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Alterations in Platelet Functions During Aging: Clinical Correlations with Thrombo-Inflammatory Disease in Older Adults

Donya Mohebbi, BS¹, David Kaplan, MD¹, McKenzie Carlisle, PhD³, Mark A. Supiano, MD², and Matthew T. Rondina, MD^{1,3}

¹Divisions of General Internal Medicine, Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, Utah, 84132

²Divisions of Geriatrics, Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, Utah, 84132

³Program in Molecular Medicine, University of Utah Health Sciences Center, Salt Lake City, Utah, 84132

Abstract

Platelets possess a dynamic functional repertoire that mediates hemostatic and inflammatory responses. Many of these functions are altered in older adults, promoting a pro-thrombotic and pro-inflammatory milieu and contributing to an increased risk of adverse clinical events. This review summarizes, drawing primarily from human studies, key aspects of aging-related changes in platelets. The relationship between altered platelet functions and thrombotic and inflammatory disorders in older adults is highlighted. Established and developing anti-platelet therapies for the treatment of thrombotic and inflammatory disorders are also discussed in light of these data.

Keywords

Platelet; Monocyte; Inflammation; Aging; Thrombosis

INTRODUCTION

Platelets are highly specialized effector cells that rapidly respond to sites of vascular injury or endothelial damage. While the hemostatic roles of platelets are well recognized, emerging data demonstrate that platelets possess diverse and dynamic functions that also mediate inflammatory and immune responses. These functions are germane to disease processes prevalent among older adults and likely influence susceptibility to thrombotic and inflammatory disorders, including vascular diseases and infectious syndromes, such as

Corresponding Author: Matthew T. Rondina, M.D., University of Utah, Department of Internal Medicine, 50 North Medical Drive, SLC, Utah 84132, (801) 585-0950 (phone), (801) 585-1393 (fax), matt.rondina@u2m2.utah.edu. **Alternate Corresponding Author:** David Kaplan, M.D., University of Utah, Department of Internal Medicine, 50 North Medical Drive, SLC, Utah 84132, (801) 585-0950 (phone), (801) 585-1393 (fax), david.kaplan@hsc.utah.edu.

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Author Contributions

All authors contributed significantly to the conception and design of this work (MTR, Donya Mohebbi, David Kaplan), drafting and revising the article critically (Matthew T. Rondina, Donya Mohebbi, CK, McKenzie Carlisle, Mark A. Supiano), and/or final approval of the version being submitted (Matthew T. Rondina, Donya Mohebbi, CK, McKenzie Carlisle, Mark A. Supiano). Each author will also be involved in the final approval of the manuscript version to be published.

sepsis. This review will summarize key aspects of aging-related changes in platelets in the context of inflammatory and thrombotic pathologies in older adults. In light of these emerging data, established and developing anti-platelet therapies for two thrombotic and inflammatory disorders are also discussed.

Aging Increases the Risk of Thrombotic and Inflammatory Disorders

Thrombotic diseases (including ischemic heart disease, peripheral vascular disease, stroke, and venous thromboembolism (VTE)) are among the most common causes of death worldwide. Among thromboembolic disorders, coronary artery disease (CAD) is the leading cause of death for adults ≥ 75 years, with more than 80% of all CAD-related deaths occurring in this population¹. Older age is also a risk factor for VTE, including deep vein thrombosis (DVT) and pulmonary embolism (PE), and VTE-related mortality². While estimates vary with differences in population characteristics and study methods, the incidence of VTE rises exponentially with aging. Estimates suggest that rates of VTE increase from approximately 1/10,000 in those aged < 40 years to 1/1,000 (~10-fold increase) in adults ≥ 75 years and older³. VTE may also be more closely linked to cardiovascular disease risk than previously appreciated, suggesting the possibility of shared risk factors and mechanisms⁴. Consequently, as the life expectancy increases and the proportion of adults ≥ 65 years rises, the burden of thrombotic disease in the elderly will likely become even greater.

Older adults are also at increased risk for acute systemic inflammatory and infectious syndromes, such as sepsis, acute lung injury, and acute respiratory distress syndrome⁵. For example, in a large longitudinal study, patients ≥ 65 years accounted for more than 60% of all sepsis cases and had a 13-fold higher relative risk of developing sepsis compared with younger patients⁵. In this dataset, case-fatality rates also increased linearly with age⁵. While comorbid conditions, frailty, and immunosenescence are implicated in this higher risk, exaggerated and/or dysregulated platelet functions are also likely contributors.

Platelet Functions Bridge Thrombosis and Inflammation

Platelets are anucleate blood cells specialized for rapid hemostatic reactions directed at sites of vascular injury⁶. The central role of platelets in thrombus initiation and propagation has long been recognized⁶. Nevertheless, initially thought to be merely circulating cell fragments with a relatively fixed repertoire of functional responses, more recent discoveries demonstrate that platelets are more versatile and dynamic effector cells that bridge thrombotic and inflammatory continua (Figure 1 and⁶). Platelet activation, which can be induced by thrombin, collagen, platelet-activating factor (PAF), microbial agents and/or toxins, and other agonists, leads to numerous platelet responses, including activation of integrin $\alpha_{IIb}\beta_3$ and upregulation of surface p-selectin, secretion of antimicrobial factors, chemokines, and cytokines, and enhanced aggregation with other cells⁶.

Human platelets possess adrenergic and dopaminergic receptors and avidly take up and secrete catecholamines⁷. While plasma catecholamine levels fluctuate dynamically, platelet catecholamine levels accumulate in a more stable, progressive pattern. Catecholamine secretion induces platelet activation and aggregation and may also potentiate the effects of other platelet agonists, including beta-thromboglobulin (β -TG) and platelet factor 4 (PF4). In settings of sympathoadrenal activation, including cardiovascular disease and sepsis, increases in circulating and platelet catecholamine levels may potentiate platelet functional responses.

Activated platelets aggregate with other platelets (homotypic aggregation) and with leukocytes, including neutrophils, monocytes, and lymphocytes (heterotypic aggregation).

Platelet-leukocyte aggregation induces the secretion of numerous proteins, peptides, biologically active lipids, and eicosanoid mediators by activated platelets⁶. In addition, platelet-leukocyte aggregation leads to the upregulation of pro-inflammatory gene synthesis by leukocytes, further contributing to thrombotic and inflammatory responses⁸⁻¹⁰. Increased platelet-leukocyte aggregates have been detected in patients with acute coronary syndromes, stroke, sepsis, acute lung injury, and other diseases^{6, 9, 11, 12}. Dysregulated platelet-leukocyte interactions may contribute to injurious thrombotic and inflammatory responses in older patients, leading to an increased risk of adverse clinical outcomes.

Activated platelets serve as a platform for the induction and amplification of thrombus formation through numerous direct and indirect mechanisms. Platelet activation results in the transport of negatively charged phospholipids to the platelet surface membrane, forming a site for assembly of the tenase and prothrombinase complexes (which include activated factor(F) FVa, FVIIIa, and FXa)¹³. Platelets are also major reservoirs for pro-thrombotic, fibrinolytic, and inflammatory mediators. These key factors, many of which are stored within platelet granules and released upon activation, include fibrinogen, von willebrand factor (vWF), and plasminogen-activating inhibitor (PAI)-1. Fibrinogen and vWF both promote hemostasis and thrombosis (whether adaptive or maladaptive) while PAI-1 enhances fibrinolysis.

Older adults may have comorbid conditions (e.g. cancer, immobility, diabetes, obesity, etc.) that increase their risk of thrombo-inflammatory disorders. Nevertheless, underlying, age-related changes in platelets are also likely contributors. While these changes remain only partially understood (and thus present important opportunities for well-designed experimental and clinical studies), established and emerging evidence support the supposition that the platelet molecular signature and associated functional responses are altered during aging, contributing to thrombotic and inflammatory syndromes in older adults.

Intrinsic Platelet Activation, Aggregation, and Secretion are Enhanced in Older Adults

The classic platelet functions of activation and aggregation are altered during aging. For example, plasma levels of beta-thromboglobulin (β -TG) and platelet factor 4 (PF4), factors secreted upon platelet activation, are increased in healthy older adults^{14, 15}. β -TG and PF4 are key effector molecules implicated in the pathophysiology of vascular wall and systemic inflammatory disorders and wound repair⁶.

Bleeding time, a measure of platelet aggregation responses (i.e. greater aggregation associated with fast clot formation and shorter bleeding time), decreases with aging, an indirect demonstration that platelets are hyperreactive in older adults^{16, 17}. Similarly, adults 60 years and older have reduced platelet aggregation thresholds in settings of stimulation with adenosine diphosphate (ADP) and collagen, compared to younger subjects^{14, 18, 19}. Platelet alpha 2-adrenergic receptor density and receptor-mediated inhibition of adenylyl cyclase, which mediate platelet aggregation, are also reduced in healthy older adults, contributing to a propensity towards platelet activation and homotypic aggregation responses^{20, 21}. Older adults also have decreased platelet surface prostacyclin (PGI_2) receptor density, increased platelet resistance to PGI_2 inhibition, and increased urinary excretion of prostacyclin metabolites^{17, 22}. PGI_2 is an eicosanoid that inhibits platelet aggregation and leads to vasodilation. Similarly, in experimental and human studies endothelial and platelet nitric oxide production decreases in aging, further leading to heightened platelet activation, atherogenesis, and thrombosis^{23, 24}. Interestingly, enhanced platelet activity in older healthy subjects correlates with higher basal platelet

polyphosphoinositide content and thrombin-stimulated platelet polyphosphoinositide turnover, patterns indicative of age-related increases in platelet lipid concentration¹⁵. These observations demonstrate that in humans, aging is associated with increases in platelet transmembrane signaling, prostaglandin synthesis, and cyclooxygenase activity. Taken together, these findings provide evidence that the structure, intra-cellular content, and function of platelets are significantly altered in older adults, thus promoting a pro-thrombotic, pro-inflammatory milieu (Figure 2).

Enhanced Platelet-Monocyte Interactions in Older Adults May Promote Injurious Thrombo-Inflammatory Responses

In addition to platelet activation and aggregation, monocyte phenotype and function are altered in older adults, resulting in amplified platelet-monocyte interactions and downstream thrombo-inflammatory effects. Circulating monocytes are innate immune cells that rapidly respond to infectious and inflammatory cues to produce numerous regulatory and pro-inflammatory molecules. These molecules include interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)- α ^{25, 26}. Of these, IL-6 has been particularly implicated as a central mediator of dysregulated inflammatory pathways in aging²⁷. IL-6 serum concentrations increase with aging, independent of confounding illnesses²⁸. IL-6 mediates inflammatory activities and also upregulates the synthesis of hemostatic factors, such as fibrinogen²⁷. IL-6 may also directly activate platelets, leading to platelet aggregation and secretion of arachidonic acid metabolites such as thromboxane A2 and β -TG²⁹.

Monocyte surface antigens differentiate cellular sub-populations. The two major subsets are commonly defined based on the expression of the CD14 (also the receptor for lipopolysaccharide (LPS)) and CD16 (Fc γ RIII receptor) surface antigens. In healthy individuals, the majority of circulating monocytes (~95%) display the cell surface antigen CD14 and the remaining, smaller fraction of monocytes also express CD16, a pattern typically referred to as 'classical' CD14^{high}/CD16^{low} (or CD14⁺⁺/CD16⁻). The remaining 'non-classical' monocytes are referred to as CD14^{low}/CD16^{high} (or CD14⁺/CD16⁺). Differences in the receptors and functional responses of these two monocyte subtypes are worth briefly noting as they provide biological links to thrombotic and inflammatory disorders. CD14^{high}/CD16^{low} monocytes possess the Chemokine (C-C motif) receptor 2 (CCR2), CD62L (L-selectin), and FC γ RI. In contrast, CD14^{low}/CD16^{high} monocytes lack CCR2 but possess higher levels of the major histocompatibility complex (MHC)-II as well as the receptor FC γ RII and, accordingly, synthesize greater amounts of pro-inflammatory cytokines³⁰. During acute settings of systemic activation and increased platelet-monocyte aggregation (such as sepsis), the population of circulating CD14^{low}/CD16^{high} monocytes is expanded, potentially contributing to exaggerated cytokine generation and injurious thrombo-inflammatory responses^{30, 31}.

Clinical studies suggest, however, that even in the absence of acute inflammatory syndromes, aging is associated with a shift towards non-classical, pro-inflammatory monocytes (e.g. CD14^{low}/CD16^{high}). For example, in a cohort of 181 healthy adults aged 18–88 years, non-classical CD14^{low}/CD16^{high} monocyte counts increased with age and, consistent with phenotypic changes described above, demonstrated altered surface protein and chemokine receptor expression²⁵. These findings were consistent with results from a smaller study of nursing home residents where isolated monocytes from older adults produced higher levels of pro-inflammatory cytokines and/or exhibited imbalanced cytokine production³². Importantly, *ex vivo* incubation of freshly isolated monocytes with platelets from older, healthy adults leads to higher levels of IL-6 and monocyte chemotactic protein-1 (Rondina MT, Campbell RC, et al; unpublished observations). Thus, taken together, these clinical data suggest that the chronic inflammatory condition that often exists in older adults

may be due, in part, to shifts in monocyte sub-populations, a greater propensity towards interactions with platelets, and exaggerated, downstream, pro-inflammatory gene synthesis (Figure 2). While more investigations are needed, these molecular changes may have direct links to the increased risk of thrombotic and inflammatory events in older adults.

Platelet Hemostatic Factors are Increased in Older Adults

Aging is associated with alterations in many plasma coagulation factors that are stored, synthesized, and/or released by platelets. For example, platelet alpha granules contain fibrinogen, factor V, and vWF and upon activation release these and other mediators into the systemic milieu. Fibrinogen binds to activated $\alpha\text{IIb}\beta 3$ integrin on the platelet surface, allowing for platelet activation and aggregation. Plasma fibrinogen levels increase with age, with an approximate 10 mg/dL incremental rise per decade in healthy subjects³³. Increased fibrinogen levels are correlated with an increased risk of stroke and myocardial infarction³⁴ and may also predispose older adults to VTE³⁵. In similar fashion, vWF levels also increase with aging^{36, 37}. vWF, which is produced constitutively in megakaryocytes and stored within platelets, binds collagen at areas of damaged endothelium or sub endothelium, thus contributing to the development of atherosclerotic plaque. vWF also binds to Factor VIII (FVIII), providing a stable platform for continued propagation of thrombin generation. FVIII levels also increase with aging and are associated with an increased risk of sub clinical cardio vascular disease and overt thrombosis³⁸.

Platelet-associated components of the fibrinolytic pathway are substantially altered in older adults. For example, plasma levels of PAI-1, the major inhibitor of fibrinolysis, increase with aging³⁹. While platelets are not the only source of PAI-1, platelets synthesize, store, and release large amounts of functional PAI-1 in a signal-dependent fashion⁴⁰. Obesity, which is more common in older adults, may also increase PAI-1 levels, thus further enhancing the risk of thrombotic events. Interestingly, transgenic mice that overexpress a stable variant of human PAI-1 demonstrate spontaneous coronary artery thrombosis that only occurs in older mice (i.e. in an age-dependent fashion)⁴¹. While mouse models may not always precisely recapitulate human physiology, these observations provide intriguing mechanistic insights and support clinical observations in older adults.

Taken together, these findings highlight the numerous soluble thrombo-inflammatory factors that are substantially altered in older adults (Table 1). Platelets and platelet precursors (megakaryocytes) synthesize and/or internalize these factors and, in response to activating signals (e.g. damaged endothelium, bacterium or bacterial toxins, cytokines, and other agonists), rapidly release them into the systemic circulation. Thus, platelets in older adults may be “primed” for exaggerated responses, enhancing susceptibility to adverse clinical outcomes in settings of acute vascular and systemic inflammatory syndromes.

Clinical and Therapeutic Considerations Targeting Dysregulated Platelet Responses in Older Adults

As noted above, the prevalence of thrombotic and inflammatory disorders increases markedly in older adults. While comorbid conditions may predispose the elderly to these disorders, aging-associated changes in platelet phenotype and function, increased levels of platelet hemostatic factors, and enhanced interactions with leukocytes and endothelial cells also contribute to these disease processes. Established and emerging therapies that inhibit platelet activation, aggregation, and adhesion continue to evolve. A brief review of anti-platelet agents (APAs) and their role in two thrombo-inflammatory disorders, ischemic stroke and sepsis, is provided here. We have chosen to focus on these two diseases given their high prevalence, morbidity, and mortality in older adults.

Anti-Platelet Agents in Ischemic Stroke

Alterations in platelet phenotype and function underlie the pathophysiology of ischemic stroke⁹ and APAs remain the cornerstone of therapy in preventing recurrent stroke. Current, FDA-approved APAs for secondary stroke prevention (Table 2) include aspirin, clopidogrel, and dipyridamole⁴². These drugs inhibit platelet activation and aggregation and improve clinical outcomes. While current guidelines generally accept any of these APAs for secondary stroke prevention, the combination of aspirin plus extended-release dipyridamole is recommended over aspirin alone in the 2012 American College of Chest Physician guidelines, particularly if cost is not an issue⁴².

Several key aspects of these APAs are worth mentioning. Mechanistically, aspirin blocks cyclooxygenase-1 (COX-1) dependent synthesis of thromboxane while clopidogrel irreversibly inhibits the P2Y₁₂ ADP receptor. Through inhibition of ADP-mediated platelet aggregation, clopidogrel, but not aspirin, also blocks platelet surface P-selectin, platelet-leukocyte aggregate formation, and the presence of tissue factor on the surface of platelets and leukocytes^{43, 44}. Through these and other mechanisms, clopidogrel may attenuate downstream thrombo-inflammatory processes in addition to its direct inhibition of platelet aggregation. Consistent with these experimental data, clinical trial data from CAPRIE suggest that compared to aspirin 325mg daily, clopidogrel (at a dose of 75mg daily) is more effective at reducing the composite endpoint of stroke, myocardial infarction, or vascular death (5.32% vs. 5.83%, relative risk reduction of 8.7%, p=0.043) without significant increases in bleeding risk⁴⁵.

Dipyridamole inhibits phosphodiesterase enzymes, leading to increases in extra cellular adenosine levels. While a potent anti-platelet agent, dipyridamole also has selective anti-inflammatory properties. For example, *in vitro* investigations using co-incubated human platelets and monocytes demonstrate that dipyridamole (at concentrations similar to those achieved clinically), attenuates IL-8 and MCP-1 synthesis⁴⁶. In these same investigations, aspirin did not block pro-inflammatory gene synthesis. These experimental observations support clinical trial data suggesting that compared to aspirin as monotherapy, the combination of aspirin plus extended-release dipyridamole results in significant reductions in adverse clinical events, including vascular death, stroke, and myocardial infarction⁴⁷. Moreover, aspirin plus extended-release dipyridamole have similar safety and efficacy profiles relative to clopidogrel monotherapy⁴⁸. Aspirin plus clopidogrel is generally not a recommended therapy for secondary stroke prevention. This combination increases bleeding risk without offering any significant clinical benefits over aspirin or clopidogrel monotherapy.

Anti-Platelet Agents in Septic Syndromes

Septic syndromes, including sepsis, severe sepsis, and septic shock, are the 10th leading cause of death among older adults. Septic syndromes are characterized by pervasive platelet activation, adhesion, and aggregation. As a result, pre-formed and newly-synthesized platelet mediators (IL-1 β , tissue factor, PF4, β -TG, p-selectin, etc.) are released into the systemic milieu, contributing to exaggerated inflammation, micro- and macro-vascular thrombosis, organ failure, and death. While still incompletely understood, age-associated platelet hyperreactivity, along with expansion in non-classical monocyte populations, may strongly influence the increased incidence, severity, and mortality from sepsis in older adults⁵. Consistent with this supposition, older adults with the highest circulating levels of TNF- α and IL-6 (pro-inflammatory cytokines produced upon platelet-monocyte aggregate formation) had the highest risk of sepsis due to pneumonia, independent of medical conditions, steroid use, or smoking status⁴⁹.

Given the important role that platelets play in the pathophysiology of septic syndromes, recent studies have examined whether APAs improve clinical outcomes in both experimental and clinical sepsis. In a rat model of endotoxin-induced systemic inflammation, clopidogrel reduced the synthesis of TNF- α and IL-6, and attenuated liver and lung inflammation⁵⁰. Similarly, in acid-induced and transfusion-related lung injury models, platelet depletion as well as inhibiting platelet adhesion and activation, protects against lung injury^{51, 52}. In clinical studies, pre-hospital aspirin therapy was associated with significantly lower rates of acute lung injury/acute respiratory distress syndrome (ALI/ARDS), which is commonly caused by sepsis (incidence of ALI/ARDS of 17.7% vs. 28.0% for pre-hospital, aspirin non-users, $p < 0.05$)⁵³. Similarly, a larger retrospective analysis examined mortality rates in more than 1600 critically ill patients admitted to an ICU, based on the in-hospital prescription of APAs for the secondary prevention of vascular disease. Overall, patients who received an APA (approximately 25% of the entire cohort) had significant mortality reductions, without any increase in bleeding complications⁵⁴. Studies in critically ill patients receiving blood transfusions, another common risk factor for ALI/ARDS, have also found benefit with APAs⁵⁵.

Thus, these emerging data highlight an intriguing new potential therapeutic role for APAs in the prevention of ALI/ARDS. Furthermore, in older patients, who often have an increased bleeding risk, low-dose aspirin offers the possibility of reducing adverse clinical events in critical illness without causing significant harm. A large, prospective randomized clinical trial evaluating the role of aspirin for the prevention of ALI in hospitalized, high risk patients is currently ongoing⁵⁶.

Conclusions

In conclusion, aging is associated with altered and dysregulated platelet functions, leading to platelet hyperreactivity, enhanced aggregation and interactions with other cells, and an increased risk of injurious thrombo-inflammatory events. Nevertheless, our understanding of the molecular and phenotypic changes in platelets with aging remain only partially understood, limiting advances in therapeutic options. Currently approved anti-platelet agents have clear roles in cardiovascular disease prevention and treatment and emerging roles in acute systemic inflammatory syndromes such as sepsis and ALI/ARDS. Ongoing research is needed to fill these knowledge gaps and identify novel therapies that safely and effectively reduce the burden of thrombo-inflammatory disease in older adults.

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Figure 1.

Human platelets have a rich repertoire of dynamic functions that span thrombotic and inflammatory pathways. Panel A: In response to agonists such as thrombin, adenosine diphosphate (ADP), lipopolysaccharide (LPS), and other pathogens or toxins, platelets are activated. Platelet activation results in numerous functional responses that include the expression of surface ligands, homotypic and heterotypic binding, protein synthesis, the release of pro-thrombotic and pro-inflammatory mediators, and signaling to leukocytes and endothelial cells. These responses and interactions are discussed in the text. Panel B: Immunocytochemistry (ICC) image demonstrating freshly isolated human platelets activated with thrombin and adhering to fibrinogen. In this image, the green stain identifies actin filaments, the red stain identifies fibrin strands (white arrowheads), and the magenta stain identifies the binding of wheat germ agglutinin (WGA) to sialic acid residues within platelets (white arrows). Panel C: The functional responses of activated platelets are pivotal in acute thrombotic disorders, such as stroke. Illustrated here are activated platelets interacting with and binding to monocytes and red blood cells (RBC) to form a thrombotic occlusion within the vascular lumen.

Figure 2.

Aging is associated with platelet hyperreactivity, leading to increased expression of platelet surface ligands, exaggerated platelet and leukocyte aggregation, and enhanced release of pro-thrombotic and pro-inflammatory mediators. These changes contribute to the increased risk of adverse thrombo-inflammatory events in older adults and are discussed in the text.

Table 1

Summary of select age-related changes discussed in the manuscript text. A brief description of several of the key functions is also provided.

	Description and Function	Change During Aging
Platelet factor 4 (PF4)	Secreted by platelets upon activation; pro-coagulant factor; chemoattractant for neutrophils and fibroblasts	Increased in older adults
Interleukin-6	Pro-inflammatory cytokine; produced by monocytes bound to and activated by platelets; in some settings may activate platelets	Increased in older adults
Fibrinogen	Released by activated platelets; binds activated α IIB β 3 integrin on platelet surface; induces platelet aggregation	Increased in older adults
von Willebrand Factor (vWF)	Released by activated platelets; binds collagen at areas of damaged endothelium or sub-endothelium; binds Factor VIII allowing for thrombin generation	Increased in older adults
Plasminogen-activating inhibitor-1 (PAI-1)	Major inhibitor of fibrinolysis; synthesized, stored, and released by platelets	Increased in older adults

Table 2

Mechanisms of Action of Antiplatelet Agents and 2012 American College of Chest Physician (ACCP) Guidelines for the Prevention of Recurrent Stroke

Antiplatelet Agent	Primary Mechanism of Action	Recommended Dose	Grade of Evidence ^a	Comments
Aspirin	Inhibits cyclooxygenase-1 (COX-1)-dependent synthesis of thromboxane A ₂	50–325mg once daily	1A	Aspirin as monotherapy or in combination with extended-release dipyridamole
Dipyridamole	Inhibits phosphodiesterase enzymes that break down cAMP and cGMP; increases extracellular adenosine levels	200mg twice daily	1A	Combination of aspirin with extended-release dipyridamole preferred in some settings over aspirin monotherapy
Clopidogrel	Thienopyridine that specifically and irreversibly inhibits the P2Y ₁₂ ADP receptor	75mg once daily	1A	As monotherapy, particularly in patients allergic to aspirin

^aGrade definitions: 1A, strong recommendation, high evidence; 1B, strong recommendation, moderate quality evidence; 2B, weak recommendation, moderate quality evidence. (ADP: adenosine diphosphate, cAMP: cyclic adenosine monophosphate, cGMP: cyclic guanosine monophosphate)