Chapter

Cellular and Molecular Characteristics of Vascular Damage in Giant Cell Arteritis, the 'Unmet Needs' for Targeted Treatment

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Abstract

Giant cell arteritis (GCA) is a primary systemic vasculitis characterized by systemic inflammation and vascular insufficiency of large and medium blood vessels which may lead to end-organ damage in patients age 50 and older. Standard corticosteroid treatment of GCA significantly improves the intima-media thickness while having less influence on vascular endothelial dysfunction. GCA morbidity may be related to both cardiovascular complications and corticosteroid toxicity. Therefore, we aim to discuss 1) characteristic aspects of vascular damage, 2) several mechanisms that cause vascular dysfunction, intima-media 'nodular' thickness, progressive narrowing of the arterial lumen and vascular blockage in the context of systemic inflammation, thrombosis and of the cardiovascular complications in GCA and 3) new therapeutic glucocorticosteroid-sparing (GS) agents which might be a more productive way of avoiding the invalidating or life-threatening cardiovascular complications of GCA.

Keywords: giant cell arteritis, mispositioned inflammation, vascular remodeling, von Willebrand factor, thrombosis, GS-saving therapeutic agents

1. Introduction

Vasculitis is a heterogenous group of conditions characterized by inflammation of the vessel wall resulting in narrowing or occlusion of the vessel lumen, aneurysm formation and impairment of downstream organ functions [1–3]. Vasculitis is classified according to the predominant size of the vessels involved into large, medium or small vessel vasculopathy [1–3].

Giant cell arteritis (GCA) is an autoimmune disease of the blood vessels, a disease where the immune system not only fails to protect but actively damages the blood vessels [4]. It is the most frequent primary vasculitis in adults. It manifests in patients mid age and older (odds 1:500 in this age group) [1]. The estimated annual incidence worldwide is 2.4 to 32.8 cases per 10^4 [3, 5]. GCA is a large-vessel vasculitis which has tropism for thoracic aorta and its extracranial branches [3, 6]. It affects mostly ethnic groups of northern European descent [7], especially female

gender (3:1 ratio) [7, 8], and immune response polymorphisms associated with HLA-DRB1*04 alleles [9]. In a genome-wide association study collected from over 2000 GCA patients of European ancestry [9], two independent signals in the human leukocyte antigen MHC HLA II class region were reported recently to be strongly correlated with GCA [9]. Plasminogen and prolyl 4-hydroxylase subunit alpha 2 (an enzyme involved in collagen synthesis) have important roles in vascular damage and neoangiogenesis [9] and gene variants of both proteins are related to high GCA risk, suggesting a role of these factors in the underlying pathologic mechanisms of GCA [9]. The nongenetic etiopathogenesis of GCA is also not completely understood [1]. The role of environmental factors (the incidence of the disease increases seasonally) [7], viral (herpes-infected mice develop large vessel arteritis [10]) and bacterial infectious agents was reported [1, 7, 10].

The arterial adventitia (the external arterial layer where the GCA inflammation starts before it spreads inward) is rich in dendritic cells (DCs) [4]. These cells get activated by the interaction of their own toll-like receptors (TLRs) with pathogen-associated molecular patterns (PAMPs) [11]. Dendritic cells are immunosurveillance cells which belong to the vessel wall, and, when activated, synthesize proinflammatory cytokines leading to activation of GCA pathogenic cascade [12–14]. To date and to our knowledge, no unique triggering pathogen has yet been singled out in GCA [1, 4].

GCA has a large spectrum of clinical manifestations [1], and many times it goes undiagnosed until a considerable amount of damage is done and complications occur [1]. People with GCA often are referred to several specialists: ophthalmologist (for partial/total visual loss), neurologist or ORL (for severe headaches and jaw pain), rheumatologist (polymyalgia reaction); when in fact GCA is a problem caused by inflammation in arteries of large and medium size [1, 6]. Ischemic complications of GCA and other systemic complications might be resulting in significant comorbidities and death [8]. If treated promptly and properly, the life expectancy does not change [15].

Temporal artery biopsy (TAB) is a gold standard for diagnosis of GCA [1]. TAB reliability might be reduced by the segmented pattern in which the lesions occur in the blood vessel [16, 17] and by longer time treatment (over 2 weeks) [1, 18].

Due to the fact that imaging testing might not be readily available, the use of biomarkers in laboratory testing for GCA is highly valuable but there are no specific diagnostic or prognostic blood biomarkers yet found for GCA [1]. The main criteria used to diagnose GCA are the elevated inflammatory markers [1] usually associated with for ischemic events. Acute phase reactants frequently have high blood levels: erythrocyte sedimentation rate (ESR) and more sensitive C-reactive protein (CRP), elevated platelets and white blood cell numbers, elevated blood von Willebrand factor (VWF) [1, 19, 20]; and in addition, GCA is associated with normocytic normochromic anemia [1]. Elevated serum level of the proinflammatory cytokine interleukin 6 (IL6) is critical in GCA pathogenesis and perhaps the most sensitive marker in GCA [21].

GCA is usually evaluated together with another related condition, polymyalgia rheumatica (PMR) [22]. PMR is not a vasculitis, but its relationship to GCA requires discussion because in some patients PMR evolves into GCA and 40–50% of GCA patients have polymyalgic symptoms [23]. In 20% of PMR patients with subacute presentation (proximal stiffness located in the shoulder area), abnormal blood work and high ESR and CRP, the disease will progress to GCA [22]. Imaging and pathology studies have shown subclinical arteritis may be much more common with PMR patients [18]. It is possible these two conditions represent two distinct phenotypes of the same pathological entity [23].

The standard therapy for GCA patients is glucocorticosteroid treatment [18] because it makes GCA symptoms better immediately, especially in ischemic complications when it is lifesaving or avoids a permanent invalidity (partial/total visual loss) [21]. For instance, heightened vascular inflammation progressively affects more vessels, compromising carotid vessels and other branches from aorta, that supply blood to vital organs (GCA-related cerebrovascular events [24, 25] and GCA-related myocardial infarction [26]) [8]. GCA affects the eye as well, and patients can have sudden and painless visual loss [27]. Because of the risk of progressive visual loss, GCA is an ophthalmology emergency for which the treatment is high-dose methylprednisolone pulse therapy (1000 mg i.v. for 3 days) within 24 h of the onset of GCA symptoms, which works as an effective 'emergency' treatment option to prevent evolving optic (and progressively central nervous system) [28] involvement [18]. If treatment is delayed more than 24 h, the blood supply to the eye can be cut off for enough time that restoration of vision might not occur. IV pulse therapy is followed with oral GS therapy [18].

In all, chronic administration of GS insures 90% of GCA patients will have GS-related side effects [29]. Therefore, a lot of research nowadays is focused on what treatment we can give instead of or in combination with GS to minimize side effects of GS in GCA, and in all the other types of vasculitis where we depend on GS for the immediate beneficial effects as well [30]. Ongoing clinical trial investigations are showing promising preliminary results. Most of the newer targets are not yet used in clinical practice because of toxicity, poor efficiency or because are under development, but nevertheless these biologics are key in deciphering the pathogenic mechanisms of GCA.

In this study we aim to (1) describe the characteristic aspects of vascular damage; (2) discuss several known mechanisms that cause vascular disfunction in GCA; (3) overview new therapeutic GS-sparing agents which might be a more productive way of avoiding the invalidating or life-threatening cardiovascular complications of GCA. We focus on the current knowledge updates about the GCA pathogenic mechanisms subsidiary to ischemic complications of the disease and their targeted treatment.

2. Characteristics of vascular damage in GCA arteries

Healthy arteries have a large lumen, the arterial wall histology comprises three distinct layers. The adventitia is the external layer, separated from the medial layer by the external elastic lamina, the medial muscular layer is separated from the internal layer by the internal elastic lamina and the intima is composed of unistratificated endothelial cells (ECs) [3]. Positive temporal artery biopsy (TAB) is the gold standard of GCA diagnosis [31], however due to the segmented pattern in which the lesions occur a TAB in a segment without lesion will generate a false negative GCA test. The typical histopathology of GCA arteries highlights transmural granulomatous inflammation. It consists of transmural mononuclear cell infiltration, mostly T cells, macrophages, numerous multinucleated giant cells (seen in 75% of cases), surrounded by low number B cells in all the layers of the artery [16]. The destruction of the internal elastic lamina, vascular smooth muscle cells (VSMCs) from the media to the intimal layer, and intima hyperplasia (IH) [1] are pathognomonic for GCA. IH protrudes in the lumen, progressively causing the occlusion of the vessel. Intraluminal thrombosis is reportedly seen in about 10–20% of GCA cases, probably occurring more frequently in the conditions of high shear in artery stenosis in GCA ischemic complications. A negative TAB does not exclude GCA [1].

The kind of inflammation pattern the vascular wall will take depends entirely on the initial injury events. In GCA, the site of inflammation is restricted the blood vessel wall [4, 11]. Typical GCA pathological findings involve local vascular inflammation, granulomatous infiltrate, and segmented lesions alternating with healthy segments [16]. GCA is characterized by a highly specific tropism to medium and large arteries of the upper extremities, neck and head in older people [1, 11, 32]. On the other hand, the fact that PMR and GCA have similar clinical presentation and a percentage of patients with GCA also have PMR suggest that these two clinical presentations might be two distinct phenotypes of the same pathological process [23].

Intriguingly, topography and histological structure dictate whether GCA lesions are likely to occur. GCA develops mainly in the 2nd-5th extracranial branches of the carotid arteries and upper extremities branches of the proximal aorta that have a highly visible internal elastic membrane and vasa vasorum. Temporal arteries are the most affected [1], and the vertebrobasilar [28] and the ophthalmic arteries can be affected as well [33]. Intracranial branches of the cervical arteries are small vessels that are not prone to GCA as their elastic membranes are very thin to inexistent and lack vasa vasorum because they are nourished through diffusion not through vasa vasorum [34, 35].

2.1 Vasculitis initial events

Under physiological conditions, the vascular wall mainly acts as a barrier between circulating immune cells and surrounding tissues. Besides its role in blood transport, blood gas regulation and preservation of wall integrity, the arterial network also has an immunosurveillance function [4]. The only immune cell known to be present in the healthy vascular wall is the intramural DCs, found in an immature state, characterized by the expression of C-C motif chemokine receptor 6 (CCR6) and a low density of Toll-like receptors (TLRs) [14].

Under pathological conditions, the large arteries turn into a target of autoimmune disease [4]. The main immunopathogenic mechanisms in the GCA arteries are progressing from the outside to the luminal side of the arterial wall, from the adventitia, where mural DCs reside, towards the intima [4].

2.1.1 The crucial role of dendritic cells in GCA pathogenesis

To date it is well known that DCs are the antigen-presenting cells (APCs)-belonging to the vessel wall-responsible for the initial steps in GCA pathogenesis [14]. Arteries use their wall-embedded sentinel cells, the dendritic cells (DCs) [14], to intervene and generate inflammatory responses [11]. This ability of large arteries to control localized and systemic inflammation using indigenous cell populations is a critical trigger element in this primary vasculitis [11, 12, 14]. Vascular DCs are mostly localized at adventitia/media border [12, 14]. In the presence of unknown, diverse, and non-specific PAMPs, resident DCs activate and break the "immune privilege" [4] in GCA arteries [12], DCs initiate the pathogenic cascade [11] and DCs sense the danger from a distance through PAMPs interactions with their specific toll-ligand-receptors (TLRs) [11]. In contrast to physiological conditions, in GCA arteries, activated DCs at the adventitial/media border fail to leave the artery lesion site, meaning they don't migrate to a lymph node, are retained in the granulomatous infiltrates in the wall of the arteries [14], amplifying a mispositioned inflammation reaction [14].

The TLR cellular distribution in the GCA arteries is as follows: (1) immune cells: dendritic cells, T cells, monocytes, macrophages, and to a lower extent B cells (2) vascular cells: endothelial cells and vascular smooth muscle cells [11]. GCA artery

TLR fingerprint consist of high amounts of TLR2, TLR4 and TLR8, intermediate levels of the TLR1, TLR5, and TLR6 and lower or absent for TLR3 and TLR9 [11]. TLRs of vascular DCs are implicated in the strong tissue tropism of GCA [11].

In GCA, most severe inflammatory effects occur at the intima/media border, adjacent to the internal elastic lamina from the outside in, from the adventitia to the intima direction [14], but sometimes the inflammation is initiated in a tiny area in the vasa vasorum in intra-adventitial small vessels [31].

2.1.2 Recruitment, proliferation, and polarization of T cells into GCA arteries

Already activated adventitial DCs produce cytokines and chemokines (C-C motif chemokine ligand) CCL 19, 20, and 21 that trigger the recruitment of CD4+ T cell subpopulation, in proximity to vascular DCs [4, 36]. They in turn proliferate and synthesize chemokines (including CD4, CD61), creating an inflammatory environment [12]. Under the influence of the DCs and modulated by immune checkpoints [36], CD 4+ T cells subpopulation [37] differentiate into T helper 1 (Th1) cells and T helper 17 (Th 17) cells [38]. Next, T cells polarize into two T cell lineages defined by the production of their marker cytokines Th1 cells start producing interferon gamma (IFN_{γ}), while Th17 cells produce interleukins IL17 and IL21 [38]. IFNv-releasing T cells axis and IL17/21 T cells axis, respectively, have different immunomodulatory effects [4].

IL-12 and IL 23 stimulate Th1 and Th17 responses, respectively, both of which are believed to be involved in promoting systemic and vascular inflammation, progression, and maintenance of inflammation [39, 40].

Interleukin 6 is the cytokine that controls the balance between proinflammatory Th1 and Th17 cells and the regulatory T cells, particularly involved in GCA pathogenesis [4, 41]. Regulatory T cells normally ponder or inhibit the immune system response. The disturbance of T cell homeostasis is probably related to an imbalance in the amount of serum IL6 [42]. In patients with GCA, IL 6 is upregulated in the inflamed arteries and in circulation [5, 42]. Serum IL6 corelates with disease activity and is decreased when GCA is in remission [42]. T proinflammatory cells Th1 and Th17 are in excess [38], while regulatory T cell numbers are inhibited by excess interleukin 6 (IL 6) [42].

Upon T cell activation in presence of an antiself attack [14], inhibitory checkpoints such as T-cell–inducible immune checkpoint programmed death-1/programmed death ligand-1 (PD-1/PD-L1) pathway are instrumental to minimize potential immunopathology [36]. PD1/PD-L1 pathway has a role in maintenance of tolerance, protective immunity, preventing autoimmunity disease, and protection against collateral vascular damage [36]. PD-1 is expressed on activated T and DCs cells [36] and its coupling by its ligands PD-L1 or PD-L2 induces T cell receptor (TCR)-activation cascade in a Src homology region 2 domain-containing phosphatase 2 (SHP2) manner [36], resulting in immunosuppression. An aberrant PD-1/PD-L1 checkpoint in DCs (low amount of both PD1/PDL1) and T cells of GCA patients [36] is responsible for the observed DC-mediated hyperactivation of T cells, polarizing T cells to Th1 and Th17 [36]. The immunotolerance defect in regulatory T cells in GCA patients [41] is characterized by a stimulatory instead of inhibitory function of PD1-mediated immune checkpoint in GCA patients [36], followed downstream by a FOXP3 transcription factor defect in the regulatory T cells locally in GCA arterial lesions [41] which leads to decrease in the number of FoxP3+ T regulatory cells and hyperstimulation of proinflammatory T cells. In addition, the regulatory T cell population can be influenced with an IL6 receptor antagonist [41] which stresses the necessity of IL6 in these processes. Importantly, IL17 is controlled rapidly by glucocorticoids [4]. IFNy is resistant to corticoids, also to aspirin and NOTCH inhibitors [4, 43].

2.1.3 Monocytes differentiation/macrophages role in GCA-related vascular damage

Under an increased level of interferon-gamma (IFNy) produced by CD+4 T cells, monocytes and macrophages are recruited in the arterial wall [44]. IFNv primarily targets macrophages leading to their fusion together into giant cells (GC) [4, 44]. IFNv concentration was found to be elevated in arterial tissue from GCA patients with ischemic disturbances, including visual loss [44]. In addition, they reported patients with PMR and fever had elevated IL2 production [44].

Using *ex vivo* cultures of temporal artery biopsies, Cid et al., 2006 demonstrated that the role of IFNy is to induce the production of C-C motif chemokine ligand 2 (CCL2) (ligand of CCR2 receptor) by VSMCs [45, 46]. CCR2, the corresponding receptor, is also expressed by monocytes, binding of CCR2 on monocytes leads to the recruitment of monocytes and their differentiation inside all layers of the arterial wall [46]. INFy induces the production of C-X-C motif chemokine ligand (CXCL9, 10, 11) by VSMCs [46], linked to the recruitment of cells expressing CXCR3 which is expressed by Th1 cells and CD8+ cells [4]. This will initiate a positive feedback loop since T cells are being activated and produce more IFNy [4, 46, 47].

Macrophages have different functions depending on which vascular layer they are going to be trapped in (1) adventitial CD 68+ TGF β1+ macrophages produce proinflammatory cytokines IL1β and IL 6 [4, 11, 48]; (2) intimal-media junction macrophages secrete metalloproteinases to clear cellular debris. They are also responsible for unintended, pathological elastic membranes digestion; (3) macrophages in the intima layer have roles in cellular outgrowth [49]. Intima-located macrophages led to production platelet-derived growth factor (PDGF) [50] which is needed for dedifferentiation, proliferation and migration of VSMCs [50, 51] and vascular endothelial growth factor (VEGF) which is needed for neoangiogenesis [52].

IL1β and IL 6 levels are influenced by GS in GCA [43], in contrast, IL12 is mostly resistant to GS therapy [4, 18]. The lack of reaction to GS suggests a need for better therapeutic strategies to interfere with these pathologic cascades.

Monocytes and macrophages accumulate in high numbers generating granulomatous inflammation of large and medium arteries and, under stimulatory influence of INFv, they form giant cells (GC) by fusing together [4, 5].

In physiological conditions, GC are the body defense response against a foreign body or a kind of irritant, for instance, a splinter in the finger, the body will produce GCs to break up this irritant and remove it. In the case of GCA, 'the foreign body' is not known. It has been stated by some authors that it might be the arteriosclerotic plaque [53]. Macrophage multinucleated giant cells in pathological conditions of GCA are a unique cell population that produce different mediators leading to the destruction and faulty reconstruction of the arterial wall [4, 54].

3. Vascular remodeling of healthy arteries to GCA arteries

Regarding the GCA pathophysiology, studies have observed many interchangeable features dictated by the 'confused' immune system and by the cellular populations of the vascular wall itself mediated by blood factors. Upon the destructive actions of GCs, the vessel initiates a faulty PDGF- [50] and VEGF- [55] dependent maladaptive reparatory mechanism [55].

GCs' proteases digest the vascular wall at the level of the internal elastic lamina [54, 56, 57]. For instance, media-intima junction GCs are producing metalloproteinases (MMP-9 and MMP2) [57] as was demonstrated recently [54, 57], and also

other mediators. VEGF [55] is linked to neoangiogenesis and the recruitment of proinflammatory T cells via Notch/Jagged 1 dependent pathway [4, 5], reactive oxygen species (ROS) [54], and the other inflammatory mediators with proteolytic activity that cause breakage of the internal elastic lamina. The vascular response is a result of the two-way interaction between the hyperactive immune cells and the activated vascular cells. These processes lead to: (1) release of additional growth factors; (2) release of vWF from ECs Weibel-Palade bodies [19, 58–61]; (3) release of macrophage factors [13, 44, 48]; (4) media thickening in response to the immune insult [50] and deposition of extracellular matrix proteins (i.e. collagen); (5) intima myofibrotic hyperplasia (IH) [50]; (6) release of angiogenic factors in the vasa vasorum [62]; and finally, (7) upregulation of the proteinases [54] and downregulation of their inhibitors which causes the intima elastic membrane to tear [54], destroying locally the vessel wall.

Most importantly, the increased production of such mediators as PDGF [50, 51] and endothelin-1 [63, 64], initiates a faulty vascular repair process, leading to the activation, dedifferentiation, proliferation, and migration of the VSMCs from the arterial media to the intima. This leads to myofibrous intimal hyperplasia and "nodular" media thickening with granulomatous giant multinucleated cells infiltration, and neoangiogenic vasa vasorum [44, 62] characteristic to GCA pathology [51]. Typically, VSMCs turn from a contractile cell into a dedifferentiated, secretory, and migratory cell [5, 65]. Activated and injured vascular smooth muscle cells (VSMCs) produce growth factors (including PDGFs [55], TGF-β and ET-1 [63, 64]) that promote further myofibroblast dedifferentiation, proliferation and migration [4]. Reversely, pharmacologic blockage of the PDGF receptor or blockage ET-1 receptors [64] results in reduced IH in cultured GCA arteries [51, 64].

In all, hyperplastic cell outgrowth in the lumen of medium sized artery through autoimmune vascular remodeling progressively narrows the lumen, resulting in vascular stenosis and ischemia in the distal organs these vessels normally irrigate. The eye and the brain are at highest risk. The invalidating or life-threating consequences are severe, possibly visual loss or even stroke. Patients with GCA who have ocular ischemic complications have higher blood concentrations of ET-1 and other EC biomarkers, highlighting these biomarker potential role in thromboembolic vascular disturbances [63, 64].

3.1 The crosstalk between immune cells/vascular cells, faulty vascular repair and thrombosis in primary vasculitis

More and more evidence suggests the presence of crosstalk between mispositioned vascular inflammation [4] and thrombosis [5, 61, 66]. In the past the underlying molecular mechanisms of vasculitis have been overlooked, but more recently, it has become increasingly evident that inflammatory diseases of the blood vessels are associated with arterial [25, 67] and venous thrombosis [66]. In GCA, certain risk factors will seriously increase the likeliness of thrombotic events, including, but not limited to systemic inflammation, localized vascular inflammation, endothelial dysfunction and treatment-related complications [5, 68]. The higher risk of thrombosis during active disease underlines the role of inflammation in thrombogenesis [61, 68]. Furthermore, GCA patients have a greater risk of thromboembolic complications due to the advanced age, and to the other risk factors that they concomitantly have, such as hypertension, smoking, hypercholesterolemia, previous arterial thrombotic events, family history of thrombosis, and the presence of additional cardiovascular risk factors [25, 28, 61, 68]. This may contribute to the choice of administrating antithrombotic therapy: low dose aspirin 75–250 milligram per day prevents cerebrovascular events and ocular symptoms [69]. Antiplatelet therapy

influences arterial disease events [69], while anticoagulants and immunosuppressive medication have a debatable effect [21, 70].

3.1.1 Immune cells response in vasculitis

A growing body of evidence supports the above-mentioned 'outside-in' hypothesis that vascular inflammation is initiated and perpetuated in the adventitia in GCA and contributes progressively to medial and intimal remodeling [14]. Once the immune barrier is broken [12], the vessel expresses cell surface adhesion molecules in the vasa vasorum [55] and inflammatory mediators [5]. In result, the monocytes migrate towards the intima of the blood vessels [53]. Th1 and Th17 T-cells pathogenic pathways promote the production of IFN-ɣ and, respectively IL-17 [38, 38]. Th17 differentiation via its effector IL17 pathways induce chronic inflammation [71]. B cell differentiation contribute less to the formation of granulomatous structures [4, 5]. Cytokines like TNF alpha, interleukins IL1β and IL6 are promoting not only systemic signs in primary vasculitis (fever, malaise, weight loss, fatigue) but they also favor a prothromboembolic state [61, 67]. GCA complications comprise arterial [67] and, although less frequent, venous involvement, both DVT and PE [66]. In mouse models of DVT, it has been demonstrated high IL 6 during thrombogenesis [72], while inhibition of IL6 reduces expression of CCL2 which leads to a low recruitment of monocytes at the site of thrombosis of the vessel wall and the post-thrombotic syndrome [72]. IL6 is triggering an amplification and recruitment of monocytes which is in relation to the ability to express in excess cell adhesion molecules in vasa vasorum [4]. One puzzling observation from published data is that IL 6 pleiotropic effects on immune and vascular cells assert disease activity in GCA, however, when IL6 expression in the temporal artery is low, the IL6-induced angiogenic response is decreased without a protection mechanism against ischemic events in GCA patient [42]. Acute phase proteins are elevated: serum amyloid A (SAA), CRP, ESR, WBC count and platelet count [1]. GCA patients having ocular complications have significantly decreased level of SAA, CRP and ESR [27] as well as high VCAM1 levels [73]. GCA patients during relapses had significantly higher levels of SAA, CRP, ESR and WBC counts [73, 74].

Hyperactivated T cells and macrophages organize the granulomatous lesions in the vessel wall [44], destroy the media layer, the inflamed artery initiates an abnormal vascular repair program [11], inducing ischemic organ damage through intimal hyperplasia and luminal occlusion changes [5, 36]. Elevated IFNv was demonstrated to be correlated with neoangiogenesis [4, 62], and IH [46], two critical processes in vascular remodeling in GCA artery. Persistance of IFNv correlates with chronical arterial inflammatory disease [4]. Clinical variations in GCA are correlated with local expression of cytokine mRNA (elevated IL1β, IL6, TGF, IL2, INFy, IL17, 13). Heterogeneity of immune response and targeting of the arterial tissue in a TLR-specific manner explain the diverse clinical manifestations inside GCA spectrum of diseases [4, 11].

3.1.2 Vascular wall response to vasculitis

In GCA pathogenesis it is thought that the vascular wall response has the same impact as the immune cell response [4].

3.1.2.1 Arterial luminal vs. vasa vasorum endothelial cells response in GCA

Vascular endothelial dysfunction was previously reported in GCA [53]. It is associated with elevated blood levels of proinflammatory endothelial factors that

have important roles in the pathogenesis of GCA: endothelin 1 (ET-1, 64], and cell adhesion molecules [19, 64, 75], and von Willebrand factor (VWF) [19, 20, 53, 59]. The presence of the proinflammatory and procoagulant factors at GCA lesions sites is indicative of the extensive crosstalk between immune system and vascular cells [61]. The contribution of the blood vessel wall to the GCA pathogenesis is stressed by the fact that symmetrical, collateral vessels are much more likely to be affected (implicating GCA strong tissue tropism [4] in contrast to, for instance, atherosclerotic diffuse display which also manifests in this age group [53]) as demonstrated in these patients by PET scans/CT angiography imaging [17, 73].

Thrombin, the main protease in the coagulation cascade, also has numerous effects on the endothelium, i.e. thrombin-induced expression of chemokines that trigger binding of platelets and monocytes to the endothelial surface [76] and increased permeability across endothelium [77]. By these and many other mechanisms [78], thrombin is coupling coagulation and inflammation [61, 78, 79]. The stimulatory effects of thrombin on ECs and platelets occur mainly through activation of the protease-activated receptors (PARs) [79]. PARs are seven-transmembrane G protein coupled signaling proteins [80]. PAR1 is the prototype for a family of four related receptors [79]. PAR1 is the key mediator of thrombin's effect on human ECS [79]. Thrombin activates PAR1 receptors which couple to Gαq/11 and Gα12/13 that upregulate the vWF and P-selectin secretion from the ECs storage granules named Weibel Palade bodies (WPBs) [79, 81, 82]. Soluble P-selectin is an adhesive molecule also stored in WPBs [81]. Relevantly, P-selectin secreted by luminal endothelium of the carotid artery in a murine vascular damage model was reported to be involved in monocyte trafficking and neointima formation [65].

One of the mechanisms that may cause endothelial dysfunction in primary vasculitis is the excess of proinflammatory cytokines that are depressing endothelial function [31]. Also, the inflammatory microenvironment is directly leading t0 endothelial cell toxicity. In addition, healthy and pathologically damaged cells are intercommunicating and interconnecting, for instance monocytes and GC interactions close to the vessel wall trigger endothelial damage. Another role of the endothelium in inflammation is in leukocyte trafficking (P-selectin, E-selectins expression) [55] and the expression of cell adhesion molecules in the vasa vasorum [55]. Transcript levels for markers of endothelial activation: VWF, ICAM1, VCAM1, CD31, VE-cadherin (and of myofibroblasts smooth muscle cells actin (SMA)) measured by RT-PCR in the tissues in a GCA mouse model [36] were found to be elevated by up to fourfold when PD-1 was blocked with anti-PD-1 Ab when compared with control IgG or vehicle-treated [36] immune checkpoint inhibition led to intimal hyperplasia, angiogenesis and nodular thickening of the media. Inflammation-induced effects caused by the endothelium in systemic inflammation disease include but are not limited to: (1) increased expression of procoagulant factors: VWF, plasminogen activator inhibitor 1 (PAI1), platelets activated factor (PAF), vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule (ICAM1) and tissue factor (TF) and (2) and inhibition of anticoagulation pathways and fibrinolysis activity: endothelial protein C receptor (EPCR), tissue plasminogen activator (tPA), thrombomodulin (TM), prostaglandin I 2 (PGI2), that are causing thrombotic tendency [61, 83]. In patients with visual disturbances there were reported high VCAM1 levels compared with GCA patients that did not have visual disturbances [73]. VCAM1 was also significantly correlated with large vessel envolvement [73].

Weibel-Palade bodies (WPBs) are the secretory granules of vascular ECs [81]. The main resident of WPBs is vWF [81, 84]. vWF is pro-inflammatory and prothrombotic agent which plays a central role in morbidity and mortality associated with systemic inflammation and cardiovascular disease. The fact that elevated vWF

in the circulation is a marker of inflammation-induced activation of ECs is well established [82, 85], but why do activated endothelial cell release von Willebrand factor in the context of the pro-inflammatory microenvironment in vasculitis, in particular in GCA, by which mechanism, is not completely understood; it was suggested it is part of the reparatory process. When an immune checkpoint is inhibited, endothelial cells are bigger in size and increased VWF expression and secretion was reported [36], indicative of endothelial cell maladaptive reaction. Moreover, VEGF derived from macrophages and the other immune cells have a stimulatory influence on Weibel-Palade bodies' secretion of VWF and angiopoetin-2 [86] involved in neointima formation. Intriguingly, it was reported that PDGF released by vascular dendritic cells [55] -on top of the above-described proinflammatory features- regulates vWF gene promoter.

vWF is an important molecular link coupling thrombosis and inflammation. It was found to contribute to systemic and vascular inflammatory manifestations of GCA. VWF levels are elevated in GCA patients circulation up to three-folds [19]. Highest vWF values are recorded at the onset of the disease [19], high blood VWF levels are persistent throughout the active disease period and remain elevated in some patients a long time after corticosteroid treatment [19, 59]. According to Persellin et al. elevated blood vWF values are not due to impairment of vWF formation or storage but to increased ECs secretion of normal von Willebrand factor, as shown by electrophoretic analysis of high molecular weight vWF polymers pattern [20]. High active VWF levels reflect vascular distress that predicts the course of the disease towards vasoocclusive problems [20]. Intraluminal thromboses were seen in 10% in TAB+ GCA, but in fact, the rate might be higher and hidden by the concomitant hyperplastic reaction in the intima. Several studies proposed VWF could be a parameter to monitor treatment or a parameter for diagnosis when the acute phase reactants (ESR) are normal in treated GCA patients. The fact that elevation of VWF is persistent throughout the steroid treatment suggests that GS treatment has little effect on the underlying endothelial disease. A significant percentage of patients receiving steroid treatment are developing irreversible vascular occlusive complications episodes (%) even after receiving GS treatment [67].

Several studies investigated the role of vWF in the formation of the hyperplasic intima (IH) [58, 60]. In one study, matching TABs and matching blood collection was done for VWF measurement. Increased vWF deposition in hyperplastic neointima mirrored high plasma vWF levels [19]. These data and other published data- that increased levels of vWF are associated with hyperplasia in grafts [87], and no occurrence of atherosclerotic plaques in vWF deficient pigs [88] -suggests a role for vWF in vascular remodeling and faulty vacular repair [60, 75]. Indeed, in a recent study by Lagrange et al. [58] it was found that vWF/LRP4/integrin α vβ3 axis stimulates proliferation of VSMCs: (1) vWF binds through its A2 domain to the VSMCs LRP4 receptor; (2) crosstalk LRP4 receptor-integrin α vβ3; (3) integrin α vβ3 activates Src signaling leading to vWF-dependent VSMCs proliferation [58]. Relevant to the aim of this review, their new findings provide new insights into the pathogenic mechanisms that drive pathological hyperplasia of the GCA arterial vessel wall. Moreover, the vWF/LRP4/integrin α vβ3 axis may represent a novel therapeutic target to inhibit VSMC proliferation, and, at least partially, prevent the maladaptive reparatory process in GCA [58].

At high shear-rates-which is the case in our pathogenetic context in the artery stenosis provoked by vascular remodeling in select ischemic complications of GCA, the inactive, globular circulating vWF unfolds into a highly active HMW elongated conformation. The active, elongated vWF can bind platelets via its repetitive A1 domain forming'beads on a string' conformations the incipient steps of thrombus

formation [82]. von Willebrand factor, then, has a two-way pathogenic mechanism to actively participate in GCA artery occlusion, on one hand, because at the site of the autoimmune vascular lesion, VWF, released from systemic inflammation thrombin-activated vascular ECs, initiates platelet adhesion, and changes the thrombotic propensity [19, 59], and on the other hand, amplifies maladaptive vascular response via vWF/integrin axis [58], based on these published data it is highly probably that the rate of intraluminal thrombotic events is higher than expected and the rate of TIA in GCA patients is also probably to be higher. It is worth mentioning here that episodes of transient visual loss precede permanent visual loss in 44% of cases [27]. To conclude, these two roles of vWF in GCA arteries are most likely associated with poor outcome of cerebral, coronary, or ocular ischemic complications of GCA disease [67]; leading in some people to type 2 GCA-related myocardial infarction [25, 26]. The highest VWF levels were recorded in GCA patients with positive temporal arteries biopsy when associating ocular symptoms [20], further studies are needed for vWF role in the ischemic complications of GCA. Reversely, in PMR, they reported lower levels of VWF than in GCA, which might be indicative of a lower degree of endothelial dysfunction, in PMR it correlates with the severity of the clinical signs, "more fuel on the fire" in the course of the disease [20]. For all these purposes, accurate criteria for active GCA disease are needed.

GCA relapsing cases or cases unresponsive to corticosteroid therapy that also have high blood VWF levels, high ESR, eye symptoms, would be candidates for testing new prospective therapeutics that either block vWF release from ECs activated by inflammatory cytokines in GCA [19] or block the mitogenic effect of vWF on IH [58].

3.1.2.2 Vasa vasorum ECs response to vasculitis

In response to activated vascular DCs in the adventitial layer, the invasion of multiple types of immune cells is currently thought to occur through the vasa vasorum endothelium [4]. The question to ask is why and how endothelial cells of the tiny adventitial vessels allow the immune cells to break into the vascular wall of the GCA arteries. The role of vasa vasorum ECs is major in GCA pathogenesis though the molecular details are still cryptic. In PMR and GCA as well, adventitial macrophages stimulated by DCs produce IL6 and IL1 which are detected from the early stages, when temporal artery is histologically apparent normal and the INFy expressing T cells are still absent from the vascular wall [11, 13]. At these early stages it is expected that vasa vasorum ECs are activated, express selectins and have a role in increasing wall permeability. In the same time, new vasa vasorum are formed, not only in the adventitia, but across all layers of arterial wall. Their role is to transport the invading immune cells.

3.1.3 Platelets response to vascular inflammation

Platelets are activated by the following activated EC factors: (1) increased thromboxane A2; (2) increased von Willebrand factor and (3) decreased prostaglandin I2 [83]. Platelets are activated by the pro-inflammatory cytokines expressed by ECs and immune cells, by PAF, and by thrombin [83]. When activated, platelets secrete P-selectin and directly interact with endothelial cells [83]. Activated platelets interact with monocytes and neutrophils through the NF-KB mediated pathway [4, 83]. Activated platelets release pro-inflammatory cytokines or chemokines (like IL1 and CD 40) [4, 83]. Activated platelets are involved in microparticle-mediated inflammation [83].

3.2 Thromboembolic complications in GCA patients-clinical features and pathophysiological data subsidiary to vascular dysfunction

3.2.1 Arterial thrombotic complications in GCA

Most common clinical features in GCA patients are the ischemic symptoms: headache, jaw claudication and visual symptoms. If not promptly treated, GCA can lead to systemic complications: aortic aneurysm and rupture, and to ischemic complications of GCA: myocardial infarction, stroke, and blindness. Patients with GCA are experiencing these syndromes due the progressive vessel stenosis/occlusion of the affected arteries, secondary to vascular damage and IH. Several pathogenic mechanisms could explain the increased risk of thromboembolic complications in patients with GCA, including immune and vascular cell aging [89], stasis, endothelial dysfunction, hypercoagulability, and decreased fibrinolysis, the features of inflammatory-derived thrombosis.

3.2.1.1 Cranial symptoms-involvement of intra carotid artery and vertebrobasilar branches

Cranial symptoms are classically associated with GCA: new onset headaches, the most common initial symptom, typical in temporal area but can be diffuse/nonspecific, persistent throughout the day, partly responsive to analgesics; scalp tenderness seen in 50% of patients; usually noticed while brushing hair; temporal artery abnormalities pulse; jaw claudication is seen in 50% of patients, the most specific symptom of arteritis, is a mandibular pain brought on by speech and mastication, relieved when stopping the activities, highly suggestive of GCA, strongly associated with positive TAB. In rare cases, muscles of the tongue and swallowing may be affected.

3.2.1.2 Stroke in GCA

Most strokes in the investigated GCA patients were found in the vertebrobasilar and internal carotid artery territory [33, 90]. The reported rate of stroke/transient ischemic attack (TIA) is approximately 5–20% [25, 33]. The underlying mechanism of cerebrovascular ischemia is related to the vascular dysfunction that is characteristic of GCA. More recent GCA studies [28, 33, 67] reported a 2.8% -7% incidence of ischemic stroke. As mentioned on previous section, a lot of inflammatory cells collect around internal elastic lamina but intracranial arteries lack an internal elastic lamina that being one of the reasons stroke is not seen as a severe manifestation of GCA in that territory.

In a cohort study evaluating the thrombotic risk in GCA patients vs. control it was found an increased risk of cerebrovascular accidents like in the other studies, and also peripheric arteritis and myocardial infarction [25]. The incidence rate ratio for CV events was 1.68 [25]. There is a significantly increased risk of thromboembolic disease in GCA during active disease; the risk for thrombotic events was reportedly the highest in the first month from the onset of the disease hazard ratio 4.92 (95% CI 2.59–9.34. The risk for CV risk was much decreased at a follow-up (hazard ratio of 1.70) (95% CI 1.51–1.91) [25]. Although patients with GCA have an increased risk of cerebrovascular accidents, long-term survival study concluded that GCA patients' mortality is not higher than in the general population if treated properly [34].

3.2.1.3 Visual symptoms-internal carotid artery branches involvement

Blindness is the most severe thromboembolic event experienced by 15–20% of GCA patients, usually at onset [6, 27]. It is rarely reversible. Visual loss is abrupt and painless and the most feared consequence of GCA by the clinicians. GCA is an ophthalmology emergency which requires 'emergency' IV pulse therapy with high dose prednisolone followed by oral therapy to prevent progression in the affected eye and extension to the contralateral eye [21].

Transient monocular visual loss (TMVL) or amaurosis fugax means a person cannot see through one or both eyes, a symptom of poor blood supply to the eye(s). TMVL is seen in 10–15% of patients. If left untreated 50% of cases are rapidly progressing to permanent visual loss (VL). Unilateral VL is a strong risk factor for VL in the contralateral eye which can occur in more than 50% of cases within 2 weeks if left untreated [27].

VL is usually due to arteritic anterior ischemic optic neuropathy: occlusive arteritis of the posterior ciliary branches of the ophthalmic artery which are the main arterial supply of the optic nerve [91]; it accounts for 85% of all VL cases in GCA [27, 91]. VL can also be due to central retinal artery occlusion or posterior ischemic optic neuropathy [27, 33, 91]. Other ocular symptoms in GCA might be ophthalmoplegia and diplopia from ischemia of the extraocular muscles and blurry vision [27].

Intriguingly, one of the acute phase reactants, an innate immunity pattern recognition receptor, pentraxin 3(PTX3) accumulates at the site of active vascular remodeling, more so in GCA patients with recent ocular ischemic events ischemia [52], indicative of thrombo-inflammation manifestations in GCA vessels that supply the eye, as shown by immunohistochemistry and measurement of plasma levels of PTX3 [52].

3.2.1.4 Extracranial/large vessel involvement

Extracranial/large vessel involvement refers to involvement of aorta and its major branch vessels. About 25–30% of GCA cases have clinically evident large vessel involvement [90] but PET scans and CT angiography have demonstrated that subclinical large vessel involvement is present in a significant percentage of cases [90]. The GCA vasculopathy may evolve to aneurysm formation and vascular rupture of aorta and stenosis/occlusion of its branch vessels [68]. Clinically, these patients may present extremity claudication, absent peripheral pulses, abdominal pain, masked HTA, dizziness depending on affected vessels. Because of risk of vascular stenosis, it is needed an evaluation of the blood supply. If decreased blood supply is found, the question is if this is part of GCA occlusive complications or related to artherosclerosis which is almost universal in people in this age group that develop GCA or both. Leg vessels are less involved in GCA than the arm, the neck, the brain, or the eye vessels, but vascular complications can occur in the leg too, just less frequently. Blood pressure and pulses discrepancies of 15-20 mg between left and right extremities might raises question of large vessel arteritis involvement and might hide hypertension.

3.2.2 Venous thrombotic complications in GCA

In a cohort study of circa one thousand GCA patients, an increased risk of venous thromboembolism was observed (both DVT and pulmonary embolism), during the early active, uncontrolled phase of the disease [66].

Nevertheless, with appropriate health care, giant cell arteritis has a relatively good prognosis.

4. Vascular remodeling is the 'unmet need' in GCA treatment-standard therapy and future perspectives

4.1 Glucocorticosteroids treatment-advantages and disadvantages

The glucocorticosteroids (GS) remain the drug of choice for the GCA treatment. In presence of the characteristic clinical signs, GCA is diagnosed by using the Westergren method, high erythrocyte precipitate is indicative of this inflammatory condition and the indication is to start the GS therapy without delay and followed up with a biopsy of the temporal artery [29]. GS treatment is started with about 1 mg/kg/day prednisone and then is tapered at 3, 6, 9 and 12 months-a long taper with intend to withdraw GS around 12–24 months [29]. GCA disease, if treated properly, has an excellent prognosis, but it is difficult for most people to be tapered off prednisone entirely. Most GCA patients must take low dose prednisone daily for months and years. GCA patients are elderly people, a prednisone hit on top of a frail constitution leads to higher disease toll compared to if the disease would occur earlier in life.

About 50% patient relapse after a mean follow up of 7 months at a mean prednisone dose of 4 mg/day [29]. Furthermore, GS are generating side effects, about 90% of patients receiving prednisone for GCA will have at least one GCA-related side effect after 1 year [29].

The question to ask is what we can add or substitute for GS to get tide control of the disease and address the unmet need of vascular luminal changes. GS are easing the symptoms quickly, by blocking inflammatory responses, probably correlated with the rapid decrease in the serum IL 6 and blockage of activation, proliferation, and polarization of the T cells, in particular of Th17 cells. Therefore, GS are still the best available treatment for the induction and remission of GCA, but, unfortunately, they fail to resorb the vessel wall infiltrates or to attenuate the underlying vascular dysfunction pathogenic mechanisms. It is not well known how GS intervene on systemic inflammation's vascular component in GCA, but for instance Cid et al. 2000 reported that GS treatment was not sufficient to completely abrogate the expression of adhesion molecules, [55] in their patients, indicating a persistent exposure of ECs and VSMCs cells to a remaining inflammatory microenvironment despite the rapid symptomatic improvement achieved at follow-up after GS treatment. Moreover, GS have no effect on the restoration of regulatory T cells, and mild effect on TH1 polarization [21, 29]. Another interesting study on 40 patients with TAB+ GCA treated with prednisone, in which they had a randomization for follow-up TABs at 3, 6,9 and 12 months, has shown that in 50% of cases, positive TAB histopathologic signs were still present after one year of GS treatment, showing GS have little to zero effect on vascular remodeling [21, 92, 93]. Vascular remodeling remains the unmet need of current GCA therapy [43, 93]. Therefore, it is very important to develop new strategies to spare GS in GCA. Some of the drugs proposed in the past are toxic or ineffective [5].

The other drug that is recommended for almost all GCA patients is low dose aspirin (85 mg a day) because it decreases the risk of developing subsequential visual loss or cerebrovascular events in giant cell arteritis [69], and it addresses any thrombogenic tendency in blood vessels supplying the eye and the CNS. Some more recent studies challenge the benefit of using aspirin in GCA treatment [5]. The use of otherconventional anticoagulant therapy for the thromboembolic complications of GCA remains controversial and is not recommended [5, 70].

Up to more recent date, the only GS-saving agent was methotrexate [5]. It was demonstrated in a metanalysis gathering phase III randomized blind controlled trials on 161 patients that methotrexate decreases the risk of GCA relapses and is able of GS-saving effect [43].

4.2 Experimental models of GCA

In most to-date published studies, the biochemical assays were conducted on cultured human arteries collected by TAB from GCA patients compared with healthy cultured arterial cells, according to protocol from healthy subjects unrelated to GCA and on peripheral blood collected from GCA patients.

Several murine models of large vessel vasculitis are currently available [5, 43]. Some murine models are KO for genes which encode proteins that have crucial roles in GCA pathogenesis. IL1 rn−/− mice are lacking the gene encoding for the IL 1 receptor antagonist and it was found that these mice develop T-cell dependent vasculitis [5, 43]. Others used herpes virus-infected mouse models of vasculitis [10]. Another model involved microsurgery and physical contact of the murine aorta with an elastase to destroy the vascular wall and mice developed aortitis [5, 43].

The most interesting systemic murine model developed by Weyand group [4] uses implant of human temporal artery (allowing dissection of specific GCA pathogenetic mechanisms) or infusion of human peripheral blood monocytes from GCA patients in severely immunodeficient mice [38]. The model of subcutaneous engraftment of human TAB+ GCA arteries in severely immunodeficient mice opened the possibility to test new biologics therapy effects on immune cells and its afferent GCA artery, both cells and GCA artery originating from the specific patient in the murine model of GCA, maybe allowing in future studies collection of predictive data on how a specific GCA patient would react to the administrated drug of choice [4].

4.3 GS-sparing agents for GCA treatment

4.3.1 Tocilizumab and GiACTA study

One of the most important therapeutic targets for the treatment of GCA disease is related to IL6.

Tocilizumab is a monoclonal antibody targeted against IL6 receptor α [94]. IL6 is the cytokine that controls the balance between regulatory T cells, Th17 and Th1 which is particularly involved in GCA pathogenesis. Collectively, IL6 published data leaded to the breakthrough Giant cell Arteritis Actemra (GiACTA) study was published in 2017 by Stone et al. [94]. GiACTA is a global, randomized, double blind, double placebo-controlled Phase III trial evaluating efficacy and safety of tocilizumab in active GCA in which there were compared 4 groups: in two groups people where receiving IL6 receptor antagonist tocilizumab every week or every other week in association with prednisone over 6 months or 1 year; in the two placebo groups patients were receiving prednisone either 6 months or 1 year [94]. The primary outcome measurement at one year was the sustained remission (56% of the patients in weekly tocilizumab group and 53% in those receiving every other week tocilizumab group compared to only 14% in the short-course prednisone without tocilizumab group, of a total of 251 patients) [95]. GiACTA results demonstrates the superiority of tocilizumab to placebo non-dependent on the duration of prednisone, sustained remission, excluding CRP concentration normalization [94].

GiACTA lead to the FDA approval of tocilizumab in 2017 as first and only specific therapy to treat GCA in the USA and Europe [30, 95], in combination with protocol-defined dose of GS. Tocilizumab successful clinical trials indicate that blockage of IL-6-dependent inflammatory pathways strongly inhibits systemic inflammation as well as PMR and ocular syndrome in GCA patients [30, 95]. Tocilizumab prescription is particularly useful in corticodependance and severe adverse reactions to GS (osteoporosis, diabetics, HTA) [30, 95].

There were a few reports of what happens after tocilizumab withdrawal. In an effort to optimize the tocilizumab treatment duration, a multicenter prospective open label study investigated the risk of relapse associated with tocilizumab discontinuation after new GCA patients received 4 infusions of tocilizumab at weeks 0, 4, 8 and 12 wks [5, 43]. They observed that this treatment can be very effective, but after tocilizumab termination, at least in some patients (25%), it was seen relapse revival [43]. Same, in a long-term follow-up of the GiACTA study confirmed on larger number of patients, in which the treatment was stopped at one year, the relapse revival decrease was seen and it was more comparable between groups after two years follow-up [43, 93]. Importantly, there is no proof of tocilizumab efficacy on vascular remodeling. One of the molecular effects of IL-6 blockade was reported by Terrades-Garcia et al. who investigated the molecular effects of tocilizumab; for this study temporal arteries from 13 GCA patients and 8 controls were cultured with or without tocilizumab. After 5 days of culture, tocilizumab selectively induced a decrease in CXC 13 chemokine mRNA expression in cultured arteries, and they concluded disruption of B cell homeostasis may partially account for the therapeutic effects of tocilizumab (ACR meeting 2016), with no significant changes in other chemokines [5]. Further studies are needed to identify predictive factors of relapses.

4.3.2 Therapeutical targets-updates and controversies

METOGia is a currently conducted, randomized controlled clinical study comparing administration tocilizumab for one year with one-year treatment of methotrexate in association with protocol-controlled prednisone [43].

A new IL1R antagonist (anakinra) effects in GCA treatment is currently under clinical trial, with promising perspective [43].

Activated T cells could be moderated by inference at immunoinhibitory checkpoints [36]. A potential drug intervention is to control excessive TH cell activation and invasion along arterial wall by using abatacerpt. Abatacept is a recombinant fusion protein made from fragment of human Ig1 fused to a domain of cytotoxic T-lymphocyte-associated antigen 4 (which is usually expressed on stimulated T cells) used in vasculitis with positive results, mild efficacy [96], mild GC-sparing effect [5]. There is an ongoing Phase III clinical trial [93].

Blocking IL-12/23 by binding to their common p40 subunit, with another monoclonal antibody ustekinumab has according to one report a positive influence in relapsing GCA [97]. Ustekinumab administered after rapid decrease in GS dose did not prevent disease relapse in one recent small study [98], it still has an open trial label for comparative multicenter study comparing GS alone treatment to GS and ustekinumab in refractory GCA [43].

Macrophages' activation various pathways (mediated by IFN-y, TNFα, CSF-2/CSF-2R (CSF-2: colony-stimulating factor 2), IL 6/IL-6 receptor) [2] should be therapeutically targeted in GCA to prevent blood vessel destruction [54, 56] and the faulty vascular reparatory remodeling [2, 38, 54, 57, 62]. For instance, mavrilimumab is another agent under clinical trial for GCA treatment, targeting macrophage CSF-2/CSF-2R [43].

One of the most promising targets are Janus Kinase (JAK) inhibitors which are pursuing to block the signaling pathways of cytokines. JAKs are kinases that are involved in the signaling of different cytokines. The JAK inhibitors would possibly block different cytokines at the same time. It will be interesting to see whether blocking JAKs in GCA artery will block the signaling of both vascular inflammation (IL6-mediated pathway) and vascular remodeling (IFNy-mediated pathway) at the same

time. This very interesting concept was first demonstrated by the Weyand research group, by blocking concomitantly vascular inflammation and vascular remodeling, with tofacitinib (an inhibitor of Jak1 and 3) as shown in *ex vivo* studies by Zhang et al. 2017 [36]. SELECT-GCA is an ongoing Phase III clinical trial investigating Upadacitinib, another JAK inhibitor for active GCA, at new onset or relapse [43, 93].

4.3.3 Future therapeutical strategies and developments

In terms of therapeutic strategy, the question to ask is which targeted therapy has more GS-saving effects, and also which of them reduces vascular dysfunction and vascular remodeling, which is still the "unmet need" in GCA treatment. For these purposes, we could target by blocking different molecules for instance, endothelin 1, PDGF, mTor (rapamycin) [43]. TLR-induced activation of dendritic cells attracts and retain more dendritic cells and promote the activation of TH1 and TH17 cells, one of the putative therapeutic development would be TLR blockage [5]. vWF and its crosstalk with LRP4/integrin $\alpha_v\beta3$ axis could also constitute a future target for new therapeutics (monoclonal antibody against VWF to prevent pathological hyperplasia of the GCA arterial wall [58], or as possible future research perspective and therapy objective one could investigate could be using a small molecule to inhibit Weibel-Palade bodies secretion from arterial ECs [82, 99] and therefore control ECs mediators' availability.

The impact of targeted GCA treatment on vascular inflammation and vascular remodeling, associated with vascular complications, needs to be further evaluated [22, 95] for more insight into the vascular inflammation and vascular repair unique features specific to GCA.

5. Discussion and conclusions

In this review study, we discussed several cellular and molecular pathogenetic mechanisms of vascular damage characteristic to GCA, that might occur during the progress of disease, especially during the active phase of the disease.

The paradigm in terms of GCA physiopathology is that inflammation starts in the adventitial layer with the activation of the vascular DCs which shifts the situation to the point where there are multiple types of immune cells recruited, proliferating, and differentiating in the vessel wall, causing together with inflamed vascular cells an erroneous repair of the arterial wall. It is unlikely that DCs are the one cells driving these processes, given the multitude of cell functions the arterial wall's ECs play in complicated processes of vascular inflammation, hemostasis/thrombosis, and vascular repair, resulting in a distinct GCA-specific vasculopathy most commonly term used in the field is GCA-related vascular remodeling. There are three ECs populations in GCA artery: arterial luminal ECs, vasa vasorum ECs and capillary ECs formed *de novo* in the intima and media layers (which showed be avascular in a normal arteries) of the diseased artery. These three types of ECs are activated in a sequential manner, probably their activation is subordinated to the invading immune cells, but not to all. For instance, vasa vasorum ECs are activated after vascular DCs are activated but ECs activation most probably precedes the activation of T cells. The invading cells must get in the vessel wall through the vasa vasorum. Activated ECs provide the means for invasion by mobilizing, preformed contents of storage granules WPBs. These secreted ECs mediators are released in a timely manner to fulfill proinflammatory, chemoattractant and neoangiogenic roles, or increased endothelial permeability functions.

Biomarkers have the potential to detect the disease that is missed by TAB/imaging. Several large multi-centers clinical trials being done recently [43, 71, 95, 96] led to the discovery of new potential biomarkers to monitor disease activity and relapses, which is a new critical development in the field. Some of the recently published data imply that testing several blood acute phase reactants can optimize earlier diagnosis and the ability to predict flares and complications [73, 93].

In addition, our study underlines the importance of the candidate targets for novel therapeutics. In the more severe complications of this disease-as blindness or stroke-the underlying GCA-related vascular damage does not respond to GS, as previously reported by several independent studies. A multistep treatment for GCA should be envisioned which involves first line: steroids, especially when people with GCA are particular ill; and secondly, efficient medication to control vascular dysfunction (for instance to lower proinflammatory cytokine levels, to lower the levels of circulating active vWF in parallel). From the variety of GCA treatments that are being investigating a few have the potential to improve outcomes and reduce the need for steroids. The availability of new drug tocilizumab was received with a lot of enthusiasm it is the only FDA approved drug specific for GCA treatment. Tocilizumab is effective to control GCA symptoms, allows rapid GS tapering, and persistent remission with a low dose GS after 6 mo followup, however after tocilizumab discontinuation the relapse-free survival (%) decreases, at least in some patients. Tocilizumab poses certain challenges for clinicians regarding biomarkers follow-up of patients, since tocilizumab is repressing both CRP and ESR; therefore, making careful anamnesis, physical examination, and clinical judgement even more important part of the disease assessment. 20 adverse events were considered directly related to drug; danger with tocilizumab administration was reported in the instance of infection in patients receiving tocilizumab [43, 93], with pneumonia and no CRP and ESR rise, [43] signifying that more careful assessing of the disease activity and infections in the patients treated with tocilizumab is required [43]. Further studies are needed to determine the optimal duration of treatment and maintaining of dosing and to further reduce the risk of relapse [93]. An important note to make is that molecular pathogenic pathways promoting GCA disease are changing with the disease progression under treatment [93]. This situation is frequent in clinical practice and requires adequate follow-up and adapted therapeutic strategies [93].

Hopefully, future research will bring us closer to the goal of identifying new therapy for active and/or refractory GCA, which used in substitution or addition to steroids will provide tide control of the disease, addressing not only vascular inflammation but also vascular remodeling, skewed thrombotic propensity and luminal changes in GCA patients at the brink of having VL, or a stroke or other ischemic event at the initial onset of the arterial disease or in evolution.

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