Review: Laboratory markers quantifying prothrombin activation and actions of thrombin in venous and arterial thrombosis do not accurately assess disease severity or the effectiveness of treatment

Frederick A. Ofosu
Canadian Blood Services and Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Summary
Thrombin is normally produced for hemostasis and physiological wound healing. Increased thrombin production in vivo, cell activation and inflammation mediated in part by thrombin are hallmarks of both arterial and venous thrombosis. Thrombin generates (pro) coagulant, mitogenic, inflammatory and anticoagulant responses by interacting with a variety of cells in vivo. Both direct and indirect thrombin inhibitors are effective drugs for preventing and treating the consequences of arterial and venous thrombosis. For these reasons, measurements of the production and activities of thrombin in vivo have the potential for gauging the extent of thromboembolism and the responses of patients to anticoagulant, antiplatelet and anti-inflammatory drugs. However, a critical review of published information suggests that measurement of thrombin production and activity in patients at risk for and in patients with significant thrombosis generally does not provide information useful for clinical decision-making. This lack of clinical utility of levels of thrombin production in vivo may arise from two causes: the inability of the measurement to differentiate between physiological (hemostatic) and disease-related (pathological) sources and/or causes of thrombin production in vivo, and the inability of antithrombotic treatment modalities to permanently eliminate the stimuli that cause increased thrombin production evident in venous and arterial thrombosis.

Keywords
Thrombin, prothrombin fragment 1 + 2, arterial and venous thrombosis, platelets

Introduction
Evidence for increased production and actions of thrombin in vivo is invariably found in the plasmas of individuals at high risk for clinically significant venous or arterial thromboembolic disease (1–5). Increased production and actions of thrombin may even be stronger in individuals with deep vein thrombosis and/or pulmonary embolism, acute coronary syndrome, myocardial infarction (MI), or ischemic stroke (1–5). When used to treat both venous and arterial thromboembolism, the clinical effectiveness of drugs known to inhibit prothrombin activation (i.e. thrombin production) in vivo and in vitro, or directly inhibit (inactivate) thrombin in vitro, or accelerate thrombin inhibition in vitro clearly supports a role for thrombin in driving the processes that result in both venous and arterial thrombosis. Drugs proven to be clinically effective in randomized placebo-controlled trials include agents described as indirect (e.g. heparin) and direct (e.g. hirudin) thrombin inhibitors, a drug proposed to solely inhibit thrombin production (e.g. fondaparinux), oral anticoagulants (e.g. warfarin), and anti-inflammatory drugs (e.g. aspirin). The demonstrated clinical effectiveness as antithrombotic drugs (see below) of strong anticoagulants (e.g. heparin and hirudin), weaker anticoagulants [e.g. low-molecular-weight heparins (LMWH), dermatan sulfate, and fondaparinux], an anti-inflammatory drug (aspirin) antiplatelet drugs (e.g. aspirin, ADP receptor and GPIIb/IIIa antagonists) and statins (cholesterol-lowering medication) highlight the combined contributions of increased coagulation, cell activation and the consequent inflammatory processes in vivo to arterial and venous thrombosis. A focus on thrombin production and actions in vivo may, therefore, not provide the most relevant information for clinical decision making related to the diagnosis, prevention or treatment of thrombosis.

The two major inhibitors of thrombin in undiluted human plasma are antithrombin and heparin cofactor II (6–8), and the...
half-life of thrombin in undiluted human plasma is approximately 30 seconds (8). Both antithrombin and heparin cofactor II are serpins which form covalent complexes involving the catalytic centre of thrombin in reactions accelerated by up to four orders of magnitude by the glycosaminoglycans heparin and heparan sulfate (antithrombin) and dermatan sulfate (heparin cofactor II) (6, 7). The description of these three glycosaminoglycans as indirect thrombin inhibitors reflects their catalytic nature (i.e. their cofactor roles) on the two serpin-mediated inhibition of thrombin in plasma. In contrast, direct inhibitors, such as hirudin and hirulog inactivate thrombin in reactions that are independent of any cofactors (9, 10). Anti-inflammatory drugs are not known to interact with thrombin and are, therefore, not direct or indirect thrombin inhibitors.

In view of the important role ascribed to thrombin in the establishment and progression of both venous and arterial thrombosis (1–5), it is attractive to consider the possibility that the concentrations of thrombin in vivo are related to and/or can be used to gauge the extent of the thromboembolic illness. However, it should be noted here that prothrombin activation in vivo normally increases with age in the healthy population (5, 11). Therefore, the levels of thrombin production and actions in individuals at risk for or with thrombosis may not accurately reflect the rates of progression of thromboembolism in view of increases in production of thrombin associated with normal aging as well as vascular disease processes. How thrombin production of normal aging influences prothrombin activation which is attributable to reactions that ultimately result in venous and arterial thromboembolism is not known. Further, the concentration of thrombin in vivo cannot be used to reliably estimate the clinical effectiveness of measures used either to prevent disease progression in patients, or to treat patients with thromboembolic illnesses. One reason is that not all effective antithrombotic drugs inhibit prothrombin activation or the actions of thrombin in vivo. Antithrombotic drugs do not decrease the concentrations of prothrombin fragment 1 + 2 in healthy volunteers (which represent the thrombin required for hemostasis) or eliminate the stimuli for increased thrombin production associated with arterial or venous thrombosis (see below). Further, thrombin is just one of several key players involved in the complex reactions that may ultimately result in venous and arterial thromboembolism. Other key players include inflammation, platelets, monocytes, and endothelial cells (12–17). These cells respond to stimulation by thrombin and to stimulation by some mediators of inflammation (16, 17). Based on the above considerations, only a limited facet of the processes that may result in clinically significant thromboembolism can be attributed to increased thrombin production and actions in vivo.

This review will first consider the risk factors for venous thrombosis and arterial thrombosis, and the relationships between thrombin and the risk factors for venous and arterial thrombosis. It will next consider how these risk factors may be related to the cell modulators (including thrombin) thought to actively participate in the progression of the thromboembolic disease processes. How drugs that modify the course of venous and arterial thromboembolism influence prothrombin activation and the actions of thrombin in vivo will also be considered. Finally, some of the reasons why measurements of thrombin production and actions in vivo do not necessarily provide good insights on either the level of risk present, the course of thromboembolic disease, or the clinical effectiveness of prophylactic or therapeutic antithrombotic drugs will be presented.

Risk factors for venous and arterial thromboembolism

Venous thromboembolism

It is appropriate when discussing high risk factors for developing venous and arterial thromboembolism that the related molecular markers in the patients’ plasmas consistent with increased coagulation, fibrinolysis, inflammation, platelet, leukocyte and endothelial cell activation are also presented. As summarized in a recent review, the major risk factors for venous thromboembolism [deep vein thrombosis (DVT) and pulmonary embolism (PE)] include age, major trauma (especially fracture of a hip, pelvis or leg), a recent abdominal, knee or hip replacement surgery in individuals over forty, and inherited thrombophilia (18). About 100 people per 100,000 develop venous thromboembolism (VTE) in North America each year. The rate of VTE is highest in adults aged over 80 (up to 6-fold higher than the rate seen in the general population) and is least in children under 15 (< 5 cases per 100,000) (18). Normal aging is associated with increased prothrombin activation or thrombin production in vivo. This age-related increase in coagulation in vivo is not necessarily associated with evidence consistent with generalized increased inflammation (e.g. increased concentrations of C-reactive proteins in plasma) in the healthy people studied (5, 11). Unlike normal aging, major trauma and major surgery increase prothrombin activation in vivo but in the context of significant inflammation in these patients (19–21). The chronic inflammation of osteoarthritis and rheumatoid arthritis necessitating total knee and hip replacement is associated with high concentrations of prothrombin fragment 1 + 2 and thrombin-antithrombin (increased coagulation), D-dimer (increased fibrinolysis that usually accompanies increased coagulation in vivo), and C-reactive protein (increased generalized inflammation) in the patients’ pre-operative plasmas (5, 12, 22). Without prophylaxis, up to 50% of patients over 40 who undergo elective knee and hip replacement surgery develop post-operative thrombosis (18). The significant stasis associated with orthopedic surgery combined with the pre-existing hypercoagulable state in the patients, the trauma and inflammation caused by the surgery contribute to the high rates of post-operative DVT (18–21). Prophylaxis with aspirin, heparin or LMWH, alone or in combination with oral anti-coagulants, can reduce post-operative DVT by up to 70% (18, 22, 23).

Previous VTE, malignancy, existing arterial disease (e.g. congestive heart failure and paralytic stroke) and inherited thrombophilias (antithrombin, protein C or protease S deficiency, factor V Leiden, prothrombin G20210A mutation and high factor VIII:C or factor IX:C) are also associated with a moderate risk for developing apparently unprovoked VTE (18, 24). As discussed further below, Hron et al. reported that the age at which the first unprovoked DVT develops, the prothrombin G20210A mutation, and high factor IX levels are significantly associated
with a high endogenous thrombin potential, i.e. increased pro-
thrombin activation \textit{ex vivo} in response to low concentrations of tissue factor. Prior to initiating treatment, plasmas of patients with confirmed venous thromboembolism have higher concent-
trations of D-dimer, prothrombin fragment 1 + 2 and thrombin-
antithrombin than plasmas of aged-matched healthy controls (25–27). Additionally, plasmas of cancer patients show evidence for increased coagulation, fibrinolysis and inflammation \textit{in vivo} (28), as do plasmas of diabetic patients (30). Diabetic patients have a moderate risk of VTE and the annual incidence rates for VTE in diabetic patients all age categories after 35 are higher than are found in non-diabetic patients (29, 31). Further, plasmas of diabetic patients have elevated markers reflecting increased coagulation, platelet, endothelial cell and leukocyte activation \textit{in vivo} (28, 30). An approach for estimating the relative contribu-
tions of increased \textit{in vivo} coagulation, cell activation and inflam-
mation to unprovoked and post-operative thrombosis is to com-
pare the relative efficacies of the agents used to treat or prevent VTE, and their effects on coagulation, cell activation and inflam-
mation \textit{in vivo} (see below).

\textbf{Arterial thromboembolism}

The major risk factors for propagating the clinical consequences of arterial thrombosis include age, peripheral arterial disease, hyperlipidemia, unstable and stable angina, transient ischemic attack, a previous stroke, arterial fibrillation, hypertension and diabetes (29, 33, 34). Both the prevalence and incidence of arte-
rial thrombosis also increase exponentially with age. This exponen-
tial age-related increase in arterial thrombosis is probably due to age-related increases in the prevalence of hypertension, hyperlipidemia/dyslipidemia and glucose intolerance as well as increases in the plasma concentrations of prothrombin fragment 1 + 2, C-reactive protein and pro-inflammatory cytokines (34). In addition to the above, elevated D-dimer concentrations in plasma are strongly associated with ischemic heart disease and heart failure (35–37).

Diabetic adults die from MI and stroke at twice the rates of non-diabetic adults (29). Retinopathy (ultimately leading to blindness) and end stage renal disease occur at approximately 20 and 25 times, respectively, the rates seen in non-diabetic individu-
als (29). Associated with diabetes are increased coagulation, fi-
brinolysis and endothelial cell activation \textit{in vivo} (30, 32). In-
creased inflammation, endothelial cell activation and increased coagulation are also seen in plasmas of patients with atrial fibril-
luation, unstable angina, and MI (36–41). Markers of increased inflammation, e.g. increased concentrations of C-reactive pro-
tein, interleukin (IL)-6 and tumor necrosis factor (TNF)-α are present, as are increased concentrations of prothrombin frag-
ment 1 + 2 (thrombin production), thrombin-antithrombin, fibrin-
olysin (increased concentrations of plasma D-dimer) (36–41).

Thus, the increased thrombin production seen in conditions associated with venous and arterial thrombosis (1–5, 34–36, 38–40) occurs in the context of increased generalized inflam-
mation, activation of platelets, endothelial cell and white cells (32, 36, 37). One may obtain a better estimate of the relative con-
tributions of increased \textit{in vivo} coagulation, platelet activation and inflammation to the pathogenesis of atherosclerosis by ap-
preciating how aspirin and other antiplatelet drugs, anti-inflam-
matory drugs, unfractionated heparin, LMWH, oral anticoagu-
\textit{How anticoagulants, antiplatelet and anti-
inflammatory drugs influence the course of
thrombosis and laboratory markers of thrombin
formation and actions}

The most direct way currently available to measure steady-state levels of prothrombin activation, i.e. thrombin production \textit{in vivo} is to measure the concentrations of prothrombin fragment 1 + 2 endogenous to plasmas. Activation of prothrombin by prothrom-
binase generates equimolar prothrombin fragment 1 + 2 and thrombin. Prothrombin fragment 1 + 2 may be further processed \textit{in vivo} as prothrombin fragment 1 and prothrombin fragment 2 were found in urine. Prothrombin fragment 1, and not prothrom-
bin fragment 1 + 2, was found in all thirty-seven of urine specimens studied. Further, prothrombin fragment 2 was also found in 22 of the same 37 urine specimens studied (42, 43). Four- to five-
fold higher concentrations of prothrombin fragment 1 and pro-
thrombin fragment 2 were found in the urine of 14 normal preg-
nant women during the third trimester (43). While this may be in-
ferred, generation of prothrombin fragment 1 and prothrombin fragment 2 from prothrombin fragment 1 + 2 \textit{in vivo} has not yet been reported. The relationships between the concentrations of plasma prothrombin fragment 1 + 2 and urinary prothrombin fragment 1 and prothrombin fragment 2 have not been estab-
lished. Further, neither urinary prothrombin fragment 1 nor pro-
thrombin fragment 2 has been evaluated in large numbers of healthy individuals or in individuals either at risk for or with thromboembolic illnesses. Therefore, their clinical utility is un-
known. Prothrombin activation \textit{ex vivo} has more recently been measured using a variety of approaches to obtain information that may be related to the hemostatic state of individuals (44–46). The endogenous thrombin potential and modifications measure the total amount of thrombin that can be generated and inhibited over time in plasmas activated with a variety of coagulant stimu-
lants. Among the parameters that clearly influence the endogenous thrombin potential are heparin, LMWH, use of oral anticoagu-
\textit{Downloaded from www.thrombosis-online.com on 2017-06-19 | IP: 54.191.40.80
For personal or educational use only. No other uses without permission. All rights reserved.}
healthy subjects, patients at risk for developing venous or arterial thrombosis, or patients treated with antithrombotic drugs have not yet been reported. Therefore, additional work remains to be done to explore the clinical utility of the endogenous thrombin potential.

A recent prospective study has explored the utility of thrombin generation ex vivo for identifying patients at low risk for recurrent VTE in 914 patients who had developed their first idiopathic VTE (DVT or PE) after they had discontinued their oral anticoagulant therapy. The reliability of the ex vivo thrombin generation assay used was demonstrated in the reproducible concentrations (correlation coefficient 0.46) of thrombin generated in paired patient samples collected several months apart (48). VTE recurred in 100 (11%) of the 914 patients, and the mean maximum thrombin generated in the patients with recurrent VTE was 420 ± 111 nM compared to 349 ± 108 nM in patients who did not experience recurrent thromboembolism (48). Using > 400 nM thrombin as the index of high risk for recurrence, the relative risk for recurrence in over four years was 0.42 for patients whose plasmas generated between 300 and 400 nM thrombin, and 0.37 for patients whose plasmas generated less than 300 nM thrombin. The % cumulative probability of recurrence over five years for patients whose plasmas generated < 400 nM thrombin was 10% compared with 22% for patients whose plasmas generated > 400 nM thrombin ex vivo (48). Exciting as these observations are, the patients whose plasmas generated < 400 nM thrombin ex vivo still had a 10% chance for VTE recurrence five years after their first idiopathic DVT (48). This cumulative rate of DVT in the low risk group is 20-fold higher than the DVT rate of the general North American population (18).

The specific ways one could measure some of the actions of thrombin in vivo include measurement of the concentrations of thrombin-antithrombin and thrombin-heparin cofactor II (inhibition of thrombin), and the products generated when thrombin generated in vivo activates platelets, endothelial cells, or leukocytes. Ternary complexes of vitronectin with thrombin-antithrombin and thrombin-heparin cofactor II have been reported for normal plasmas (49). The concentrations of thrombin-antithrombin-vitronectin in normal plasmas are up to ten-fold higher than the binary thrombin-antithrombin (49). The binary thrombin-heparin cofactor II complex has not yet been reported in normal plasma. The utility of the ternary complexes of thrombin with its two plasma inhibitors in the diagnosis and assessment of the progression of thromboembolic diseases is not known as the concentrations of ternary complexes of thrombin with its inhibitors in plasmas of patients with arterial or venous thrombosis have not been reported. Thrombin also releases fibrinopeptides A and B from fibrinogen, converting fibrinogen into soluble fibrin that in turn serves as a cofactor for factor XIII activation by thrombin. Therefore, the concentrations of fibrinopeptide A and/or soluble fibrin endogenous to plasmas could also be used to estimate the actions of thrombin towards its principal substrates in plasma. Neither the concentrations of the binary form of thrombin-antithrombin nor soluble fibrin in pre-operative plasmas identified patients with high risk for developing venous thrombosis after elective orthopedic surgery (5, 22).

The concentrations of thrombin fragment 1 + 2 found in the plasmas of blood donors aged 16–75 vary between 0.7 and 1.2 nM (5, 11). They vary from 1.1–1.7 nM and 1.2–1.8 nM for men aged 45–65 who do and do not have ischemic heart disease, respectively (35). This study found no relationship between the age-adjusted concentrations of prothrombin fragment 1 + 2, thrombin-antithrombin, factor VII:C, or APTT, or APC ratio and the incidence of ischemic heart disease. In contrast, the relative odds of the incidence of ischemic heart disease increased with the D-dimer concentrations (35). Another recent study also found no relationship between the pre-operative plasma levels of prothrombin fragment 1 + 2, thrombin-antithrombin or factor VIIa and the operative thrombosis in over 300 patients with severe arthritis who had elective knee or hip replacement surgery (5). Based on these concentrations of prothrombin fragment 1 + 2, the endogenous concentrations of thrombin in most plasmas are high enough to cause significant platelet activation in vivo, as 1.0 nM of thrombin activates platelets in platelet-rich plasmas (50). The observation that < 5% of platelets in normal blood are activated (50) may reflect the natural antithrombotic properties of the normal endothelium that depend on the prostacyclin, ADP hydrolase and thrombomodulin synthesized by endothelial cells. Both endothelial cell-derived prostacyclin and ADP hydrolase can effectively suppress the responses of platelets to thrombin (51, 52). Thrombomodulin alters the substrate specificity of thrombin in a manner that favors protein C activation by thrombin. Activated protein C inhibits coagulation and inflammation in vivo (16). Activation of endothelial cells by thrombin and other agonists in vivo changes the normal anticoagulant/anti-thrombotic phenotype of the endothelium into a procoagulant/prothrombotic and inflammatory phenotype (16, 53). Free thrombin is also a potent mediator of inflammation, as thrombin activates platelets, endothelial cells and leukocytes; these cells in turn release several mediators of inflammation (16). Many of these mediators of inflammation induce tissue-factor synthesis by endothelial cells and peripheral monocytes (13). Infusion of small doses of TNF-α into healthy volunteers, for example, stimulates tissue factor synthesis and enhances in vivo coagulation and inflammation within four hours (54). Thus, coagulation and inflammation in vivo are intrinsically linked processes (13).

Another measure of the intricate links between inflammation and coagulation in vivo is readily apparent in patients with chronic and severe osteoarthritis requiring elective hip or knee surgery. The pre-operative plasmas of these patients have significantly higher concentrations of prothrombin fragment 1 + 2 and C-reactive protein than age-matched controls (5, 11, 12). Patients with inflammatory bowel disease or chronic heart failure, two diseases associated with development of venous thromboembolism, have high levels of TNF-α, IL-6, and IL-1β, tissue factor and von Willebrand factor (31, 55, 56). The high level of TNF-α in these patients’ plasmas, as well as in the plasmas of post-surgery patients (21) could contribute to thrombosis. The high plasma concentration of von Willebrand factor is an index of endothelial cell activation (15, 55).

**Venous thrombosis**

Two cautions are necessary before considering how agents used to prevent venous thrombosis after elective orthopedic or post-trauma surgery influence in vivo thrombin production and the ac-
The studies to be considered here cover a period of 30 years or more, and the techniques used to replace hips or knees as well as post-operative management of the patients have changed considerably. The second caution is that either by accident of history or design, many manufacturers of LMWHs sponsored several of the studies to determine their efficacy in the past twenty years. Therefore, considerably more patients have been evaluated using a variety of LMWHs than each of low-dose heparin, warfarin, aspirin or pneumatic compression (23, 57–59).

As noted above, up to 50% of patients over forty with chronic arthritis develop venous thrombosis after elective knee or hip replacement surgery (18, 23). Prophylaxis with heparin, LMWH, combinations of heparin and oral anticoagulants, LMWH and oral anticoagulants (18, 23) or aspirin (56–60) reduces the incidence of post-operative thrombin by up to 70%. With the exception of aspirin, all prophylactic regimens also reduce the incidence of post-operative PE by at least 60% (18, 23, 57–59, 61). Prophylaxis with these agents similarly reduces the incidence of post-operative venous thrombosis in patients over 40 with fractured hips, knees or legs. Based on the results of more recent studies, it appears necessary for patients who have knee or hip replacement to receive ongoing prophylaxis for at least 30 days with LMWH or oral anticoagulants in order to reduce the development of venous thrombosis after they were discharged from hospital (60). Prolonged use of aspirin (for up to 6 weeks) apparently also reduced the incidence of venous thrombosis after patients were discharged from hospital (61).

Used to prevent post-operative thrombosis, unfractionated heparin or LMWH reduces the surgery-related increases in the plasma concentrations of prothrombin fragment 1 + 2, thrombin antithrombin, and D-dimer for approximately three days to the levels seen in the pre-operative plasma (60–63). This initial post-operative increase in thrombin production may be required for hemostasis. Surprisingly, the concentrations of prothrombin fragment 1 + 2 then increase even beyond the levels seen immediately post-operatively by day 7 and subsequently (60, 63). This markedly increased thrombin production persists for at least six weeks (60). The additional thrombin production seen subsequently may primarily be required for the necessary wound healing and tissue repair after the surgery. The extent to which thrombin that is produced for wound healing contributes to post-operative thrombosis is not known. Nonetheless, it is evident that prophylactic use of LMWHs does not significantly influence the massive increases in thrombin production seen beyond the immediate post-operative period (60, 63).

A more recent study involving 1,947 patients evaluated a multimodal approach for preventing thromboembolism after total hip replacement. All the patients received intravenous heparin during surgery (before femoral preparation), pneumatic compression, knee-high elastic stockings, early mobilization, and chemoprophylaxis with aspirin (83%) or warfarin (17%) for four to six weeks (61). It is unclear which specific component(s) of the novel strategies used contributed to the very low rate (2–5%) of post-operative DVT and PE (0.5%) observed when the patients thus treated were evaluated after three months (61). Nonetheless, it is evident that aspirin (an antiplatelet and anti-inflammatory drug) administered to these patients for up to six weeks, may have contributed to the extremely low level of post-operative DVT observed in this study (61), since aspirin by itself reduces the risk of post-operative venous thrombosis (57, 59). Hirudin and hirulog have also been used to prevent venous thrombosis after high risk surgery and both were highly effective (64, 65).

Treatment of VTE is initiated with heparin or LMWH for 2–5 days followed by oral anticoagulant therapy for at least three months after the therapeutic range [international normalized ratio (INR) 2–3] is attained (18, 25, 48, 66, 67). The function of the heparin/LMWH is to immediately inhibit coagulation, and this goal is achieved in the short term (25, 27, 66, 67). The oral anticoagulants are used to achieve a more effective suppression of coagulation in vitro and this is also achieved in most patients (66, 67). In spite of the marked suppression of thrombin production and activities seen, it is not clear why DVT recurrences in up to 11% of the patients thus treated (24, 48, 68).

**Arterial thrombosis**

Results from several large randomized placebo-control trials and meta-analyses reporting the primary and secondary prevention of fatal and non-fatal MI and stroke have demonstrated the clinical utility of the combination of antiplatelet drugs and that of anti-coagulant and antiplatelet drugs for maximizing clinical efficacy. Most of these reports appeared in 1999 or later. The first of these randomized studies compared the effectiveness of heparin plus aspirin with hirudin plus aspirin on death, MI, angina and the need for revascularization procedures in patients with acute myocardial ischemia (69). This study, which enrolled over 10,000 patients, reported hirudin to be superior to heparin in preventing cardiovascular death, MI and refractory angina. However, the superior clinical benefits of hirudin were achieved at a cost, namely an increase in life-threatening blood loss which required transfusion (69). The second study was a meta-analysis in which 17,157 patients with acute coronary syndrome without ST elevation were randomized to receive aspirin and unfractionated heparin or aspirin and LMWH or aspirin alone (for up to 3 months). The combination of aspirin and heparin and that of aspirin and LMWH for seven days, reduced by >50% the number of deaths associated with the use of aspirin alone. Thus, heparin and LMWH were equivalent in this regard (70). Continued use for three months of heparin and aspirin or LMWH and aspirin conferred no additional clinical benefit compared to the long-term use of aspirin alone. Furthermore, it was estimated that 12 patients/1,000 treated for up to three months with the LMWH would experience a 2.2-fold higher major bleeding risk versus the aspirin group (70).

The Antithrombotic Trialists Collaboration published the results of another large meta-analysis that reviewed 287 studies involving 135,000 patients in comparisons of anti-platelet therapy versus placebo control, and 77,000 patients in comparisons of different anti-platelet therapies (71). This meta-analysis concluded that aspirin or other oral anti-platelet drug protects most at-risk patients from occlusive events (i.e. primary and secondary prevention). The at-risk patients included individuals with acute MI, unstable or stable angina, previous MI, stroke or cerebral ischemia, peripheral artery disease or atrial fibrillation. The study also concluded that while at least 150 mg aspirin may be required for initial loading, 75–150 mg daily of aspirin was an ef-
fective regimen for long-term use. The study also concluded that more research is required to confirm whether adding a second anti-platelet drug to aspirin provides additional benefits in some clinical circumstances (71).

Another class of drugs that is effective in primary and secondary prevention of arterial thrombosis is statins. An MRC/BHF Heart Protection Study evaluated the clinical effectiveness of simvastatin in a randomized control trial involving over 20,000 high-risk individuals. A particular strength of this study was that in addition to simvastatin (versus placebo), the patients on aspirin, anti-hypertensives, β-blockers or ACE inhibitors continued receiving whatever medication they were on. A second strength of this study was its duration (5 years). There was a significant reduction of all-cause mortality associated with the use of simvastatin. This was due to a highly significant (18%) reduction in coronary death rate (72, 73). There were also highly significant (~26%) reductions in the first event rates for non-fatal MI or coronary death, and for coronary or non-coronary revascularization. It is noteworthy that these reductions were seen after one year of treatment with simvastatin, and that the reductions then persisted for at least five years (72). Furthermore, the clinical benefits of simvastatin were also evident in the subgroup of nearly 6,000 patients with diabetes mellitus; and the benefits were also seen in patients with hypertension, patients receiving aspirin, β-blockers, ACE inhibitors or vitamins (72, 73). The two studies demonstrated that this statin therapy reduced the incidence of both coronary events and ischemic stroke, even among individuals who did not have high cholesterol concentrations when they enrolled in the study. The beneficial effects were also seen in individuals with pre-existing cardiovascular disease who did not present evidence for coronary disease (73). A second statin, namely atorvastatin, similarly reduced in nearly 3,000 diabetic patients the incidence of major cardiovascular events, any cardiovascular events, and all cause mortality (74). This randomized control trial had to be terminated two years earlier than expected because of the favourable outcome seen in patients receiving the atorvastatin (74). It should be noted that a meta-analysis involving 13,024 patients treated with statins for three months or less found no evidence for clinical efficacy (combined end points of death, MI and stroke) (75).

The penultimate large meta-analysis to be considered consisted of 11 randomized control trials involving 35,970 patients randomized for treatment with a direct thrombin inhibitor (hirudin or bivalirudin) or heparin for up to seven days, and the patients were then monitored for at least 30 days (76). Each trial included in this meta-analysis involved at least 200 patients. Use of direct thrombin inhibitors was associated with a 20% lower risk of death or MI at the end of the treatment. The clinical benefit of direct inhibitors was seen in patients with acute coronary syndromes and patients who had percutaneous coronary interventions (76). Curiously, the benefit seen with hirudin and bivalirudin (both bivalent direct thrombin inhibitors) was not seen with monovalent thrombin inhibitors (76). Further, there was a greater risk, of major bleeding with hirudin than bivalirudin or heparin (76).

The final two meta-analyses involve studies with patients who have peripheral artery disease (77). Patients with peripheral artery disease have markedly high risk of MI and stroke, and are six times more likely to die within ten years than patients without this condition (77). Use of aspirin (80–325 mg/day) reduced by 23% the composite end point of cardiovascular death, MI or stroke in the over 9,200 patients enrolled in this randomized control trial (77). In the second study, 6,452 patients with established peripheral artery disease were randomized to receive 75 mg of clopidrogrel (a precursor of platelet ADP receptor antagonist that is generated in vivo after clopidrogrel ingestion) or 325 mg of aspirin. Clopidrogrel was associated with a 24% risk reduction (78).

Based on the foregoing, antiplatelet drugs (aspirin, ADP receptor antagonists or anti-glycoprotein IIb/IIIa antibodies), anticoagulants (heparin, oral anticoagulants, hirudin, bivalirudin) and statins are beneficial for primary and secondary prevention of arterial thrombosis. Combinations of two or more classes of these drugs may confer additional clinical benefits in arterial thrombosis. How these effective treatments for arterial thrombosis influence the markers of in vivo coagulation, fibrinolysis and inflammation has not yet been studied extensively.

The results of studies that generally involve relatively small numbers of patients are presented below. In one study, 16 patients with lower limb ischemia received 60 mg of alprostadil-PGE, by intravenous infusion lasting two hours for a total of four weeks. This antiplatelet treatment decreased the plasma concentrations of fibrinopeptide A but curiously not that of prothrombin fragment 1 + 2 (79). Continuous infusion of prostacyclin in 15 patients with pulmonary hypertension decreased the concentrations of P-selectin and increased that of thrombomodulin in the plasma (80). Thus, a single prostacyclin infusion partially corrected the endothelial injury present in this group of patients.

In another study involving 22 patients with heart failure, 10 mg of atorvastatin for 16 weeks significantly decreased the levels of soluble TNF receptor-1 and C-reactive protein in the patients’ plasmas. However, this treatment did not reduce the elevated levels of IL-6 and brain natriuretic peptide in the plasmas of patients with heart failure (81). In a study involving 21 patients with end stage renal disease, supplementation of their dialysis bags with 4,500 anti-factor Xa units of a LMWH for 60 days in a three-month period significantly decreased inflammation (a 26% reduction of C-reactive protein) and IL-6 (55% reduction) (82). The largest study to date reported how treatment of arterial thrombosis influences the various molecular markers involved 555 patients with coronary artery disease. The patients received a LMWH by two daily subcutaneous injections (120 IU/kg) for 5–7 days. Half the patients were then randomized to receive a placebo while the other half received twice-daily doses of the LMWHs for three months. The longer-term use of the LMWH significantly decreased coagulation in vivo (marked suppression of plasma factor VIIa, prothrombin fragment 1 + 2 and D-dimer levels). Reactivation of coagulation, however, was evident in patients in both the placebo and active treatment arms when administration of the LMWH was stopped. Prolonged use of the LMWH, however, did not reduce the level of inflammation in the patients (unaltered levels of C-reactive protein, IL-6 and fibrinogen) (83).

In the final studies of 24 patients, use of atorvastatin (20 or 40 mg) for six and 24 weeks by patients with hypercholesterolemia.
significantly reduced the plasma concentrations of C-reactive proteins and soluble amyloid A. In contrast, 40 and 80 mg of simvastatin had no effects on the two liver-derived markers of inflammation (84). Both statins had minimal effects on the levels of IL-6 or intercellular adhesion molecule-1 (ICAM-1) (84). Based on the foregoing, making firm concluding statements about how agents shown to be clinically effective in arterial thrombosis influence coagulation, fibrinolysis or inflammation in the patients may be premature.

Low clinical utility of measurement of thrombin production in vivo for identifying patients at greatest risk for thrombosis or the efficacy of drugs

Evidence has been presented for increased production and actions of thrombin in individuals with high and moderate risk developing venous and arterial thrombosis (1–5). Patients with osteoarthritis and atherosclerosis of varying severity also present evidence consistent with significantly increased in vivo interactions between thrombin and platelets, endothelial cells, and leukocytes in vivo (3, 5, 12, 14, 15, 29–32, 34, 35, 37–41) and for increased inflammation (30, 32, 36–41, 84). However, the levels of thrombin produced in vivo have not been found to be useful for identifying patients with high, moderate, or low risk for developing either arterial or venous thrombosis (1, 5, 22, 35, 61, 65, 67, 68, 81–83). It should be noted, however, that three studies have reported the utility of prothrombin fragment 1+2 concentrations for identifying patients with high risk for thrombosis or death (4, 36, 84). As discussed, heparin and LMWH, hirudin and bivalirudin are highly effective antithrombotic drugs in the context of preventing and treating the potentially devastating consequences of venous and arterial thrombosis (17, 23, 25–27, 60, 61, 64, 65, 67). The two families of indirect thrombin inhibitors (heparin and LMWH) and direct thrombin inhibitors can, in the short term, clearly inhibit both production and actions of thrombin in vivo (23, 61, 63, 64, 66, 68). It is also clear that none of these drugs can permanently shut off the triggers of hypercoagulability that may lead to increased thrombin production in the patients (55, 60, 62, 63, 66, 67, 82–84). This probably explains the need for the concomitant use of oral anticoagulants and heparin or LMWH in venous thrombosis (25), and the combined use of aspirin or other antiplatelet drugs with heparin, LMWH, hirudin or hirulog in arterial thrombosis (69, 71, 73, 76). The inability of heparin/LMWH to permanently shut off the triggers of hypercoagulability can probably not be attributed solely to the elimination of the drugs from the patients’ blood. Using more sensitive tests for measuring heparin and LMWHs in undiluted plasmas, the antithrombin (catalytic) activity of heparin/LMWH can be detected up to 17 hours after a single injection of either drug (85, 86).

Dermatan sulfate, a glycosaminoglycan isolated from porcine intestinal mucosa, effectively prevents post-operative thrombosis, and has also been used as the initial treatment of established DVT [87 and references therein]. Dermatan sulfate which catalyzes thrombin inhibition by heparin cofactor II and is a weak anticoagulant, relative to heparin (7), is the major anti-thrombotic glycosaminoglycan isolated from human thoracic aorta and saphenous veins (88). Whether the prophylactic or therapeutic use of dermatan sulfate is associated with the inhibition of prothrombin activation or increased inhibition of thrombin by heparin cofactor II in vivo has not been reported. A synthetic pentasaccharide based on the sequence of heparin pentasaccharides that bind antithrombin with high affinity has been used to prevent post-operative thrombosis and to treat venous thrombosis (89, 90). Its principal catalytic activity in vitro is to accelerate the inhibition of factor Xa by antithrombin (6) and it may weakly catalyze the inhibition of thrombin by heparin cofactor II. When this pentasaccharide is used to treat or prevent thromboembolism, its ability to inhibit the conversion of prothrombin into thrombin and prothrombin fragment 1+2 in vivo has not been reported.

Thrombin is normally produced in vivo for hemostasis; further, thrombin production in vivo normally increases significantly with age, even in the absence of significant inflammation with normal aging (5, 22). Also noteworthy is that neither prophylactic dose of heparin nor LMWHS inhibits thrombin production in healthy volunteers (85, 91). It is therefore reasonable to suppose that neither heparin nor LMWH can inhibit thrombin production in vivo at the sites where thrombin is produced solely for normal hemostasis. Why do heparin and LMWHS not inhibit the production of thrombin for normal hemostasis? Interestingly, either drug can at least temporarily inhibit prothrombin activation and suppress some actions of the additional thrombin produced in vivo in patients with acute DVT, acute coronary syndrome, MI or stroke and for up to three days following knee or hip replacement surgery. This state of hypercoagulation in patients with acute venous and arterial thrombosis returns after the heparin or LMWH is stopped (25, 27, 60, 63). Beyond this initial post-operative period, however, heparin no longer inhibits prothrombin activation to the pre-operative levels and a period of markedly elevated thrombin production persists for at least six weeks after orthopedic surgery (60, 63). Curiously, heparin or LMWH continues to suppress the generation of thrombin-anti-thrombin after orthopedic surgery even when it cannot inhibit the generation of prothrombin fragment 1+2 in vivo (60, 63, 92, 93). It is unclear why heparin/LMWH does not effectively inhibit the additional thrombin production which is probably required for tissue repair/wound healing after orthopedic (and presumably other) surgeries. The nature of the stimuli and sites where additional thrombin is produced for post-operative tissue repair/wound healing are not yet defined. Are some of the sources of the stimuli or the stimuli required for post-operative tissue repair/wound healing also present in patients with atherosclerosis who are at risk for acute coronary syndrome?

While no definitive answers to any of the above question can now be given, the clinical effectiveness of aspirin in preventing post-operative venous thrombosis and that of aspirin and statins in reducing the progression of arterial thrombosis provide some hints as to the potential sources of the stimulus for increased (pathophysiological) thrombin production present in arterial and venous thrombosis. As discussed earlier, aspirin (an antiplatelet and a general anti-inflammatory drug) effectively prevents the progression of arterial thrombosis and prevents venous thrombosis after high risk surgery (57–59, 61, 70, 71, 78). The effectiveness of aspirin in arterial thrombosis is significantly improved when it is combined for a short time with an anticoagulant (hepa-
The demonstration of any anticoagulant function of heparin in patient plasmas may not be necessary for the antithrombotic action of heparin to become manifest in man. Negus et al. reported in 1980 a double-blind randomized study that intravenous administration of an ultra-low dose of heparin (1 IU/kg/h) for three to five days during and after orthopedic surgery was highly effective in reducing the frequency of post-operative DVT. Specifically, only 4% of the patients in the heparin arm of this study developed venous thrombosis or PE while 22% of the patients in the placebo arm did (96). Clearly, no anticoagulant heparin would be detectable in the plasmas of the patients receiving this ultra-low dose of heparin. In spite of the probable absence of measurable heparin activity in the plasmas of these patients, this ultra-low dose of heparin effectively prevented venous thrombosis and PE after surgery (96). The antithrombotic effectiveness of dermatan sulfate and fondaparinux in man (87, 89, 90), agents with significantly weaker anticoagulant properties than heparin/LMWH support the notion that demonstrably strong in vivo and ex vivo anticoagulant activity may not be necessary requirements for effective antithrombotic activity. Until answers to some of the questions posed in the last two paragraphs are known, attempts to determine levels of in vivo thrombin production and actions suitable or relevant for making reliable prognosis on venous and arterial thrombosis in man may remain both difficult and non-productive.

Acknowledgement
The author is a recipient of operational funds from Canadian Blood Services and a grant-in-aid from Heart and Stroke Foundation of Ontario.

References