SYMPOSIUM

VII WORKSHOP ON AUTOIMMUNITY

OTHER ANTIPHOSPHOLIPID ANTIBODIES AND THEIR ROLE IN ANTIPHOSPHOLIPID SYNDROME

ANTI-CD74 ANTIBODIES: BIOMARKERS FOR AXIAL SPONDYLOARTHRITIS

Grifols-sponsored symposium.
Wendelsheim, Germany.
November 22nd-23rd, 2017

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VII WORKSHOP
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CONTENTS

Other antiphospholipid antibodies and their role in antiphospholipid syndrome

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Guys' and St. Thomas' NHS Foundation Trust (GSTT) in London is a reference center for systemic autoimmune diseases. It was here that Professor Graham Hughes, the prestigious rheumatologist who reported on antiphospholipid syndrome (APS, also known as Hughes syndrome) worked until his retirement in 2005 and with whom the speaker had the opportunity of working for 8 years. GSTT is currently a major reference center for APS: it provides care for some 1600 patients with the disease and is an institution where immunologists carry out clinical work, as is usually the case in the UK.

Diagnosis of APS is based on specific tests that may be sophisticated or very rarely used and must therefore must be carried out in dedicated, highly reliable laboratories.

DEFINITION, DIAGNOSTIC CRITERIA, AND MANIFESTATIONS OF APS

APS is a clinical picture characterized by the following:

- **Venous and arterial thrombosis**. Venous and arterial thromboses are a unique characteristic of this syndrome, in contrast with hereditary thrombophilias, such as protein C or protein S deficiencies, which usually involve venous thrombosis only.

- **Complications of pregnancy or "obstetric antiphospholipid syndrome"**. These manifest mainly as early miscarriage (before week 10), fetal death (after week 10), and problems due to placental insufficiency—especially intrauterine growth restriction and preterm birth—and pre-eclampsia. Pre-eclampsia in particular is characterized by maternal proteinuria and hypertension, constitutes a severe condition, and, if labor is not induced, carries a risk of both maternal and fetal death.

Laboratory diagnosis of APS is currently based on the presence of 2 types of antiphospholipid antibodies (aPL). The first is anti-beta2-glycoprotein-1 (aB2GP1), which targets the main antigen in APS, and the second is anticardiolipin antibody (aCL). A third criterion for laboratory diagnosis is detection of the inappropriately named "lupus anticoagulant" (LA), which is not really an antibody, but leads to prolongation of certain coagulation times, in particular dilute Russell viper venom time (dRVVT) and activated partial thromboplastin time (aPTT), both of which indirectly reflect the presence of antibodies against membrane phospholipids, which play a key role throughout the coagulation cascade. The use of the term "lupus anticoagulant" (thus called because it was first detected in a patient with lupus) is somewhat unfortunate, since it does not refer to an "anticoagulant" and is not really associated with lupus. This could be confusing, in particular for specialists who are not well acquainted with this entity.

Other clinical manifestations of APS that are not included in the classification criteria but are

commonly observed in clinical practice include microangiopathy or small vessel thrombosis, the most relevant types being renal microangiopathy, cardiac microangiopathy (myocarditis), and cutaneous microangiopathy. In the case of renal microangiopathy, it may be necessary to take a biopsy specimen from the area affected in order to differentiate the condition from inflammatory glomerulonephritis, especially in patients with lupus. This diagnostic measure is very important, since each of these 2 conditions requires a completely different treatment: anticoagulants in the case of renal microangiopathy and immunosuppressants in inflammatory glomerulonephritis. Therefore, a diagnostic error could considerably worsen the prognosis of a patient with APS. Similar observations can be made for myocarditis, which may be confused with inflammatory myocarditis, although treatment is different. Lastly, in more severe cases, cutaneous microangiopathy may lead to prominent skin ulcers on the lower limbs, extensive cutaneous necrosis, and livedo reticularis.

Other relatively frequent laboratory and clinical manifestations of APS have been observed, although their etiology does not seem to be based on thrombotic mechanisms. The main laboratory abnormality is thrombocytopenia, which is usually moderate, around 100,000 platelets/μL; this can fall to < 20,000 platelets/μL in 5% of patients. Another such manifestation is hemolytic anemia, which is generally characterized by hemoglobin values of 9-10 g/dL, although these can fall to 4-5 g/dL in hemolytic crises. Other common manifestations include the following: arterial hypertension, even in young patients; involvement of the heart valves, mostly the mitral and aortic valves, owing to progressive thickening of the affected valve, although only 3% of patients eventually undergo a valve replacement; chronic headache, which patients generally describe as "persistent and very limiting"; epileptic seizures (in one study, 27% of children with epilepsy were found to have aPL [1]); chorea; and transverse myelitis.

Table 1 shows the clinical manifestations of APS that are not included in the classification criteria.

CRITERIA FOR THE DIAGNOSIS AND TREATMENT OF ANTIPHOSPHOLIPID SYNDROME

We have seen that the criteria for classifying APS include positive results for 2 aPL (aB2GP1 and aCL) and the presence of LA. It is important to remember

Table 1. Common clinical manifestations of APS that are not included in the established diagnostic criteria

— Renal, cardiac, and cutaneous microangiopathy
— Thrombocytopenia
— Hemolytic anemia
— Arterial hypertension
— Heart valve involvement
— Epileptic seizures
— Chorea
— Transverse myelitis

that in order to determine the presence of LA in patients anticoagulated with acenocoumarol or the new oral anticoagulants, we must use a specific test, the so-called Taipan snake venom time (TSVT) test, which, despite being the only reliable approach in these cases, is, regrettably, unavailable in most laboratories.

Treatment of APS is of 2 types: 1) **primary prophylaxis**, which is indicated for aPL-positive patients who have not yet experienced thrombotic episodes (eg, a woman with obstetric complications and a positive aPL antibody titer; and 2) **secondary prophylaxis**, which is administered to prevent new episodes of thrombosis in patients who have already experienced a thrombotic event or to prevent recurrent thrombosis.

TREATMENT OF THROMBOSIS

In the case of a patient who does not fulfill the criteria for classification of APS because he/she has low or intermittently positive aPL titers, thrombosis can be treated as in the general population. On the other hand, in the case of a patient with definitive APS (persistently positive moderate or high aPL titers) and venous thrombosis, anticoagulation therapy (acenocoumarol or warfarin) should be administered until an international normalized ratio (INR) of 2.0 to 3.0 is achieved. The new, direct oral anticoagulants can be used in patients experiencing their first episode of venous thrombosis at 20 mg/d for rivaroxaban or 5 mg/d for apixaban. If an APS patient with these characteristics experiences arterial thrombosis, then we must consider more intense anticoagulation, which could necessitate an INR of 3.0 to 4.0 or the administration of vitamin K antagonists (with an INR of 2.0 to 3.0) combined with low-dose acetylsalicylic acid (75-150 mg/d). However, such extreme approaches remain open to debate. These recommendations are based on a systematic review in which it was seen that the risk of a new episode of arterial thrombosis in these patients was higher than the risk of bleeding [2]. Furthermore, it was seen that when the INR is maintained at around 3.0, the risk of recurrence of thrombosis is quite low [2].

SERONEGATIVE ANTIPHOSPHOLIPID SYNDROME

The recently coined term "seronegative antiphospholipid syndrome" refers to those patients whose symptoms are suggestive of APS but whose aPL titer is negative. This term can lead to error if it is not applied rigorously. A meticulous diagnosis of seronegative APS includes the following criteria: 1) recurrent thrombosis and/or gestational morbidity, especially in the second and third trimesters; 2) absence of other identifiable diseases; 3) other clinical manifestations of APS that do not form part of the diagnostic criteria (see Table 1); and 4) negative titers for the 2 conventional aPL for diagnosis of APS and negative LA titers (**Table 2**).

In practice, the most relevant clinical manifestation for the diagnosis of seronegative APS is recurrent fetal death during the second and third trimesters, since spontaneous abortions may be due to various causes (anatomic or hereditary). In the case of a history of recurrent thrombosis, it is necessary to rule out any other type of thrombophilia in order to confirm a diagnosis of seronegative APS. Otherwise, the presence of manifestations not included in the diagnostic criteria, eg, thrombocytopenia or mitral valve thickening, helps to confirm a diagnosis of seronegative APS. Lastly, a diagnosis of seronegative APS could be due to the fact that the patient is positive for other aPL that are not routinely analyzed because

Table 2. Criteria for the diagnosis of seronegative antiphospholipid syndrome.

— Recurrent thrombosis and/or complications during pregnancy
— Absence of other identifiable diseases
— Presence of manifestations of APS not included in the diagnostic criteria for APS
— Negative aPL and LA titers and positive aPL titers that do not form part of the standard diagnostic criteria

they have been reported more recently and the laboratory techniques used to detect them have not yet been standardized. However, despite these obstacles, detection of "emerging" aPL (ie, those not included in the criteria for classification of APS) is essential in patients suspected of having seronegative APS, since both confirmation of diagnosis and administration of appropriate treatment depend on it. The criteria for classification of APS could be revised in the near future. It may be possible to include other aPL—the topic of this talk—for which there is sufficient evidence of their involvement in the pathogenesis of thrombosis and/or obstetric morbidity.

ANTIPHOSPHOLIPID ANTIBODIES FOUND IN SERONEGATIVE ANTIPHOSPHOLIPID SYNDROME

Table 3 shows other aPL whose potential association with the main symptoms of APS has been investigated. These include antiphosphatidylserine antibodies (aPS), antiprothrombin antibodies (aPT), anti-aPS/prothrombin complex antibodies (aPS/PT), IgA anti-B2GP1 antibodies (lgA aB2GP1), and anti–domain 1 B2GP1 antibodies (aB2GP1-D1).

ANTIPHOSPHATIDYLSERINE ANTIBODIES

A review of the literature to date reveals that the only statistically significant evidence of an association between aPS antibodies and APS is that reported by Khogeer et al [3] in 2015. The authors analyzed a cohort of 212 patients with thrombotic and/or obstetric complications by comparing various parameters for aPS with those of other aPL and found a specificity of 87% for aPS, with 70% of aPS-positive patients confirmed as having APS. Nevertheless, it must be said that many of the patients studied were also positive for aCL and/or anti-B2GP1.

In 2016, another interesting finding was reported by Mekinian et al [4], whose study included patients with obstetric complications, 65 patients with aPL that were not part of the classification criteria, 83 with definitive APS, and 31 healthy controls. aPS were present in 73% of patients, who were also positive for conventional aPL, compared with 88% in patients with negative conventional aPL titers. While this difference was not statistically significant, it does suggest that, in practical terms, the presence of aPS could help in the diagnosis of seronegative APS.

ANTIPROTHROMBIN AND ANTIPHOSPHATIDYLSERINE/PROTHROMBIN COMPLEX ANTIBODIES

aPT and aPS/PT are detected using enzyme-linked immunosorbent assay (ELISA). Although aPT and aPS/PT are both well-differentiated antibody populations, they can coexist and, within aPL, are the antibodies that have been most associated with positive

Table 3. Antiphospholipid antibodies found in seronegative antiphospholipid syndrome.

— Antiphosphatidylserine (aPS)
— Antiprothrombin (aPT)
— Anti–aPS/prothrombin complex (aPS/PT)
— IgA anti-beta2-glycoprotein 1 (IgA aB2GP1)
— Anti–domain 1 of beta2-glycoprotein 1 (aB2GP1-D1)

LA titers. In this sense, the more important of the two is aPS/PT.

A systematic review carried out at GSTT [5] examined 38 studies on aPT and 10 studies on aPS/PT, with a total of 7000 patients (APS and controls). The authors specifically analyzed the risk of thrombosis associated with each of the antibodies and found that both types were associated with a significant increase in the risk of arterial and venous thrombosis, although this risk was 5 times higher in the case of aPS/PT than in aPS (*odds ratio* [OR] 5.11, 95% confidence interval [CI] 4.2-6.3 vs 1.82, 95% CI, 1.44-2.75, respectively). Despite this evidence, tests to detect and evaluate these 2 types of antibody are not currently requested.

A study carried out at 8 centers in 7 countries [6] analyzed serum from 247 patients in order to evaluate the association between the presence of the IgG isotype of aPS/PT (aPS/ PT IgG) and APS revealed that this parameter was positive in 51% of patients diagnosed with APS and also in 9% of those who were not diagnosed with APS (patients with "seronegative APS"), with a sensitivity of 51% and a specificity of 91%.

Finally, in a very recent study [7] involving 157 patients (51 with APS and 106 with other autoimmune diseases), the authors concluded that the combination of aB2GPI and IgG/IgM anti-PS/PT antibody tests have a high positive predictive value for the diagnosis of APS and a strong correlation with the aPL score. This combination could be a firstline approach in clinical practice for simple and definitive identification of patients with APS and a high risk of thrombosis.

The results of these studies enable us to conclude that determination of lgG aPS/PT could considerably facilitate diagnosis of APS and might even be included as a laboratory parameter in a future revision of the diagnostic criteria for APS.

LGA ANTI-BETA2-GP1 ANTIBODIES

Detection of the IgG and IgM isotypes of aB2GP1 is routinely requested for confirmation of the diagnosis of APS. However, recently, and in light of the number of cases of seronegative APS, detection of lgA aB2GP1 is also starting to be requested.

A study carried out at GSTT [8] compared the findings for serum samples from 3 groups: 40 patients with APS and positive titers for the 3 types of conventional antibodies; 40 patients with seronegative APS; and 200 healthy controls. Nine specific ELISAs were used to determine the IgG, IgM, and IgA isotypes of aCL, aB2GP1, and aB2GP1-D1, with the cutoff point for positivity for each assay being the 99th percentile in the healthy population. One of the key results obtained was the fact that a positive titer was detected

for at least 1 of the aPL that did not form part of the classification criteria analyzed, that is, in 62.5% of the serum samples from the first group and—even more relevant—in 10% of the serum samples from the APS-negative group, with a quarter (2.5%) corresponding to IgA aB2GP1.

In a study carried out by a Spanish group whose results were published in 2017 [9], the objective was to evaluate a positive lgA aB2GP1 titer as a factor favoring the development of clinical events (thrombosis and gestational complications) in asymptomatic APS patients. Two groups of APS patients were followed up for 5 years: one group (n = 244) had positive titers for lgA aB2GP1 and negative titers for aCL (both lgG and lgM), whereas the other (n = 221) had negative titers for all 3 types of antibodies. At the end of follow-up, 45 patients (9.7%) had developed clinical manifestations of APS (38 in the lgA aB2GP1–positive group [15.6%] vs 7 in the group for which all 3 types were negative [3.2%]). The difference was statistically significant ($p < 0.001$). In the aB2GP1 lgA–positive group, the rate of APS-related events was 3.1% per year (similar to that reported for conventional aPL), compared with a rate of 0.6% per year in the control group. The most common event was arterial thrombosis (25 patients, 55% of the total events observed), which particularly affected the lgA aB2GP1–positive group (21 patients vs 4 in the control group, $p = 0.001$). The authors concluded that a positive lgA aB2GP1 titer was the main independent risk factor for clinical events in patients with asymptomatic APS, especially arterial thrombosis, and that determining this titer could identify up to 8% of APS patients with negative titers for the conventional aPL. This finding is relevant, because arterial thrombosis is more devastating than venous thrombosis: it can lead to cerebrovascular accidents, myocardial infarction, and peripheral artery disease, with potentially very limiting sequelae. Furthermore, while not the main objective of the study, the authors found that older age, male sex, and a positive aB2GP1-D1 titer were risk factors for these events.

ANTI-BETA2-GP1 DOMAIN 1 ANTIBODIES AND OTHER EMERGING ANTIPHOSPHOLIPID ANTIBODIES

B2GPI comprises 5 domains. In the case of a conformational change, domain 1 (D1), to which aB2GP1 bind, is more exposed. The antibodies that act against D1 of B2GP1 have been identified (aB2GP1-D1), and specific assays have been applied to detect their IgA, IgG, and IgM isotypes, as in the study by Pericleous et al [10] in 2016. The authors concluded that evaluation of anti-DI IgG and IgA could prove useful when determining the thrombotic risk of patients with APS or who carry aPL. However, this evaluation does not identify new patients, since all those who have positive aB2GP1-D1 titers also have positive titers for conventional aB2GP1.

Zohoury et al [11] recently reported the results of an interesting study whose objective was to describe the profile of nonconventional aPL that might be used in the diagnosis of what is now known as seronegative APS. The authors used 11 nonstandardized tests in 68 patients with seronegative APS (negative titers for conventional aPL in 2 cases), and with only 4 of these tests, were able to obtain a cumulative 30.9% of new diagnoses. The tests were measurement of IgG or IgM isotypes of antiphosphatidylethanolamine (aPE) and the IgG isotype of anticardiolipin/vimentin antibodies (aCL/Vim), both of which are new candidate aPL for the diagnosis of APS, and of the abovementioned IgG and IgM isotypes of aPS/PT and aPS antibodies. Combining these tests with

those used to evaluate IgA aB2GP1 could, in theory, lead to the diagnosis of approximately 37% more cases.

GLOBAL SCALE FOR THE EVALUATION OF THROMBOTIC RISK IN ANTIPHOSPHOLIPID SYNDROME AND ANTIPHOSPHOLIPID ANTIBODY PROFILE

In 2015, Sciascia et al [12] reported the results of a study whose objective was to evaluate the clinical relevance (especially in relation to the risk of thrombosis) of the Global Anti-Phospholipid Syndrome Score (GAPSS). The scale was originally developed by Atsumi et al [13], although it did not include the cardiovascular risk factors that favor thrombosis (eg, smoking, obesity, arterial hypertension). In contrast, the scale developed by Sciasia et al includes not only these risk factors, but also the patient's antibody profile, since prognosis of APS is affected by the aPL titer and the type of aPL. Thus, it has been demonstrated that, for example, prognosis is worse with a positive titer for aB2GP1, aCL, and LA (triple positive) than with a positive titer for only 1 of these antibodies. Similarly, as mentioned above, positive titers for LA are associated with a greater risk of thrombosis. The most likely antigen for LA is aPS/PT complex. The current GAPSS, which has been validated, calculates the risk of thrombosis based on 6 parameters: positive titers for aCL (IgG/IgM), 5 points; positive titers for aB2GP1 (IgG/IgM), 4 points; positive titers for aPS/PT (IgG or IgM), 3 points; positive titers for LA, 4 points; hyperlipidemia, 3 points; and arterial hypertension, 1 point.

It is noteworthy that, thanks to the process for validation of the GAPSS, the score on the scale is greater in patients with thrombotic complications than in patients with obstetric complications and that it is also greater in patients with arterial thrombosis than in patients with venous thrombosis. Similarly, it is higher in patients with recurrent APS and in patients with positive lgG aPS/PT titers, which are associated with a greater risk of thrombosis. Of note, a GAPSS score greater than 16 points has a poorer prognostic value in thrombotic APS [12].

RECOMMENDATIONS AND CONCLUSIONS

- Additional screening of patients with seronegative APS should be based on tests for lgA aCL, IgA aB2GP1, lgG aPS/PT, and lgM aPS/PT.
- In the near future, the analytical diagnostic criteria for the classification of APS could include assessment of lgG and lgM aPS/PT, since these are closely associated with thrombosis.
- The clinical relevance of aPS/PT is much greater than that of aPE and aPT alone.
- dRVVT and aPTT are determined to detect positive LA titers. Treatment with oral vitamin K antagonists modifies these coagulation times. Therefore, in anticoagulated patients, TSVT should be determined to detect the presence of LA. However, given that this test is performed in very few laboratories, aPS/PT (associated with positive LA titers in 88.7% of cases) should be measured in these patients to avoid interrupting anticoagulation.
- Patients with seronegative APS should be tested for aPL not included in the classification criteria for APS.

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VII WORKSHOP
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Anti-CD74 antibodies: biomarkers of axial spondyloarthritis

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INTRODUCTION

Spondyloarthropathy, or spondyloarthritis (SpA), constitutes a group of disorders characterized by chronic inflammation of the joints of the spinal column and other joints, as well as by chronic inflammation of specific extra-articular structures. The prevalence of SpA in the general population is estimated to be 0.45-1.8% [1-4]. While the underlying cause of the inflammatory process responsible for SpA remains unclear, the disorder is thought to arise from an interaction between the intestinal microbiome and an aggressive host immune system [5].

The concept of axial SpA (axSpA) was presented some years ago [6]. axSpA includes disorders that mainly affect the axial joints (spondylitis, sacroiliitis), generally in association with inflammation of the tendons (enthesitis) and uvea (uveitis), as well as with lesions affecting the heart, lungs, and other organs. The most common form of axSpA is ankylosing spondylitis (AS), which is characterized by the formation of bony bridges in the joints of the spinal column and progressive fusion of the vertebrae leading to ankylosis, although this is not the case in all patients. AS also involves a loss of bone mass leading to dysfunction of the spinal column and the emergence of typical deformations (hypokyphosis, hypolordosis).

Diagnosis of axSpA usually takes a mean of 7-10 years after onset of symptoms, because one of its basic manifestations, back pain, is very common in the general population [7,8]. Therefore, early diagnosis of ax-SpA is challenging, and very few diagnostic tools are available to meet the challenge. Diagnosis of axSpA is usually based on the genetic marker human leukocyte antigen (HLA) B27, although determination of the marker is only useful in some European and Asian countries and the in USA, where it has been shown to have 70-90% sensitivity and variable specificity (the marker is present in up to 10% of healthy subjects) [9]. Diagnosis of axSpA is also based on imaging studies, specifically radiography and magnetic resonance imaging (MRI). In the case of radiography, the characteristic changes that reflect the disease process, eg, progressive joint space narrowing and fusion of the vertebrae or sacroiliac joints, can be observed after a mean of 3 years in men and more than 10 years in women. Until some time ago, MRI was thought to be a specific tool for the early diagnosis of axSpA. However, over the years, it was seen not only to be more expensive than other diagnostic tools, but also to have a specificity that depends to a large extent on the evaluator's experience [10].

In the year 2009, the *Assessment of SpondyloArthritis international Society* (ASAS) published a review that established classification criteria for axSpA in patients aged < 45 years at the onset of symptoms who had been experiencing back pain for more than 3 months [6]. These criteria began to be used as diagnostic criteria in clinical practice. According to the review (**Figure 1**), in patients with the above-mentioned characteristics and MRI scans or radiographs compatible with axSpA, that is, images

showing acute active inflammation suggestive of sacroiliitis associated with axSpA or radiographic images of sacroiliitis according to the modified New York criteria, the diagnosis of axSpA is confirmed based on at least 1 of the following clinical criteria: inflammatory back pain, joint disease, enthesitis (heel), uveitis, dactylitis, psoriasis, Crohn disease/ulcerative colitis, good response to nonsteroidal anti-inflammatory drugs, family history of axSpA, positive HLA-B27 titer, and high C-reactive protein value. In contrast, in patients with the same characteristics and a positive HLA-B27 titer, the presence of at least 2 of these clinical criteria is considered necessary.

In 2 subsequent studies, one in Belgium [11] and the other in the Netherlands [12] (*SPondyloArthritis Caught Early* [SPACE] cohort), which aimed to generate proposals on the diagnosis of axSpA with sufficient sensitivity and specificity, the criteria mentioned were combined in different ways, and new criteria were included. However, despite the clearly favorable contribution of these initiatives, the objective sought was not reached. This ambiguity in the diagnostic process led the presenter of this talk and a group of colleagues to identify specific antibodies that could be used as biomarkers to ensure an early diagnosis of axSpA [13].

AUTOANTIBODIES AGAINST CD74

With the aim of identifying new biomarkers of the disease, a protein array covered with 37,830 antigens expressed in *Escherichia coli* was used against serum samples from 5 patients with radiographic axSpA and 55 controls with other diseases or healthy blood donors [13]. After various steps, the most frequently identified antigen using this screening tool was the

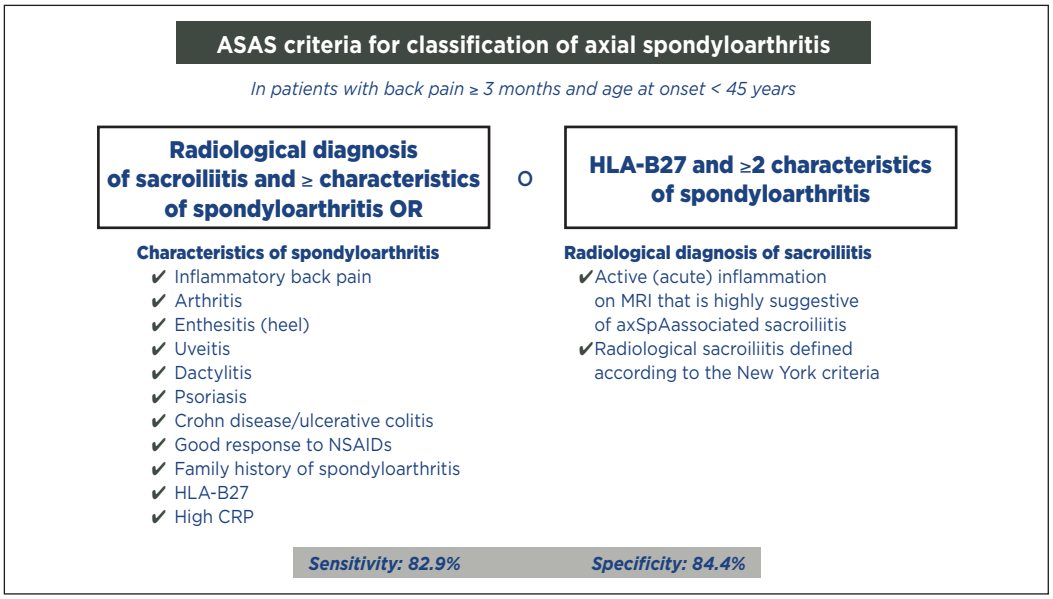

Figure 1. ASAS criteria for the classification of axial spondyloarthritis [6].
axSpA, axial spondyloarthritis; CRP, C-reactive protein; HLA, human leukocyte antigen; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug.

protein CD74 (cluster of differentiation 74), which was detected in 4 of the 5 samples from patients with axSpA and in only 1 of the 55 control samples.

CD74 acts as a receptor of macrophage migration inhibitory factor (MIF) in cells expressing the major histocompatibility complex (MHC) class II. Also known as the invariant chain of MHC class II molecules, CD74 protects immature MHC class II against nonspecific binding. The CD74 receptor forms a complex with the CD44 molecule, and both proteins must be present for MIF to carry out its functions, including modulation of expression and secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNFa) and modulation of the activity of inhibitors of cyclooxygenase 2 (a target of NSAIDs) or their role in apoptosis [14]. The action of CD74 on the surface of these cells is very short in duration, and its presence has also been detected on the surface of many other cells, including many types of tumor cells. Lastly, research published in 2010 [15] showed that the serum concentration of MIF was significantly higher in patients with axSpA (especially those with AS) than in healthy subjects (12.2 ± 7.7 ng/l vs 7.5 ± 3.7 ng/l, respectively; $p < 0.01$); therefore, the authors postulated that the presence of MIF could be associated with the inflammatory activity of axSpA, although this extreme hypothesis has not been proven to date.

In order to confirm the results obtained by screening with the protein array, the following phase of the study involved the evaluation of 117 patients with a 10 to 52–year history of axSpA [13]. The primary objective was to evaluate the frequency of positive results for detection of anti-CD74 antibodies using tests developed by the investigators themselves. Another objective of the study was to find correlations between the various candidate biomarkers and factors such as treatment, disease activity, radiographic changes, and mobility scale scores.

In the first evaluation, anti-CD74 antibodies were detected using enzyme-linked immunosorbent assay (ELISA) with IgG and IgA. The positive values found were similar with both types of immunoglobulin: around 48% with IgG and 56% with IgA (**Figure 2**).

A year after the study began, a retrospective analysis of cohort data revealed more marked progression of sacroiliitis in the modified Stoke Ankylosing Spondylitis Spine Score (SASSSm) in patients with a positive IgA anti-CD74 titer than in patients with negative titers. According to the SASSSm, 33 of 66 IgA-positive patients (50%) had reached grade 4 sacroiliitis compared with 13 of 51 IgA-negative patients (25%). This finding could point to a correlation between positive IgA-type anti-CD74 antibody titers and radiological disease progression. However, it is noteworthy that the association between a positive IgA titer and progression of sacroiliitis also correlated with treatment based on TNFa inhibitors, which are restricted to patients with more severe disease, thus potentially acting as a confounder. Consequently, this correlation cannot yet be considered definitive. Nevertheless, it must be stressed that the association between the presence of IgA-type anti-CD74 antibodies and disease progression according to the SASSSm is consistent with the results reported in the *Effects of Non-Steroidal Anti-lnflammatory Drugs on RAdiographic Damage in Ankylosing Spondylitis* (ENRADAS) study [16]. Consequently, future research is warranted.

THE "INTER SPA" STUDY

Full data have not yet been published for the international multicenter study Inter SpA (*Sensitivity*

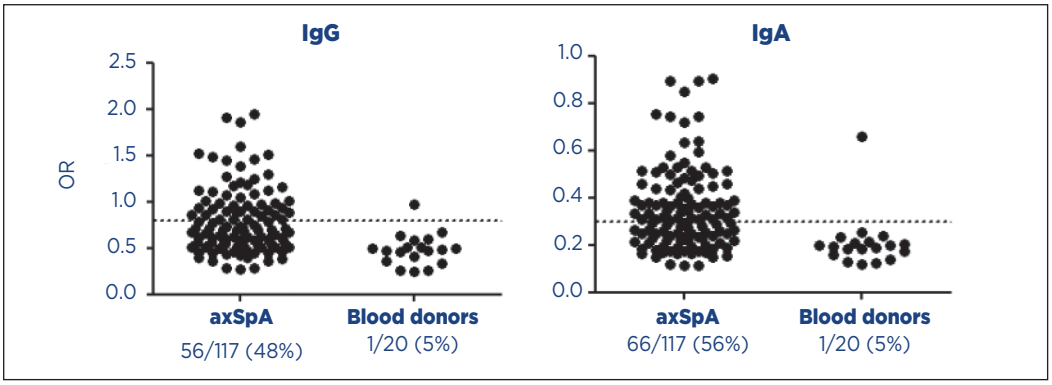

Figure 2. Evaluation of anti-CD74 antibodies in patients diagnosed with axial spondyloarthritis of more then 10 years' duration. Personal report from the speaker, together with Maria Köler and Joaquim Georgi, Kellos Ostseeklinik Damp, Germany.
asSpA, axial spondyloarthritis; OR, odds ratio.

and Specificity of Autoantibodies against CD74 in Early Axial Spondyloarthritis), although preliminary results are available [17]. The main objective of the study was to compare the sensitivity and specificity of anti-CD74 antibodies and HLA-B27 in patients with a < 2-year history of axSpA. The secondary objectives were to determine whether the presence of anti-CD74 antibodies, either alone or in combination with HLA-B27, could replace MRI of the sacroiliac joints as a diagnostic criterion and to verify whether the results obtained correlated with the activity and clinical characteristics of the disease.

Participants were selected from patients with a < 2-year history of low back pain and an indication for MRI and HLA-B27 testing. The initial proposal was to recruit a minimum of 100 patients, although 124 had been included at the time of this presentation. The study involved MRI of the sacroiliac joints and testing for anti-CD74 antibodies and HLA-B27. The results were compared with those of 100 blood donors.

Table 1 shows the demographic characteristics of the patients and the preliminary results. More than two-thirds of the patients recruited had MRI evidence of sacroiliitis and/or were HLA-B27–positive. The results of testing for IgG-type anti-CD74 antibodies were not very conclusive, with positive titers in approximately 24.5% of patients with a pathological MRI scan, 23% of patients who met the ASAS criteria, and 5% of blood donors. In patients with evidence of disease on their MRI scan, the likelihood ratio was 4.9 for IgG-type anti-CD74 antibodies; this increased to 9.4 for HLA-B27. In contrast, findings for IgA-type anti-CD74 antibodies were robust: positive results were detected in 65% of patients with evidence of sacroiliitis on their MRI scan or who fulfilled the ASAS criteria and in only 3% of blood donors. Furthermore, the likelihood ratio for IgA-type antibodies was 21.5, which was much higher than that of HLA-B27.

While preliminary, these results strongly suggest that joint measurement of IgG- and IgA-type anti-CD74 antibodies could increase sensitivity and specificity in the early diagnosis of axSpA. These results are even more interesting if we take into account the likelihood ratios reported by Rudwaleit et al [18] in 2005 for the tools used to diagnose axSpA at the time (**Table 2**).

Table 1. Demographic characteristics and preliminary findings from patients in the InterSpA study [17].

Item	Results (n = 124)
Mean age	29 years
Mean time with inflammatory back pain	12.6 months
Sex	70 men, 54 women
Sacroiliitis on MRI (results from local and independent expert evaluators)	67.7 %
Presence of HLA-B27	69.3 %
Sensitivity for: sacroiliitis/ASAS criteria/control group (%) – IgA anti-CD74 – IgG anti-CD74 – HLA-B27	 64.6/65.4/3.0 24.4/23.1/5.0 75.0/80.7/8.0
Likelihood ratio taking into account disease criteria according to MRI/ASAS – IgA anti-CD74 – IgG anti-CD74 – HLA-B27	 21.5/21.8 4.9/4.6 9.4/10.1

ASAS, Assessment of SpondyloArthritis international Society; HLA, human leukocyte antigen; MRI, magnetic resonance imaging.

Table 2. Likelihood ratio for various tools used in the diagnosis of axSpA in 2005, according to Rudalweit et al [18].

Data published	Likelihood ratio
Increased acute phase reactant values	2.5
Inflammatory back pain	3.1
Heel pain (enthesitis)	3.4
Peripheral arthritis	4.0
Dactylitis	4.5
Positive family history	6.4
Acute anterior uveitis	7.3
HLA-B27–positive	9.0
Positive MRI criteria	9.0
Grade 3 sacroiliitis	20.0

CONCLUDING REMARKS

Given that the tools currently used for the evaluation of axSpA are limited to MRI and determination of HLA-B27, it is of considerable interest to have other markers that can predict disease progression and thus enable us to modify treatment. Anti-CD74 antibodies could fulfill such a role. Prospective studies should be performed to enable us to understand whether positive anti-CD74 titers could predict the appropriateness

of TNFa inhibitors, whether their presence indicates greater radiological disease progression, or whether periodic assessment would show us when concentrations had stabilized. Other issues that should be raised include whether or not positive titers for these antibodies are associated with other manifestations of axSpA, such as psoriasis, uveitis, and colitis, or with specific habits, such as smoking or special diets.

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