The Current and Future Landscape of Urinary Thromboxane Testing to Evaluate Atherothrombotic Risk

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Biomarker testing for efficacy of therapy is an accepted way for clinicians to individualize dosing to genetic and/or environmental factors that may be influencing a treatment regimen. Aspirin is used by nearly 43 million Americans on a regular basis to reduce risks associated with various atherothrombotic diseases. Despite its widespread use, many clinicians are unaware of the link between suboptimal response to aspirin therapy and increased risk for inferior clinical outcomes in several disease states, and biomarker testing for efficacy of aspirin therapy is not performed as routinely as efficacy testing in other therapeutic areas. This article reviews the clinical and laboratory aspects of determining whole-body thromboxane production, particularly as it pertains to efficacy assessment of aspirin responsiveness.

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KEY WORDS

Aspirin • Urinary thromboxane • Atherothrombotic disease • Platelet function • Personalized medicine

Biomarker testing for efficacy of therapy is an accepted way for clinicians to individualize dosing to genetic and/or environmental factors that may be influencing a treatment regimen. Classic examples include determining glucose or hemoglobin A_{1C} levels prior to modulating diabetes therapy,[1] determination of biomarkers such as low-density lipoprotein cholesterol or C-reactive protein when making a decision to place certain patients on statin drugs,[2,3] and measuring natriuretic peptides in congestive heart failure.[4] However, biomarker testing for efficacy of aspirin therapy is not performed as routinely as efficacy testing in other therapeutic areas. Aspirin is used by nearly 43 million Americans on a regular basis,[5] prescribed by clinicians to reduce risks associated with various atherothrombotic diseases. Despite its widespread use, many clinicians are unaware of the link between suboptimal response to aspirin therapy and increased risk for inferior clinical outcomes in several disease states.[3,6] This article reviews the clinical and laboratory aspects of determining whole-body thromboxane production, particularly as it pertains to efficacy assessment of aspirin responsiveness.

Platelet Pathobiology in Atherothrombotic Diseases: the Role of Aspirin

Arterial thrombosis is the final pathogenic mechanism of many acute ischemic events, including acute myocardial infarction (MI) and sudden cardiac arrest. This process involves complex interactions within the atherosclerotic artery, including endothelial injury, vasospasm, and platelet activation. Plaque rupture exposes thrombogenic subendothelial components, which leads to platelet deposition and further activation.[7]

Aspirin has several important effects on the human body, most notably the reduction of pain, fever, and inflammation. Aspirin's capacity to prevent blood clotting as a platelet function inhibitor has been employed as an important treatment and prevention modality in atherothrombotic disease. The ability of aspirin to produce these myriad effects is mediated by reducing the production of prostaglandins and thromboxane via the irreversible inactivation of the cyclooxygenase (COX) enzyme, which is required for thromboxane synthesis. Aspirin irreversibly acetylates a serine residue in the COX-1 enzyme, a unique function not served by other drugs in the nonsteroidal anti-inflammatory drug (NSAID) class.[8]

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Aspirin therapy has been reported to reduce cardiovascular events by up to 40%. However, use of aspirin is associated with higher frequencies of gastrointestinal bleeding and hemorrhagic stroke. Moreover, in certain individuals, the expected degree of aspirin responsiveness is muted. Given both these considerations there is the potential for great clinical utility in identifying individuals who will most likely benefit from antiplatelet therapy with aspirin versus patients in whom aspirin therapy may not be sufficient.

A substantial body of literature exists examining aspirin-insensitive thromboxane production and its association with atherothrombotic risk. Various terms have been used to describe in vivo and in vitro phenomena, in which the expected degree of aspirin responsiveness is not manifested. The in vivo clinical phenomenon is variably called *aspirin nonresponse*, *aspirin treatment failure*, or *aspirin resistance after the use of aspirin*, and has parallels (but is not synonymous) with the in vitro laboratory phenomenon of *aspirin resistance*. The laboratory endpoint addressed in this article is physiologic *aspirin-insensitive thromboxane biosynthesis*, a term preferred by some authors.[9-11]

Thromboxane Metabolism: Critical Links for Risk Assessment

Antiplatelet medications are frequently used in the prevention of stroke, MI, and vascular thrombotic diseases due to the fundamental role of platelet aggregation in a variety of atherothrombotic processes. Modulation of the prostaglandin thromboxane A_2 (TxA_2) pathway is one of the pivotal routes of activation involved in stimulating platelet aggregation. The COX enzyme regulates the conversion of arachidonic acid to thromboxane. This enzyme exists in two forms: COX-1, the constitutive form found in all tissues, and COX-2, which is induced during inflammatory states.[12,13] Both COX-1 and COX-2 metabolize arachidonic acid to prostaglandin H_2, the common substrate for TxA_2. Synthesis of TxA_2 in platelets is mediated by the COX-1 enzyme, which must be functional for stimulating the production of TxA_2 from arachidonic acid. COX-2 has been identified in human atherosclerotic plaques, cells associated with chronic inflammation such as monocytes/macrophages, and in newly formed platelets.[14,15] These nonplatelet sources may contribute to thromboxane production through

production of prostaglandin H_2, which is considered a possible bypass mechanism to the effects of low-dose aspirin therapy (Figure 1). Additionally, it has been suggested that the presence of naturally occurring isoprostanes may contribute to platelet activation and subsequent irreversible aggregation when platelet agonists such as TxA_2 occur in subthreshold concentrations.[16]

The importance of TxA_2 is demonstrated by the reduction in risk of acute MI or death in patients with acute coronary syndrome (ACS) following administration of low-dose aspirin, which irreversibly inhibits the production of TxA_2 in platelets. Production of platelet-derived TxA_2 has been completely inhibited by daily doses of aspirin as low as 100 mg.[17]

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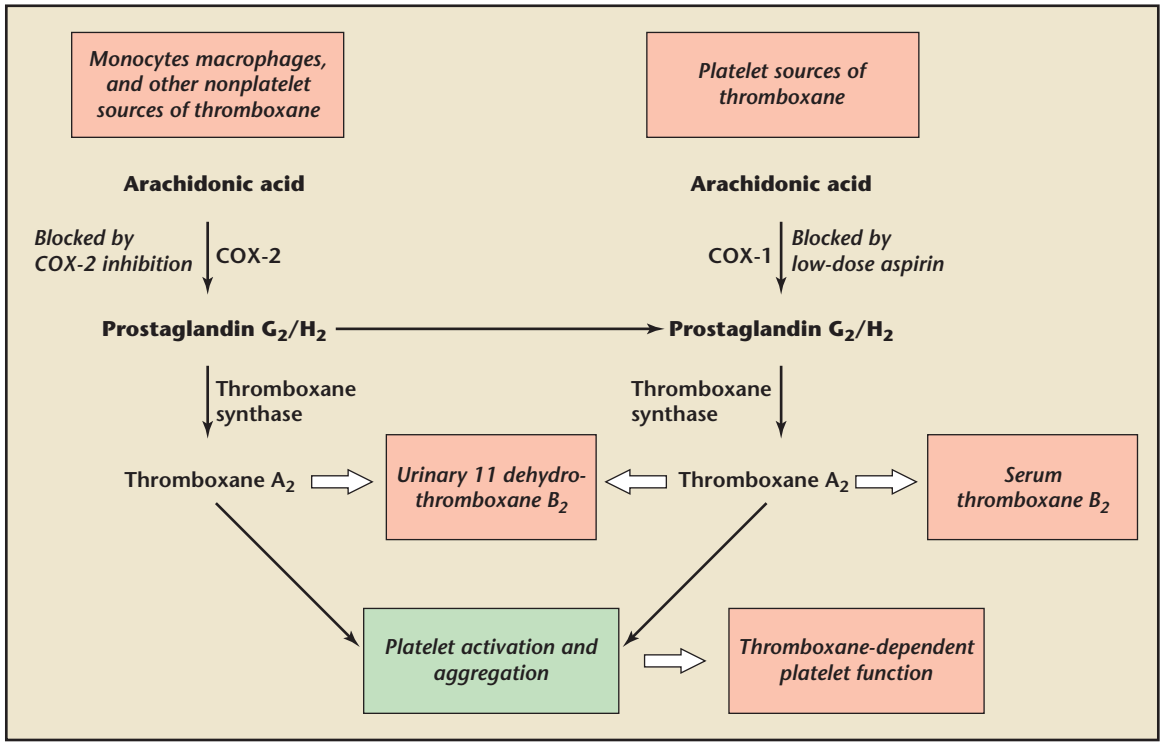
TxA_2 has an extremely short half-life, converting to two stable but inactive metabolites: 11-dehydro-thromboxane B_2 (TxB_2) and 2,3-dinor-11-dehydro-TxB_2. Excretion of TxB_2 in the urine has been shown to reflect in vivo platelet activation. Elevated concentrations of TxB_2 have been noted in patients with various high-risk phenotypes.[18-20]

Quantitation of urinary TxB_2 ($UTxB_2$) offers an advantage over platelet activation markers measured in plasma or blood because urine measurements are not subject to interference from in vitro platelet activation, which commonly occurs as a result of preanalytical variables such as local vein trauma or insufficient anticoagulation during phlebotomy.[21,22] Measurement of $UTxB_2$ may be performed in patients to assess the effectiveness of specific inhibition in TxA_2 production, along with identification of patients' ability to benefit from antiplatelet therapy, and their associated risk for developing future cardiovascular events (Figure 1).

How Urinary Thromboxane Testing Varies From Other Forms of Platelet Testing

The laboratory measurement of aspirin "failure" has been achieved to date by examining one of two pathways: (1) TxA_2 production, or (2) thromboxane-dependent platelet function. Though thromboxane is central to both measurement techniques, each method provides a different facet of information that may be complementary to the other. For example, little or no serum thromboxane may indicate that aspirin is effectively eliminating COX-1 production of thromboxane by the platelet, whereas elevated levels of urinary thromboxane in

Figure 1. Pathway of thromboxane production and antiplatelet effects of aspirin. Reproduced with permission from Hankey and Eikelboom.[23]



the same individual may indicate the continuing presence of elevated risk due to systemic thromboxane production not addressed by the patient's current aspirin dose.

Thromboxane A_2 Production

TxA_2 production can be determined by measuring stable metabolites of TxA_2, such as TxB_2 in the serum (or plasma) and 11-dehydro-TxB_2 in the urine. The measurement of serum or plasma thromboxane is specific to the platelet's capacity to produce thromboxane, whereas measurement of urinary thromboxane is nonspecific and is reflective of the entire body's production of thromboxane.

Thromboxane-dependent Platelet Function

Tests of platelet function that are dependent on platelet thromboxane production include agonist-induced platelet aggregation measured by light or optical transmission (turbidimetric aggregometry in platelet-rich plasma), electrical impedance (whole blood platelet aggregometry), or semiautomated platelet aggregometry assay (eg, VerifyNow P2Y12 Test; Accumetrics, San Diego, CA; TEG–Platelet Mapping; Mayo Medical Laboratories, Rochester, MN; Plateletworks; Helena Laboratories, Beaumont, TX). Additionally, the semiautomated PFA-100 (Siemens Medical Solutions, Malvern, PA) relies on thromboxane production; however, unlike the previously mentioned platforms, it does not specifically activate the COX-1 pathway by the addition of arachidonic acid. Finally, the bleeding time is an in vivo test of platelet function that is also dependent, in part, on platelet thromboxane production but is not frequently used because it is highly operator dependent and results are poorly reproducible.

Urinary 11-dehydro-TxB_2: Stable Metabolite to Assess Thromboxane Metabolism

One approach to quantify the activity of aspirin has been to measure the levels of the byproducts of COX-1 enzyme activity. Reduced levels of these products would normally be expected as a result of aspirin administration. TxA_2 cannot be used for measurement because it is a highly unstable compound with a short half-life of 30 seconds.[24] TxA_2 is rapidly converted in vivo to the more stable TxB_2, which is subsequently converted by the liver into two major metabolites: 2,3-dinor-TxB_2 and 11-dehydro-TxB_2. Both metabolites, along with total TxB_2, are excreted unchanged in the urine.[25] The small amount of unchanged total TxB_2 in urine and 2,3-dinor-TxB_2 levels are likely more reflective of renal TxB_2 biosynthesis than of platelet activity. Additionally, measurement of serum TxB_2 can be prone to artifact. In contrast, 11-dehydro-TxB_2 is a stable metabolite of TxB_2 that can be measured noninvasively in the urine and can thus serve as an indirect measure of TxB_2 activity in vivo.[26]

Aspirin use has been shown to cause a dose-dependent reduction in urinary levels of 11-dehydro-TxB_2. Use of standardized controls circumvents the variability of the assay among testing laboratories. The assay requires a 2-mL urine sample and is normalized to the patient's urine creatinine (therefore, results are expressed as pg $UTxB_2$/mg creatinine). This assay is advantageous because it is noninvasive and is normalized with standard controls.[27] Currently, a US Food and Drug Administration (FDA)-cleared and commercialized assay uses a monoclonal-linked immunosorbent assay (ELISA) technology (vs the previously available polyclonal assay used for research). Both ELISAs utilize primary antibodies that predominantly recognize 11-dehydro-TxB_2 but exhibit variable cross-reactivity with other related TxB_2 species, such as 11-dehydro-2,3-dinor-TxB_2.[28] DeFilippis and colleagues[29] examined the two ELISA assays along with a third method for $UTxB_2$ detection (liquid chromatography-mass spectrometry [LC-MS]) in a well-characterized cohort of patients with acute MI. They found that the newer-generation monoclonal ELISA (but not the older polyclonal ELISA, nor LC-MS) was capable of differentiating patients with atherothrombotic acute MI from those whose infarction was not caused by atherothrombotic disease.[29] The authors concluded that 11-dehydro-2,3-dinor-TxB_2 may play an important role in the pathophysiology of atherothrombosis, particularly in individuals on adequate aspirin therapy. Moreover, they suggest that, "given the great clinical importance of the accurate diagnosis and treatment of atherothrombosis, the investigation of such an analyte is warranted."

The manufacturers of this FDA-cleared assay studied apparently healthy adults before and after receiving controlled doses of aspirin. Based on the resulting frequency of $UTxB_2$ levels, they established a cut-off value to assess an adequate aspirin effect as $\leq$ 1500 pg $UTxB_2$/mg of creatinine. The assay is indexed to urinary creatinine concentration to control for varying urine concentrations based on hydration.[30-31] This cut-off was reconfirmed in subsequent studies that investigated healthy and diseased populations before and after

aspirin treatment. After aspirin ingestion, $UTxB_2$ levels below the cutoff (< 1500 pg/mg) were classified as good aspirin effect, whereas levels above 1500 pg/mg were considered poor aspirin effect. Though this established cutoff value was used to determine the presence or absence of an expected aspirin effect, the actual concentrations of the measured metabolite were used to assess atherothrombotic risk in a quartile fashion.

Patient Populations With Potential to Benefit From Urinary Thromboxane Testing

Patients in Need of Cardiovascular Disease Risk Reduction

There are strong suggestions from three major clinical trials that evaluation of 11-dehydro-TxB_2 production may be a more important surrogate marker for clinical response to aspirin therapy than isolated arachidonic acid-induced platelet aggregation. A nested case-control study of 970 patients with a history of aspirin use enrolled in the Heart Outcomes Prevention Evaluation (HOPE) trial[20] had baseline measurements taken of urinary 11-dehydro-TxB_2 levels as a marker of overall thromboxane generation. At the 5-year follow-up, patients with lower response to aspirin treatment (ie, levels in the highest quartile) had a significantly greater risk for a composite endpoint of nonfatal MI, nonfatal stroke, or cardiovascular death compared with those in the lowest quartile (adjusted odds ratio [OR] 1.8; 95% confidence interval [CI], 1.2-2.7) with significant increases in acute MI and cardiovascular-related death.

In the aspirin-treated patients from the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) study,[32] baseline urinary $UTxB_2$ concentrations in the highest quartile were associated with an increased risk of stroke, MI, or cardiovascular death compared with results in the lowest quartile (adjusted hazard ratio 1.7; 95% CI, 1.1-2.6).

The Reduction in Graft Occlusion Rates (RIGOR)[33] study evaluated 229 subjects treated with aspirin following coronary artery bypass graft (CABG) surgery. Although arachidonic acid-induced platelet aggregation was inhibited by $> 99\%$ at 6 months, 31% of subjects still had elevated urinary $UTxB_2$ excretion. In multivariate analysis, there was a statistically significant association between $UTxB_2$ excretion $\geq$ 450 pg/mg creatinine and graft occlusion (OR 2.59).

A more recent study tested 287 non–aspirin-treated patients who presented with ACS and underwent percutaneous coronary interventions. Levels of $UTxB_2$ were determined before and after aspirin treatment to determine a possible association of aspirin-free baseline levels with adverse events. High aspirin-free baseline levels were associated with a higher incidence of poor aspirin response after treatment. Though aspirin-free baseline levels did not predict adverse events at 1 year, levels of $UTxB_2$ in the upper quartiles after aspirin treatment were associated with a more than twofold increased odds of adverse events, consistent with the findings from the CHARISMA study.[34]

Special Populations

Patients With Atrial Fibrillation With Low $CHADS_2$ or CHA_2DS_2-VASc Score. There have been many trials researching the optimization of therapy to prevent embolization in atrial fibrillation due to the high risk of disabling stroke in patients who develop this increasingly prevalent cardiac rhythm disorder. The recommendations from these trials clearly point toward anticoagulation or antiplatelet therapy, depending on the individual's risk. However, the highest quality evidence and recommendations have been geared toward patients with higher estimated risk based on other comorbidities (as predicted by $CHADS_2$ [Congestive heart failure, Hypertension, Age $\geq$ 75 y, Diabetes mellitus, prior Stroke or transient ischemic attack] or CHA_2DS_2-VASc [Congestive heart failure, Hypertension, Age $\geq$ 75 y, Diabetes mellitus, prior Stroke or transient ischemic attack, Vascular disease, Age 65-74 y, female Sex] scores).

For patients with $CHADS_2$ scores of $\geq$ 2, available evidence strongly suggests treatment with anticoagulation. Only the occasional exception will be managed using antiplatelet therapy such as aspirin. Conversely, a good deal of clinical uncertainty surrounds the management of patients with atrial fibrillation and low risk scores. In these patients, the benefits of anticoagulation may not always outweigh the lower risk of stroke in certain categories.[35] Determining which patient subgroups may be more responsive to aspirin therapy could be beneficial to these patients.

Patients with atrial fibrillation and a $CHADS_2$ score of 1 are considered to be at intermediate risk of stroke ($\sim$ 2% per year) and, according to recent guidelines, should be treated with oral anticoagulant therapy or aspirin (75-325 mg/d). Anticoagulant therapy is generally preferred to aspirin in most patients with a $CHADS_2$ score of 1, although in certain groups (eg, patients with a high risk of falling or of lower

gastrointestinal bleeding) aspirin may be the preferred therapy. Determining which patients will be best served by this strategy as well as what dose of aspirin remains a matter of controversy.

The issue of whether aspirin is the preferred choice in low-risk patients ($CHADS_2 = 0$) has not been well addressed. The individual trials that compared aspirin with placebo enrolled very few patients with a $CHADS_2$ of 0, but meta-analyses of these trials suggest that the risk of stroke was reduced by approximately 20% with aspirin use (but the introduction of aspirin to these patients included the potential for harm).[36] It is important to consider that these studies have not been done stratifying patients based on their potential degree of responsiveness to aspirin using laboratory testing, and there may therefore be an opportunity to refine knowledge and improve management in this population

Patients who are identified by systemic thromboxane generation as poor aspirin responders are considered to be in a state of enhanced oxidative stress that may play an important role in their maintaining active platelet function irrespective of COX-1 inhibition...

Type 2 Diabetes. Ames and colleagues[11] demonstrated that patients with diabetes have a 50% higher urinary 11-dehydro-TxB_2 excretion (both baseline and after administration of aspirin) than healthy control subjects, suggesting that suspected platelet hyperactivation and/or alternative sources of thromboxane generation may contribute to the development of more atherothrombotic sequelae in this population.[37,38] Patients who are identified by systemic thromboxane generation as poor aspirin responders are considered to be in a state of enhanced oxidative stress that may play an important role in their maintaining active platelet function irrespective of COX-1 inhibition, perhaps by an increase in systemic generation of thromboxane from nonplatelet sources.

Chronic Kidney Disease. There is a paucity of data concerning the efficacy of aspirin therapy in patients with kidney disease. Proper guidance for physicians who manage renal patients is lacking regarding which patients might benefit from aspirin therapy. Some observational studies have suggested that aspirin use may be associated with increased cardiovascular mortality or adverse cardiovascular events, but these same studies show a decrease in the risk of stroke.[39-41] A Cochrane meta-analysis that included randomized trials of nondialysis chronic kidney disease (CKD) and end-stage renal disease patients found that antiplatelet agents, compared with no treatment or placebo, reduced the risk of MI but not all-cause mortality, cardiovascular mortality, or stroke.[42] The trials reviewed consistently demonstrated that, in CKD, antiplatelet agents increased the risk of major and minor bleeding. In a post hoc analysis of the Hypertension Optimal Treatment (HOT) trial, Jardine and colleagues[43] suggested that aspirin therapy produces greater absolute reduction in major cardiovascular events and mortality in hypertensive patients with CKD than those with normal kidney function, and that the increased risk of major bleeding appears to be outweighed by significant benefits.

This is a sizeable at-risk population about whom the experts are undecided regarding when and how best to deploy aspirin therapy. Because the inhibition of prostaglandin synthesis by COX-1 inhibitors may worsen elements of renal function in patients with compromised renal hemodynamics, there is a reflex among nephrologists to be wary of aspirin and NSAIDs as a class. However, because the protective effects of aspirin against various lethal nonrenal diseases are so well appreciated, consensus opinion from nephrologists still recommends that "that individual treatment decisions be based upon consideration of patients' individual risks, potential benefits, and preferences. The prescription of low-dose aspirin (81 mg/d) is probably safe in most patients with CKD and those on chronic dialysis. These recommendations are consistent with the Kidney Disease Outcomes Quality Initiative clinical practice guidelines."[44] Knowing the potential degree of aspirin responsiveness of a given patient might assist nephrologists and others who manage patients with renal disease to make better-informed decisions on the optimization of primary and secondary disease prevention strategies in this high-risk and growing population.

Cardiothoracic Surgery. CABG is performed in patients with severe coronary artery disease to reduce risks of future atherothrombotic disease, specifically fatal and nonfatal MI. Paradoxically, however, the CABG procedure is associated with its own thrombotic risks of perioperative MI, stroke, pulmonary embolism, and bowel infarction.[45] These risks are of concern to the patient, the referring cardiologist and, of course, the operating cardiothoracic surgeon. Although antiplatelet drugs such as aspirin and clopidogrel reduce thrombotic events, they add to the risk of excessive bleeding during and after

surgery, with associated higher rates of blood transfusion, postoperative tamponade, and reoperation for bleeding.

Historically, bleeding concerns have led to the recommendation that aspirin be discontinued 3 to 5 days before surgery in patients undergoing elective CABG. However, this general approach is no longer recommended according to the 2011 American College of Cardiology Foundation/American Heart Association guideline for CABG, which recommends that aspirin be started or continued preoperatively.[46] Unfortunately, the recommended dosing range for aspirin is quite large (100-325 mg) and there is no guidance in this document or other consensus guidelines on how best to choose a specific dose of aspirin using available clinical information or laboratory testing. Clearly, the wide threefold dose range in the administration of a drug recommended as a Class I intervention represents a pharmacologic missed opportunity to reduce perioperative CABG morbidity and mortality by identifying the patients for whom aspirin works and at what dose. This same incongruity referring to wide aspirin dose ranges continues throughout the guidelines as they address issues related to postoperative aspirin administration in this high-risk population.

We know from work by Gluckman and colleagues[33] that we can identify aspirin-insensitive individuals who may benefit from alternate or more aggressive therapy in the perioperative CABG phases. This group's analysis from the RIGOR study showed that TxA_2 generation (as measured by $UTxB_2$) and shear-dependent platelet hyper-reactivity (as measured by PFA-100 collagen and adenosine diphosphate closure time) act as independent risk factors for early saphenous vein graft thrombosis. This indicates that there are pathways that are independent of platelet COX-1 activity that are not inhibited effectively by aspirin. Yet the timing, dosing and interruption of antiplatelet therapy perioperatively in CABG remain an area of substantial debate and research, with new concerns about bleeding being raised in this generation of dual antiplatelet antagonism.[47] Fortunately, there are researchers who advocate a personalized approach to the patient's drug regimen, which uses measurements of antiplatelet drug activity to predict the risk of excessive postoperative bleeding in patients.[48]

Heart Failure and Ventricular Assist Devices. The decision of whether to use antiplatelet and/or antithrombotic therapy in heart failure patients who do not have coexisting atrial fibrillation is controversial. The Warfarin and Aspirin in Patients in Reduced Cardiac Ejection Fraction (WARCEF) trial[49] noted that among patients with reduced left ventricular ejection fraction who were in sinus rhythm, there was no significant overall difference in the primary outcome between treatment with warfarin or aspirin. The reduced risk of ischemic stroke that was seen in those on warfarin was offset by an increased risk of major hemorrhage. The authors' conclusion was that the choice between warfarin and aspirin should be individualized, once again pointing toward the need to assay whether aspirin works for an individual patient. However, significant guidance based on thromboxane metabolite measurements has yet to be reviewed in the literature.

Ventricular assist devices are implanted in patients with end-stage heart failure to provide both short- and long-term hemodynamic support. Unfortunately, both bleeding and thromboembolic complications are introduced due to the severely disturbed flow conditions generated by these devices. Patients supported by ventricular assist devices are treated with systemic anticoagulation and antiplatelet agents to reduce the risk of thrombotic complications such as device thrombosis and embolic stroke. However, initiating anticoagulation too early or being too aggressive in this approach increases the risk of bleeding complications both in the early perioperative period (exacerbated by the coagulopathic effect of cardiopulmonary bypass [CPB]) and long-term postoperatively (via the development of an acquired form of von Willebrand disease). Rossi and colleagues,[50] among a set of recommendations based on an extensive review of the existing literature, suggest that using patient laboratory results to titrate aspirin and clopidogrel doses to a specifically targeted percentage of inhibition, permitted doses of aspirin to be lowered to as low as 25 mg/d while not disturbing the bleeding/thrombosis balance in these patients.

... aspirin therapy appears to be underutilized in patients with HIV.

Human Immunodeficiency Virus. Mechanisms for increased cardiovascular risk in human immunodeficiency virus (HIV)-1 infected adults are not yet fully understood, but platelet activation and immune activation leading to a prothrombotic state are proposed as significant contributors. However, aspirin therapy appears to be underutilized in patients with HIV.[51] O'Brien and colleagues[52] have shown in a pilot study that low-dose aspirin may be a potential hot-spot for intervention for

HIV-1-infected subjects on antiretroviral therapy in order to blunt platelet and immune activation, as well as inflammation. Their work has shown that the basal level of median urinary concentration of 11-dehydro-TxB_2 was significantly higher in HIV-1-infected subjects compared with control subjects (9626.8 vs 7295.2 pg/mL; $P = .02$). After 1 week of aspirin, 11-dehydro-TxB_2 decreased significantly in both groups but remained significantly higher in the HIV-1-infected group (2255.7 vs 1422.6 pg/mL; $P = .04$).

Pediatric Heart Disease. In children undergoing surgery for cardiac defects, CPB is associated with significant perioperative thrombotic events.[53] Additionally, systemic arterial to pulmonary artery shunt thrombosis is an uncommon but life-threatening complication of pediatric cardiac surgery. Despite trials suggesting benefits, the precise role of aspirin in reduction of occlusion of shunts unfortunately is not fully defined in the literature.[54] Children undergoing cardiac surgery are also at increased risk of venous thrombosis but the ability of aspirin to prevent venous thrombosis has not been formally tested. Thus, there are many features of pediatric cardiac surgery that lend themselves to increased knowledge about aspirin's potential to prevent critical arterial and/or venous thromboses. Cholette and colleagues[54] investigated the issue of thrombosis and potential aspirin resistance in pediatric patients undergoing cardiac surgery with CPB. These investigators found aspirin therapy effectively inhibits ex vivo platelet function and suppresses in vivo TxA_2 production in pediatric patients undergoing cardiac surgery. They noted that aspirin resistance as measured by aspirin resistance units was not associated with a greater risk of early symptomatic thrombosis but that resistance, if measured using a urinary TxB_2 assay, was indeed predictive of thrombotic events in all treated subjects, though this finding only approached statistical significance in the high-risk group. The authors suggested that this trending of urinary TxB_2 may prove to be predictive when larger numbers of high-risk patients are able to be tested.

Deep Vein Thrombosis and Venous Thromboembolism Prophylaxis

For the initial primary prevention of venous thromboembolism, anticoagulants are considered the preferred first-line therapy for both medical and surgical patients. For extended prophylaxis (after 10 days of initial treatment with a low molecular weight heparin), recent evidence suggests that there is no difference in outcomes between aspirin and dalteparin in a surgical population,[55] so it might be reasonable to consider aspirin therapy for extended prophylaxis, especially when the use of anticoagulants is contraindicated or not feasible. For secondary prevention, aspirin might be considered after completion of initial anticoagulant therapy for venous thromboembolism.[56-58] Clarification of the aspirin effectiveness of an individual patient may help the clinician make a more structured decision in these cases where protracted prophylaxis is required and injected or oral anticoagulation is not a preferred option for the patient (Table 1).

… aspirin therapy effectively inhibits ex vivo platelet function and suppresses in vivo TxA_2 production in pediatric patients undergoing cardiac surgery.

Forward Thinking: Future Implementation and Research

A key consideration in advancing broader use of urinary thromboxane testing is prospective trials that demonstrate improved clinical outcomes with testing and subsequent therapeutic adjustments. Building on the encouraging data from well-characterized retrospective cohorts, prospective data will allow for integration of urinary thromboxane testing into practice standards as a noninvasive, readily accessible means of identifying at-risk individuals and titrating antiplatelet therapy, and even optimizing non-antiplatelet therapy in those with poor responsiveness to aspirin or other antiplatelet agents. Also important to the incorporation of any diagnostic test into clinical practice are subanalyses of future studies that demonstrate an economic advantage conferred by testing, using cost or quality-of-life adjustments, now commonly accepted as cost-justification models. The optimal frequency of monitoring for aspirin response should be determined, with consideration made for the natural progression of disease states and the retooling of therapy that advancing disease requires.

Discussion

The subject of aspirin response is more complex than a simple explanation of how aspirin functions and determining if it has achieved its target acetylation, blocking subsequent thromboxane production.

TABLE 1

Unmet Clinical Needs in Disease States: Potential Benefits Conferred by Assessment of Individual Patients' Aspirin Responsiveness

Disease State	Unmet Clinical Need	Benefit of Detection of Aspirin-insensitive Thromboxane Production	Study
Atrial fibrillation with low $CHADS_2$ score	Thromboprevention without increased bleeding side effects in low-risk patients	Reducing bleeding risks in patient at very low risk for stroke	Turagam MK et al,[35] Hart RG et al[36]
Type 2 diabetes	Higher risk of CVD but apparent underprescription of aspirin therapy	Determining which diabetes subpopulations might benefit from personalized aspirin dosing or other therapies	Ames PR et al[11]
CKD	Higher risk of CVD but also higher risks of NSAIDs on marginal renal function	Studies needed to determine which CKD patient overall benefit of aspirin will outweigh risks	
Cardiothoracic surgery	Early SVG thrombosis	Urinary thromboxane levels serve as predictor of early SVG thrombosis	Gluckman TJ et al[33]
Heart failure/ ventricular assist devices	High frequency of bleeding and thrombosis	Further optimization of aspirin dosing and/or interval	
HIV	High risk of CVD with apparent underutilization of aspirin	Reduction of prothrombotic and inflammatory states	Burkholder GA et al,[51] O'Brien M et al[52]
Pediatric cardiac surgery	Significant perioperative thrombosis	Identify aspirin nonresponders and adjust surveillance accordingly	Cholette JM et al[54]
DVT prophylaxis	Questionable efficacy of aspirin for the primary prevention of VTE in medical and surgical patients	May allow for determination of subpopulations for whom this therapy is reasonable or preferred over anticoagulation, such as patients undergoing extended prophylaxis	

$CHADS_2$, Congestive heart failure, Hypertension, Age ≥ 75 y, Diabetes mellitus, prior Stroke or transient ischemic attack; CKD, chronic kidney disease; CVD, cardiovascular disease; DVT, deep vein thrombosis; HIV, human immunodeficiency virus; NSAIDs, nonsteroidal anti-inflammatory drugs; SVG, saphenous vein graft; VTE, venous thromboembolism.

Aspirin response in a clinical setting has become synonymous with the risk of the aspirin-treated patient having an atherothrombotic event. Is an individual's aspirin treatment enough to protect that individual from an atherothrombotic event, or is he or she at continued risk of an event despite aspirin treatment? Aspirin, at doses that almost completely suppress thromboxane production in most healthy patients, does not always achieve the same degree of thromboxane suppression in various disease states. Albeit to date, review articles on aspirin effect do not provide clear direction for diagnostic testing to adjust for individual aspirin response, Hennekens and colleagues[59] suggested that "if the in vitro response to arachidonic acid is robust (demonstrating lack of aspirin effect), this information may be useful in addressing compliance issues or adjusting the aspirin dosage." As several studies reviewed in this paper have shown, testing of $UTxB_2$ has indeed been able to identify individuals on aspirin treatment who are at increased risk for an atherothrombotic event.

Would more complete thromboxane suppression be beneficial in these disease states? If so, what approaches can be taken to reduce

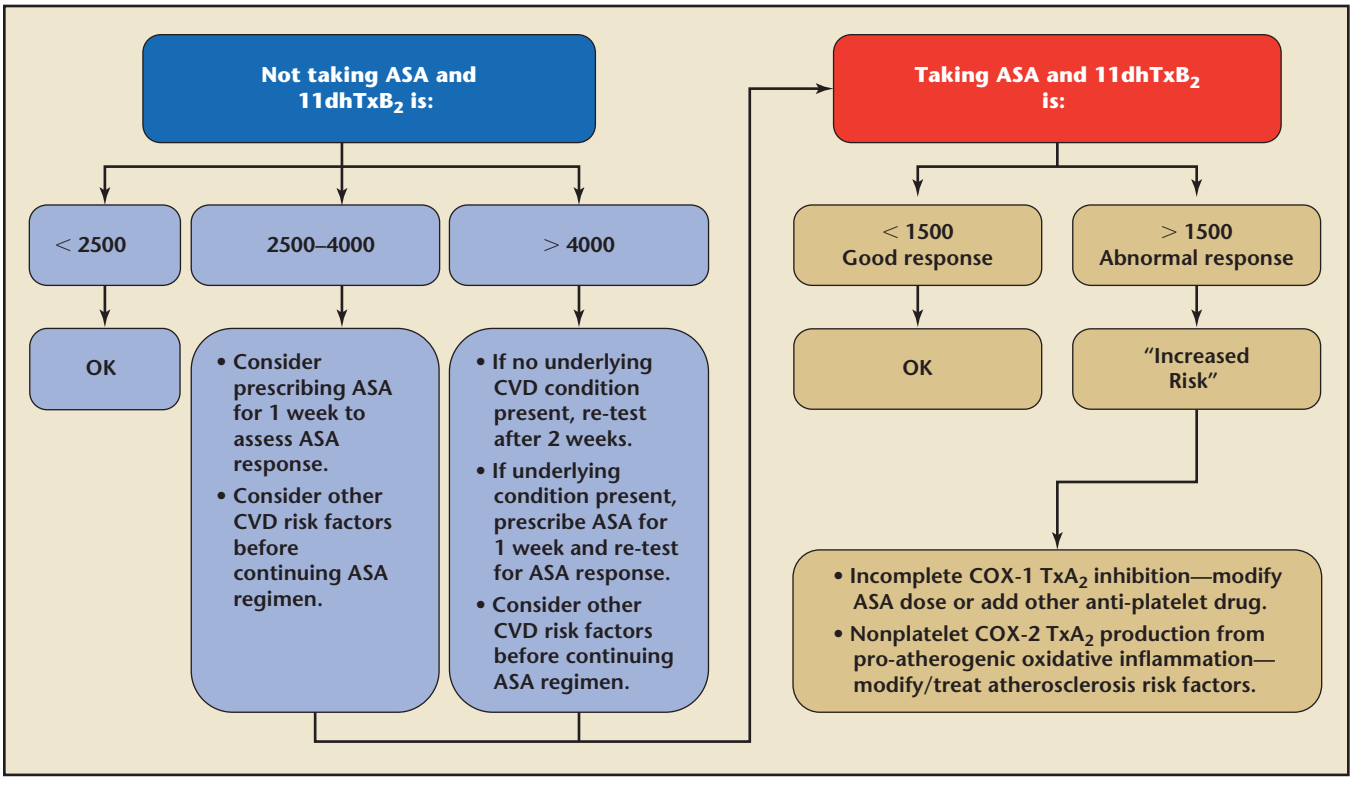
overall whole-body thromboxane burden? Dragani and colleagues[60] have shown that, in patients with essential thrombocythemia who are taking daily aspirin, the addition of a COX-2 inhibitor to aspirin decreases both urinary excretion of a thromboxane metabolite (marker of endogenous production) and ex vivo thromboxane production in serum (measure of total platelet synthetic capacity) by approximately 30%. These studies corroborate that COX-2 contributes to endogenous thromboxane production in this disease state. However, it is important to note, the addition of the COX-2 inhibitor did not abolish thromboxane production, suggesting the role of still additional mechanisms contributing to thromboxane production.

Strategies to optimize sensitivity of diagnosis and therapy remain an intriguing area of research. Aspirin in the primary and secondary prevention of atherothrombotic disease states is customarily prescribed taken once daily. Therefore, for the treatment-refractory patient, the first treatment modification made is often a change in drug dose. However, we know that, traditionally, the aspirin-dosing interval for most indications (such as fever) is much more frequent, with doses sometimes as close as 4 hours apart. Should our focus therefore be on a different element of aspirin pharmacotherapy? Pascale and colleagues[10] have shown, using the disease model of essential thrombocytopenia, that thromboxane biosynthesis is better controlled by modulating the aspirin interval as opposed to the dose or formulation. Modulation of thromboxane outside of aspirin dosing has also been demonstrated; Lev and colleagues[61] showed that the introduction of omega-3 fatty acids effectively reduced platelet reactivity and improved the response to aspirin. In the Indian Polycap Study,[62] the investigators demonstrated that statin treatment lowers levels of thromboxane production independent of aspirin treatment. Clinicians may express doubt about the phenomenon of aspirin resistance but when you look at their clinical behavior they clearly acknowledge its existence by providing incremental antiplatelet therapy to their patients' regimens.

However, if daily low-dose aspirin really does remain effective prevention for most, it would be unwise to broadly and blindly recommend either an increased quantity or increased frequency of dosing due to the well-known side effects of aspirin. Therefore, determination of individual responsiveness to aspirin represents an important advance in personalized medicine for its potential to more effectively address long-term prevention of atherothrombotic events.

Because not all patients receive the same protective benefits from low-dose aspirin therapy, guidance should be developed to help optimize antiplatelet therapy in those

Figure 2. Clinical interpretation of 11-dehydro-thromboxane B_2 ($11dhTxB_2$) results. ASA, aspirin; COX, cyclooxygenase; CVD, cardiovascular disease; TxA_2, thromboxane A_2.



with poor aspirin responsiveness. Although large-scale prospective clinical trials have not yet been performed, some clinicians have proposed an algorithm to aid in tailoring patient therapy based on the clinical literature presently available.

Using urinary 11-dehydro-TxB_2 testing, one could assess patients prior to, and after, initiation of aspirin therapy to assess for degree of inhibition of thromboxane biosynthesis, and, if suboptimal, adjust therapy strategies accordingly to minimize downstream risks (Figure 2).

The major clinical impact of this protocol is that consistently high baseline 11-dehydro-TxB_2 levels in subjects not taking aspirin may justify further investigation for underlying cardiovascular risk factors including poor aspirin response. In addition, the presence of high post-aspirin 11-dehydro-TxB_2 levels will identify patients with increased risk of atherothrombotic disease. This suggests the need of a comprehensive multifaceted management plan that would include both antiplatelet as well as antiatherosclerotic treatments.

Conclusions

The world faces growing epidemics of coronary artery disease, type 2 diabetes, and CKD, all of which present unique challenges in the prevention and management of the cardiovascular risks associated with these conditions. Improving patient care globally in the next decade must address methods to reduce atherothrombosis without increasing morbidity and mortality from bleeding risks. Assessing patients for their potential to respond well to preventative treatments such as aspirin therapy can reduce this disease burden by individualizing therapy to the appropriate patients. Though future clinical investigation is necessary to strengthen this argument, the present scientific literature suggests that testing $UTxB_2$ levels is an appropriate first step for identifying individuals at increased risk for downstream atherothrombotic events associated with cardiovascular disease. ■

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MAIN POINTS

- Arterial thrombosis is the final pathogenic mechanism of many acute ischemic events, including acute myocardial infarction (MI) and sudden cardiac arrest. Aspirin has several important effects on the human body, most notably the reduction of pain, fever, and inflammation. Aspirin's capacity to prevent blood clotting as a platelet function inhibitor has been employed as an important treatment and prevention modality in atherothrombotic disease.
- Antiplatelet medications are frequently used in the prevention of stroke, MI, and vascular thrombotic diseases due to the fundamental role of platelet aggregation in a variety of atherothrombotic processes. Modulation of the prostaglandin thromboxane A_2 (TxA_2) pathway is one of the pivotal routes of activation involved in stimulating platelet aggregation. The importance of TxA_2 is demonstrated by the reduction in risk of acute MI or death in patients with acute coronary syndrome following administration of low-dose aspirin, which irreversibly inhibits the production of TxA_2 in platelets.
- There are strong suggestions from three major clinical trials that evaluation of 11-dehydro-TxB_2 production may be a more important surrogate marker for clinical response to aspirin therapy than isolated arachidonic acid-induced platelet aggregation.
- The current scientific literature suggests that testing urinary TxB_2 levels is an appropriate first step for identifying individuals at increased risk for downstream atherothrombotic events associated with cardiovascular disease.

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