ABNORMAL LABORATORY RESULTS

Anti-Xa assays

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Key words
anticoagulation, low molecular weight heparins, thromboembolism

Aust Prescr 2013;36:98–101

SUMMARY

The plasma anti-Xa assay is a laboratory test that indirectly measures the activity of heparins. It is predominantly used for monitoring patients treated with low molecular weight heparins, particularly when dosing at the extremes of weight and in patients who are pregnant, critically ill or have renal impairment.

This monitoring is controversial as there is a poorly defined therapeutic range in different clinical settings and with different dosing regimens. Consequently, the timing of blood tests and their interpretation is problematic, often resulting in empirical dosing strategies.

Limitations to the assay include its lack of availability. The assay is not available in many hospitals. Its use is also restricted by the lack of Australian consensus guidelines that assist clinicians to adjust doses in response to the result of the assay.

The monitoring of prophylactic doses of low molecular weight heparins is seldom indicated.

Introduction

Heparins are commonly used anticoagulants. Treatment may be with an unfractionated heparin or a low molecular weight heparin.

Unfractionated heparin is routinely monitored by measuring the activated partial thromboplastin time. When low molecular weight heparins were first marketed regular monitoring was not recommended. While this is generally the case for prophylactic use, some patients require their treatment to be monitored.

As low molecular weight heparins are a mixture of molecules of varying length, their concentration is difficult to measure. Instead a pharmacodynamic observation, the anti-Xa activity, is used as a surrogate.

Mechanism of action of heparins

The anticoagulant properties of low molecular weight heparins and unfractionated heparin are derived from their interaction with antithrombin, a naturally occurring anticoagulant protein. Antithrombin suppresses coagulation by inactivating proteins involved in the coagulation cascade, primarily thrombin and clotting factor Xa. Heparins bind to antithrombin, inducing a change in the molecule that results in a many-fold increase in its anticoagulant activity.

The specific binding of any size heparin molecule to antithrombin is sufficient for inactivation of factor Xa. Unfractionated heparin also inactivates thrombin (coagulation factor IIa) as larger molecules are necessary for this process.

Dosing

The low molecular weight heparins have a purported predictable dose–response relationship and a half-life that permits once- or twice-daily dosing.[1,2] These properties help facilitate simple fixed or weight-based (mg/kg) dosing and enable outpatient treatment without the need for routine monitoring. This is reflected in the product information of enoxaparin and dalteparin.

The need for monitoring low molecular weight heparins

Patients with renal disease and obesity were predominantly excluded from the drug development studies. There is little evidence to guide the management of patients with extreme values of renal function or body weight. Data are also limited in newborns, children, pregnant women and the critically ill.

Many clinicians recognise the limitations of the fixed or weight-based dosing strategies. They reduce the recommended doses in an effort to minimise the likelihood of an adverse event or opt for monitoring to guide their choice of dose.[3]

Measuring anti-Xa activity

Low molecular weight heparins predominantly affect the activity of factor Xa, so it is appropriate to monitor them with an anti-Xa assay. The measured anti-Xa activity is considered to be directly proportional to the plasma concentration. Fondaparinux and danaparoid are two other drugs that inhibit factor Xa and their activity can also be measured using an anti-Xa assay.

The recommended method is the chromogenic procedure.[1] The patient's plasma is added to a known amount of excess factor Xa. If a heparin is present in the plasma, it will bind to antithrombin and form a

complex with factor Xa. The amount of residual factor Xa is inversely proportional to the amount of heparin in the plasma. The residual factor Xa is detected by adding a substrate that mimics the natural substrate of factor Xa. This is cleaved by the residual factor Xa, releasing a coloured compound (chromophore) that can be detected by a spectrophotometer. The quantity of chromophore released is inversely proportional to the activity of the heparin present. Each chromogenic substrate release is measured against a calibration curve that is specific to each heparin (or heparinoid). Recently multicalibration kits have become commercially available. Results are expressed as units/mL or units/L of anti-Xa activity.

The assay is not widely available and is reported to be poorly standardised between laboratories. There can be wide variations in the results obtained from the same plasma sample.[4] Antithrombin deficiency affects the assay, however this is rare.

Sampling

If the monitoring of anti-Xa activity is deemed necessary, sampling should occur as soon as possible after starting or adjusting treatment. The low molecular weight heparins have a half-life of four to six hours in average adults and a steady state will occur within one day. The half-life will be prolonged in renal impairment, but this should not detract from an assessment of these patients who are at risk of bleeding.

A maximum plasma activity or concentration above the target range increases the risk of bleeding. To estimate this peak concentration (C_{max}), the recommended sampling time is four hours after the dose. This time will often misrepresent the true C_{max} due to inter-individual variation in pharmacokinetic parameters. In some patients the peak concentration can be reached in one hour, however a reasonable representation can be gained between three and five hours after the dose. Sampling outside this time window will affect the ability to interpret the results. Often blood cannot be collected at the preferred time, so the result needs to be extrapolated to the 'true' C_{max}. This can be difficult and when in doubt the clinician should take another sample after the next dose.

Trough monitoring has been suggested. If trough monitoring is indicated the sample should be taken 12 hours after the dose, immediately before the next dose.

Therapeutic range

As with all anticoagulation, clinicians seek a therapeutic range that minimises the risk of bleeding and embolic events. The most robust data for enoxaparin come from the Thrombolysis in Myocardial Infarction 11A trial where peak anti-Xa concentrations greater than 1.0 IU/mL increased the incidence of bleeding.[5] A later study found that patients with a peak concentration less than 0.5 IU/mL had a three-fold increase in re-infarction and mortality when compared to patients with a concentration between 0.5 and 1.2 IU/mL.[6] When using enoxaparin at a twice-daily dose, the clinician should therefore aim for a peak concentration between 0.5 and 1.0 IU/mL although some guidelines recommend 0.5–1.2 IU/mL or 0.6–1.0 IU/mL.[1] A recent study suggested that a 50% reduction in adverse events would occur if the trough (C_{min}) is less than 0.5 IU/mL provided that the peak (C_{max}) is above 0.5 IU/mL.[7]

The suggested peak activity range for once-daily treatment is 1.0–2.0 IU/mL and 0.2–0.4 IU/mL for prophylactic use, albeit without supporting evidence.[2,8] As evidence supports the link between bleeding and a peak concentration greater than 1.0 IU/mL, the higher range for once-daily treatment is fraught with danger if a patient has severe renal impairment with reduced ability to eliminate the drug.

The target anti-Xa range for a peak concentration for dalteparin is listed in the product information as 0.5–1.5 IU/mL. Although clinical studies are lacking to support this range, it is assumed the concentrations above this range are linked to bleeding. For treatment doses, the reported therapeutic range for anti-Xa activity of danaparoid is 0.5–0.8 IU/mL.

The evidence for all therapeutic ranges originates from studies in arterial disease. Few data exist that define a separate range for venous disease.

When are anti-Xa assays indicated?

There is a developing consensus that monitoring is advisable in patients who have renal impairment, are pregnant or obese.[2] In these patients the pharmacokinetics of the drugs are altered when compared to otherwise healthy adults.

Pregnancy changes renal function and the distribution of fluid, which affects the clearance and distribution of the drugs, and makes predicting a therapeutic dose more difficult.[9] As warfarin is commonly contraindicated during early pregnancy, low molecular weight heparins with accompanying anti-Xa monitoring are recommended for indications such as recurrent deep vein thrombosis and in pregnant women with mechanical heart valves. In high-risk patients, trough anti-Xa monitoring is often used to ensure constant anticoagulation although there is no consensus on the target concentration.

Monitoring is also indicated in patients who receive extended therapy or do not have the expected

response, for example, those who thrombose or bleed during therapy. Anti-Xa monitoring should be considered in patients at high risk of bleeding as, unlike unfractionated heparin, the anticoagulant effects of low molecular weight heparins are not so readily reversible.

Renal impairment

Low molecular weight heparins are polar, hydrophilic drugs that are approximately 80% renally eliminated. In patients with renal impairment, accumulation could potentially occur with standard doses.[1,10] This increases the risk of bleeding.[10,11] Numerous studies of enoxaparin have shown higher peak and trough anti-Xa activity in patients with renal impairment.[8] However, the size of the risk has never been quantified in suitably powered studies, leaving a range of 0.5–1.0 IU/mL as the best dosing guide. According to the product information for dalteparin, monitoring only needs to occur after the patient has received three to four doses. However, drug accumulation will occur if the patient has severe renal impairment so it would be prudent to monitor before this time.

The table shows a summary of US monitoring guidelines.[2,8]

Obesity

The dosing and monitoring of low molecular weight heparins in obese patients is contentious. As the drugs are hydrophilic they are predominantly distributed in plasma and lean tissue and do not easily partition into adipose tissue. The clearance of low molecular weight heparins correlates with lean body mass, therefore the addition of adipose weight into the weight-based dose calculation is difficult to justify.[12]

Dosing based on total body weight may result in excessive concentrations so physicians often introduce an arbitrary dose adjustment that has never been formally evaluated. One method is to 'cap' the dose (for example 100 mg for enoxaparin), regardless of the patient's total body weight, however capping is likely to result in sub-therapeutic concentrations. Despite suggestions that anti-Xa monitoring should only be considered in the morbidly obese,[8] monitoring peak activity in adults with a total body weight more than 100 kg is justifiably common practice.

Table **Anti-Xa monitoring according to renal function**[2,8]

Renal function (mL/min) *	Anti-Xa monitoring
Severe impairment (<30)	Always
Moderate impairment (30–60)	For extended therapy (more than 48 hours)
Normal (>60)	Not required †

* Calculated using the Cockcroft-Gault equation
† Factors such as obesity need to be considered

Does anti-Xa activity change with different heparins?

Heparins have different molecular weights and consequently differing anti-IIa and anti-Xa activity. Unfractionated heparin molecules have approximately equivalent anti-Xa and anti-IIa activity. Low molecular weight heparins are approximately one-third the molecular weight of unfractionated heparin. This decreases their ability to bind to thrombin, giving an anti-Xa:anti-IIa ratio between 2:1 and 4:1.[1] It is this reduced anti-IIa activity which makes the activated partial thromboplastin time less reliable for monitoring low molecular weight heparins. At present there is no evidence to show that the differences in anti-Xa activity among the low molecular weight heparins influence clinical outcomes.[1]

Dose modification

No strategies have been evaluated in large, randomised studies to assist in dose modification once anti-Xa activity is known. A small Australian study demonstrated that the risk of bleeding is reduced when doses are individualised using anti-Xa concentrations.[13] Other dose reduction strategies for obesity and renal impairment have been proposed, but are yet to be tested against clinical outcomes.[8] Drug monitoring principles suggest that a linear dose adjustment could be used if the clearance of the low molecular weight heparins is stable, or an extension in dosing frequency if clearance is significantly reduced.

Conclusion

The anti-Xa assay is being increasingly used when treating patients with low molecular weight heparins, but a clear correlation between anti-Xa concentrations and clinical outcome is yet to be shown. The best evidence points towards a peak concentration between 0.5 and 1.0 IU/mL taken four hours after a dose. While clinical studies are pending, it is prudent to monitor anti-Xa activity in at-risk patients such as those with renal impairment, in the obese, and in pregnant women.

Measurement of anti-Xa activity assesses the activation of only a single component of the clotting system. There is a need for an alternative, simple, stable, diagnostic clotting time-based test to monitor treatment with low molecular weight heparins. New tests are promising, but require evaluation.[14]

Acknowledgements: Professor Steve Duffull and Mr Hesham Al-Sallami, University of Otago, Dunedin; Dr Raymond Banh, consultant haematologist, Mater Health Services, Brisbane; Ms Joanne Beggs, advanced scientist, Pathology Queensland, Brisbane

Conflict of interest: none declared

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SELF-TEST QUESTIONS

True or false?

7. The activity of low molecular weight heparins is monitored by measuring the activated partial thromboplastin time.

8. A fall in anti-Xa activity, in a patient treated with low molecular weight heparins, is associated with an increased risk of bleeding.

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