CHAPTER 1

Introduction to this thesis

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Systemic vasculitides

Vasculitis is an inflammatory process that occurs within the vessel wall as the primary site of inflammation. The vasculitic process may affect vessels of any type or any size including capillaries, venules, arterioles, veins and arteries. The inflammation may result in damage and fibrinoid necrosis in the vessel wall, and when arteries are involved this process may result in irreversible changes of arterial walls such as stenosis, occlusion, dilation or aneurysm formation. When capillaries and venules are affected by the vasculitic process, weakening of vessel wall may ensue often leading to rupture of vessel wall and bleeding to surrounding tissues [1,2].

Disease manifestations of vasculitic syndromes are heterogeneous and usually include constitutional symptoms due to the systemic inflammatory process as well as dysfunction of organs and systems whose supplying vessels are affected by the vasculitic process [3]. The involvement of multiple organs and/or systems characterizes a systemic vasculitis while when only one organ is affected by the vasculitic process it is regarded as a single organ vasculitis [4]. Systemic vasculitis is considered primary when there is no known etiological factor identified but systemic vasculitis may be associated with infectious diseases (e.g., hepatitis C or HIV infection), drugs (e.g., anti-thyroid drugs, hydralazin and minocyclin), drug abuse (e.g., vasculitis associated with levamisole-adultered cocaine), systemic autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid vasculitis and Sjögren's syndrome) and cancer [5,6].

Several classification systems of systemic vasculitides have been developed since the first proposal in 1952 by Dr. Perla Zeek that included: hypersensitivity angiitis, allergic granulomatous angiitis, rheumatic arteritis, periarteritis nodosa and temporal arteritis [7]. In 1993, the first International Chapel Hill Consensus Conference (CHCC) aimed to develop a consensus for the names and definitions of the most common forms of vasculitis. Ten

vasculitic syndromes were included in the first CHCC and their definition was based on disease manifestations and histopathologic features [8]. More recently, a second International CHCC was held in order to update the initial vasculitis nomenclature and definition system based on advances in the understanding of epidemiology and pathophysiology of vasculitis. Systemic vasculitides are classified according to the size of vessels predominantly affected in large vessel vasculitis (LVV), medium vessel vasculitis (MVV) and small vessel vasculitis (SVV) (Table 1) [9].

Table 1. Names of vasculitides adopted by the 2012 International Chapel Hill Consensus Conference, adapted from [9].

Large vessel vasculitis (LVV)
Takayasu arteritis (TAK)
Giant cell arteritis (GCA)
Medium vessel vasculitis (MVV)
Polyarteritis nodosa (PAN)
Kawasaki disease (KD)
Small vessel vasculitis (SVV)
Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)
Microscopic polyangiitis (MPA)
Granulomatosis with polyangiitis (Wegener's) (GPA)
Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA)
Immune complex SVV
Anti-glomerular basement membrane (anti-GBM) disease
Cryoglobulinemic vasculitis (CV)
IgA vasculitis (Henoch-Schönlein) (IgAV)
Hypocomplementemic urticarial vasculitis (HUV) (anti-C1q vasculitis)
Variable vessel vasculitis (VVV)
Behçet's disease (BD)
Cogan's syndrome (CS)
Single-organ vasculitis (SOV)
Cutaneous leukocytoclastic vasculitis
Cutaneous arteritis
Primary central nervous system vasculitis
Isolated aortitis
Others
Vasculitis associated with systemic disease
Lupus vasculitis
Rheumatoid vasculitis
Sarcoid vasculitis
Others
Vasculitis associated with probable etiology
Hepatitis C virus–associated cryoglobulinemic vasculitis
Hepatitis B virus–associated vasculitis
Syphilis–associated aortitis
Drug–associated immune complex vasculitis
Drug–associated ANCA–associated vasculitis
Cancer–associated vasculitis
Others

SVV is divided in two subgroups based on the deposition of immune deposits in the vessel wall as follows: antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis characterized by paucity or absence of immune deposits, and immune complex small vessel vasculitis with moderate to marked immune complex deposition on vessel walls. Furthermore, additional categories of vasculitis have been added to the current classification system such as variable vessel vasculitis, single-organ vasculitis, vasculitis associated with systemic diseases and vasculitis with probable etiology have been added (Table 1) [9].

The International CHCC nomenclature and definitions of vasculitis are not meant to be used as diagnostic or classification criteria. Thus, the diagnosis of a systemic vasculitis in patients with suggestive signs and symptoms needs to be confirmed by biopsy findings, serological markers (e.g. ANCA test, serum cryoglobulins) or by imaging studies as appropriate [1,3]. The American College of Rheumatology (ACR) in 1990 developed classification criteria for seven common forms of vasculitis: Takayasu arteritis (TAK), giant cell arteritis (GCA), polyarteritis nodosa (PAN), granulomatosis with polyangiitis (GPA) (formerly Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (EGPA) (formerly Churg-Strauss syndrome), IgA vasculitis (formerly Henoch-Schönlein purpura) and hypersensitivity vasculitis [10-16]. The 1990 ACR criteria for systemic vasculitides were developed to identify a homogeneous group of patients for inclusion in epidemiologic and therapeutic studies and not intended to be used as diagnostic tools in clinical practice [5,6]. The sensitivity of the 1990 ACR criteria for systemic vasculitides ranged from 71.0% to 95.3% while the specificity ranged from 78.7% to 99.7%. The criteria for EGPA, GCA and TAK were shown to present the best sensitivity and specificity [17].

In 2006, Watts et al. developed and validated an algorithm to categorize patients with ANCA-associated vasculitis (AAV) and PAN for

epidemiological studies. The four-step algorithm included patients with a clinical diagnosis of AAV or PAN. The ACR and Lanham criteria for EGPA and the ACR criteria for GPA were applied first, surrogate markers of GPA were used to distinguish GPA form microscopic polyangiitis (MPA), then MPA was classified using the CHCC definition and surrogate markers for renal vasculitis. Finally, the CHCC was used to classify patients with PAN [18]. More recently, an international multicenter collaboration is underway to develop a single set of validated diagnostic and classification criteria for systemic vasculitides [19].

Without appropriate therapy, systemic vasculitides are associated with a high mortality rate. Advances in the management of these conditions have converted systemic vasculitides into relapsing and remitting chronic diseases with periods of active disease and remission. In systemic vasculitides, significant morbidity may arise from permanent damage secondary to disease activity and therapy as well. In clinical practice, a systematic evaluation of patients with systemic vasculitis is important to check all possible organs and systems involved by the vasculitic process and to avoid missing new disease manifestations [20]. Currently, the Birmingham Vasculitis Activity Score (BVAS) is the most widely used instrument to measure disease activity, especially in SVV and in MVV [21]. BVAS was developed in 1994 for use in collaborative clinical trials in vasculitis, it was modified in 1997 to create a second version [22]. In 2001, BVAS was adapted to produce a specific instrument to evaluate disease activity in patients with GPA (BVAS/WG) and in 2009 BVAS was modified and validated again to create a third version with 56 items from 9 organs and systems [23,24]. Due to different disease manifestations, BVAS is not adequate to evaluate disease activity in TAK. Kerr's criteria and more recently the Indian Takayasu Activity Score (ITAS) have been used for this purpose [25,26].

High mobility group box-1

High mobility group box-1 (HMGB1) is a nuclear non-histone protein with 215 amino acid residues that comprises three distinct domains: two tandem HMG box domains (box A and B) and a 30 amino acid-long C terminal tail. Nuclear HMGB1 binds to DNA, modulates chromosomal architecture and regulates DNA transcription, repair and recombination [27]. Although HMGB1 is abundantly found within the nucleus, HMGB1 may also be translocated into the cytoplasm, where it is responsible for mediating cellular autophagy and also acts as a cytosolic sentinel for immunogenic nucleic acids by binding to DNA or RNA [28-30]. In addition, HMGB1 may be released by dying cells or secreted by activated cells to the extracellular environment. The derangement of cell permeability from necrotic cells results in the passive release of HMGB1 whereas apoptotic cells retain HMGB1 in the nucleus due to post-translational modifications that affect chromatin binding. However, when late apoptosis ensues (secondary necrosis) HMGB1 may be finally released by apoptotic cells [28]. Immunologically active cells can secrete HMGB1 upon activation by cytokines or TLR ligands. After cell activation, lysine residues of HMGB1 are acetylated and this chemical modification signals migration into the cytoplasm inside vesicles that merge with the plasma membrane and eventually HMGB1 is secreted into the extracellular milieu [28,31].

Once outside the cell HMGB1 acts as an alarmin or a danger-associated molecular pattern (DAMP) and displays different functions by binding to different receptor such as the receptor for advanced glycation-end products (RAGE), Toll-like receptor (TLR)-2, TLR-4 and TLR-9 [28,29]. The interaction between extracellular HMGB1 and its receptor triggers signaling events that mediate HMGB1 functions on cellular activation, differentiation and proliferation, cytokine production, chemotaxis and angiogenesis. Moreover, HMGB1 may complex with other molecules including interleukin

(IL)-1β, DNA, RNA, LPS and nucleosomes in order to act synergistically in the activation of their receptors [32].

Post-translational modifications of HMGB1 may also affect its functions. The immunological activity of HMGB1 is affected by the redox state of cysteine residues at positions 23, 45 and 106. When HMGB1 is in its fully reduced state (i.e., all-thiol HMGB1) it only induces chemotaxis by binding to CXCL12 and activating CXCR4. However, HMGB1 triggers cytokine production by the interaction with TLR4 when HMGB1 is partially oxidized with a disulfide bond formed at position 23 and 45, with a free thiol at position 106. The fully oxidized form of HMGB1 cannot induce either chemotaxis or cytokine production [31,33,34].

Due to its multiple functions in activating the immune system, the role of HMGB1 in the pathogenesis or as a biomarker has been evaluated in several systemic inflammatory and autoimmune diseases, in atherosclerosis, cancer and infectious disease [28,32,31,35]. In systemic lupus erythematosus (SLE), increased levels of HMGB1 were found in serum and in urine samples of patients with active systemic disease and nephritis, respectively [36,37]. Increased expression of HMGB1 was found in the cytoplasm and in extracellular sites of renal tissue from patients with lupus nephritis [37,38]. Furthermore, the release of HMGB1 is increased in the skin of SLE patients and this release is increased by ultraviolet B exposure and is related to the number of apoptotic cells [39]. In rheumatoid arthritis (RA), an increased concentration of HMGB1 is found in synovial fluid in comparison with patients with osteoarthritis while CD68-positive cells express HMGB1 in the synovium of RA patients [40]. Serum levels of HMGB1 in RA are associated with IL-6 levels, swollen joint count and acute phase reactants [41].

Patients with active idiopathic inflammatory myopathies present high cytoplasmic expression of HMGB1 in muscle fibers, infiltrating macrophages and in vascular endothelial cells, but this expression is dramatically

decreased after 3-6 months of high dose prednisolone [42]. Increased serum HMGB1 levels were found in patients with Sjögren's syndrome, systemic sclerosis, ankylosing spondylitis and juvenile idiopathic arthritis [43-46].

In systemic vasculitides, up to the start of this thesis, serum HMGB1 levels had been evaluated in patients with Kawasaki's disease (KD), Behçet's disease (BD), GPA and MPA. In KD, serum HMGB1 levels were very high in the early acute phase of the disease and decreased in the late acute phase and convalescent phase of KD [47]. Furthermore, serum HMGB1 levels were predictive of the clinical response to intravenous immunoglobulin [48]. In BD, serum levels were higher in patients than healthy controls and patients with intestinal involvement of BD presented the highest levels of HMGB1 [49]. In patients with AAV, serum HMGB1 levels had been evaluated in three cross-sectional studies. Firstly, serum HMGB1 levels were associated with disease activity in GPA but not MPA and a significant correlation between serum HMGB1 and BVAS was found in GPA [50]. Higher serum HMGB1 levels were found in GPA patients with predominantly granulomatous manifestations compared with GPA patients with predominantly vasculitic manifestations. A significant correlation was found between the volume of pulmonary granuloma and serum HMGB1 [51]. Finally, another study showed higher serum HMGB1 levels in AAV patients with biopsy-proven glomerulonephritis compared with those patients with a normal kidney biopsy. A significant decrease in serum HMGB1 levels was observed in seven patients who underwent a second kidney biopsy in remission 6-9 months after baseline [52].

Aims of this thesis

In this thesis we aimed to evaluate HMGB1 as a biomarker of disease activity in systemic vasculitides including GPA, MPA, TAK, GCA and Behçet's disease (BD). Furthermore, we also aimed to check associations between

serum HMGB1 levels and specific organ and system involvement and to assess the impact of therapy on serum HMGB1 levels in systemic vasculitides. Firstly, we reviewed the subject of HMGB1 in vascular diseases including systemic vasculitides and atherosclerotic disease in **chapter 2**. As previous cross-sectional studies had already evaluated serum HMGB1 levels in AAV patients (i.e. GPA and MPA), in **chapter 4** we decided to evaluate serum HMGB1 levels in a longitudinal study with AAV patients to assess fluctuations of HMGB1 at disease presentation, during remission, prior to and during relapses. We hypothesized that serum HMGB1 levels could be predictive of disease relapses in AAV. Moreover, we checked associations between serum HMGB1 levels at presentation and specific organ or system involvement in AAV and the presence of anti-HMGB1 antibodies in AAV patients with active disease. In **chapter 5**, we evaluated whether serum levels of HMGB1 and soluble RAGE (sRAGE) could be associated with subclinical atherosclerotic disease or with therapy in GPA. In this study, we also evaluated the *in vitro* effect of atorvastatin on the release of HMGB1 by human umbilical vein endothelial cells (HUVEC) activated with lipopolysaccharide (LPS). In **chapter 6**, we evaluated whether urinary HMGB1 levels were increased in AAV patients with active nephritis compared to healthy controls and whether levels decreased after achieving remission. In this study, we also evaluated associations between urinary HMGB1 levels and BVAS, urinary monocyte chemoattractant protein-1 (MCP-1), CD4+ T cells and effector memory T cells in urine and peripheral blood. To introduce this study, we wrote a review about the role of mononuclear cells, especially T cells, in endothelial damage in AAV in **chapter 3**. The role of serum HMGB1 as a biomarker of active disease in LVV (i.e. TAK and GCA) was evaluated in chapter 7 and the role of HMGB1 levels in BD was addressed in **chapter 8**. Finally, the summary and conclusions from all findings described in these studies are given in **chapter 9**.

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