The Clock Is Ticking as the Clot Thickens

Edward M. Conway

According to the United Nations Population Division, the number of people who are over the age of 65 will approach 1.5 billion, or 16% of the population, by the year 2050. The fastest-growing segment of the population is the “oldest old,” i.e., age ≥80. In North America and Europe, this group will jump from ~10 million in 2010 to almost 40 million by 2050. These staggering demographic changes will increasingly challenge health care systems, as the numbers of individuals requiring medical care will progressively rise.

Delineating the mechanisms underlying the age-dependent increased risk of VTE and atherothrombosis is tantamount to identifying risk factors and developing effective preventative and therapeutic strategies. Unfortunately, advances in this field have been limited. The challenge of discerning independent age-related risk factors for thrombosis in populations of individuals who often have comorbidities or are receiving multiple medications has prompted investigators to turn to animal models. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Hemmeryckx et al.9 have laid new and important groundwork by using a unique mouse model to explore vascular thrombosis in the context of aging.

Mice in which the gene encoding the circadian rhythm modulator brain and muscle ARNT-like protein-1 (Bmal1) is inactivated (Bmal1 knockout [KO]) display features of premature aging, including osteoporosis, infertility, and cataracts.7,8 Although other mouse models of premature aging exist, none have undergone as intensive a “thrombosis workup” as was performed by Hemmeryckx et al.6 These investigators determined that the prematurely aged mice with a defective “clock” mechanism exhibit imbalances in the hemostatic system that favor thrombosis, including higher plasma levels of prothrombotic factors VII, VIII, and fibrinogen; a shortened prothrombin time; and raised platelet counts. Further promoting a diathesis to clot, transcripts for the natural anticoagulant molecules thrombomodulin and endothelial protein C receptor were reduced in the Bmal1 KO aortas. Plasma levels of C-reactive protein, an inflammatory marker of arterial disease in humans, were also elevated in aging Bmal1 KO mice, although in vivo studies did not support a role for inflammation in this model. Strikingly, however, was the observation that aortic endothelial function was severely compromised in the Bmal1 KO mice, reflected by attenuation of basal and acetylcholine-induced nitric oxide (NO) bioavailability and vasorelaxation. Based on previous studies with mice specifically lacking endothelial cell Bmal1,8 it is likely that endothelial dysfunction is in fact, a key factor underlying the prothrombotic phenotype of the Bmal1 KO mice. Compelling evidence of the relevance of the age-related changes in endothelial function and hemostatic balance was finally provided by well-designed in vivo studies. These showed that Bmal1 KO mice spontaneously develop near-occlusive venous sinus thrombi, manifest by priapism, and exhibit increased susceptibility to injury-induced thrombosis in both arterioles and venules.

Studies in humans, although often confounded by small numbers, are in line with those of Hemmeryckx et al.6 As with the Bmal1 KO mice, aging in humans correlates with progressive endothelial dysfunction (Figure 1). Arteries become stiff and dilated, in part because of degeneration of elastic fibers and increased collagen content.9 Vessels exhibit impairments in endothelial dependent dilation because of reduced bioavailability of NO and endothelial nitric oxide synthase (eNOS).10 In the Bmal1 KO mice, this is believed to be due to blunted Akt signaling in the aging arteries, with diminished phosphorylation of eNOS and thus reduced NO release.6,7 However, a progressive decline in expression of active eNOS may also be due to an age-dependent reduction in endothelial production of the cellular deacetylase sirtuin (SIRT)-1 which modulates eNOS acetylation/activation-associated NO production.11 Brachial artery endothelial cells from elderly patients express less SIRT-1 and release greater amounts of proinflammatory cytokines and markers of oxidative stress. These changes may be reversed with agents that...
interfere with nuclear factor-κB nuclear translocation, thereby improving endothelial function, vasodilatory performance,\textsuperscript{12,13} and the capacity to overcome prothrombotic stresses.

Other age-dependent risk factors in humans for atherothrombosis and adverse cardiovascular events have been identified and include, for example, elevated circulating levels of d-dimers and plasmin-antiplasmin complexes.\textsuperscript{14} Platelet activity was not affected in the Bmal1 KO mice, but in aging individuals, it has been reported to be enhanced as their phospholipid content increases and the platelets become more resistant to inhibition by prostacyclin.\textsuperscript{15} For VTE risk, there are fewer data establishing links between age-dependent elevated coagulation protein levels—reported for fibrinogen; factors VII, VIII, IX, and XI; and von Willebrand factor—and age-related disease.\textsuperscript{16} Although validation in larger populations is required, VTE risk for individuals over the age of 65 is reported to be moderately increased in those with elevated plasma levels of factor VIII and von Willebrand factor\textsuperscript{17} and for carriers of the genetic defects factor V<sub>Leiden</sub> and prothrombin PT20210A.\textsuperscript{18} The relevance of other genetic defects that confer the highest risk of VTE to young and middle-aged people, i.e., functional deficiencies of antithrombin, protein C, and protein S, has not been evaluated in the aging population. Although intuitively, one might expect that excess thrombin generation would predispose individuals at any age to VTE,

Figure. Prothrombotic changes in the aging vasculature. In this cartoon, the left panel reflects healthy, closely apposed endothelial cells (pink) showing 2 of the many vasculoprotective systems. Thrombomodulin (TM) and endothelial cell protein C receptor (EPCR), which themselves play direct roles in inflammation, act as key cofactors in thrombin (IIa)-mediated generation of the antiinflammatory and anticoagulant activated protein C (APC). Phosphorylated endothelial nitric oxide synthase (eNOS-P) induces the release of nitric oxide (NO), which suppresses coagulation, inflammation, and platelet activation and promotes endothelial cell health and vascular integrity. With aging (right panel), numerous changes occur, only a few of which are shown. Vascular permeability increases as endothelial cells become dysfunctional. Reactive oxygen species (ROS) promote oxidative changes with increased nuclear translocation of nuclear factor-κB (NF-κB), reduction in phosphorylation of SIRT-1 (SIRT-1-P) and Akt (Akt-P), and dampened release of bioavailable NO. Circulating levels of the procoagulant factors prothrombin, factor VII, factor VIII and fibrinogen are increased. Expression of TM and endothelial cell protein C receptor are reduced, with consequent suppression of generation of APC. Platelet numbers are increased. Although not shown in the Bmal1 knockout mice, it is likely that tissue factor (TF) exposure from the subendothelium is increased, facilitating the activation of coagulation and the generation of excess thrombin, which would be expected to confer a heightened risk of fibrin clot formation. Elevated fibrin degradation products (FDPs) and d-dimers confirm increased thrombin production with subsequent fibrinolysis due to the transformation of plasminogen to plasmin via tissue-type plasminogen activator (t-PA) released from endothelial cells.
this conclusion may not be valid. In that respect, centenarians with heightened activation of prothrombin, factor VII, factor IX, and factor X were entirely healthy and without a history of vascular thrombotic disease,19 highlighting the importance of seeking alternative mechanisms by which aging predisposes to thrombosis.

In addition to Bmal1, it will be important to examine the role of other “clock” regulators in age-related thrombosis. Indeed, mice that lack Per2 also exhibit impaired endothelial function with reduced NO production and increased release of cyclooxygenase-1-derived vasoconstrictors.20 How and why these genes modify endothelial function and hemostatic system activity remains a mystery. Similarly, other mouse models of aging require extensive thrombosis workups, hopefully to uncover novel mechanistic insights. Good candidates to evaluate would include the klotho mutant mice that lack fibroblast growth factor 23 (Fgf-23) and exhibit evidence of vascular disease21 or others that have mitochondrial defects22,23 or progeroid syndromes24 that are known to be linked to age-related human vascular disease.

With the accelerated demographic shift toward an aging population, the clock is rapidly ticking. Physicians will soon be overwhelmed with elderly patients experiencing a range of clotting disorders that will complicate their care and place increasing strains on health care systems throughout the world. The work of Hemmeryckx et al6 will hopefully promote further research in this critically important field.

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References

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