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Anti-Xa assays: What is their role today in antithrombotic therapy?

ABSTRACT

Although some suggest anti-Xa assays should be the preferred method for monitoring intravenous unfractionated heparin therapy, which method is best is unknown owing to the lack of large randomized controlled trials correlating different assays with clinical outcomes. This article provides an overview of heparin monitoring and the pros, cons, and clinical applications of anti-Xa assays.

KEY POINTS

Intravenous unfractionated heparin treatment is typically monitored by the activated partial thromboplastin time (aPTT), with a therapeutic target defined as the range that corresponds to an anti-Xa level of 0.3 to 0.7 U/mL.

Monitoring unfractionated heparin is important to achieve a therapeutic target within the first 24 hours and to maintain therapeutic levels thereafter.

The heparin anti-Xa assay is unreliable for unfractionated heparin monitoring when switching from oral factor Xa inhibitor therapy to intravenous unfractionated heparin. In such cases, the aPTT is preferred.

Most patients receiving low-molecular-weight heparin do not need monitoring, but monitoring should be considered for pregnant women with prosthetic heart valves, using an anti-Xa assay specific for low-molecular-weight heparin.

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Should clinicians abandon the activated partial thromboplastin time (aPTT) for monitoring heparin therapy in favor of tests that measure the activity of the patient's plasma against activated factor X (anti-Xa assays)?

Although other anticoagulants are now available for preventing and treating arterial and venous thromboembolism, unfractionated heparin—which requires laboratory monitoring of therapy—is still widely used. And this monitoring can be challenging. Despite its wide use, the aPTT lacks standardization, and the role of alternative monitoring assays such as the anti-Xa assay is not well defined.

This article reviews the advantages, limitations, and clinical applicability of anti-Xa assays for monitoring therapy with unfractionated heparin and other anticoagulants.

UNFRACTIONATED HEPARIN AND WARFARIN ARE STILL WIDELY USED

Until the mid-1990s, unfractionated heparin and oral vitamin K antagonists (eg, warfarin) were the only anticoagulants widely available for clinical use. These agents have complex pharmacokinetic and pharmacodynamic properties, resulting in highly variable dosing requirements (both between patients and in individual patients) and narrow therapeutic windows, making frequent laboratory monitoring and dose adjustments mandatory.

Over the past 3 decades, other anticoagulants have been approved, including low-molecular-weight heparins, fondaparinux, parenteral direct thrombin inhibitors, and

direct oral anticoagulants. While these agents have expanded the options for preventing and treating thromboembolism, unfractionated heparin and warfarin are still the most appropriate choices for many patients, eg, those with stage 4 chronic kidney disease and end-stage renal disease on dialysis, and those with mechanical heart valves.

In addition, unfractionated heparin remains the anticoagulant of choice during procedures such as hemodialysis, percutaneous transluminal angioplasty, and cardiopulmonary bypass, as well as in hospitalized and critically ill patients, who often have acute kidney injury or require frequent interruptions of therapy for invasive procedures. In these scenarios, unfractionated heparin is typically preferred because of its short plasma half-life, complete reversibility by protamine, safety regardless of renal function, and low cost compared with parenteral direct thrombin inhibitors.

As long as unfractionated heparin and warfarin remain important therapies, the need for their laboratory monitoring continues. For warfarin monitoring, the prothrombin time and international normalized ratio are validated and widely reproducible methods. But monitoring unfractionated heparin therapy remains a challenge.

Although other anticoagulants are now available, unfractionated heparin is still widely used

UNFRACTIONATED HEPARIN'S EFFECT IS UNPREDICTABLE

Unfractionated heparin, a negatively charged mucopolysaccharide, inhibits coagulation by binding to antithrombin through the high-affinity pentasaccharide sequence.[1–6] Such binding induces a conformational change in the antithrombin molecule, converting it to a rapid inhibitor of several coagulation proteins, especially factors IIa and Xa.[2–4]

Unfractionated heparin inhibits factors IIa and Xa in a 1:1 ratio, but low-molecular-weight heparins inhibit factor Xa more than factor IIa, with IIa-Xa inhibition ratios ranging from 1:2 to 1:4, owing to their smaller molecular size.[7]

One of the most important reasons for the unpredictable and highly variable individual responses to unfractionated heparin is that, infused into the blood, the large and negatively charged unfractionated heparin molecules bind nonspecifically to positively charged plasma proteins.[7] In patients who are critically ill, have acute infections or inflammatory states, or have undergone major surgery, unfractionated heparin binds to acute-phase proteins that are elevated, particularly factor VIII. This results in fewer free heparin molecules and a variable anticoagulant effect.[8]

In contrast, low-molecular-weight heparins have longer half-lives and bind less to plasma proteins, resulting in more predictable plasma levels following subcutaneous injection.[9]

MONITORING UNFRACTIONATED HEPARIN IMPROVES OUTCOMES

In 1960, Barritt and Jordan[10] conducted a small but landmark trial that established the clinical importance of unfractionated heparin for treating venous thromboembolism. None of the patients who received unfractionated heparin for acute pulmonary embolism developed a recurrence during the subsequent 2 weeks, while 50% of those who did not receive it had recurrent pulmonary embolism, fatal in half of the cases.

The importance of achieving a specific aPTT therapeutic target was not demonstrated until a 1972 study by Basu et al,[11] in which 162 patients with venous thromboembolism were treated with heparin with a target aPTT of 1.5 to 2.5 times the control value. Patients who suffered recurrent events had subtherapeutic aPTT values on 71% of treatment days, while the rest of the patients, with no recurrences, had subtherapeutic aPTT values only 28% of treatment days. The different outcomes could not be explained by the average daily dose of unfractionated heparin, which was similar in the patients regardless of recurrence.

Subsequent studies showed that the best outcomes occur when unfractionated heparin is given in doses high enough to rapidly achieve a therapeutic prolongation of the aPTT,[12–14] and that the total daily dose is also important in preventing recurrences.[15,16] Failure to achieve a target aPTT within 24 hours of starting unfractionated heparin is associated with increased risk of recurrent venous thromboembolism.[13,17]

Raschke et al[17] found that patients prospectively randomized to weight-based doses of intravenous unfractionated heparin (bolus plus infusion) achieved significantly higher rates of therapeutic aPTT within 6 hours and 24 hours after starting the infusion, and had significantly lower rates of recurrent venous thromboembolism than those randomized to a fixed unfractionated heparin protocol, without an increase in major bleeding.

Smith et al,[18] in a study of 400 consecutive patients with acute pulmonary embolism treated with unfractionated heparin, found that patients who achieved a therapeutic aPTT within 24 hours had lower in-hospital and 30-day mortality rates than those who did not achieve the first therapeutic aPTT until more than 24 hours after starting unfractionated heparin infusion.

Such data lend support to the widely accepted practice and current guideline recommendation[8] of using laboratory assays to adjust the dose of unfractionated heparin to achieve and maintain a therapeutic target. The use of dosing nomograms significantly reduces the time to achieve a therapeutic aPTT while minimizing subtherapeutic and supratherapeutic unfractionated heparin levels.[19,20]

THE aPTT REFLECTS THROMBIN INHIBITION

The aPTT has a log-linear relationship with plasma concentrations of unfractionated heparin,[21] but it was not developed specifically for monitoring unfractionated heparin therapy. Originally described in 1953 as a screening tool for hemophilia,[22–24] the aPTT is prolonged in the setting of factor deficiencies (typically with levels < 45%, except for factors VII and XIII), as well as lupus anticoagulants and therapy with parenteral direct thrombin inhibitors.[8,25,26]

Because thrombin (factor IIa) is 10 times more sensitive than factor Xa to inhibition by the heparin-antithrombin complex,[4,7] thrombin inhibition appears to be the most likely mechanism by which unfractionated heparin prolongs the aPTT. In contrast, aPTT is minimally or not at all prolonged by low-molecular-weight heparins, which are predominantly factor Xa inhibitors.[7]

HEPARIN ASSAYS MEASURE UNFRACTIONATED HEPARIN ACTIVITY

While the aPTT is a surrogate marker of unfractionated heparin activity in plasma, unfractionated heparin activity can be measured more precisely by so-called heparin assays, which are typically not direct measures of the plasma concentration of heparins, but rather functional assays that provide indirect estimates. They include protamine sulfate titration assays and anti-Xa assays.

Protamine sulfate titration assays measure the amount of protamine sulfate required to neutralize heparin: the more protamine required, the greater the estimated concentration of unfractionated heparin in plasma.[8,27–29] Protamine titration assays are technically demanding, so they are rarely used clinically.

Anti-Xa assays provide a measure of the functional level of heparins in plasma.[29–33] Chromogenic anti-Xa assays are available on automated analyzers with standardized kits[29,33,34] and may be faster to perform than the aPTT.[35]

Experiments in rabbits show that unfractionated heparin inhibits thrombus formation and extension at concentrations of 0.2 to 0.4 U/mL as measured by the protamine titration assay,[27] which correlated with an anti-Xa activity of 0.35 to 0.67 U/mL in a randomized controlled trial.[32]

Assays that directly measure the plasma concentration of heparin exist but are not clinically relevant because they also measure heparin molecules lacking the pentasaccharide sequence, which have no anticoagulant activity.[36]

As long as unfractionated heparin is an important therapy, laboratory monitoring is needed

ANTI-Xa ASSAY VS THE aPTT

Anti-Xa assays are more expensive than the aPTT and are not available in all hospitals. For these reasons, the aPTT remains the most commonly used laboratory assay for monitoring unfractionated heparin therapy.

However, the aPTT correlates poorly with the activity level of unfractionated heparin in plasma. In one study, an anti-Xa level of 0.3 U/mL corresponded to aPTT results ranging from 47 to 108 seconds.[31] Furthermore, in studies that used a heparin therapeutic target based on an aPTT ratio 1.5 to 2.5 times the control aPTT value, the lower end of that target range

was often associated with subtherapeutic plasma unfractionated heparin activity measured by anti-Xa and protamine titration assays.[28,31]

Because of these limitations, individual laboratories should determine their own aPTT therapeutic target ranges for unfractionated heparin based on the response curves obtained with the reagent and coagulometer used. The optimal therapeutic aPTT range for treating acute venous thromboembolism should be defined as the aPTT range (in seconds) that correlates with a plasma activity level of unfractionated heparin of 0.3 to 0.7 U/mL based on a chromogenic anti-Xa assay, or 0.2 to 0.4 U/mL based on a protamine titration assay.[32,34–36]

Nevertheless, the anticoagulant effect of unfractionated heparin as measured by the aPTT can be unpredictable and can vary widely among individuals and in the same patient.[7] This wide variability can be explained by a number of technical and biologic variables. Different commercial aPTT reagents, different lots of the same reagent, and different reagent and instrument combinations have different sensitivities to unfractionated heparin, which can lead to variable aPTT results.[37] Moreover, high plasma levels of acute-phase proteins, low plasma antithrombin levels, consumptive coagulopathies, liver failure, and lupus anticoagulants may also affect the aPTT.[7,25,32,36–41] These variables account for the poor correlation—ranging from 25% to 66%—reported between aPTT and anti-Xa assays.[32,42–48]

Outcomes are best when the target aPTT is achieved rapidly

Such discrepancies may have serious clinical implications: if a patient's aPTT is low (subtherapeutic) or high (supratherapeutic) but the anti-Xa assay result is within the therapeutic range (0.3–0.7 units/mL), changing the dose of unfractionated heparin (guided by an aPTT nomogram) may increase the risk of bleeding or of recurrent thromboembolism.

CLINICAL APPLICABILITY OF THE ANTI-Xa ASSAY

Neither anti-Xa nor protamine titration assays are standardized across reference laboratories, but chromogenic anti-Xa assays have better interlaboratory correlation than the aPTT[49,50] and can be calibrated specifically for unfractionated or low-molecular-weight heparins.[29,33]

Although reagent costs are higher for chromogenic anti-Xa assays than for the aPTT, some technical variables (described below) may partially offset the cost difference.[29,33,41] In addition, unlike the aPTT, anti-Xa assays do not need local calibration; the therapeutic range for unfractionated heparin is the same (0.3–0.7 U/mL) regardless of instrument or reagent.[33,41]

Most important, studies have found that patients monitored by anti-Xa assay achieve significantly higher rates of therapeutic anticoagulation within 24 and 48 hours after starting unfractionated heparin infusion than those monitored by the aPTT. Fewer dose adjustments and repeat tests are required, which may also result in lower cost.[32,51–55]

While these studies found chromogenic anti-Xa assays better for achieving laboratory end points, data regarding relevant clinical outcomes are more limited. In a retrospective, observational cohort study,[51] the rate of venous thromboembolism or bleeding-related death was 2% in patients receiving unfractionated heparin therapy monitored by anti-Xa assay and 6% in patients monitored by aPTT ($P = .62$). Rates of major hemorrhage were also not significantly different.

In a randomized controlled trial[32] in 131 patients with acute venous thromboembolism and heparin resistance, rates of recurrent venous thromboembolism were 4.6% and 6.1% in the groups randomized to anti-Xa and aPTT monitoring, respectively, whereas overall bleeding rates were 1.5% and 6.1%, respectively. Again, the differences were not statistically significant.

Though some have suggested that the anti-Xa should be the preferred monitoring assay for intravenous unfractionated heparin therapy,[29,41] the ideal assay has not been established by large-scale randomized controlled trials correlating different assays with meaningful clinical outcomes.[8,33] Nevertheless, anti-Xa assays are considered the most accurate method of monitoring unfractionated heparin in cases of heparin resistance or lupus anticoagulant, and in other clinical circumstances (**Table 1**).[56–58]

Heparin resistance. Some patients require unusually high doses of unfractionated heparin to achieve a therapeutic aPTT: typically, more than 35,000 U over 24 hours,[7,8,32] or total daily doses that exceed their estimated

weight-based requirements. Heparin resistance has been observed in various clinical settings.[7,8,32,37–40,59–61] Patients with heparin resistance monitored by anti-Xa had similar rates of recurrent venous thromboembolism while receiving significantly lower doses of unfractionated heparin than those monitored by the aPTT.[32]

Lupus anticoagulant. Patients with the specific antiphospholipid antibody known as lupus anticoagulant frequently have a prolonged baseline aPTT,[25] making it an unreliable marker of anticoagulant effect for intravenous unfractionated heparin therapy.

Critically ill infants and children. Arachchillage et al[35] found that infants (< 1 year old) treated with intravenous unfractionated heparin in an intensive care department had only a 32.4% correlation between aPTT and anti-Xa levels, which was lower than that found in children ages 1 to 15 (66%) and adults (52%). In two-thirds of cases of discordant aPTT and anti-Xa levels, the aPTT was elevated (supratherapeutic) while the anti-Xa assay was within the therapeutic range (0.3–0.7 U/mL). Despite the lack of data on clinical outcomes (eg, rates of thrombosis and bleeding) with the use of an anti-Xa assay, it has been considered the method of choice for unfractionated heparin monitoring in critically ill children, and especially in those under age 1.[41,44,62–64]

While anti-Xa assays may also be better for unfractionated heparin monitoring in critically ill adults, the lack of clinical outcome data from large-scale randomized trials has precluded evidence-based recommendations favoring them over the aPTT.[8,34]

LIMITATIONS OF ANTI-Xa ASSAYS

Anti-Xa assays are hampered by some technical limitations:

Samples must be processed within 1 hour to avoid heparin neutralization.[34]

Samples must be clear. Hemolyzed or opaque samples (eg, due to bilirubin levels > 6.6 mg/dL or triglyceride levels > 360 mg/dL) cannot be processed, as they can cause falsely low levels.

Exposure to other anticoagulants can interfere with the results. The anti-Xa assay may be unreliable for unfractionated heparin monitoring in patients who are transitioned from low-molecular-weight heparins, fondaparinux, or an oral factor Xa inhibitor (apixaban, betrixaban, edoxaban, rivaroxaban) to intravenous unfractionated heparin, eg, due to hospitalization or acute kidney injury.[65,66] Different reports have found that anti-Xa assays may be elevated for as long as 63 to 96 hours after the last dose of oral Xa inhibitors,[67–69] potentially resulting in underdosing of unfractionated heparin. In such settings, unfractionated heparin therapy should be monitored by the aPTT.

Assays that directly measure plasma heparin exist but are not clinically relevant

TABLE 1

Settings in which anti-Xa monitoring is preferred

Critically ill children and adults
Presence of lupus anticoagulant
Pregnancy
Deficiencies of contact system factors (factor XII, kallikrein, high-molecular-weight-kininogen)
Special populations receiving low-molecular-weight heparin
Suspected heparin resistance:
> 35,000 U of unfractionated heparin required over 24 hours, or total daily dose exceeding estimated weight-based requirement
Antithrombin deficiency
Increased heparin clearance
Concomitant use of aprotinin and nitroglycerin

ANTI-Xa ASSAYS AND LOW-MOLECULAR-WEIGHT HEPARINS

Most patients receiving low-molecular-weight heparins do not need laboratory monitoring.[8] Alhenc-Gelas et al[70] randomized patients to receive dalteparin in doses either based on weight or guided by anti-Xa assay results, and found that dose adjustments were rare and lacked clinical benefit.

However, the use of low-molecular-weight heparin-specific anti-Xa assays should be considered for certain patients (**Table 2**).[8]

The suggested therapeutic anti-Xa levels for low-molecular-weight heparins are:

- 0.5–1.2 U/mL for twice-daily enoxaparin
- 1.0–2.0 U/mL for once-daily enoxaparin or dalteparin.

TABLE 2

Indications for monitoring low-molecular-weight heparin

Children
Very elderly (age > 85)
Extreme body weight (< 40 kg, and > 144 kg in the case of enoxaparin)
Chronic kidney disease (creatinine clearance 15–30 mL/min)
Pregnancy (especially with a mechanical heart valve)

Levels should be measured at peak plasma level (ie, 3–4 hours after subcutaneous injection, except during pregnancy, when it is 4–6 hours), and only after at least 3 doses of low-molecular-weight heparin.[8,71] Unlike the anti-Xa therapeutic range recommended for unfractionated heparin therapy, these ranges are not based on prospective data, and if the assay result is outside the suggested therapeutic target range, current guidelines offer no advice on safely adjusting the dose.[8,71]

Anti-Xa assays are more expensive than the aPTT and are not available in all hospitals

Measuring anti-Xa activity is particularly important for pregnant women with a mechanical prosthetic heart valve who are treated with low-molecular-weight heparins. In this setting, valve thrombosis and cardioembolic events have been reported in patients with peak low-molecular-weight heparin anti-Xa assay levels below or even at the lower end of the therapeutic range, and increased bleeding risk has been reported with elevated anti-Xa levels.[71–74] Measuring trough low-molecular-weight heparin anti-Xa levels has been suggested to guide dose adjustments during pregnancy.[75]

Clearance of low-molecular-weight heparins as measured by the anti-Xa assay is highly correlated with creatinine clearance.[76,77] A strong linear correlation has been demonstrated between creatine clearance and anti-Xa levels of enoxaparin after multiple therapeutic doses, and low-molecular-weight heparins accumulate in the plasma, especially in patients with creatine clearance less than 30 mL/min.[78] The risk of major bleeding is significantly increased in patients with severe renal insufficiency (creatinine clearance < 30 mL/min) not on dialysis who are treated with either prophylactic or therapeutic doses of low-molecular-weight heparin.[79–81] In a meta-analysis, the risk of bleeding with therapeutic-intensity doses of enoxaparin was 4 times higher than with prophylactic-intensity doses.[79] Although bleeding risk appears to be reduced when the enoxaparin dose is reduced by 50%,[8] the efficacy and safety of this strategy has not been determined by prospective trials.

ANTI-Xa ASSAYS IN PATIENTS RECEIVING DIRECT ORAL ANTICOAGULANTS

Direct oral factor Xa inhibitors cannot be measured accurately by heparin anti-Xa assays. Nevertheless, such assays may be useful to assess whether clinically relevant plasma levels are present in cases of major bleeding, suspected anticoagulant failure, or patient noncompliance.[82]

Intense research has focused on developing drug-specific chromogenic anti-Xa assays using calibrators and standards for apixaban, edoxaban, and rivaroxaban,[82,83] and good linear correlation has been shown with some assays.[82,84] In patients treated with oral factor Xa inhibitors who need to undergo an urgent invasive procedure associated with high bleeding risk, use of a specific reversal agent may be considered with drug concentrations more than 30 ng/mL measured by a drug-specific anti-Xa assay. A similar suggestion has been made for drug concentrations more than 50 ng/mL in the setting of major bleeding.[85] Unfortunately, such assays are not widely available at this time.[82,86]

While drug-specific anti-Xa assays could become clinically important to guide reversal strategies, their relevance for drug monitoring remains uncertain. This is because no therapeutic target ranges have been established for any of the direct oral anticoagulants, which were approved on the basis of favorable clinical trial outcomes that neither measured nor were correlated with specific drug levels in plasma. Therefore, a specific anti-Xa level cannot yet be used as a marker of clinical efficacy for any specific oral direct Xa inhibitor.

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