Review

Systemic vasculitides: a critical digest of the most recent literature

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ABSTRACT

Herewith we provide our annual digest of the recent literature on systemic vasculitides. In this manuscript, we reviewed all the articles published during the last 12 months on large-, medium- and small-vessel vasculitis and selected the most relevant studies regarding the epidemiology, pathogenesis and management of systemic vasculitis. In particular, we focused the attention on giant cell arteritis, ANCA-associated vasculitis and cryoglobulinaemia.

Introduction

We hereby provide our annual digest of the recent literature on systemic vasculitides, which we made by performing a Medline search of English language articles published in the PubMed database from January 2013 to date. The following key words: *vasculitis*, *giant cell arteritis*, *Takayasu arteritis*, *antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis*, *microscopic polyangiitis*, *granulomatosis with polyangiitis* (formerly *Wegener's*), *eosinophilic granulomatosis with polyangiitis* (formerly *Churg-Strauss*) and *HCV-related cryoglobulinaemia* formed the data sources. We reviewed all the articles and selected the most relevant manuscripts with regard to classification, epidemiology, pathogenesis and management of systemic vasculitis.

Systemic vasculitis are a heterogeneous group of diseases with a clinical picture that may vary from mild disorders to multisystem life-threatening conditions. In the last few months, a number of steps forward have been made in the understanding of pathogenesis, clinical picture, treatment and outcome.

Following the previously published paper (1), this review will provide an annual update on the significant original contributions in the field of vasculitis.

Novelties in large-vessel vasculitis

Pathogenesis

The aetiopathogenesis of large-vessel vasculitis (LVV) still remains unclear. These diseases seem to be caused by the interaction of genetic, environmental and immune factors; indeed it is believed that LVV develop in a genetic predisposed subject from an exaggerated immune response against a not already identified infectious antigen. Both humoral and cellular immune systems have been implicated in the pathogenesis of giant cell arteritis (GCA). GCA has long been considered as a Th1-mediated disease characterised by granuloma formation in the wall of affected arteries and a high level of INF-γ; however, in GCA lesions we can also find Th17 cells which secrete IL-17, a pro-inflammatory cytokine involved in many autoimmune diseases. Recent studies have confirmed the implication of both Th1 and Th17 lymphocytes in the pathogenesis of GCA with different roles in the vasculitic process. At the beginning of the disease, IL-6 drives the differentiation of T cells into Th17 and, at the same time, it reduces the induction of anti-inflammatory T regulatory (Treg) cells. In GCA patients, Th17 cells are increased not only in blood but also in the wall of the affected arteries, while Treg cells are decreased. Corticosteroids suppress IL-6, IL-17 and IL-23 production, but vasculitis could persist as a Th1-dependent disease characterised by high serum level of IL-12 and INF-γ and a higher resistance to steroids (2). Another recent study shows that the homeostasis of B cells is highly disturbed in patients with a new diagnosis of GCA and/or polymyalgia rheumatica (PMR). The presence of high levels of autoantibodies in the serum of patients with GCA and PMR suggested the idea that there could be a B-cell activation in

these patients. The number of B cells is decreased during active disease and in particular it seems that effector B cells (Beff) are redistributed in a still unidentified site during the active disease and return to normal values during steroid induced remission. B cells in GCA and PMR produce not only autoantibodies, but also cytokines such as IL-6 contributing to the development of Th17 cells which have a pathogenetic role in GCA and PMR. Since B cells seem to take part in the development of GCA, it would be very interesting to understand whether B-cell depletion therapy could be useful in controlling disease activity (3). In the last years, many researchers have focused on the study of the genetic background in GCA, and several genes involved in the genetic predisposition to this vasculitis have been identified. Assuming that Th17 cells have a crucial role in the inflammatory process at the basis of GCA, Marquez *et al*. tried to understand if polymorphisms at IL-17A gene could confer the risk of developing GCA. They analysed five single-nucleotide polymorphisms (SNPs) and showed that 3 of these (rs4711998, rs2275913, rs7747909) were linked with GCA development. Rs2275913 is the SNP most strongly associated with GCA; it is located in the IL-17 promoter and causes an increase in IL-17 secretion (4). IL-33 is another cytokine that has been recently demonstrated to have a pathogenetic role in GCA. Márquez *et al*. investigated whether the genetic variant of IL-33 gene is involved in GCA genetic predisposition. They analysed four European cohorts of GCA patients (1363 patients) and showed that IL-33 rs7025417 polymorphism could be considered as a genetic risk factor for GCA (5). Finally, since GCA is more frequent in females, Márquez *et al*. supposed that genes located on the X chromosome could have a role in GCA predisposition. They studied the genetic variant of IRAK1 e MECP2 which are two genes strongly associated with several autoimmune diseases such as primary Sjögren's syndrome, systemic lupus erythematosus, systemic sclerosis and rheumatoid arthritis. They analysed 627 female biopsy-proven GCA, but no association emerged between any IRAK1 and MECP2 genetic variants and GCA (6). As described for GCA, Th1 and Th17 cells seem to have a pathogenetic role also in Takayasu arteritis (TAK). Th1 and Th17 responses are involved in the development of inflammatory processes underlying TAK and are strictly correlated with disease activity. Contrary to what happens in GCA, in TAK glucocorticoids suppress Th1 cytokine production leaving the Th17 ones unchanged (7).

Clinical aspects and diagnosis

The similarities between GCA and TAK might be much more numerous than it was thought in the past. The use of fluorodeoxy-glucose positron emission tomography (FDG-PET) in the evaluation of patients with LVV has not only demonstrated that large vessels are involved in GCA more frequently than it was reported in literature with a particular predilection for the aorta and subclavian arteries, but also revealed a high rate of large-vessel involvement typical of TAK in patients above the age of 50 presenting a clinical picture of GCA. Therefore, it could be supposed that GCA and TAK might be a part of the spectrum of the same disease. Moreover, these data suggest the necessity of a revision of the current classification criteria for GCA and TAK, according to which they are classified as two distinct diseases on the basis of differences in age, signs, symptoms and vascular involvement (8). The need for new classification or diagnostic criteria for GCA is also underlined in a recent study by Muratore *et al*. that compares the features of patients with large-vessel GCA (LV-GCA) with those of patients with classic cranial symptoms of GCA (C-GCA). LV-GCA has a predilection for the proximal branches of the aorta such as the subclavian, axillary and proximal brachial arteries. Patients with LV-GCA seem to be younger at diagnosis than those with C-GCA, they less frequently complain of typical cranial and visual symptoms and present unspecific and subtle onset symptoms, including symptoms of vascular insufficiency, with a consequent delay in diagnosis. Furthermore, in LV-GCA the temporal artery biopsy (TAB) is often negative and a vascular imaging technique is required to confirm the diagnosis. These patients need a higher cumulative dose of glucocorticoids, more immunosuppressive therapy and they also have more disease relapses and a higher prevalence of aortic aneurysm in comparison with those with C-GCA. GCA thus appears as a disease with a great variety of clinical manifestations; indeed these patients could present typical cranial symptoms associated with a positive TAB or less specific clinical manifestations, because of a predominant large-vessel involvement, but they could also manifest both clinical features. Moreover, this study underlined that the 1990 ACR classification criteria for GCA are inadequate to identify the subset of patients with LV-GCA; in these cases the help of a vascular imaging technique, and in particular FDG-PET, is essential for a correct diagnosis. It is undeniable that new classification criteria, including imaging findings, should be developed in order to be able to recognise all variants of GCA (9). Another important aspect of LVV is the correct classification of isolated aortitis, defined as an isolated increased FDG-PET uptake of the aorta associated with constitutional symptoms or occurring in absence of specific clinical manifestations. We routinely consider these patients as affected by GCA or TAK on the basis of the age of disease onset, but it is not already clear if isolated aortitis should be considered as a part of GCA or TAK or as a completely different disease. Talarico *et al*. have recently reviewed the data of all patients seen in two Italian Rheumatology unit in the last two decades with a suspicion of GCA and compared epidemiological and clinical data of patients with isolated aortitis with those of patients affected by typical GCA. They observed many statistical significant differences: patients with isolated aortitis are younger than GCA patients (62 *vs*. 78.4), they are more frequently male and none of them presented typical GCA symptoms. This study shows that patients with isolated aortitis have different clinical and epidemiologic features in comparison with patients with GCA, but further

studies are necessary to understand if isolated aortitis should be still considered as a variant of GCA or TAK or if it should be classified as a different disease (10). The gold standard test for the diagnosis of GCA still remains TAB, even though a negative TAB does not exclude the diagnosis of GCA, as it has been evaluated that 13–44% of GCA patients have a negative TAB. Several factors could negatively influence the result of TAB, such as the typical segmental inflammatory involvement of the temporal artery (skip lesions), the length of the biopsy specimen and the duration of pre-treatment with corticosteroids. TAB is usually performed on the most symptomatic side, but, according to the literature, both unilateral and bilateral TAB could be performed. To date, some research has been done to establish the usefulness of bilateral TAB, but the results are still conflicting. However, in a recent study, the rate of discordant biopsy results in 250 suspected GCA patients who underwent initial bilateral TAB has been investigated. 62 patients had GCA diagnosis confirmed by the biopsy; the rate of discordant biopsy was 4.4%, with 11 unilaterally positive biopsies. From these results, initial bilateral temporal biopsies seem to be able to improve the diagnostic certainty of GCA, although the diagnostic advantage of this choice should be weighed against the time of surgical procedure and discomfort of the patient, the potential higher risk of complications and eventually the cost-effectiveness of the procedure; at present, initial bilateral biopsy is not a procedure to be recommended for routine clinical practice (11). In the last few years, ultrasound has gained growing attention in the diagnosis of LVV. This imaging technique can be helpful in the evaluation of both temporal arteries and large vessels such as axillary, subclavian and common carotid arteries. Ultrasound evaluation of temporal arteries of GCA patients shows a hypoechoic, not compressible halo ("halo sign") due to the oedema of the inflamed arterial wall while the ultrasound evaluation of large vessels affected by LVV (both GCA and TAK) shows an arterial wall thickening higher than 1 mm. Moreover, ultrasound could be used to detect LVV long-term complications such as arterial stenosis and aneurisms (12). Since the halo sign is considered a high specific sign for GCA, in a recent study Germanò *et al*. tried to understand if colour duplex sonography-guided TAB (CDS-TAB), performed at the site of halo, might decrease the rate of false-negative TAB results. To this end, they compared CDS-guided TAB with standard TAB in a prospective cohort of 112 patients with suspected GCA. No significant differences were observed in the two groups; they thus concluded that CDS-guided TAB does not improve the sensitivity of TAB in the diagnosis of GCA (13). Although TAB is the gold standard for the diagnosis of GCA, the high frequency of false-negative results makes it difficult for clinicians to establish which patients are affected by GCA on a histopathological basis. It is well known that patients with suspected GCA have to be empirically treated with corticosteroids to avoid the possible catastrophic consequences of a delayed treatment, such as blindness and stroke. In fact, as shown by Khoi *et al*., in the case of a suspected GCA, the physician has to make the treatment decision on the basis of the patient's clinical features, and the steroid treatment, that had been previously started because of the clinical suspicion of GCA, should never be changed in the presence of a negative TAB (14).

Several studies confirm the role of FDG-PET in the diagnosis of LVV. The most largely adopted method to assess the presence and the grade of severity of LVV is the qualitative one which analyses FDG-PET uptake of the vessel wall and then visually compares it with that of a reference structure, such as the liver. FDG-PET detects metabolic changes in the arterial wall, making it possible to diagnose GCA at an early stage; however, the role of FDG-PET in the follow-up of these patients is still not clear (15, 16). Other imaging techniques, such as computed tomography angiography (CTA), magnetic resonance imaging (MRI) and Doppler sonography are instead able to demonstrate the affected vessel anatomical changes (wall thickening, contrast enhancement, aneurisms and stenosis) which occur when the inflammatory process is well established. Prieto-Gonzalez *et al*. evaluated the effect of glucocorticoid therapy on the CTA findings of LVV in patients with GCA. To this end they repeated a CTA in 35 GCA patients after a year of steroid therapy and they observed that wall thickening was still present in 68% of patients, while contrast enhancement disappeared in 15 out of 16 patients in whom it was demonstrated at the first evaluation. They also observed a decrease in wall thickening and in the number of affected segments and no development of new lesions and/or aortic dilatations in any analysed patients. These data suggest that signs of LVV at CTA improve with steroid treatment; however, further investigations are necessary to explain the clinical significance of persisting wall thickening in these patients, since it could represent a persistent subclinical vascular inflammation, which, in the long term, might lead to relapses, to the development of aneurisms or stenosis, or it could alternatively be the result of fibrosis and vascular remodeling, as suggested by the disappearance of contrast enhancement (17). Siemonsen *et al*. have recently focused their attention on a controversial aspect of GCA: the involvement of intracranial vasculature in the vasculitic process. For this purpose, 28 patients with suspected GCA underwent 3T MRI and a dedicated MR imaging protocol was used to assess the presence of vasculitic changes (intramural contrast enhancement and wall thickening) in intracranial arteries. The involvement of intracranial arteries was observed in at least half of the analysed patients, indicating that the inflammation of intracranial arteries can represent a rare feature of a subset of GCA patients, but the real clinical significance of this observation is not yet fully understood and further studies are required (18).

Many recent studies have been dedicated to the search of a specific biomarker for the assessment of disease activity in TAK; currently, TAK activations are monitored by acute-phase reactants, even though these tests are not always

precise or reliable indicators of disease activity. Alibaz-Oner *et al*. studied levels of pro-inflammatory cytokines in TAK patients and observed an increase of IL-6, IL-8 and IL-18 in patients with active disease, suggesting that these cytokines might be potential biomarkers for the assessment of TAK activity (19). Liu *et al*. evaluated the possible role of N-terminal pro-brain natriuretic peptide (NT-proBNP) as a biomarker of disease activity, severity and progression. NT-proBNP is a well-known marker of cardiovascular diseases, but higher levels have also been detected during inflammatory diseases. NT-proBNP levels could thus increase in TAK because it is an inflammatory condition and because vasculitic lesions might result in an increase of large artery stiffness, and consequently of left ventricular afterload with stimulation of BNP release. In this study, NT-proBNP levels of 68 TAK patients were compared with those of 90 control subjects and resulted higher in patients with active disease and in those with severe disease, compared with patients with mild disease. These data prove that NT-proBNP might be a useful biomarker for the assessment of TAK activity and severity, but further studies are required to confirm these results. (20)

Therapy

Corticosteroids are the cornerstone in the therapy of LVV, but a long-term therapy with glucocorticoids could cause several adverse events. For this reason, a steroid-sparing drug could be introduced in the treatment of patients who experience severe glucocorticoid side effects and in those who need prolonged corticosteroid therapy for relapses of the disease, although there is no strong evidence on the use of immunosuppressant drugs in these diseases. Indeed, a recent meta-analysis shows that the addiction of immunosuppressant to steroids brings no benefit either in terms of efficacy or toxicity in GCA. In particular, this study demonstrates that anti-tumour necrosis factor (TNF)-α agents (infliximab and adalimumab) are not useful to improve the outcome of these patients, and that a low dose of methotrexate (10-15mg/week) has a modest role in reducing relapses and lowering the cumulative steroid dose (21). Small, uncontrolled studies have shown some efficacy in TAK therapy, and a steroid-sparing effect of methotrexate, azatioprine, mycophenolate mofetil and anti-TNF-α. Youngstein *et al*. have recently analysed a cohort of 98 patients with TAK, looking for a clear evidence of prolonged efficacy of TNF-α antagonists and interleukin-6 receptor (IL6-R) antagonist (tocilizumab) in refractory TAK. They showed that anti-TNF-α drugs could be useful in controlling refractory disease also in long term. Further studies may help us in clarifying which is the best anti-TNF-α for TAK treatment and the optimal duration of the biologic therapy (22). Encouraging data about the efficacy of tocilizumab in the treatment of both GCA and TAK come from several case reports and small case series (23). To confirm the possible therapeutic role of tocilizumab in GCA, a large multicentre, international, randomised, double-blind controlled trial has been designed, named GIACTA; GCA patients could be enrolled in this trial until June 2015. On the basis of the pro-inflammatory properties of angiotensin II (ATII) and of the anti-inflammatory properties of angiotensin-converting enzyme inhibitors (ACEI) and of the ATII receptor blockers (ARB), Alba *et al*. tried to understand if ACEI and ARB could be useful in treatment of GCA. They analysed 187 GCA patients who were divided into three groups: patients treated with ACEI, patients treated with ARB and patients who received neither ACEI nor ARB. The three groups were compared and the treatment with ARB seemed to be associated with lower relapse rate, lower cumulative steroid dose and longer remission. The mechanisms of action of ARB in inflammatory diseases are not fully understood; it has been proved that ATII is able to stimulate the production of IL-1, IL-6 and IL-8 which are pro-inflammatory cytokines involved in GCA pathogenesis. In an experimental animal model it has also been demonstrated that the use of ACEI and ARB suppresses the development of auto-reactive Th1 and Th17 cells, which have a pathogenetic role in GCA. It is interesting that ARB, but not ACEI, might have a positive effect in the control of GCA; the possible explanation is that in blood vessels the production of ATII is catalysed not only by ACE, but also by other enzymes, demonstrating that ACEI are not able to inhibit ATII completely. This is an observational study on a small number of patients treated disomogeneously with ACEI/ARB; a randomised controlled trial is essential to confirm these data (24).

Novelties in ANCA-associated vasculitis

Pathogenesis

The aetiopathogenesis of ANCA-associated vasculitis (AAV) is not yet clearly known. According to the more unanimous etiopathogenetic model, it seems that people with a genetic predisposition, when exposed to environmental agents, might develop a chronic inflammation of vessel walls through a disregulation of the immune-response (25).

During the last few years, several genome-wide association studies (GWAS) have demonstrated a correlation between specific polymorphisms of candidate genes and a major risk in the development of AAV.

As we outlined in our previous papers (1, 26), a correlation seems to be proved between GPA and HLA-DP, SERPINA-1 (encoding α1-antytripsin), PRT3 (encoding proteinase-3, PR3, the main GPA-related autoantigen) and SEMA6A (semaphorin 6A) genes, while MPA seems to be much more correlated with poymorphisms in HLA-DQ genes. No GWAS have been conducted to date in EGPA, which is the AAV with a less known etiopathogenetic mechanism (27)

Many *in vitro* experimental data, animal models and clinical observations, confirm the direct role of ANCA in causing AAV; the pathogenetic role of B cells, T cells and alternative pathway of complement have also been elucidated.

An imbalance in CD4 T cells subsets in peripheral blood of patients with AAV is also confirmed (1). Particularly, the reduction of circulating CD4 T effector memory cells (Tem) during disease activity and their detection in urine analysis of patients with renal disease, sug-

gested their role in determining kidney damage and as a potential biomarker of renal disease activity or relapse in AAV. These results justify the new interest in anti-T cells therapies, such as Abatacept, also to improve the renal outcome in AAV (28). According to the concept of target-therapy, Chandy *et al*. also demonstrated in 2003 that CD4 Tem are characterised by high expression of surface Kv1.3 channels, unlike other naive and central memory T cells. In several animal models the selective blockade of these channels ameliorated the disease without compromising the protective immune response to acute infections. In fact, Kv1.3 is required for the expression of pro-inflammatory cytokines and its absence could lead to an increase in the expression of anti-inflammatory cytokines such as IL-10. This could be a novelty in terms of new targeted therapies (1). The importance of B cells in the immunopathogenesis of AAV is underscored by animal models that demonstrate the pathogenetic role of ANCA, but also by the effectiveness of Rituximab (a B cell depleting therapy against CD20) in the induction of the remission and in the maintenance therapy (29). Regulatory B cells also play a significant role in maintaining immunological tolerance. Several studies have demonstrated that patients with active AAV express low levels of CD5, a surface molecule that through the B cell receptor (BCR) maintains immunological tolerance.

Lepse *et al*. (30) and Todd *et al*. (31) have recently conducted two independent studies in which they both underlined that CD5 Breg are only numerically diminished in AAV compared to healthy controls, but there are no abnormalities in their function. According to this, Unizony *et al*. conducted another study on 197 AAV patients trying to evaluate the utility of CD5 Breg cells as biomarkers of AAV. Since, all patients came from the RAVE trial (Rituximab for ANCA-associated Vasculitis), the percentage of peripheral CD5 Breg reflected disease activity only in patients treated with Rituximab (and not in patients treated with cyclophosphamide and azathioprine). Moreover, they did not perform intracellular staining for IL-10 and this represented an important limitation of the study. Then, the sole staining for CD5 as a putative surrogate marker for Breg cells did not identify a subgroup of B cells with clear potential for clinical use. Adequate phenotyping of B cells is necessary to evaluate their role as biomarker in AAV (32).

According to the role of Rituximab and the B cells pathway in AAV, a new potential target in such diseases could be BAFF (33-35).

BAFF, also known as BLyS, is a member of the TNF family that plays a crucial role in B-cell development by promoting B-cell survival and transition from the immature to mature B-cell stage. The binding of BAFF to its high affinity receptor activates signalling pathways that lead to the expression of genes essential for B-cell survival. Moreover, BAFF can also augment Th1 responses *in vivo*. While cells of the monocyte/macrophage lineage seem to be the primary source of BAFF production *in vitro*, also neutrophils, under certain stimulatory conditions, can express and release BAFF. According to this, based evidence of increase serum and tissue levels of BAFF in AAV, BAFF seems to be a promising target for the treatment of these diseases (36). In contrast, BAFF as a potential biomarker in AAV appears to be less promising as compared to more traditional markers of the disease. In fact, even if Xin *et al*. demonstrated an elevated BAFF level in patients with anti-MPO AAV, especially in correlation with disease activity, BAFF levels were not related to MPO-ANCA levels (37).

Another important novelty in the aetiopathogenesis of AAV is the formation of neutrophil extracellular traps (NETs) or NETosis. NETs are decondensed chromatin filaments decorated with histones and neutrophil anti-microbial proteins that are normally involved in the capture of various microbes as part of the innate immune defense. Deregulation of the normal formation and degradation of NETs represents a source of intracellular antigens that can contribute to the pathogenesis of several autoimmune diseases such as AAV (38-40). A few years ago, Kain and colleagues provided evidence that a new type of ANCA autoantibody, anti-LAMP-2 (directed against lysosomial membrane-protein-2) may have a pathogenetic role in AAV. Sha Tang *et al*. (41) conducted a study in which not only did they confirm the role of anti-LAMP-2 antibodies in activating neutrophils to release NETs, but they also demonstrated the capacity of NETs to trigger vasculitis and perpetrate the autoimmune response against neutrophil components. Moreover, since LAPM-2 is critical for autophagy, they also hypothesised that autophagy-related signalling may be involved in anti-LAMP-2 antibody-induced NETs formation. Therefore, a defective apoptotic cell clearance and consequent excessive NETs formation could be a potential novel mechanism for the induction of inflammation in active AAV (42-43).

Moreover, novel mutations identified in single genes vasculopathies could help in unraveling the pathogenesis of adult onset primary systemic vasculites (44-45). Gain of function mutation in gene TMEM173, encoding the stimulator of interferon genes (STING), have been identified in vasculopathy with onset in infancy with vascular and pulmonary syndrome (44). Furthermore loss of function mutations in CECR1, encoding adenosine deaminase 2, have been associated with vasculopathies resembling on occasions polyarteritis nodosa (45).

Therapy

Systemic vasculitides are a complex set of heterogeneous conditions whose natural history has been significantly modified by current therapies. Nowadays about 70% of patients with small-vessel vasculitis survive up to 5 years. The therapies available, that combine immunosuppressive agents and supportive management in fact, minimise systemic and local inflammation and can preserve organ function. Nevertheless, a definitive treatment for AAV is not available at the moment and, even if the induction of remission is almost always achieved, the risk of relapses and the harm due to a poor control of an active disease continue to challenge both patients and clinicians (46).

According to the European League Against Rheumatism (EUVAS), patients with AAV respond to different treatment protocols, depending on their disease activity (47). Moreover, therapeutic decisions should be modified according to the context in which the disease occurs in individuals and eventually, to the entity of severity changes during the course of the disease.

The role of conventional immunosuppressive agents remains important. Cyclophosphamide (CYC) and glucocorticoids (GC) continue to be the gold standard for the remission induction of multi-system primary small vessel vasculitis; on the contrary, methotrexate (MTX) and GC are recommended for the induction of remission in non-organ or non-life threatening AAV. Finally, EULAR recommends the use of azathioprine (AZA), leflunomide (LFM), mycophenolate mofetil (MMF) or MTX as remission-maintenance therapy (48).

Since the use of some traditional drugs (such as GC and CYC) is associated to serious side effects and new information are available about AAV pathogenesis, the scientific community is striving to identify alternative drugs with greater efficacy, but less toxic effects.

According to the role of B cells in determining AAV, a promising new drug in the treatment of these diseases is Rituximab, a chimeric murine human monoclonal IgG1 antibody directed against CD20 lymphocytes. As reported last year (1), the results from RAVE and RITUXIVAS studies on the role of Rituximab in the induction of remission in AAV, seem to be confirmed; the French Vasculitis Study Group (FVSG) claims that RTX may be prescribed as first-line therapy to induce remission of GPA and MPA with the same indications as CYC, especially in patients in whom it would be advisable to avoid CYC because of its high gonadal toxicity and carcinogenity, or an ongoing infection (49-50). Moreover, Charles *et al*. conducted a retrospective study on 80 patients in which RTX seemed to be superior for flaring patients and also as a maintenance treatment (51). In accordance with this study, several other trials have been conducted to define the role of this drug in controlling and preventing relapses. Miloslavsky *et al*. recently perfomed a randomised double-blind placebo-control trial in which retreatment of AAV relapses with RTX and GC appears to be a safe and effective strategy, regardless of previous treatments (52). A similar result comes from another recent study conducted by Azar *et al*. on 89 patients with relapsing GPA. This study compared two groups of patients: the first one (n=47) received a conventional maintenance agent (AZA, MTX, MMF) in conjunction with RTX and GC, while the other group (n=42) received no additional immunosuppressive agent after the induction of remission. Without any difference in serious adverse events, the addition of a conventional maintenance agent to RTX and GC decreased the incidence of relapses (53). Regarding the maintenance of the remission in AAV, in October 2012, Guillevin *et al*. started a study in which they claimed the major efficacy of RTX against AZA (MAINRISTAN) also as AAV maintenance therapy. The results of this first study confirm that RTX may help to maintain remission (54) and to strengthen this observation. Currently, another study (MAINRISTAN2) (55) is still ongoing with promising results especially for patients positive for anti-proteinase 3 ANCA (56).

Despite these new acquisitions and the encouraging results, some crucial concerns which many authors are dealing with, remain unclear. The modalities of RTX prescription and use are still very heterogeneous. Several studies seem to support a repeat-dose RTX schedule as a more efficient maintenance therapy as compared to on-demand schedule (57-58); but another unmet need we have to consider is the risk of secondary hypogammaglobulinaemia and consequent serious infections (59-60). At the moment, we can only take advantage of the FVSG recommendations to front these problems and wait for the discovery of new biomarkers that could help us to understand the state of activity of the disease and the risk of adverse events (61-62).

Another novel therapeutic option proposed according to the pathogenetic role of B cells in AAV, is Belimumab, a fully humanised monoclonal antibody that acts against BAFF and has already shown success in human systemic lupus erythematosus (63). A phase III multi-centre multinational randomised double-blind study (BREVAS) is now ongoing to evaluate the efficacy and safety of Belimumab in combination with AZA for maintenance of remission in GPA and MPA (64).

As we already outlined, the data available are still insufficient to recommend the use of RTX and other anti-B cells therapies for EGPA (1); however RTX could be considered as an option for refractory cases, particularly when characterised by predominant vasculitic manifestations and MPO-ANCA positivity (47).

Two new drugs might become available for EGPA: Mepolizumab (MPZ) and Omalizumab (OMZ). MPZ is a humanised anti-interleukin-5 (IL-5) monoclonal antibody successfully used for the first time in EGPA in 2010. During these years, MPZ has shown to be able to induce remission in most cases of EGPA and to be well tolerated, with a corticosteroid-sparing effect. However, patients suffered relapses at cessation of MPZ, suggesting that patients with EGPA may require long-term treatment. Some novelties could come from a phase III double-blind randomised placebo-controlled clinical trial that is still ongoing whose aim is to investigate the efficacy and safety of MPZ in EGPA patients receiving standard of care therapy (65).

OMZ, a humanised anti-IgE monoclonal antibody, could be beneficial in EGPA patients with persistent asthma after the induction of remission (66).

According to the role of T reg cells in AAV pathogenesis, another novel promising therapy that is currently being validated, is abatacept (an anti-CTLA4-IG).

This molecule is comprised of the ligand-binding domain of CTLA4 plus modified Fc domain derived from IgG1. By containing CTLA4, abatacept blocks the engagement of CD28 with its ligand, thereby inhibiting T cell activation. Since blockage of T cells activation might impact GPA disease pathogenesis, Langford *et al*. con-

ducted an open-label standardised trial to investigate the safety and efficacy of Abatacept in non-severe relapsing GPA. They found that abatacept was well tolerated and led to disease remission and prednisone discontinuation in a high percentage of patients, suggesting that abatacept warrants further study as a therapeutic option for mild form of relapsing GPA (67). A phase III multi-centre randomised double-blind placebo-control study to evaluate the efficacy and safety of abatacept to achieve sustained GC-free remission in these patients is now ongoing (68).
Since also the alternative pathway of the complement has shown a pathogenetic function in AAV, and particularly in necrotising and crescentic glomerulonephritis (NCGN), Xiao *et al*. evaluated the possible therapeutic role of an antagonist of the complement in AAV. Specifically, they investigated CCX168, an oral molecule undergoing phase II evaluation (69) that blocks C5aR/CD88, and they found that the blockade of this complement protein might have a therapeutic benefit in patients with AAV and GN (70). These findings not only provide additional support for the important pathogenic role for complement activation in AAV, but also support the possibility that therapy directed at preventing or reducing complement activation might be a future interesting proposal for the treatment of these patients.
Finally, de Joode *et al*. confirmed the possible use of plasmapheresis not only in patients with advanced renal failure or pulmonary haemorrhage, but also as rescue therapy in patients with insufficient response to initial induction therapy or when their clinical condition or renal function worsens due to ongoing vasculitis disease activity. Their study showed a significant improvement in renal function without higher mortality or increase risk of severe infections. Overall, no difference was found also in the development of end stage renal disease during long-term follow-up after diagnosis. Therefore, the addition of plasmapheresis, should be considered in those patients with insufficient response to standard induction therapy (71).

Novelties in mixed cryoglobulinaemia
Mixed cryoglobulinaemia is a small-vessel vasculitis with characteristic cryoprecipitable immune-complexes, that may occur in the settings of chronic infections (mainly Hepatitis C virus, HCV) or without any evidence of infection (1). These two clinical entities seem to differ to some extent, particularly as far as outcome and therapeutic approach is concerned, in this section we will focus only on HCV-related cryoglobulinaemic vasculitis (HCV-CV).

Pathogenesis
Since the discovery of HCV, the knowledge of the pathogenesis of HCV CV has grown in parallel with the rising understanding of the biology of the virus. It is well known that host and viral factors may contribute to the spectrum of the disease, response to treatment and viral persistence. The disease is the result of the complex interplay between viral factors, such as HCV lymphotropism, viral replication that continually trigger the immune system and host factors, such as genetic background and predisposition to autoimmune disorders (1, 26).
As far as viral factors are concerned, novel genotype 1 lymphotropic HCV variant have been identified by deep sequencing analysis. Infection by HCV could significantly enhance the development of Th17 cells. The HCV protein responsible for inducing the Th17 cells was identified as HCV-Core protein, which could enhance the STAT-3 signalling and up-regulate the expression of RORct as a Th17 master gene. This mechanism might be involved in autoimmune-related diseases in chronically infected HCV patients. Moreover, STAT-3 signalling might prove a valuable therapeutic target for HCV-related autoimmune disorders (72).
Moreover, it has been previously outlined that mixed cryoglobulinaemia might be an antigen-driven process, though the triggering antigen is unknown (1). However, the interaction between the host and the virus might result in the cryoprecipitability of the complex. In fact HCV non-enveloped core protein (HCV cp) seems to be a constitutive part of cryoglobulins and to have a direct effect on the cold precipitation process. Furthermore, novel data lend support to the concept that cryoprecipitation might be a genotype-dependent process (73).
Host factors, such as genetic background, explain why only a minority of chronically HCV-infected patients develop the full blown MC syndrome, even though a much higher number of subjects have detectable cryoglobulins. Recently, a genome-wide significant association with cryoglobulin-related vasculitis was identified with single nucleotide polymorphisms near NOTCH4 and MHC Class II genes (74).
It is already known that IL28 B genotype is an independent predictor of response to interferon-based therapy in MC (1). Last year, Sansonno *et al*. strengthened this observation, outlining that a particular polymorphism (C/C genotype) of IL28 B is more prevalent in subjects with CV than in those without. In addition, this variant is distinguished biologically by a higher frequency of restriction of B cell response and clinically by a higher risk of cryoglobulinaemic nephropathy and B cell malignancies, while acting as an independent predictor of a sustained virological response to antiviral therapy (75).

Clinical aspects and diagnosis
Preliminary classification criteria for CV have been recently defined (76). These criteria have been developed with modern statistical methodology and validated in large and independent series of real patients. They request the mandatory positivity of cryoglobulins on at least two determinations two weeks apart and a number of clinical features to be fulfilled, comprising data collected by history (standardised questionnaire items), clinical assessment (clinical items) and laboratory parameters (lab items) (76-77). Last year, a new large multinational cohort of subjects was selected for validation of the criteria, confirming its good sensitivity and specificity for CV (78).

Therapy
The growing knowledge of host and virus interaction and the availability of long-term observational data on large

series of CV patients, have unravelled that, although immunosuppressive drugs remain strongly recommended, particularly in life-threatening disease manifestations. Viral eradication should be the ultimate goal in every CV patient, since viral clearance is associated with better outcome (79) and the use of immunosuppressive medications is an independent predictor of ominous outcome, regardless of disease severity (80). It must be taken into account, however, that mixed cryoglobulins are an independent predictor of non-response to dual, interferon-based (IFN-based), antiviral treatments (79). These observations, on the one hand, strengthen the importance of strategies based on combination regimens with sequential or synchronous administration of antiviral and immunosuppressive medications, and on the other hand, highlight the need for newer strategies for viral eradication in CV patients (81). Triple IFN-based antiviral combination is more effective than dual antiviral treatment, however, higher risk of adverse events should be taken into account in patients with CV, who are probably more at risk of complications of triple combination (82-83). Newer antiviral combination, IFN-free regimens, have recently proved to be very effective with a very good safety profile, and now need to be challenged in an HCV-CV patient population (84-85).

List of abbreviations

AAV: ANCA associated vasculitis
ACEI: angiotensin-converting enzyme inhibitors
ANCA: antineutrophil cytoplasmic antibodies
ARB: ATII receptor blockers
AZA: azathioprine
BAFF: B cell Activating Factor
Beff: effector B cells
BCR: B cell receptor
CDS-TAB: colour duplex sonography-guided TAB
C-GCA: cranial symptoms of GCA
CTA: computed tomography angiography
CV: cryoglobulinaemic vasculitis
CYC: cyclophosphamide
EGPA: eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss)
FDG-PET: fluoro-deoxy-glucose positron emission tomography
FVSG: French Vasculitis Study Group
GC: glucocorticoids
GCA: giant cell arteritis
GPA: granulomatosis with polyangiitis (Wegener's)
GWAS: genome-wide association studies
HCV: hepatitis C virus
INF-γ: interferon gamma
LFM: leflunomide
LV-GCA: large-vessel GCA
LVV: large-vessel vasculitis
MMF: mycophenolate mofetil
MRI: magnetic resonance imaging
MTX: methotrexate
NETs: neutrophil extracellular traps
NT-proBNP: N-terminal pro-brain natriuretic peptide
PMR: polymyalgia rheumatic
SNPs: single-nucleotide polymorphisms
TAB: temporal artery biopsy
TAK: Takayasu arteritis
Treg: T regulatory (cells)

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