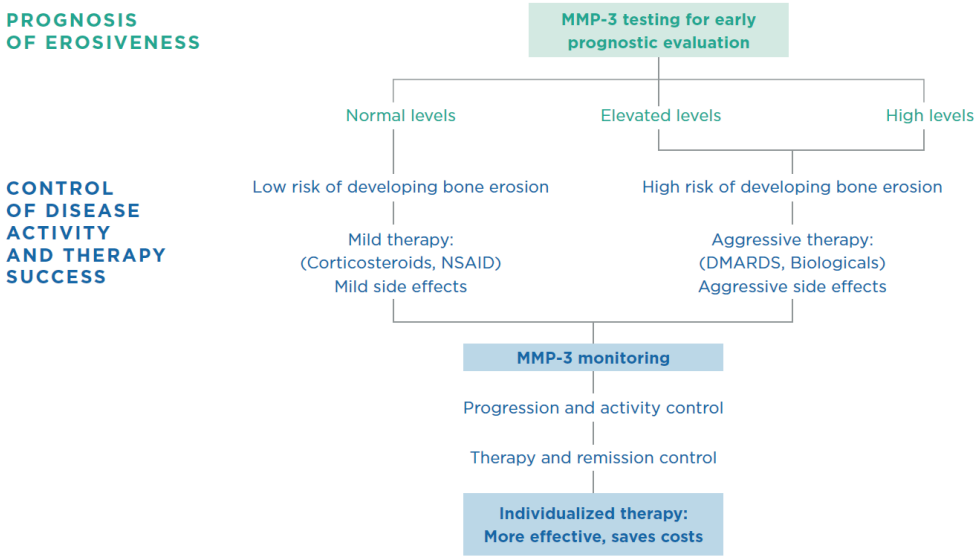

Figure 2. Algorithm for MMP-3. NSAID, nonsteroidal anti-inflammatory drug; DMARD, disease-modifying antirheumatic drug.

- Treatments for symptom relief:
  - Nonsteroidal anti-inflammatory drugs (NSAIDs)
    - Cyclo-oxygenase (COX) inhibitors 1 and 2
    - COX-2 inhibitors
  - Analgesics
  - Corticosteroids

- Treatments that prevent joint damage
  - DMARD: methotrexate
  - Biologics (bDMARD):
    - Biological response modifiers: tumor necrosis factor inhibitors (anti-TNF), antibodies against interleukin 6 (anti-IL6), antibodies against CD20 in B lymphocytes (anti-CD20).

The advent of the biologics anti-TNF, anti-IL6, and anti-CD20 has made it possible to control the disease in a high number of patients.

OPTIMIZATION OF PATIENT MANAGEMENT

The tools most widely used to evaluate the efficacy of treatment in patients with RA are the so-called activity indexes, of which there are several, such as the Simplified Disease Activity Index (SDAI), and a subsequent version, the Crohn Disease Activity Index (CDAI), which makes it possible to measure the grade of disease without the need to assess C-reactive protein levels, as is the case in the SDAI. We can also use the Health Assessment Questionnaire (HAQ), which is one of the main self-reporting instruments by which the patient provides data on his/her functional capacity. The instrument comes in abbreviated versions, such as the HAD-DI (disability index). The Routine Assessment of Patient Index 3 (RAPID 3) includes 3 self-reported measures: physical function, pain, and global evaluation of disease. However, the most widely used instrument is the Disease Activity Score 28, which comes in 2 versions: DAS28 (based on ESR) and DAS28-CRP (based on CRP). The more commonly used of the two is the DAS28-CRP, which analyzes 28 painful and inflamed joints, assesses inflammation based on CRP, and asks the patient about the course of the disease during the previous 7 days using a visual analog scale. The score ranges from 0 to 9.4: scores below 2.67 represent low disease activity, scores between 2.67 and 4.09 moderate activity, and scores above 4.09 high activity. At each visit, the results of the DAS28-CRP are compared with those of the previous visit to evaluate the response to treatment.

bDMARD constitute a therapeutic revolution in areas such as rheumatology, gastroenterology, and dermatology. The disadvantages of these drugs are their cost and adverse effects. Experience in their use has shown that the target of treatment is inappropriate in one-third of patients (primary failure). In addition, up to 60% of those who initially respond lose their response over time (secondary failure)[12]. Both types of failure have been attributed to various causes, such as inadequate serum concentrations of drug, differences in the mechanism of action, and the formation of antibodies to the drug (immunogenicity).

The fact that biologics are potentially immunogenic protein macromolecules could have negative implications for efficacy. Therefore, it is very important to monitor therapy in order to know the serum drug levels and antidrug antibody titers, as well as to anticipate and identify possible causes of failure of therapy and to manage patients in remission. This information helps with objective decision making on changes in treatment regimens.

Therefore, monitoring enables the following:

- Administration of personalized medicine
- Improved patient quality of life
- Maximum efficiency in the use of these drugs
- Cost savings

Monitoring is backed by many expert groups and associations both in the USA and in Europe. It can improve the treatment strategy and reduce the risk of inappropriate doses or drugs and the risk of adverse effects[13-16]. One example is the guidelines of the French Society for Rheumatology, which recommend follow-up of first-line treatment with anti-TNF agents (drug levels, antidrug antibody titers) in order to help select second-line biological treatment after the failure of the first line[17].

Various commercially available products can be used to monitor biologics. Some are services provided by laboratories, and others are kits that enable assessment to be performed in the hospital. The Promonitor® ELISA kit determines drug levels in blood and levels of free antibodies against the drug. The drug and antibody levels are measured after administration of a dose of the drug and immediately before the following dose. Various situations can arise (**Figure 3**). The clinical response (DAS28-CRP) may be good, with the drug in the therapeutic range and no antidrug antibodies. Therefore, the dose can be reduced or the frequency of administration increased. In the case of a poor clinical response, with the drug in the therapeutic range and no antidrug antibodies, we may be facing a primary failure, because the target may not be TNF, with the result that we should consider switching to another drug, such as anti-IL6 or anti-CD20. Subtherapeutic levels may also be detected, although with no antidrug antibodies, possibly because the drug is eliminated rapidly by the patient. Therefore, it will be necessary to increase the dose and the frequency of administration. If antibodies are present when the drug is detected as a foreign body, then we are faced with a secondary failure and must consider switching to another anti-TNF agent. If the antibody titer is very high, there may be a risk of infusion reaction.

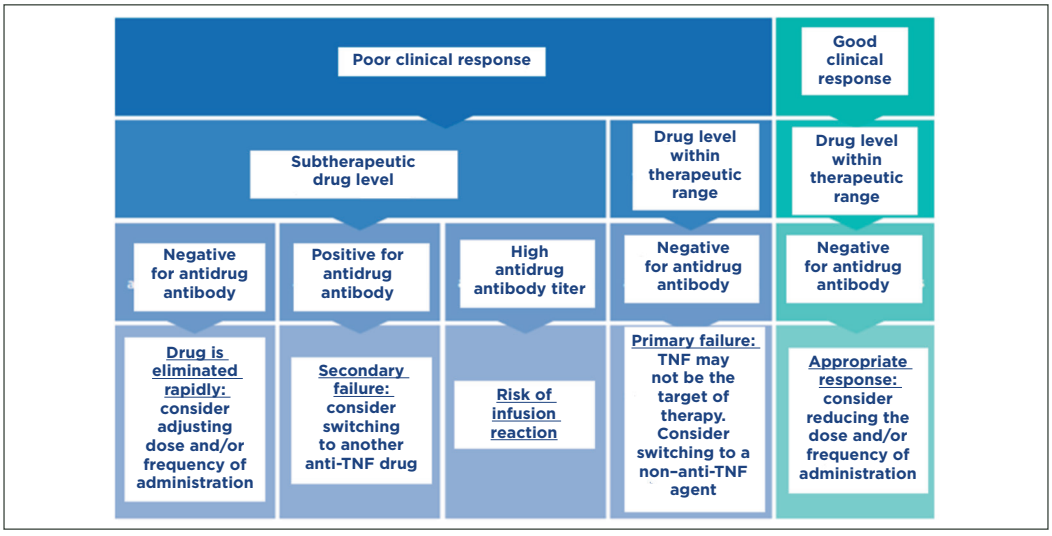

Figure 3. Patient management algorithm.

This strategy of analyzing drug levels and antidrug antibody titers can be carried out reactively or proactively. The proactive approach is used when the patient responds to treatment and involves 2 or 3 analyses per year to anticipate failure of response, thus enabling rational use of the drug, with dose adjustments, switches, and discontinuation. Reactive analysis, in contrast, is used when the patient does not respond to treatment, as in a primary failure or failure of maintenance; this is when drug levels and antidrug antibody titers are assessed.

The Promonitor® kit has received support in scientific articles and abstracts presented at international conferences. In February 2018, a total of 115 abstracts and 57 articles had been published. Of these, 30 were in rheumatology, 12 in analytical validation, 6 in dermatology, 5 in gastroenterology, 3 in pharmacoeconomics, and 1 in ophthalmology. We can detect the drug levels and antidrug antibody titers for infliximab, adalimumab, etanercept, rituximab, golimumab, vedolizumab, ustekinumab, and tocilizumab. The assays can be performed directly in the hospital laboratory or via the service provided by Grifols. In the future, it will be possible to use a simple test that can be performed in the doctor's office (Promonitor Quick®).

Both reference drugs and biosimilars can be quantitatively assessed[18].

RECOMMENDATIONS AND CONCLUSIONS

- Various tools can be used to make an early diagnosis of RA.
- Determination of RF and ACPA values plays a key role in identifying patients with RA.
- The marker MMP-3 helps to determine disease activity and to monitor treatment.
- The Promonitor test® makes it possible to follow up therapy with biologics.

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Edificio MAPFRE - Avenida de Burgos, 12
Planta 16, izquierda - 28036 Madrid
Tel: (+34) 913 453 308 - Fax: (+34) 913 430 672
admin@contentednet.com

IV SYMPOSIUM ON AUTOIMMUNITY
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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

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

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