

Venous Thromboembolism in Wegener's Granulomatosis

Wegener's granulomatosis (WG) is a potentially organ- or life-threatening, chronic inflammatory and autoimmune disease of unknown etiology characterized by necrotizing granulomatous lesions, glomerulonephritis, and an autoimmune vasculitis predominantly affecting small vessels, i.e., arterioles, capillaries, and venules. WG usually starts as granulomatous disease of the respiratory tract (so-called localized) before it converts to a systemic disease (generalized) in most patients with the emergence of antineutrophil cytoplasmic autoantibodies specific for "Wegener's autoantigen" proteinase 3 (PR3-ANCA) and PR3-ANCA associated vasculitis¹.

In vitro studies support the concept that PR3-ANCA interact with neutrophil granulocytes, causing premature intravascular neutrophil activation and degranulation, with subsequent endothelial damage and further leukocyte recruitment to the site of vasculitis². Moreover, murine PR3-ANCA generated in PR3/elastase-deficient mice induce panniculitis *in vivo* upon passive transfer into mice challenged with intradermal tumor necrosis factor- α injection. Stronger pulmonary and renal inflammation is seen in lipopolysaccharide-primed mice in the presence of PR3-ANCA in this model³. Yet it remains unresolved how the immune response to PR3 is induced and sustained.

Danger-associated molecular patterns (DAMP) such as monosodium urate crystals induce dendritic cell maturation, antigen presentation, T cell activation, and cytokine production^{4,5}. Intriguingly, PR3 also induces dendritic cell maturation *in vitro* via the protease-activated receptor-2 and evokes a stronger Th1-type T cell response in WG as compared to healthy and disease controls⁶. In many animal models break in tolerance and organ-specific autoimmunity is induced in the presence of a "proinflammatory environment" and sustained by neof ormation of lymphoid-like structures in inflamed target organs⁷. WG-granulomata contain clusters of PR3+ cells (neutrophils/monocytes) surrounded by antigen-presenting cells, Th1-type CD4+CD28- effector memory T cells, and maturing B cells and plasma cells suggestive of lymphoid-like tissue neof ormation⁸. Thus, in many respects the immune response to PR3 in WG resembles an uncontrolled response to DAMP.

To date, there have been few reports on venous thromboembolism (VTE) in WG⁹⁻¹¹. A high incidence of VTE in WG (7.0 per 100 person-years; 95% CI 4.0 to 11.4) has been reported in a large prospective study (WeCLOT) recently¹².

In this issue of *The Journal*, Sebastian, *et al*¹³ report on a prospective analysis of 3 rather common gene variations associated with hypercoagulability and VTE [factor V Leiden; G20210A mutation of prothrombin (factor II) gene; C677T mutation of methylenetetrahydrofolate reductase gene], anticardiolipin antibodies (aCL), and anti- β_2 -glycoprotein I (anti- β_2 -GPI) antibodies in 180 WG patients from the aforementioned study. The authors identified an increased frequency of low-titer aCL in WG. However, this finding as well as the presence of anti- β_2 -GPI antibodies did not correlate with thrombotic events. Moreover, the prevalence of the 3 mutations associated with hypercoagulability was comparable to the rate in the general population. The study emphasizes findings from earlier reports on lack of association of aCL and anti- β_2 -GPI antibodies with VTE in WG and excludes a specific role of the 3 common mutations for hypercoagulability and VTE in WG¹³. Statistical caveats include lack of power calculations to ensure cohort sizes and frequencies of the genetic exchanges to allow identification of significant differences between the cohorts. Further, aCL and anti- β_2 -GPI were not detected in close temporal relation to VTE and serial assessment of aCL and anti- β_2 -GPI titers was not pursued.

The study by Sebastian, *et al*¹³ raises a question: Which factors other than aCL, anti- β_2 -GPI, and the 3 common gene mutations associated with hypercoagulability could cause VTE in WG? The number of factors influencing coagulability is large (Table 1)¹⁴. Weidner, *et al*¹¹ excluded the following: deficiencies in proteins S and C, antiphospholipid antibodies, factor V Leiden, malignancy, surgery or trauma, pregnancy, oral contraceptives, hormone replacement therapy, immobilization, obesity, and smoking — as causes of VTE in a series of 13 patients [8 PR3-ANCA+ WG, 1 PR3-ANCA+ microscopic polyangiitis (MPA), 2 myeloperoxidase (MPO)-ANCA+ MPA, and 2 PR3-ANCA+ renal-limited vasculitides] from a cohort of 105 patients with ANCA-associated vasculitis. Another study also failed to identify hereditary or

See Frequency of aCL and genetic mutations associated with hypercoagulability in WG patients with and without history of thrombotic event, *page 2446*

Table 1. Inherited and acquired causes of venous thrombosis.

Inherited
Common
G1691A mutation in the factor V gene (factor V Leiden)
G20210A mutation in the prothrombin (factor II) gene
Homozygous C677T mutation in the methylenetetrahydrofolate reductase gene
Rare
Antithrombin deficiency
Protein C deficiency
Protein S deficiency
Very rare
Dysfibrinogenemia
Homocysteinuria in homozygous state
Probably inherited
Increased levels of factor VIII, Factor XI, Factor XI, or fibrinogen (not attributable to inflammation)
Acquired
Surgery and trauma
Prolonged immobilization
Older age
Obesity
Nephrotic syndrome
Smoking
Cancer
Inflammation
Myeloproliferative disorders
Pregnancy and puerperium
Use of contraceptives or hormone replacement therapy
Mild to moderate hyperhomocysteinemia
Antiphospholipid antibodies

From Seligson U, Lubetsky A. *N Engl J Med* 2001; 344:1222-31; adapted with permission¹⁴.

acquired hypercoagulability factors apart from factor V Leiden mutation in heterozygous state in one of 5 pediatric patients with WG¹⁰. Intriguingly, VTE was observed in 2 WG patients and 1 patient with MPA and reactivated cytomegalovirus (CMV) infection. Whereas VTE is uncommon in CMV infection, e.g., after organ transplantation, the combined effects of endothelial CMV infection and vasculitis might facilitate VTE in WG and MPA⁹. So far, the influence of CMV infection on VTE and other manifestations has not been analyzed in greater detail in WG. Moreover, the effects of genetic risk factors for WG such as HLA-DPB1*0401 and PTPN22*620W on VTE in WG have not yet been evaluated¹⁵.

Most VTE episodes reported in ANCA-associated vasculitides occurred during active disease. Moreover, VTE is seen in PR3-ANCA+ WG more frequently than in MPO-ANCA+ MPA, even when you consider that WG has a higher incidence and prevalence compared with MPA¹⁰⁻¹². Of note, “Wegener’s autoantigen” PR3 and PR3-ANCA, but not MPO, induce endothelial tissue factor (TF) *in vitro*. Endothelial TF initiates blood coagulation by the interaction with factor VII, resulting in the subsequent generation of thrombin^{16,17}. Further, antibodies to complementary PR3 (cPR3), synthesized from the antisense/noncoding strand of the PR3 gene, are detected in a subset of PR3-ANCA+ WG patients¹⁸. Recently, anti-cPR3 antibodies were reported to interact with plasminogen¹⁹.

Assuming that endothelial damage in active disease may be causally linked to VTE in WG, one might expect morphological signs of active phlebitis in patients with WG and VTE. However, studies demonstrating active phlebitis as a possible origin of VTE in WG are lacking. Although it is well known that ANCA-associated vasculitis can affect large vessels of both arterial and venous type, studies dealing with morphological analysis of medium and large-vessel involvement in

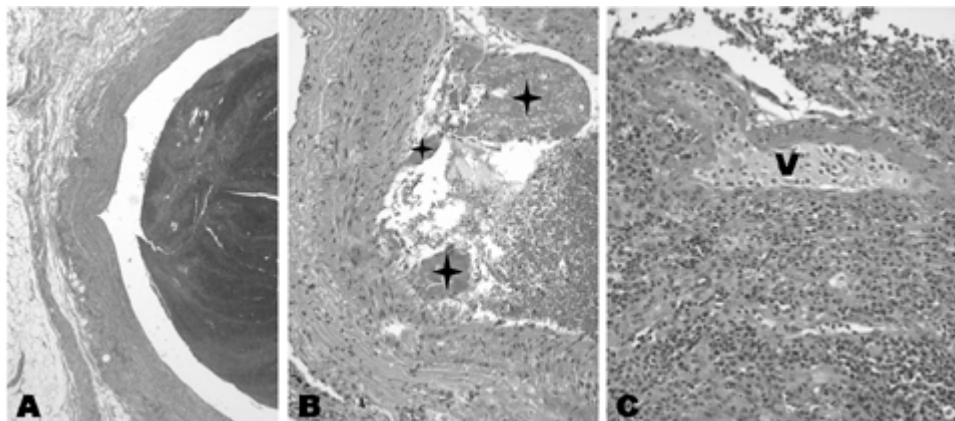


Figure 1. A. Venous thrombosis of the lower limb in a patient with WG: medium-size vein with fresh thrombus (right), but with no sign of phlebitis (H&E, original magnification $\times 25$). B. Same patient as in A: pulmonary embolism (stars) in medium-size pulmonary artery with beginning organization of thromboembolus, covered with endothelia. No vasculitis is noted (H&E, $\times 100$). C. Venulitis (V) in active WG of sinu-nasal mucosa showing prominent endothelia and subendothelial fibrinoid necrosis in a dense mixed inflammatory background (H&E, original magnification $\times 100$).

WG have mainly focused on the arterial side²⁰. Whereas venulitis is a well known phenomenon of active ANCA-associated vasculitis (Figure 1C), phlebitis of medium and large-size peripheral veins has not been convincingly demonstrated. Indeed, in our own cohort of 4 autopsy cases of VTE in WG patients, none of the affected vessels showed active phlebitis (Figure 1A and 1B). It remains to be determined how the above mentioned uncontrolled immune response, PR3, PR3-ANCA, and/or anti-cPR3 antibodies are involved in the pathogenesis of venulitis and glomerular capillary thrombosis typical of WG, and how they might give rise to the involvement of medium to large-size veins and VTE in some patients with WG. Further analysis of the impact of these and other factors on VTE in WG may yield even more promising data.

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