Review

Large- and small-vessel vasculitis: a critical digest of the 2010-2011 literature

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ABSTRACT

The last two years have been marked by significant achievement in the identification of the basic mechanisms of systemic vasculitis and in the translation of these mechanisms into targeted therapies. More specifically, new insights into the environmental, cellular, and genetic factors involved in the pathogenesis of systemic vasculitis have been provided. Consequently, several studies focused on the development of novel strategies to achieve and maintain clinical remission in small- and large-vessel vascultis, including relevant large multicentre trials, have been promoted. The highlights of these studies, their potential clinical implications and the unmet needs, which are still to be addressed, are summarised in this review.

Introduction

Rapid progress has been made during the last two years in our understanding of the etiopathogenesis of vasculitis with consequent improvements in early diagnosis, treatment and secondary prevention of these diseases. In this manuscript we will try to provide an overview of the recent advances in pathogenesis, nomenclature and treatment of large and small-vessel vasculitis. A systemic Medline search was performed using the term "giant cell arteritis" (MeSH Terms and semantic search), Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis (MeSH Terms and semantic search) and "cryoglobulinemia" (MeSH Terms and semantic search). The Medline search was focused on relevant literature contributions published in 2010 and 2011.

Recent insights into the diagnosis and classification of systemic vasculitis

During 2010, two events occurred in the field of Vasculitis nomenclature: an ambitious project aimed at modifying the Chapel Hill Consensus Conference (CHCC) nomenclature for systemic vasculitides and the proposal for novel classification criteria for cryoglobulinaemic vasculitis and other systemic vasculitis (1, 2). In this scenario, the change from the diagnostic term "Wegener's granulomatosis" to "granulomatosis with polyangiitis" (GPA) has represented a further starting example for a global revision of the existing nomenclature in vasculitis based on clinical, pathophysiological and ethical developments. In particular, the latter change has been justified both on the general rule that diagnostic terms with eponyms are less effective than more descriptive terms and on the evidence that in this particular instance Dr Friedrich Wegener was a member of the Nazi party before and during World War II (3). Moreover, the new term emphasises both of the histological distinctive features of the disease and namely the granuloma lesions and the necrotising polyangiitis (3).

As far as the classification of vasculitis is concerned, during the last two years novel classification criteria have been recently proposed for cryoglobulinaemic syndrome (CV) (2), while an international study has been undertaken for the other systemic vaculitis (1). The latter, called 'Diagnosis and Classification of Vasculitis (DCVAS)' study represents a joint effort supported by the Vasculitis Foundation, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) aimed at overcoming some of the limitations recognised in the currently adopted ACR classification criteria for vasculitis. In fact, it has been widely recognised that ACR

criteria might fail in terms of validity with respect to clinical research and practice (*i.e.* overlapping disorders (4), unclassified vasculitis (5)). For these reasons, the European League Against Rheumatism (EULAR) has organised an expert consensus to analyse current definitions, classification and diagnostic criteria in systemic vasculitis and to underline areas requiring an updating. This working group laid the foundations for revised criteria's validation and for systemic vasculitis' new definitions, by considering the traditional approaches, the presence of diagnostic autoantibodies (*e.g.* ANCA) and the new acquisition on pathogenesis (1).

Preliminary classification criteria for CV have been developed through a study which involved seventeen experts in the diagnosis and treatment of CV from twelve centres. Out of 83 questions, the experts identified 3 questions which best contributed to the predictability of the disease mainly related to purpura and viral hepatitis. A positive response to at least 2 of the 3 questions showed a specificity of 83.5% and a sensitivity of 81.9% for CV. In the second part of the study, in addition to the 3 questions chosen in the first part of the study, a set of clinical features (constitutional symptoms, articular involvement, vascular and neurologic involvement) and a set of laboratory tests (reduced serum C4, positive RF and positive serum M component) associated with CV were identified and combined in order to obtain the final classification criteria. When tested in patients with CV (HCV-related or HCV-unrelated) with serum cryoglobulins but without CV and in controls without serum cryoglobulins but with clinical or laboratory features which can be observed in CV these criteria showed a sensitivity of 88,5% and a specificity of 93,6% for CV (2). Quartuccio *et al*. have recently tested the new classification criteria for CV in a cohort of 500 patients with positive cryoglobulins. The criteria showed high sensitivity and specificity in both HCV-positive and HCV-negative patients with CV (6). Even if these criteria have been elaborated not for diagnostic purposes but for investigation and epidemiological purposes, it is likely that the efforts in improving the classification of systemic vasculitis may simplify the diagnostic algorithm of these disorders in the near future.

Concomitantly, recent advances in non invasive imaging modalities have appeared as a useful adjunct for the diagnosis of systemic vasculitis, particularly in patients with primarily large-vessel involvement (7).

Nowadays, we well know the importance of duplex sonography (DS) in the diagnostic approach of vasculitis. Fifteen years ago, Schimdt *et al*. identified hypoechogenic wall thickening around temporal artery (halo-sign), that represents an indicator of vessel wall inflammation in giant cell arteritis (GCA) (8). Recently, Arida *et al*. in a meta-analysis have shown that unilateral halo sign achieved an overall sensitivity of 68% and a specificity of 91% for the diagnosis of GCA, as well as 43% and 100%, respectively, for the bilateral halo sign. Since the diagnostic accuracy of halo sign for GCA seems to be comparable to anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis, these data suggest the need for a reassessment of the ACR criteria, evaluating a potential primary role of the halo sign (9). An interesting recent study confirmed the use of DS in GCA and reported that detected changes in the vessel wall can also be present in upper and lower limb arteries, particularly in patients without signs of systemic inflammation (10). Other promising imaging techniques are represented by 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) and Magnetic resonance imaging (MRI). Since FDG-PET is able to identify *in vivo* areas characterised by elevated glucose metabolism, such as inflammatory, infective, and neoplastic lesions, it had a major role in the diagnostic workup and in guiding treatment choice of several vasculitis (11). PET and PET/Computer tomography (CT) represent two extremely useful diagnostic tools to detect extracranial vascular involvement in GCA (11, 12), most importantly for those areas which are difficult to explore by DS. Involvement of the ascending aorta has been reported in up to 33% in a study using PET (7); moreover, sensitivity and specificity of PET/CT to detect large-vessel vasculitis is 85% and 95%, respectively, and data regarding sensitivity decrease in GCA patients treated with steroids (13, 14). Recently, Henes *et al*. have suggested the important role of PET/CT in assessing active inflammation during treatment in GCA patients, reporting a significant decrease in FDG uptake in ten patients affected by GCA and Takayasu arteritis treated with cyclophosphamide (13). Moreover, PET and PET/CT represent a crucial tool in the diagnostic work-up of GCA presenting as fever of unknown origin (FUO) (11). As far as MRI is concerned, MRI technique represents a useful instrument for GCA diagnosis, that has increased the sensitivity and specificity needed to detect vascular involvement (14).

Recent insights into the pathogenesis of systemic vasculitis

It is widely recognised that the pathogenesis of systemic vasculitis is the result of the interplay of genetic, environmental and immune cellular and humoral factors. Although the cause of systemic vasculitis remains unclear, novel insights into vasculitis underlying pathogenetic mechanisms have been achieved during the last two years especially concerning the genetic and environmental factors involved and the role of humoral and cellular immune responses (15-17). More specifically, the role of B cells in systemic vasculitis has recently been revisited specifically due to the efficacy of B-cell depletion therapy with rituximab in AAV and CV. A renewed interest in B cells has also arisen for CSS considering both the strong association between ANCA and the vasculitic manifestations of the disease and the proven increase in the production of IgG4 in active CSS patients (18, 19). The latter has been related to the inflammatory milieu and particularly to the presence of TH2 cytokines such as IL-4, IL-5 and IL-13, which may condition B-cell maturation and the switch towards IgG4 production (19).

As far as CV is concerned, the role of the interplay between B cells and environmental factors is well known. HCV plays a central role in the patho-

genesis of CV, based on a number of empirical observations such as the high prevalence of HCV antibodies and genome in the serum of MC patients, the high concentration of viral particles in the cryoglobulins, the immuno-histochemical localisation of HCV or its markers in peripheral blood mononuclear cells and in tissue samples (20). Cryoglobulins are generated by B-cells clonal expansion which finds its first step in HCV lymphotropism. However, although many steps forward have been made in the knowledge of CV, it is not clear why only a part of HCV-infected patients (40–60%) develop the cryoglobulinaemic syndrome. Recent studies have suggested that the host's genetic background plays an important role in determining the susceptibility to CV of some HCV-infected patients. On the basis of this hypothesis, Zignego *et al*. focused their attention on this aspect. They established that the abnormal presence of large immune-complexes in CV may be due either to the reduced uptake of immunoglobulins by reticuloendothelial system or to the excessive production of immunoglobulins by B-cells, so they analysed allelic variants of the low-affinity Fcγ receptor (FcγR) genes and of BAFF promoter. Indeed, low affinity Fcγ receptors mediate the clearance of circulating cryoglobulins, while BAFF, a tumour necrosis factor α (TNF-α) family member, regulates immunoglobulin secretion, B-cell differentiation and survival. From this study no significant differences emerged in the distribution of FcγR genotypes while a greater prevalence of BAFF promoter's T allele homozygosity was observed in patients with CV than in HCV infected patients without cryoglobulinaemic syndrome. It has been observed that T allele is associated with elevated serum BAFF levels and with the presence of CV. These results confirm that the host's genetic background may have a significant role in the development of cryoglobulinaemic syndrome (21).

The importance of the genetic background, environmental factor and humoral response has been outlined also for AAV. Because of the rarity of AAV, genetic studies in AAV have been challenging. In recent years, the stronger genetic associations have been described between the HLA-DPB1*0401 allele and ANCA-positive GPA and between the PTPN22*620W allele and ANCA-positive MPA and GPA (22, 23); these data show the possible link of these two alleles to a positive autoantibody status as in other autoimmune diseases (24). Many other genes have been recently investigated in AAV, in particular in GPA with contrasting data. Mahr *et al*. have studied the already known relationship between alpha1-antitrypsin deficiency variants Z and S and GPA in a large cohort of patients; with this genetic study they confirmed the data of a predisposing role of α1AT deficiency for GPA and demonstrated for the first time that the susceptibility to the disease is most strongly determined by the subset of homozygous (ZZ, SS) or compound heterozygous (SZ) genotypes (25). In another study by Morris *et al*. in 2011, the same association has been studied in 856 White Europeans with AAV by demonstrating that only the Z but not the S deficiency allele is associated with this group of vasculitis (26, 29). In summary, the evidence from genetic studies in AAV suggests that the genetic background consists of risk factors that are common to several autoimmune diseases, (such as PTPN22 R620 W). In the future, the genome-wide association studies will be a powerful tool to identify further genetic risk factors. It will then be important to link identified genetic risk factors to functional studies to fully understand the genetic basis for AAV and their subgroups or phenotypes.

In genetically susceptible individuals, environmental agents could trigger the onset of AAV; over the past 15 years many epidemiological evidences have suggested a causal role of bacterial infections. This hypothesis is confirmed by the significantly higher incidence of nasal carriers of *S. aureus* between GPS patients and by the increased nasal colonisation with the same bacterium in their relapses. In this pathogenetic pathway an important role is played by antimicrobial peptides (AMPs). Recently Hui *et al*. have revealed a dysregulation in the secretive response of nasal epithelial cells to *S. aureus* stimulation in GPA patients and the altered production of these peptides could lead to the higher bacterial colonisation rates (27). A molecular mimicry between pathogen and host proteins has often been proposed as an explanation for the association between infection and AAV. In fact, it remains difficult to understand what triggers the breakdown in immune tolerance and initial generation of ANCA.

Although ANCAs might explain the predominance of AAV vasculitis manifestations, such as glomerular nephritis in patients who express them, different pathogenic mechanisms may account for ANCA-negative patients and, in general, for non-vasculitic manifestations including granuloma lesions.

From this point of view the recent acquisition on the role of T cell in systemic vasculitis is of the pivotal importance. In renal, pulmonary and nasal samples of AAV-affected patients, activated T cells it can be demonstrated that as important components of the GPA and CSS granuloma (28, 29), starting from this evidence, an increasing number of studies have been performed to characterise the T cell-subsets implicated in AAV pathogenesis.

GPA is generally considered a Th1-type cytokine mediated disorder, indeed both in mice and humans $CD4^+$ Tcells and in particular effector memory T cells ($CD4^+$ T_{EM}) seem to give a large contribution to the formation of granuloma and to the disease progression: thanks to the lacking expression of the lymph node homing receptors CCR7 and to an increased expression of chemokine receptors (such as CCR3 and CC5), these $CD4^+$ T_{EM} cells could be recruited to sites of inflammation(30). This finding is confirmed by the observation of a lower number of circulating $CD4^+$ T_{EM} cells, most of them negative for FoxP3, in GPA during the remission phase (31). In addition, Klapa *et al*., besides the demonstration of a dysbalance between effectors and regulators T cells, found an alteration in the phenotype of T-reg cells of GPA patients by testing their *in vitro* suppressive capacity: the majority of these $CD4^+$ T_{EM} do not co-express FoxP3 and CCR4 and show

an impaired suppressive function (31). The latter seems to be due to an isoform of FoxP3 (*FoxP3Δ2* isoform) unable to block the IL-17A mRNA transcription with the subsequent conversion of T-reg in effector T cells producing IL-17 (Th17) and the formation of necrotising lesions of AAV (32).

In CSS, T cells predominantly exhibit an activated TH2 phenotype, resulting in the secretion of high levels of IL-4, IL-13,21 and IL-5 which are in turn essential for eosinophil activation, maturation, and survival. More recent studies even showed the possible cross-talk between eosinophils and TH2-type lymphocytes in CSS, via the secretion of IL-25, a potent TH2-response enhancer, by the eosinophils themselves (33).

Other cells are also apparently implicated in CSS pathogenesis, including regulatory T cells (Tr1) and Th17 cells. Although the contribute of Th17 cells it is still not well understood, there are many evidences about their role in vasculitis symptoms due to the production of a wide variety of proinflammatory cytokines, such as IL-17A, IL-17F and IL-22 which are demonstrated to be increased in active CSS (33). Besides Th2 and Th17 cells, it has also been suggested an important role for T-reg cells and for an imbalance between effector and suppressory cells in maintaining inflammatory reaction. In fact Jakiela *et al*. has recently investigated the T-reg function in CSS treated with steroids only or with steroids and immunosuppressive drugs and confirmed the correlation between Th17/Treg ratio and disease activity with some differences depending on the treatment (33). In particular a lack in controlling effector T-cells appears most evident in CSS patients treated with steroids only where both Th2 and Th17 lineages are increased also during remission.

As far as CV is concerned, T cell-mediated immune response has greater importance in the production of anti-viral cytokines but seems to have a role also in cytotoxic damage. In this regard, recently a substantial divergence in cytokine production have been claimed between subjects with HCV infection not associated with CV and patients with HCV related CV. In particular, Antonelli *et al*. have shown an emerging role for chemokines and type 1 cytokines in the pathophysiology of this vasculitis. Serum levels of interleukine-1β (IL-1β) are significantly higher in patients with MC+HCV compared to healthy controls and patients with HCV. Furthermore, high serum levels of interferon γ inducible chemokine (CXCL10) have been observed both in patients with HCV and patients with MC+HCV. Increased CXCL10 and IL-1β levels were associated with the presence of active vasculitis in MC+HCV patients (34). Furthermore, Antonelli *et al*. have demonstrated that subjects with CV associated with autoimmune thyroiditis (AT) have a different serum expression of cytokines compared with subjects only with CV. Indeed, patients with MC+HCV and those with MC+AT have higher serum levels of interleukine 6 (IL-6) and TNF-α compared with healthy controls. Moreover, circulating IL-6 levels are significantly increased in MC+AT patients with respect to CV (35). The revelations of Antonelli *et al*. seem to indicate a role of cytokine pattern in different clinical expressions of the disease. However, we need further studies to understand if the determination of these cytokine serum levels can be used as clinico-prognostic markers in MC+HCV patients (34, 35).

Recent insights into the induction and maintenance therapies of systemic vasculitis

a) Large-vessel vasculitis: induction and maintenance therapy

Glucocorticoids (GC) represent the mainstay of treatment in patients with GCA. On the basis of the EULAR recommendations, the starting dose of prednisolone/prednisone should be 1 mg/kg body weight, maintained for about four weeks and then gradually tapered; however, their long-term use is limited by concomitant appearance of side effects (36). About 25% of GCA patients have a chronic-relapsing course and may require steroids for many years. Conflicting results are available on the use of non-steroidal medications in GCA. The efficacy of azathioprine as steroid-sparing agent has been shown in a small randomised controlled trial (RCT), but the resulting data were not very significant (37). The role of methotrexate has been explored both by three RCT and case-series (38-40), that have provided conflicting results; as a matter of fact, although literature data show a benefit effect of methotrexate in controlling disease activity, the same results are not available on the prevalence of long-term GC-related side effects and this leaves unanswered questions regarding the more appropriate dose to use. So far, no controlled trials are available on the use of cyclophosphamide in GCA. Recently, Henes *et al*. (13) have published a retrospective study that has shown the efficacy of cyclophosphamide for large-vessel vasculitis (both GCA and Takayasu arteritis), moreover, documented by clinical routine diagnostics and repeated FDG- PET/CT. The anti-TNF-alpha agents infliximab (41) and etanercept (42) did not give successful results in inducing and maintaining remission in GCA, while the recognition of the pathogenetic role of interleukin-6 (IL-6) in GCA, has opened the way to exploring the effectiveness of the IL-6 receptor inhibitor tocilizumab. After the first report by Nishimoto *et al*. in 2008, further studies have reported the successful utilisation, in terms of complete clinical response and normalisation of acute phase reactans, of tocilizumab given monthly at the dose of 8mg/kg in both patients with GCA and Takayasu arteritis (43, 44).

b) Cryoglobulinaemic vasculitis: induction and maintenance therapy

The potential goals of CV treatment are essentially to eradicate HCV (etiologic therapy); to limit or suppress B lymphocytes proliferation (pathogenetic therapy) and to ameliorate symptoms reducing the damage caused by circulating immune-complexes (symptomatic therapy) (45, 46). Treatment should be tailored to the single patient focusing on the clinical history, disease manifestations, possible co-morbidities and previous therapies (47). In cryoglobulinaemic patients with life-threatening manifestations (abdominal vasculitis, haemorragic alveolitis, hyperviscosity syndrome and sometimes acute motor

neuropathy and rapidly progressive glomerulonephritis) the first line intervention is represented by high dose of corticosteroids and plasmapheresis (46). Concomitant immunosuppressive therapy with cyclophosphamide might be needed to prevent the postapheresis cryoglobulin rebound (45). Rituximab (RTX) was recently used successfully in patients with CV and severe vasculitis refractory to conventional therapies (48). The common manifestations of severe CV are: skin ulcers, peripheral neuropathy (motor or sensory refractory to symptomatic therapy) and active glomerulonephritis. In these cases the best therapeutic option is represented by RTX; it destroys CD20-positive cells which may harbour HCV and play an important pathogenetic role in cryoglobulin production. Indeed, RTX decreases serum cryoglobulin and RF levels and increases C4 levels with the disappearance of bone marrow B-cell clonal expansion (46). Glomerulonephritis responds to RTX within the first 3 months; skin ulcers usually improve within 3 months but complete healing requires a longer time. Both sensitive and motor neuropathy improve within 1-5 months with a stable or improved electromyography. RTX can increase HCV viral load without significant liver impairment; it has been given to CV patients with liver cirrhosis and led to an improvement in both MC symptoms and liver function. This effect on liver functions may be due to the reduction of the hepatic B-cell infiltrated and to the improvement of the Kupffer cell functions (47). By contrast it may induce the severe reactivation of HBV so it should be used in HBsAg-positive patients and in occult HBV-carriers (anti-HBc positive patients) only when strictly needed and in combination with antiviral therapy (49). The duration of the response to a single cycle of RTX is difficult to define because of lack of long-term follow-up data; long-term responses (more than a year) have been most frequently observed and re-treatment with RTX after relapses has proved to be effective in most cases. The possible persistence or onset of CV in patients with persistently negative serum HCV-RNA finding suggests that the autoimmune process can become independent of viral triggering and play a predominant pathogenetic role in some disease stages. So the antiviral therapy cannot be considered as a first step option for severe cases. It has recently suggested that patients with serious clinical manifestations may receive a combination therapy that is RTX, as first step, and when the efficacy and safety of it have been demonstrated, it could be administered antiviral therapy. This approach seems to reduce the time to clinical remission and to be more effective than the standard therapies (50). Antiviral therapy remains a cornerstone for the management of CV in HCV-related cases and it should be used for more stabilised patients, particularly in those with non-severe CV manifestations, such as constitutional features, purpura or arthritis, very mild renal and neurologic features. The current standard antiviral treatment is the combination of pegylated interferone alfa (Peg-INF-a) and ribavirin (RBV). The decision to treat patients with chronic hepatitis C depends on multiple parameters including a precise assessment of the severity of liver disease, the presence of absolute or relative contraindications to therapy, its previous failure or intolerance. The HCV genotype is systematically determined before treatment since it determines the duration of treatment (up to 48 weeks for HCV genotype 2 or 3 and 72 weeks for 1 or 4 (49)) and the dose of RBV (51). Peg-INF combined with RBV lead to 41-54% sustained viral response in the case of genotype 1 and approximately 80% in the case of genotypes 2 and 3. When antiviral therapy is not effective, contraindicated or not tolerated, treating patients with RTX should be considered (52). The treatment of non-HCV-related CV is usually similar to that of other vasculitis with steroids and immunosuppressives in most severe cases. In these cases treatment with RTX also seems effective (53, 54). In the future, the treatment of HCV-associated CV may be improved by the administration of an antiviral triple therapy in which the standard Peg-INF and RBV may be associated with one of the new protease inhibitors (telaprivis or boceprevir) especially in patients infected by genotype 1 HCV (55). Good results have been obtained in one study in which HCV-related CV has been treated with la ow dose of interleukine 2 (IL-2), a cytokine that promotes regulatory T cell survival and functions, without short-term detrimental effects on HCV infection (56). It can also be supposed in the future a treatment of CV patients with anti-BAFF monoclonal antibody, a molecule that regulates B cell proliferation (Belimumab).

c) AAV vasculitis:
induction and maintenance therapy

The evidence base for the management of the AAV has been recently established by the EULAR on the basis of the results of a series of randomised controlled trials conducted by the EUVAS Group during the 1990s and 2000s (57, 58). According to these guidelines, the AAV are conventionally treated with a staging approach strategy of remission induction followed by maintenance therapy. The majority of patients with AAV receive glucocorticoids combined with CYC for induction followed by AZA with a glucocorticoid taper for maintenance. Patients with mild, non-organ-threatening disease may receive MTX as an alternative to CYC, while patients with severe life-threatening disease or significant renal impairment receive adjuvant plasma exchange (57, 58). Recently, on the basis of the new acquisition about the role of B-cells in the pathogenesis of AAV, two randomised controlled trials have been published that question whether the routine use of CYC should remain the induction therapy of choice for most patients with organ-threatening AAV. Two recent trials (RAVE and RITUXVAS) have compared the oral Cyc regimen (59) and the pulse Cyc treatment (60) with Rituximab in a population of patients with severe ANCA positive vasculitis. Both the "rituximab for AAV study (RAVE)" and the "rituximab *vs*. CYC in ANCA-associated renal vasculitis (RITUXVAS) trials" showed that rituximab was not inferior to CYC for remission induction. Despite these encouraging data, there are still unanswered questions regarding the optimum dose regi-

men for rituximab and the optimum use of RTX in AAV. At present, therefore, its usage is now recommended in premenopausal women in whom the risk of permanent infertility is high and in relapsing patients who will be at greatest risk of cumulative CYC toxicity. In addition Holle *et al*. have recently showed that that RTX is less efficient refractory GPA patients with granulomatous manifestations compared to vasculitis manifestations (61).

Recently a randomised study has also compared the efficacy of Rituximab and Infliximab in a population of refractory GPA patients (62). Infliximab was given at the initial dose of 3 mg/kg, which was increased to 5 mg/kg in cases of absent or partial response, while Rituximab was administered iv at dose of 0.375g/m2 on days 1, 8, 15 and 22. The results after 12 months confirm the previous acquisition regarding Rituximab, with prolonged complete remission obtained in most of treated patients, while only 33% of patients under infliximab therapy reached the end point of remission (62). In addition, in the case of infliximab failure or relapsing, Rituximab succeeded in inducing remission in most patients. As regards other anti-TNF-α treatments, fewer data are still available; the Wegener's Granulomatosis Etanercept Trial Research Group (WGET) has recently confirmed the inefficacy of etanercept in inducing remission in GPA patients and reported an higher incidence of solid cancer (63); more promising are the results of a preliminary study on adalimumab given at doses of 40 mg every two weeks for three years with the end point of remission in GPA with renal involvement: in the small sample considered, the humanised anti-TNF-α antibody could be effective in association with standard immunosuppressive drugs (60). However, in the future, further data are awaited concerning the long-term efficacy and safety of anti-TNF-α monoclonal antibodies approach.

As far as maintenance therapy is related, the recently reported IMPROVE trial compared MMF and AZA questioning whether there were acceptable alternatives to the conventional AZA for maintenance remission in AAV (64). This randomised controlled trial (RCT) included 175 patients with new-onset AAV who had achieved remission with CYC, and were randomised to 2 g/day MMF or 2 mg/kg/day AZA. The primary end point was time to first relapse. The relapse rate was greater in the MMF group (42 (55%) out of 76) compared with the AZA group (30 (38%) out of 79) and the relapses occurred more quickly. The adverse event rate was the same, supporting the use of MMF mainly in patients intolerant to AZA. In conclusion, during the last two years, promising advances have been made in the treatment of AAV. However, only long-term follow-up data from RCTs will fully elucidate the efficacy of these novel therapeutic strategies on outcome in terms of late mortality and damage. New trials are under way, exploring the role of intermittent B cell depletion for maintenance of remission.

d) CSS:
induction and maintenance therapy

CSS has been generally considered a milder type of AAV, with a lower mortality and a higher remission rate in comparison to GPA and MPA. It is however, widely accepted that patients affected by CSS generally require corticosteroids for prolonged periods due to disease relapses or grumbling disease manifestations (*i.e*. asthma, peripheral hypereosinophilia) (65, 66). Novel insights have also recently demonstrated that CSS is a heterogeneous disorder both clinically and pathogenically (67). Thus, during the last two years a great effort has been made in order to search for novel steroid-sparing therapeutic strategies, targeted to the different pathogenetic pathways of the disease. Traditionally, treatment of CSS has been influenced by patients' prognosis assessment determined on the basis of the FFS. Patients with a FFS=0 corticosteroids alone have been proposed as induction and maintenance therapy. On the contrary, for patients with at least one poor-prognosis factor (FFS≥1), cytotoxic drugs have been employed similarly to the other AAV to induce remission. Two recent multicentre trials from the French Vasculitis Study Group have outlined that in patients without poor-prognosis factors the paradigm of treating patients with glucocorticosteroids alone needed to be revised. On the other hand, for patients with at least one poor-prognosis factor, cytotoxic drugs were always necessary to induce remission as is done for other AAV. This was mainly due to the fact that despite the achieved remission, relapses were documented in the 35% of the patients without poor-prognosis factors and 25% of all the participants needed addition of immunosuppressant and persistent glucocorticoid maintenance therapy for residual asthma (68). Again, patients with FFS≥1 required maintenance therapy with a less toxic immunosuppressant to avoid relapses (69). In the last two years therefore, an additional trial investigating glucocorticoids and azathioprine as first-line therapy in CSS without poor-prognosis factors has been promoted and is currently underway (ClinicalTrials.gov number NCT00647166). Methotrexate (MTX) has also been used safely and succesfully for the induction of remission in non-life-threatening CSS (70). However, the ability of MTX to prevent relapses in CSS appeared limited. In addition, as with any other maintenance regimen, to date, we do not know exactly for how long to continue MTX maintenance therapy even if it is widely accepted that this regimen should last at least 18 to 24 months (71).

Besides MTX, interferon-alpha (INF-α) has been also used for induction and maintenance of remission in patients with refractory CSS. The rationale of employing INF-alpha is represented by the fact that it can inhibit eosinophils degranulation and can reverse pathogenetic TH-2 mediated immune responses especially through the down regulation of interleukin-5 which has a pivotal role in driving eosinophilia. However, data on its ability to prevent relapses and its safety profile during long-term use seems unfavourable particularly, as far as cardiac toxicity is related. In a recent prospective open-label long-term observational study, 13 patients with CSS in stable remission received interferon-alpha (3 x 3 Mio. I.U/week s.c.) for maintenance of remission. Primary end-point was the incidence of

relapses. Secondary end-points were the doses of concomitant prednisolone and the frequency adverse events. After a median follow up of 64 month three patients were still on treatment with interferon-alpha all with a dose of 9 million units/week. In nine patients, interferon-alpha was discontinued for lack of efficacy (n=5), due to adverse events (n=2), or both (n=2) after median of 63 months (15–153) of therapy. A total of 3 major and 18 minor relapses occurred in 10 of the 13 patients with a median time to first relapse of 17 months (range 5–46). Four episodes of worsened asthmatic symptoms associated with a mild rise of blood eosinophils occurred in 3 patients and resolved following a transient increase of the oral prednisolone dosage. After 49 months one patient died probably due to a relapse. IFN-alpha was ceased prematurely, because of autoimmune-thyroiditis in one, depression in another and cerebral leukoencephalopathy in two patients. Overall, 18 infectious episodes with need of antimicrobial treatment were observed. The major conclusions of this study were that due to the numerous side effects and infections during long-term administration its use should be restricted to patients with contraindications against conventional immunosuppressive therapies (72).

Another novel potential therapeutic option to target the IL-5-related pathways in CSS is represented by mepolizumab a monoclonal IgG neutralising antibody against interleukin-5 (73). Recent data remarkably demonstrated that mepolizumab is an effective corticosteroid-sparing agent for patients with refractory eosinophilic asthma and with hypereosinophilic syndromes negative for the FIP1L1-PDGFRA fusion gene, which are able to reduce blood and tissue eosinophil counts (73). It has been, therefore, suggested that mepolizumab might be able to induce remission also in CSS allowing for steroid reduction. This hypothesis has been tested in two recent pilot studies including, respectively, seven (74) and ten steroid-dependent CSS patients (75). Most patients were able to taper their steroid dose, achieving a better control of the disease and a reduction in eosinophil counts; however, relapse rates were high after discontinuation of mepolizumab.

Targeting eosinophils and asthma omalizumab, a murine anti-IgE antibody, has been also successfully used in some CSS patients but it has been also viewed as a possible CSS-triggering factor probably because it allows steroid tapering, unmasking CSS “forme fruste” (18).

In a limited number of ANCA-positive patients refractory to conventional immunosuppression, on the other hand, on the basis of the experience gained from the other AAV, rituximab has been successfully used. However, it has also been suspected that rituximab has provoked immediate and severe bronchospasms in two ANCA-negative patients (76). An open-label study on rituximab use in CSS patients with renal involvement is currently underway (ClinicalTrials.gov, NCT00424749).

In conclusion, an increasing number of reports have indicated the possibility of employing novel promising drugs for inducing and maintaining remission in CSS with the specific aim of sparing steroids and limiting steroid side effects. Concomitantly a great interest has arisen in trying to distinguish CSS subgroups in order to tailor patients’ treatment according to the pathogenetic pathways underlying the different disease subtypes. From this point of view, considerable emphasis has been attributed to the pathogenetic role of B cells in ANCA-positive patients or to the IL-5 pathways in sustaining hypereosinophilia in ANCA-negative patients. However, whether the effectiveness of these new drugs might differ according to ANCA status remains to be elucidated.

Conclusions

In summary, during the last two years, novel and important steps forward have been made in the research field of systemic vasculitis. Novel pathogenetic pathways underlying these complex diseases have been unravelled opening new opportunities for targeted therapies. Novel therapeutic strategies have been proposed to achieve and maintain clinical remissions in small- and large-vessel vascultis and large multi-centre randomised controlled trials (RCTs) have been published resulting in evidence-based therapeutic approaches with higher efficacy and less toxicity compared to previous regimens.

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