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BSH GUIDELINE

Measurement of heparin, direct oral anti-coagulants and other non-coumarin anti-coagulants and their effects on haemostasis assays: A British Society for Haematology Guideline

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METHODOLOGY

This guideline was compiled according to the BSH process at [https://b-s-h.org.uk/media/16732/bsh-guidance-development-process-dec-5-18.pdf]. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org. Literature search criteria can be found in Appendix A.

REVIEW OF THE MANUSCRIPT

Review of the manuscript was performed by the British Society for Haematology (BSH) Haemostasis and Thrombosis Task Force and the BSH Guidelines Committee. It was also placed on the members section of the BSH website for comment.

INTRODUCTION

This guideline aims to update healthcare professionals working in the UK on the measurement of anti-coagulants (other than coumarins) currently licensed for use in the UK, and their effects on laboratory assays (Table 1). It provides recommendations based on the body of literature produced since the previous guidance published in 2014.[1] Direct factor (F)XIa- and direct FXIIa-inhibiting anti-coagulants are at various stages of development but not yet licensed, so are not discussed in this guideline.[2] The recent guidelines from the International Society of Thrombosis and Haemostasis Scientific Standardization Committee (ISTH/SSC) on the nomenclature to be used when describing non-vitamin K anti-coagulation[3] are followed.

Some anti-coagulants, such as unfractionated heparin (UFH) and argatroban, have been in clinical use for decades. Laboratory monitoring to guide dose adjustments has been with the widely available activated partial thromboplastin

TABLE 1 Anti-coagulants currently licensed for use in the UK (excluding vitamin K antagonists) and their modes of measurement/monitoring.

Mode of administration	Drug name	Half-life (h)	Elimination route (approx. %)	Major mode of action	Potential laboratory tests for measurement
Oral	Dabigatran	12–17	Hepatobiliary (20) Renal (80)	Direct Inhibition of FIIa	Dabigatran dTT Dabigatran ECT/ECA Dabigatran anti-FIIa
	Rivaroxaban	5–9	Hepatobiliary (66) Renal (33)	Direct Inhibition of FXa	Rivaroxaban anti-FXa
	Apixaban	8–15	Hepatobiliary (75) Renal (25)	Direct Inhibition of FXa	Apixaban anti-FXa
	Edoxaban	10–14	Hepatobiliary (50) Renal (50)	Direct Inhibition of FXa	Edoxaban anti-FXa
Intravenous or subcutaneous	Unfractionated heparin (UFH)	1–2	Low dose—Reticuloendothelial system High dose—Renal	Indirect Inhibition of FIIa and FXa	APTT UFH anti-FXa Combined UFH/LMWH anti-FXa[b] UFH anti-FIIa
	Danaparoid	17–25	Renal	Indirect Inhibition of FXa	Danaparoid anti-FXa
Intravenous	Argatroban	0.6–1.0	Hepatobiliary	Direct Inhibition of FIIa	APTT Argatroban dTT Argatroban ECT/ECA Argatroban anti-FIIa
	Bivalirudin	0.4	Renal	Direct Inhibition of FIIa	ACT
Subcutaneous	Low-molecular-weight heparin (LMWH)	3–5	Renal	Indirect Inhibition of FXa	LMWH anti-FXa[a] Combined UFH/LMWH anti-FXa[b]
	Fondaparinux	17–21	Renal	Indirect Inhibition of FXa	Fondaparinux anti-FXa

Abbreviations: ACT, activated clotting time; anti-FIIa, chromogenic anti-FIIa; anti-FXa, chromogenic anti-FXa; APTT, activated partial thromboplastin time; dTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECT, ecarin clotting time.

[a]Generic LMWH calibrator for dalteparin, enoxaparin, fragmin and tinzaparin.

[b]If locally verified.

time (APTT). However, the COVID-19 pandemic highlighted the wide variability in the sensitivity of different APTT reagents in patients with acute illness, emphasising the need for accessible and cost-effective anti-FIIa and anti-FXa assays for routine monitoring.

The introduction of specific anti-FIIa or anti-FXa-based assays has provided a means to quantitate plasma drug concentrations of the newer fixed-dose FIIa inhibitors (FIIaI) and FXa inhibitors (FXaI). When used according to licence, monitoring these direct oral anti-coagulants (DOACs) is not required, but measuring drug concentration can add value in some circumstances (Table 2). As neither drug concentration nor dose-adjustment based on the measured concentration has yet been shown to affect efficacy or safety, it is incorrect to refer to a therapeutic range. In this manuscript, the term 'expected range' is used to acknowledge this limitation.

There are many reports in the literature about the effects of anti-coagulants on measurable parameters of haemostasis. Lack of awareness of these effects, which are variable depending on anti-coagulant, timing of sampling and reagents, can cause confusion and delay diagnosis and care. Table 3 gives a broad overview of the types of impact on laboratory assays that may be seen.

All assays described must be used in accordance with requirements of ISO15189.

NEUTRALISATION OF ANTI-COAGULANT ACTIVITY PRIOR TO HAEMOSTASIS TESTING

Activated charcoal products (ACP) (tablets or filters) that adsorb some anti-coagulant activities from plasma samples have been suggested as a way of undertaking haemostatic tests while continuing anti-coagulant therapy.[4–6] The process appears to remove not only the effects of rivaroxaban, apixaban edoxaban, dabigatran, argatroban, protamine, aprotinin and polymyxin but also leaves heparin-like and coumarin anti-coagulant activity intact.[7,8] Many haemostatic parameters have been reported to be unaffected by ACP, including those associated with haemophilia, thrombophilia (including lupus anti-coagulant assays) and thrombin generation assays (TGA), making the process attractive for many diagnostic algorithms.[9–11] ACP do not remove the effects of heparin-based or coumarin anti-coagulation, and caution is required if there are high levels of FXaI present, as these may be incompletely removed.[11,12] If laboratories wish to use this approach, the impact on local assays and reagents needs to be assessed in-house and documented in accordance with the requirements of ISO15189. In general, delaying testing until off anti-coagulation is the preferred option whenever possible.

TABLE 2 Some circumstances where measurement of anti-coagulants that do not require routine monitoring may be considered.

- Renal impairment
- Use of an interacting drug
- At the extremes of body weight (e.g. below 50 kg or above 120 kg)
- In the presence of severe, acute haemorrhage
- To assess compliance
- Following suspected overdose
- Prior to an invasive procedure with a high bleeding risk when the time from the last dose is not known
- Gastrointestinal abnormalities causing impaired absorption
- Use of non-standard doses

UNFRACTIONATED HEPARIN (UFH)

Heparin is a naturally occurring glycosaminoglycan polymer that has a physiological anti-coagulant function. It exists naturally as polymers of varying sizes (20 000–50 000 Daltons), and all pharmaceutical-grade UFH is derived from porcine or bovine intestinal mucosa. Fractionation of primary polymers produces smaller molecules of varying sizes referred to as low-molecular-weight heparin (LMWH). UFH produces its anti-coagulant effect mainly through inactivation of FIIa and FXa (as well as FIXa, FXIa and FXIIa to a lesser extent) through an anti-thrombin-dependent mechanism.[13] UFH is a highly negatively charged molecule with a propensity for reversibly binding to proteins and surfaces. Pharmacokinetic limitations are caused by anti-thrombin-independent binding of heparin to plasma proteins released from platelets and endothelial cells, resulting in a variable anti-coagulant response leading not just to large interindividual variability but also to intraindividual variability influenced by the patient's inflammatory response.[14] One effect of this is to release tissue factor pathway inhibitor (TFPI), inhibiting thrombin generation in vivo, which is likely to contribute to the anti-coagulant action.[15] Therefore, the anti-coagulant effect is not directly proportional to the dose of UFH, which requires routine monitoring to optimise the balance between the required anti-thrombotic effect and excessive bleeding risk.

Tests suitable for monitoring UFH are APTT, activated clotting time (ACT) and heparin anti-FXa activity assay. None of these assess all the anti-thrombotic effects of UFH and all have limitations. Furthermore, evidence to support the widely used expected ranges by either APTT or heparin anti-FXa assay is weak.

PRE-ANALYTICAL CONSIDERATIONS

When using tri-sodium citrate blood collection tubes for UFH monitoring, there should be only a small residual air space in the tube once blood is added, achieved mainly with a predetermined vacuum.[16,17] Citrated samples containing UFH destined for APTT must be centrifuged within 1 h of collection and analysed within 4 h to avoid leakage of platelet factor-4 (PF4), leading to neutralisation of heparin.[18–20] Dextran sulphate releases heparin from its complex with PF4 so when samples are analysed by anti-FXa assay using dextran sulphate-containing reagents, centrifugation can be delayed for up to 4 h since there is only minor or no loss of heparin anti-FXa activity and little clinically relevant impact on management decisions.[20,21] Samples collected into

TABLE 3 Effects of non-vitamin K-based anti-coagulant on the more common haemostasis assays.

Assay	UFH[a]	LMWH	Fondaparinux	Danaparoid	Dabigatran	Argatroban	Bivalirudin	Rivaroxaban	Apixaban	Edoxaban
Routine screening										
PT	–/↑	–/↑	-	-	–/↑	–/↑	↑	↑/↑↑	–/↑	–/↑
APTT	↑↑/↑↑↑	–/↑	–/↑	–/↑	↑↑	↑↑	↑↑	↑	–/↑/↑↑	↑
Fibrinogen	-	-	-	-	–/↓	–/↓	–/↓	-	-	-
Thrombin time	↑↑↑	↑	-	–/↑	↑↑↑	↑↑	↑↑↑	-	-	-
D-dimer	↓↓	↓	-	-	-	-	-	-	-	-
Factor assays										
PT-based one-stage	–/↓	–/↓	-	-	↓	↓↓	↓	↓↓	↓↓	↓↓
APTT-based one-stage	↓	↓	–/↓	–/↓	↓↓	↓↓	↓↓	↓	↓	↓
Chromogenic FVIII/IX	-	-	-	-	–/↓	-	-	↓	↓	↓
Thrombophilia assay										
Protein C (clotting)	↑↑	-	-	-	↑↑	↑↑	↑↑	↑	↑	↑
Protein C (chromogenic)	-	-	-	-	-	-	-	-	-	-
Protein S (clotting)	–/↑	–/↑	↑↑	–/↑	↑↑	↑↑	↑↑	↑	↑	↑
Free Protein S antigen	-	-	-	-	-	-	-	-	-	-
Anti-thrombin (IIa)	↓	-	-	-	↑	-	-	-	-	-
Anti-thrombin (Xa)	↓	-	-	-	-	-	-	↑↑	↑↑	↑↑
APCrV	↑↑	-	-	-	↑↑	↑	↑	↑↑	↑↑	↑↑
LA assays[b]										
DRVVT ratio	–/↑	–/↑	-	↑	↑↑	↑↑	↑↑	↑↑	–/↑	↑↑
LAR-APTT ratio	↑↑	↑	–/↑	↑↑	–/↑	↑↑	↑↑	↑	↑/↑↑	↑
Quantitative										
dTT	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	-	-	-
ECT/ECA/C-IIa	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	-	-	-
Chromogenic anti-FXa	↑↑	↑↑	↑↑	↑↑	-	-	-	↑↑	↑↑	↑↑
Global										
VHA[c]	↑↑/↓↓	↑↑/↓↓	↑↑/↓↓	↑↑/↓↓	↑/↓	↑↑/↓↓	↑/↓	↑↑/↓↓	↑↑/↓↓	↑↑/↓↓
ETP	↓↓	↓↓	↓↓	↓↓	↓	↓↓	↓↓	↓↓	↓↓	↓↓
Platelets										
PFA –100/200	↑	-	-	-	-	-	-	-	-	-
LTA	-	-	-	-	↓↓	-	-	–/↓	–/↓	–/↓
Miscellaneous										
VWF antigen	-	-	-	-	-	-	-	-	-	-
VWF activity	-	-	-	-	-	-	-	-	-	-

Note: - = Most likely no effect on assay; ↑ or ↓ = Possible effect on assay (i.e. at supratherapeutic levels or could also be reagent specific); ↑↑ or ↓↓ = Likely effect at therapeutic levels; ↑↑↑ or ↓↓↓ = Substantial effect. References to the effects can be found in the main body of the text above.

Abbreviations: APCrV, activated protein C ratio with Factor V; APTT, activated partial thromboplastin time; C-IIa, chromogenic anti-FIIa; DRVVT, dilute Russell's viper venom time; dTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECT, ecarin clotting time; ETP, endogenous thrombin potential; LA, lupus anti-coagulant; LAR-APTT, lupus anti-coagulant responsive APTT (includes Silica clotting time); LTA, light transmission aggregometry; PFA, Siemens Platelet Function Analyser; PT, prothrombin time; VHA, Viscoelastic haemostatic testing; VWF, von Willebrand factor.

[a]Some routine reagents will have heparin neutralisers incorporated into them, removing a significant but potentially not all anti-coagulant effect.

[b]Refer to BSH APS guidelines.

[c]Depending on channel composition.

citrate–theophylline–adenosine–dipyridamole (CTAD) are stable for at least 4 h, even when used for an APTT.[21–23]

APTT FOR MONITORING UFH

One major limitation of the APTT for monitoring UFH is the lack of specificity. For example, a lupus anti-coagulant or deficiency of one or more clotting factors, such as FXII, may prolong the APTT. This may falsely raise the APTT into the target range despite suboptimal heparin levels. Conversely, the APTT may not be within the target range even if the heparin level is at the correct therapeutic concentration in the presence of markedly elevated levels of coagulation factors such as FVIII and fibrinogen. These are frequently elevated due to the acute-phase response that is common in patients requiring UFH.[24] Similarly, acquired anti-thrombin deficiency can be seen in critical care patients, sometimes contributing to a higher-than-expected UFH dosage requirement.[25] Furthermore, FVIII may be increased in patients with acute thromboembolic events independently of the acute-phase response, and during pregnancy.[26] This makes the APTT less sensitive to UFH, leading to the incorrect assumption that heparin levels are inadequate.[27] The target APTT range for UFH for venous thromboembolism (VTE) was established in a prospective study in 1972 which included only 234 patients (approximately 2/3 VTE and 1/3 with arterial events).[28] Although there was a low risk of recurrent thrombosis when using an APTT ratio of 1.5–2.5 times a control APTT, the evidence to support this as a target range was weak. The APTT method utilised is no longer in use and is not traceable to current methods. The target range is therefore not immediately applicable now; since APTT reagents vary markedly in their responsiveness to UFH,[29,30] the reasons for using UFH for anti-coagulation have changed and the instrument used for analysis contributes to additional variability.[31] The target APTT range[28] was later shown to correspond to 0.2–0.4 iu/mL heparin when measured using protamine titration assay or heparin anti-FXa of 0.3–0.7 iu/mL.[32] Laboratory studies without assessment of clinical outcomes have shown that establishing a target range for any particular APTT method by reference to protamine titration assay[29,30] or heparin anti-FXa[24,27,30] compensates for the variable response of APTT methods to heparin using samples from 30 to 60 patients. The limitations of the APTT for UFH monitoring are many, and even use of an APTT target range calibrated against heparin anti-FXa failed to improve interlaboratory consensus as to status of therapy (subtherapeutic, therapeutic or supratherapeutic) when three different APTT reagents were used in 44 UFH patients compared to uncalibrated APTT ranges.[33]

Any patient whose APTT is being considered for UFH monitoring should have a baseline APTT performed prior to commencement of UFH therapy. If the pre-treatment APTT is prolonged or shortened, then the APTT is unsuitable for monitoring UFH therapy for that patient. In these cases, a heparin anti-FXa assay is a better option to monitor drug response.[34]

ANTI-FXa FOR MONITORING UFH

UFH anti-FXa of 0.3–0.7 iu/mL is generally accepted as the UFH therapeutic range for treatment of VTE and other indications requiring a treatment dose, as opposed to prophylactic doses of anti-coagulation. This was derived from a single, small, randomised trial in which VTE patients requiring larger-than-average UFH doses (>35 000 units/day) were randomised to monitoring with APTT or UFH anti-FXa using therapeutic ranges corresponding to 0.2–0.4 U/mL by protamine titration.[27] The UFH anti-FXa range was 0.35–0.67 iu/mL (later rounded to 0.3–0.7 iu/mL) using an assay without dextran sulphate (Stachrom Heparin, Diagnostic Stago, France). The APTT group received higher mean daily doses of UFH than the UFH anti-FXa monitored group. Recurrent VTE within the first 12 weeks of therapy occurred in 3/65 and 4/66 in the heparin anti-FXa and APTT groups respectively. There were four bleeding events in the APTT group and one in the group monitored by UFH anti-FXa.

Calibration of the UFH anti-FXa assay should be with a UFH calibrator traceable to international standards. Commercial companies have developed combined (UFH/LMWH) calibrators to produce a single-calibrated anti-FXa assay. There is limited current peer-reviewed literature regarding comparability to separate curves, and these should be locally validated against separate curves if adopted.[35]

Dextran sulphate is added to some reagents used for heparin anti-FXa assays to disrupt binding of UFH to several plasma proteins, which may occur in vivo or in vitro. Reagents containing dextran sulphate may give higher results than those without it, at least when some of the heparin is bound to proteins in the sample.[35,36] This can lead to overestimation of heparin anti-FXa activity in cardiac surgery patients after heparin reversal by protamine.[37] On the other hand, inclusion of dextran sulphate in anti-FXa reagents protects against underestimation of the heparin available in vivo because of in vitro binding of heparin to PF4. A recent study using samples constructed by spiking UFH into normal plasma suggested using dextran-free heparin anti-FXa assays provided blood collection is performed carefully and the first tube of blood is discarded to limit the amount of PF4 produced artificially.[38] Nevertheless, there is currently no consensus on whether heparin anti-FXa assays with or without dextran sulphate should be selected for monitoring of UFH. Although the presence or absence of dextran sulphate impacts results of heparin anti-FXa assays as discussed, in a UK NEQAS survey, the interlaboratory coefficient of variation (CV) for users of different heparin anti-FXa assays (some with and some without dextran sulphate) was approximately 10% for UFH samples in the therapeutic range compared to 15%–25% for APTT results determined with multiple reagents, making the heparin anti-FXa assay a more attractive option.[36] Furthermore, use of heparin anti-FXa assays to monitor UFH achieved a faster time to therapeutic range and fewer dose adjustments per 24-h period compared to use of APTT in two studies.[39,40] A retrospective

cohort study of nearly 20 000 patients concluded that cases monitored by heparin anti-FXa were less likely to have a transfusion than hospitalised patients monitored by APTT after controlling for age, gender, other risk factors and invasive procedures.[41] Despite these theoretical and reported advantages of a heparin anti-FXa assay over APTT, a meta-analysis of 10 studies with 6677 patients found that use of APTT compared to heparin anti-FXa was not associated with increased risk of bleeding (RR 1.03, 95% CI 0.8–1.22) or an increased risk of thrombotic events (RR 0.99, 95% CI 0.76–1.30).[42] There were no differences in mortality in individual studies analysed, although the data were not considered suitable for pooled analysis. Recently, it has been reported that the heparin anti-Xa assay was the preferred method for monitoring UFH used to treat a pulmonary embolus (PE) in a study of 192 patients, identifying low incidence of recurrence or PE-associated mortality.[43] Overall, there are fewer disadvantages related to a heparin anti-FXa assay compared to APTT for monitoring UFH, and it is a better reflection of a patient's response to UFH. However, there is no strong evidence that clinical outcomes improve if heparin anti-FXa is used instead of APTT for monitoring UFH.

Lack of availability of the heparin anti-FXa assay over a 24-h period at some centres still limits the transition away from monitoring UFH with the APTT. Data provided by UK NEQAS (personal communication, December 2023) showed 540 UK sites registered for heparin monitoring using the APTT, with 125 (23%) registered for anti-FXa measurement of UFH. However, the heparin anti-FXa assay should not need to be performed as frequently as the APTT as it is less prone to interference: in stable patients on UFH, once-daily testing should be sufficient.

Recommendations

- **Blood sampling for monitoring UFH should not use tubes that are designed to be partially filled (1B).**
- **We suggest using a UFH-calibrated anti-FXa assay over an APTT for monitoring UFH therapy with a therapeutic range of 0.3–0.7 iu/mL (2B).**
- **If monitoring UFH in the absence of a heparin anti-FXa assay, a locally validated APTT ratio (patient/mean normal APTT) equivalent to heparin anti-FXa activity of 0.3–0.7 iu/mL is suggested (2C).**
- **APTT should not be used for monitoring UFH if the patient has an abnormal baseline APTT (prolonged or shortened) immediately prior to commencement of therapy (1C).**

INDIRECT FACTOR Xa INHIBITORS

Low-molecular-weight heparins

The predictable pharmacokinetic profiles of LMWH mean that when dosed according to their licence, plasma concentration monitoring is not usually required. LMWH has reduced anti-thrombin-related anti-FIIa activity relative to anti-FXa activity, and therefore LMWH anti-FXa activity is preferred when monitoring is required. LMWH anti-FXa activity is not well correlated with the risks of bleeding or thrombosis and routine monitoring does not improve clinical outcomes. The elimination of LMWH is primarily through a non-saturable renal route; therefore, monitoring LMWH anti-FXa activity could provide reassurance for minimising the risk of bleeding in patients with severe renal dysfunction.[44]

LMWH is the most used anti-coagulant during pregnancy. Pregnancy leads to physiological changes such as weight gain, altered levels of clotting factors and increased glomerular filtration rate. As these changes might be expected to affect safety and efficacy of anti-coagulation, many studies have assessed whether LMWH anti-FXa monitoring might improve clinical outcomes in pregnancy when LMWH is used at either prophylactic or treatment doses. These are summarised in a recent systematic review which concludes that adjusting LMWH doses based on calibrated anti-FXa activity does not reduce the risk of bleeding or thrombosis compared with standard, weight-based dosing.[45] There are still some specific, high-risk situations when LMWH anti-FXa monitoring is warranted, such as in pregnant women with mechanical heart valves.[46]

In children, particularly neonates and infants, the pharmacokinetic profile of LMWH is different from adults. Although there are limited data on whether LMWH anti-FXa monitoring improves LMWH safety and efficacy, paediatric guidelines support monitoring with age-dependant dosing and different weight-based dosing regimens to those for adults.[47,48] As monitoring is now standard practice, clinical trials assessing the benefit of monitoring are unlikely to be feasible.

The effect of LMWH typically peaks around 3–4 h following subcutaneous injection, following which an elimination phase is entered.[49,50] The time after a dose when samples for LMWH assay are drawn therefore matters, as it is difficult to interpret the result without this information. If bleeding risk is a concern, sampling at the trough time point, just before the next dose, is the most informative, as this provides an indication of LMWH accumulation. This is of particular relevance with renal impairment when clearance may be reduced. Peak LMWH concentration monitoring may be more useful in situations where there might be concerns about efficacy, for example, recurrent thrombosis while on treatment, but there are no data to support an optimal target.

Different LMWHs vary in size and oligosaccharide composition resulting in subtle differences in some minor anti-coagulant functions such as anti-FIIa activity. However, there is no good evidence that these differences are clinically significant. As the key anti-FXa function is mediated by the pentasaccharide sequence that is common to all LMWHs, anti-FXa concentrations do not need to be specific for different LMWHs.

A recent systematic review and meta-analysis comparing monitored versus unmonitored LMWH included six studies and 1617 patients; 724 patients in the LMWH anti-FXa monitored group and 893 patients in the unmonitored group. There was no significant difference in the incidence of bleeding events between the two groups, but the LMWH anti-FXa monitored group had a lower incidence of VTE (OR 0.44, 95% CI 0.29–0.68, $p = 0.0002$). Subgroup analysis showed that the incidence of VTE in the LMWH anti-FXa monitoring group was lower than that in the control group when the trough level was monitored (OR 0.40, 95% CI 0.25–0.63, $p < 0.0001$), while there was no significant difference between the two groups when the peak level was monitored.[51] While this work suggests that monitoring of trough levels can be correlated with thrombosis risk, other studies have not confirmed this. A range for LMWH anti-FXa activity that correlates with bleeding or thrombosis risk has not been established, therefore as previously mentioned 'expected range' is a more appropriate term.

When LMWH anti-FXa activity is measured, it should be calibrated using a chromogenic method with an LMWH calibrator, unless a combined UFH/LMWH calibration curve has been locally verified for use as described above. Target LMWH anti-FXa activity levels have not been clinically validated, and there is no standardised method for adjusting doses based on LMWH anti-FXa activity.[34,52] Furthermore, results vary depending on reagents and analyser as demonstrated by CV >15% in external quality assurance surveys.[36,53] Although target ranges have been suggested by other BSH guidelines,[46,47] the evidence in specific clinical situations is limited and these should be considered as guides for laboratories and clinicians.

LMWH has minimal effect on the prothrombin time (PT), probably due to the addition of heparin neutralisers to commercial reagents; however, very high levels of LMWH may still cause prolongation. The APTT can be slightly prolonged with LMWH in a dose-dependent manner, depending on the LMWH and the reagent used.[54] At therapeutic concentrations, LMWH would not be expected to prolong the thrombin time (TT), depending on the LMWH and reagent used, but can impact lupus anticoagulant screening.[55] Interference in lupus anti-coagulant testing should be considered even when using reagents with heparin neutralisers (Table 3). Patients should be taken off anti-coagulation or just before the next dose of LMWH to minimise these effects, if clinically appropriate.[56]

Endogenous thrombin potential (ETP) in TGA shows a negative exponential dose–response to increasing doses of LMWH with a suggestion that differences exist between LMWH preparations.[57,58] Viscoelastic haemostatic assays (VHA) will be affected by LMWH in a dose-dependent manner, with the suggestion that tinzaparin has more impact at equivalent heparin anti-FXa activity relative to other LMWHs.[59] As LMWH monitoring is not routinely required, the clinical utility of TGA and VHA are usually reserved for the research setting.

Recommendations

- **We recommend against routine laboratory monitoring of LMWH although examples of circumstances when it might be useful are given in Table 2 (1B)**
- **Routine LMWH anti-FXa monitoring during pregnancy is not required except in high-risk cases on modified dosing regimens such as those used for managing mechanical heart valves in pregnancy (1B).**
- **In neonates and children receiving LMWH, we suggest monitoring peak LMWH anti-FXa, aiming for 0.5–1.0 iu/mL, in a sample taken 4 h after subcutaneous injection once steady state has been achieved after administration of at least three doses (2C).**

Fondaparinux

The synthetic pentasaccharide, fondaparinux, is prescribed as an alternative when LMWH is not tolerated or is relatively contraindicated. It exhibits predictable pharmacokinetics and means that monitoring is not usually required, except in situations like those discussed previously (Table 2).

When fondaparinux anti-FXa activity is measured, it should be calibrated using a chromogenic method with fondaparinux calibrators and results reported in μg/mL or mg/L. The use of LMWH as the reference preparation for determining the measured anti-FXa activity of fondaparinux is problematic and should not be used.[60–62]

A therapeutic fondaparinux range has not been established, but when given as a 2.5 mg daily dose, the mean peak steady-state plasma concentration is 0.39–0.50 mg/L, 3 h post-dose. For patients of average body weight receiving 7.5 mg daily, the mean peak steady-state plasma concentration is 1.20–1.26 mg/L 3 h post-dose.[63]

In a study of laboratories participating in the College of American Pathologists Comprehensive Coagulation Proficiency Survey ($n = 898$), samples with different concentrations of fondaparinux (0, 0.4, 0.8 and 2.0 μg/mL) were distributed: prophylactic or treatment dose fondaparinux prolonged the PT by approximately 1 s and the APTT by 4 to 5 s and reduced measured FVIII levels. Fondaparinux concentrations above the expected range reduced measured FVIII from 119 iu/dL to 85 iu/dL. The APTT was prolonged in 19%, 29% and 52% of laboratories with prophylactic, treatment and above-expected-range fondaparinux levels respectively. Fibrinogen, anti-thrombin and TT assays did not show clinically significant changes.[62]

Thrombin generation assays will be affected by fondaparinux in a dose-dependent manner, although in vitro studies suggest the impact is less than with LMWH.[64] Small in vitro studies report no impact of fondaparinux on VHA in the expected range, but with significant effects at fondaparinux concentrations above the expected range.[59]

Recommendations

- **We recommend against routine laboratory monitoring of fondaparinux (1B).**
- **If measuring for one of the reasons listed in Table 2, an anti-FXa assay calibrated with a drug-specific standard should be used (1B).**
- **LMWH calibrators should not be used to report heparin equivalent units of fondaparinux (1B).**

Danaparoid

Danaparoid acts as an anti-coagulant primarily by catalysing anti-FXa activity in an anti-thrombin-dependent fashion. It is usually reserved for patients who have developed heparin-induced thrombocytopenia (HIT) or are unable to receive LMWH for another reason. Renal excretion is the main route of elimination of danaparoid, accounting for 40% to 50% of its total plasma clearance after intravenous administration.[65] There is no evidence of metabolism by the liver.[65,66] Based on danaparoid anti-FXa activity, it has a half-life of approximately 25 h.[67]

Monitoring of danaparoid anti-FXa activity is not routinely recommended. When danaparoid anti-FXa activity is measured, it should be calibrated using a chromogenic method with danaparoid calibrators. The expected danaparoid anti-FXa activity for patients prescribed 750 iu bd by subcutaneous injection is 0.2–0.4 iu/mL (6 h post-dose), and, for patients prescribed intravenous (IV) therapy, 0.5–0.7 iu/mL (5 to 10 min after an IV bolus of 2500–3750 iu) or 0.5–0.8 iu/mL (during 150–200 iu/h infusion).[67]

Danaparoid is not expected to prolong the PT, but in vitro work suggests that it could prolong the APTT and this may influence the results of APTT assays for lupus anti-coagulant (LA) at danaparoid anti-FXa activity above 0.6 iu/mL.[12,68] Dilute Russell's Viper Venom Time (DRVVT) assays are affected at levels above the expected range, and ACP does not neutralise danaparoid anti-FXa activity in plasma.[12] If unavoidable, samples for LA testing should be drawn just before the next dose of danaparoid to minimise any chance of interference.

Danaparoid is expected to impact the parameters of TGA, in a dose-dependent manner.[68,69]

Recommendations

- **We recommend against routine monitoring of danaparoid (1B).**
- **If measuring for one of the reasons listed in Table 2, an anti-FXa assay calibrated with a drug-specific standard should be used (1B).**
- **LMWH calibrators should not be used to report heparin equivalent units of danaparoid (1B).**

ORAL AND PARENTERAL DIRECT THROMBIN INHIBITORS

Dabigatran

Dabigatran levels can be measured using a suitably calibrated dilute thrombin time (dTT), ecarin clotting time (ECT), ecarin chromogenic assay (ECA) or chromogenic anti-FIIa (C-IIa) assay.[70] Routine monitoring of dabigatran is not required, as there are limited data correlating low or high levels on clinical outcomes, and expected dabigatran concentrations are described with considerable variation.[70–74] Projected steady-state trough concentrations are largely comparable for children and adults.[75]

Measuring dabigatran may be useful in patients with severe renal disease or with moderate or severe liver disease: a higher-than-expected level may suggest that an alternative anti-coagulant be used.[70,76] In patients requiring urgent surgery, being considered for thrombolysis following an acute ischaemic event, or with serious bleeding, an urgent dabigatran level may be required. A level >50 ng/mL may be used to justify the use of a reversal agent in patients with serious bleeding, and a level of <30 ng/mL is considered safe for those requiring surgery or thrombolysis.[77,78] If a quantitative assay cannot be obtained quickly, screening tests can be used.[70]

The sensitivity of the PT to dabigatran varies by the reagent used, with a PT ratio ranging from 1.31 to 1.88 at a concentration of 200 ng/mL.[70] The PT was normal (from a variety of manufacturers) in 29% of patients on 150 mg bd dabigatran in samples collected 2 to 3 h after dosing.[79]

The APTT rises rapidly in the presence of dabigatran, but the relationship is not linear in adults or children[80,81]; the APTT is relatively insensitive to changes in dabigatran concentration within the expected range and plateaus at higher levels.[81,82]

In the REVERSE-AD trial, 88/461 (19%) of patients with detectable dabigatran by ECT had a normal APTT, but all had a raised TT. The TT is exquisitely sensitive to dabigatran, up to 15× normal within the expected range[81,83,84] and even levels below 30 ng/mL usually lead to TT prolongation[70] (Table 3). After reversal with idarucizumab, TT and APTT may normalise, but rebound prolongation may be seen 12–24 h later.[84]

Recommendations

- **We recommend against routine monitoring of dabigatran (1B)**
- **If measuring is required, we suggest dTT, ECT, ECA or C-IIa calibrated for dabigatran (2B).**
- **If such an assay is not available, a TT can be performed to exclude the presence of dabigatran, even if the PT and APTT are normal (2B).**

Argatroban

Argatroban is a small-molecule direct thrombin inhibitor requiring no co-factor to bind reversibly to free and clot-bound thrombin.[85] The summary of product characteristics (SmPC) for argatroban[86] recommends a target of 1.5–3.0 times the baseline APTT, not exceeding 100 s. This was based on a clinical trial which compared argatroban with historical controls in HIT.[87] Interpretation of the APTT results in this trial is complicated by the fact that patients with prolonged APTTs at baseline could be included unless their baseline APTT was >2 times control. This predicts that an APTT within 1.5–3.0 times baseline could occur at subtherapeutic argatroban levels, which is a limitation of using the APTT for monitoring purposes. Different APTT reagents and different analysers also show varying sensitivity to argatroban,[88] and 100 s equates to an APTT ratio of <3.0 if the baseline APTT is >33 s regardless of the reagent used.[89] The APTT plateaus at high concentrations of argatroban and is influenced by high factor VIII and lupus anti-coagulants, leading many publications to highlight the unsuitability of using the PT or APTT for monitoring.[88–95] Overall, evidence to support use of an APTT with a target of 1.5–3.0x baseline to monitor argatroban is weak.

Daily measurement of argatroban levels using a suitably calibrated dTT, ECT, ECA or C-IIa assay overcomes many of the limitations of the APTT. However, there is still uncertainty over the therapeutic range, since most studies use the APTT as a guide.[89,96,97] Some outcome-based evidence to support use of argatroban concentration over APTT for monitoring came from a single-centre retrospective study of 143 patients which compared C-IIa assay (target 0.4–1.2 μg/mL, $n=75$) with APTT (target 50–80 s, $n=68$).[98] The study showed a reduction in argatroban requirement by approximately 67% when concentration was used rather than an APTT, without an increase in thrombosis and no difference in the incidence of bleeding.

Argatroban prolongs the PT, and this needs to be accounted for when switching anti-coagulation to warfarin (Table 3). A higher target international normalised ratio (INR) is described in the SmPC, which suggests that argatroban can be stopped when the INR reaches 4.0 on combined therapy; this assumes that the INR is a result of the combined anti-coagulants, so the INR should be repeated 4–6 h after argatroban is ceased (argatroban half-life being 45 min) to ensure that the INR is within the therapeutic target for warfarin.[86] ACP may be of use in these situations, although reversal of argatroban may not be complete in all patients.[99,100] Further evaluation is required before this approach can be recommended.

Recommendations

- **When monitoring, we suggest dTT, ECT, ECA or C-IIa calibrated for argatroban, daily (2B).**
- **If monitoring argatroban in the absence of an anti-FIIa assay, an APTT ratio (patient/mean normal APTT) of 1.5–3.0, 2 h after initiation is suggested (2B).**

Bivalirudin

Bivalirudin is a bivalent hirudin analogue binding simultaneously to the thrombin active catalytic site and its fibrinogen biding site with high affinity.[101] The SmPC for bivalirudin states that the ACT should be used to confirm a response to bivalirudin, but no further laboratory monitoring is required.[102]

Bivalirudin prolongs the PT, and this needs to be accounted for when switching anti-coagulation to warfarin[103] (Table 3). No target INR is described in the SmPC, but INR monitoring should be considered once treatment with bivalirudin has ceased bearing in mind the half-life of approximately 25 min with normal renal function.

There is no clinical indication for the measurement of bivalirudin, but a suitably calibrated dTT, ECT, ECA or C-IIa assay could be used.

Recommendations

- **We recommend against routine monitoring of bivalirudin (1B)**
- **If measuring is required, we suggest dTT, ECT, ECA or C-IIa calibrated for bivalirudin (2B).**

Effects of dabigatran, argatroban and bivalirudin on measuring fibrinogen

In patients on thrombin inhibitors, some Clauss fibrinogen assays show marked underestimation if reagents contain low levels of thrombin (e.g. Werfen Fib-C) or where plasma dilution is low (Siemens Multifibren U).[104,105] These underestimations may cause a pause in anti-coagulation or fibrinogen replacement to be considered, therefore laboratories should consider their choice of reagents for Clauss fibrinogen and/or their reporting of Clauss fibrinogen results in patients on oral or parenteral thrombin inhibitors. Derived fibrinogen assays should not be considered as an alternative.[19]

Recommendation

- **We recommend using a Clauss fibrinogen assay with a high thrombin concentration reagent (e.g. 100 iu/mL) in the presence of thrombin inhibitors (1B).**

DIRECT FACTOR Xa INHIBITORS

Since the last guideline in 2014, edoxaban has been added to rivaroxaban and apixaban as a licensed direct FXaI in the UK. In a similar timeframe, betrixaban was developed but was not granted a licence in the UK, and has been subsequently discontinued.[106] Given the wider therapeutic index of the FXaI, monitoring of their effects and dose titration is not routinely advised. However, due to uncertainty surrounding certain patient cohorts not represented in the trials, previous BSH guidelines made recommendations for testing in specific settings.

As part of the ENGAGE atrial fibrillation (AF)-TIMI trial comparing edoxaban to warfarin in AF patients, trough plasma concentration was measured 1 month into the trial in a subset of patients and the outcomes followed. The work conducted was based on probability analyses and suggested a relationship between trough edoxaban concentration and stroke/systemic embolism and bleeding events.[107]

A pharmacokinetic analysis of 2392 patients enrolled in the Averroes study measured trough apixaban concentration after 3 months of treatment. The mean trough concentration for those patients receiving 2.5 mg bd was reportedly 99 ng/mL (IQR 60–146 ng/mL), while the 5 mg bd group was 125 ng/mL (IQR 64–202 ng/mL). The trial was not sufficiently powered to detect an association between apixaban concentration and outcomes due to low event rates. However, post hoc analysis suggests that patients in the lowest decile of levels had a significantly greater risk of stroke than those with higher levels.[108] An association between bleeding and apixaban level was also reported.

The availability of the anti-FXa assay is useful in some clinical situations (Table 2). There are limited real-world studies evaluating FIIaI and FXaI concentrations and outcomes, and these do not provide definitive evidence for the role of monitoring. The START laboratory registry comprised data on 565 consecutive patients with AF. Analysis of this showed a link between lower FIIaI and FXaI trough concentrations and thromboembolic events. Only 10 patients (1.8%) had a thromboembolic event, and these were associated with high CHA_2DS_2-VASc scores.[109] A study in Japan recruited consecutive patients with acute ischaemic stroke or transient ischaemic attack (TIA), started on rivaroxaban or apixaban for AF between 2012 and 2017.[110] Peak and trough levels were measured after 48 h of treatment and patients were followed for a median of 360 days. Patients with bleeding events on rivaroxaban (13/156) had higher anti-FXa peak levels than those without, although levels were still within the expected range. Those with bleeding on apixaban (11/156) had higher trough and peak levels than those without bleeding. In another study of 212 patients on FIIaI and FXaI, levels were measured in the 83% with bleeding or thrombosis. Of these, 72% had concentrations in the expected range. Higher concentrations were seen in older patients, those with impaired renal function or lower body mass index.[111] The study concluded that although there was no clear benefit from FIIaI and FXaI measurements, this was useful in certain circumstances. The International Council for Standardisation in Haematology (ICSH) published guidance on the laboratory assessment of FIIaI and FXaI which included a table of expected peak and trough concentrations in AF and VTE patients,[70] reproduced in Table 4.

TABLE 4 Expected on-therapy ranges for dabigatran, rivaroxaban, apixaban and edoxaban (reproduced with permission).[70]

	Dabigatran		Rivaroxaban		Apixaban		Edoxaban	
Indication	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE
Dose	150 mg bd	150 mg bd	20 mg od	20 mg od	5 mg bd	5 mg bd	60 mg od	60 mg od
Expected peak (ng/mL)	175[a] (117–275)	175[a] (117–275)	249[b] (184–343)	270[b] (189–419)	171[c] (91–321)	132[c] (59–302)	170[d] (125–245)	234[e] (149–317)
Expected trough (ng/mL)	91[a] (61–143)	60[a] (39–95)	44[b] (12–137)	26[b] (6–87)	103[c] (41–230)	63[c] (22–177)	36[e] (19–62)	19[e] (10–39)

Note: Other approved indications for DOACs include secondary prevention of PE/VTE, and post-hip and -knee replacement, which may have alternative dosing strategies. Additionally, changes in doses may occur after initiation phase of DOAC treatment. Consultation of regional DOAC labelling information is required before interpreting or using these peak and trough DOAC concentration data.

Abbreviations: bd, twice daily; IQR, interquartile range; NVAF, non-valvular atrial fibrillation; od, once daily; PE, pulmonary embolism; VTE, venous thromboembolism.

[a]Mean (25th–75th percentile).

[b]Mean (5th–95th percentile).

[c]Median (5th–95th percentile).

[d]Median (1.5×IQR).

[e]Median (IQR).

IMPACT ON ROUTINE COAGULATION ASSAYS

Variable prolongation of the PT and APTT can be seen with the different FXaI, thought to be associated with the composition of the reagents (activators and phospholipids)

and analyser combination (Table 3). In general, FXaI have a greater impact on PT-based assays than on APTT assays; some PT reagents are insensitive to apixaban even at levels above the expected range. In the UK, many routine laboratories have similar reagent sources and although the relationship between PT and FXaI activity has been demonstrated as being mainly linear, monitoring using the PT and APTT is not recommended as values can still be within normal reference ranges even when FXaI can be detected by other techniques.[112–114]

ANTI-FXa FOR MEASURING FXaI

When FXaI anti-FXa activity is measured, it should be calibrated using a chromogenic method with drug-specific calibrators. The lower limit of quantitation (LLoQ) for measurement of FXaI by anti-FXa assay varies by reagent, analyser and drug, and should be verified locally. These standard assays are adequate for covering therapeutic fixed-dose ranges for all the FXaI and can be used as a guide when considering patient eligibility for andexanet reversal if the LLoQ is below 50 ng/mL. A lower LLoQ can be achieved if low-range protocols and low-range calibrator/control sets are used.[115]

Some reports have suggested caution when transitioning between FXaI and other anti-coagulants. For a period, there may be a cumulative effect. Under these circumstances, it has been recommended to test more frequently,[116] although interpretation should be cautious, especially if transitioning between drugs with anti-Xa activity, as no assays can be used to distinguish between them.

LABORATORY ASSESSMENT OF DIRECT FXa INHIBITOR REVERSAL AGENTS

Four-factor prothrombin complex concentrate has been the main agent in the UK for the reversal of major bleeding associated with FXaI despite not being licensed for this purpose. Laboratory manifestations of its use are likely to be correction of PT, APTT and global haemostasis assays (associated with its composition of factors II, VII, IX and X), although FXaI anti-FXa activity may not be affected.

The human recombinant FXa agent, andexanet alfa, is an option in the UK for reversal of apixaban and rivaroxaban (but not edoxaban) in life-threatening and uncontrolled gastro-intestinal bleeding.[117] The Department of Health and Social Care published a Prevention of Future Deaths report in 2020 regarding the lack of an effective antidote for the reversal of edoxaban when bleeding occurs.[118] Reports have shown that the FXaI-calibrated anti-FXa assays can be used as part of the screening process to identify eligible patients. However, FXaI activity can persist below the LLoQ of these assays, and results may not be available rapidly enough in an emergency. Furthermore, dissociated antidote may allow for FXaI anti-FXa activity to be overestimated, possibly leading to perceived failure of the reversal process.[119,120] Alternative small-molecule antidotes are in development, some of which are based on modified molecules to compete in a similar manner to andexanet. Others have a wider anti-FIIa and/or anti-FXa effect providing a global antidote.[121–124]

OTHER ASSAYS FOR MEASURING FXaI

Thrombin generation

TGA have been widely reported for use in measuring the impact of FXaI. A recent review identified 129 full-text articles on the use of TGA in the monitoring of DOACs in several clinical scenarios.[125] Overall, FXaI concentrations correlated with both pharmacokinetic and quantitative parameters although some studies could not demonstrate statistical significance between FXaI levels and ETP.[126] TGA have been reported in several cases to be normalised completely in the presence of the specific reversal agent andexanet. During its use, several parameters including ETP have been shown to rebound to above-normal levels raising concerns about a prothrombotic effect caused by binding to TFPI.[127]

Overall ETP appeared to be the single most studied parameter for measuring the effect of DOACs and potentially its reversal, but it is less clear whether normalisation of a single parameter is predictive of bleeding risk in all cases.[125,128]

Liquid chromatography–Tandem mass spectrometry

Liquid chromatography coupled with tandem mass spectrometry is an analytical chemistry technique suited to separation of complex mixtures of compounds and their metabolites. It is possible to separate out multiple anticoagulants in the same assay run (within minutes) across a wide range of concentrations. It is seen as the gold standard for quantifying FXaI anti-FXa activity, but its limited availability in NHS laboratories makes it unsuitable for routine use.[8]

Viscoelastic haemostatic assays

VHA have been reported to successfully measure both FIIaI and FXaI activity with specific FIIa- and FXa-based channels.[129] Currently, there are limited convincing data on the impact of FXaI on VHA, although newer devices and dedicated cartridges are under review.[130,131]

Point-of-care assays

The use of point-of-care INR devices to exclude the presence of rivaroxaban and edoxaban (<30 ng/mL) has been

described using the Hemochron® Signature PT/INR cartridge and the Roche Coaguchek® INR test strip.[132–134] These assays show similar limitations in specificity as laboratory PT-based assays to FXaI and should be interpreted accordingly. DOAC levels can also be measured using a urine dipstick test, appearing approximately 1 h after plasma concentrations, showing good comparability to FXaI anti-FXa assays.[135] This provides another option for excluding or differentiating between dabigatran and FXaI in an emergency. Reports have suggested that the test can confirm levels of >30 ng/mL and can be used with a reader to prevent urine colour interference.[136,137]

Recommendations

- **We recommend against routine monitoring of FXaI (1B).**
- **PT- and APTT-based assays should not be used to measure FXaI levels (1B).**
- **When measuring rivaroxaban, apixaban or edoxaban, anti-FXa assays with separate traceable calibrators and controls should be used for each drug (1B).**
- **Anti-FXa assays using LMWH calibrators should not be used to report heparin equivalent units of FXaI (1B).**
- **Anti-FXa assays should not be used to assess the effectiveness of DOAC reversal agents (1B).**

IMPACT OF FXaI ON OTHER HAEMOSTATIC INVESTIGATIONS

FXaI can have an impact on specific factor assays whether using one-stage PT, one-stage APTT or chromogenic assays to varying extents. This can be ameliorated by performing assays at higher dilutions if non-parallelism is seen.[138,139] Thrombophilia testing is also affected (Table 3) with prolonged clot-based protein C (PC) and protein S assays potentially overestimating levels and artefactually indicating activated PC resistance. Prolongation of the DRVVT assays used for lupus anti-coagulant testing is likely even with low levels of FXaI. Anti-thrombin will be overestimated in assays based on FXa-inhibition.[140] Thrombin-induced platelet aggregation studies are also affected in the presence of all FXaI.[141,142] However, fibrinolysis appears more complex with conflicting reports as to the impact of FXaI (compared to the confirmed impact of FIIaI).[143,144]

REDUCING ADVERSE EVENTS IN PATIENTS ON ANTI-COAGULATION

Laboratory clinicians and scientists should have a clear understanding of the effects of anti-coagulants on their haemostasis tests so that they may properly advise clinicians who request tests and adjust doses of anti-coagulants. This will improve patient care and avoid unnecessary tests or interventions. For instance, normal coagulation tests in a patient taking a DOAC should not be interpreted as indicating a lack of anti-coagulant effect and prolongations may not need further investigation if correctly attributed. Adverse events include interactions with other drugs. For example, inhibitors of P-glycoprotein or the cytochrome P450 pathway may increase the concentration of DOACs, while inducers can have the opposite effect.

Some of the anti-coagulants discussed have relatively narrow therapeutic indices. As they are often used in acutely unwell patients, the potential for harm through under- or overdosing is high. Critical laboratory results are those that are life-threatening and require immediate action. The ICSH includes tests used for monitoring anti-coagulation among these.[145] Most adverse events associated with anti-coagulation are potentially preventable medication errors.[146] Inadequate monitoring or failure to act on a laboratory result is frequent cause of errors, including fatality, when using UFH. Complex dosing protocols and difficulties in interpreting results have been identified as causes of errors in investigations by NHS patient safety organisations (direct communication to BSH). When monitoring an anti-coagulant, it is essential that the correct anti-coagulant is identified in the request to the laboratory. This enables the correct standard to be used and the result reported in a way that makes it clear that it is specific to that drug. Unfortunately, requests received in the laboratory sometimes do not identify the drug correctly or give any indication that the patient is on an anti-coagulant. Electronic requesting systems reduce the chance of this occurring by making specification of the anti-coagulant a compulsory field. Laboratories vary in how over-anticoagulated samples are reported, causing difficulties for frontline healthcare staff moving between NHS organisations. As this is a consequence of varying reagent sensitivity, it is most effectively mitigated by improving local training of clinical and laboratory staff.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of this guideline.

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The BSH paid the expenses incurred during the writing of this guidance.

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REVIEW PROCESS

Members of the writing group will inform the writing group Chair if any new evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be reviewed regularly by the relevant Task Force and the literature search will be re-run every 3 years to search systematically for any new evidence that may have been missed. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made, an addendum will be published on the BSH guidelines website (www.b-s-h.org.uk/guidelines).

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Obtained for Table 4.

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REFERENCES

1. Kitchen S, Gray E, Mackie I, Baglin T, Makris M, Committee, B. Measurement of non-coumarin anticoagulants and their effects on tests of Haemostasis: guidance from the British Committee for Standards in Haematology. Br J Haematol. 2014;166:830–41.
2. Tantry US, Duhan S, Navarese E, Ramotowski B, Kundan P, Bliden KP, et al. An update on novel therapies for treating patients with arterial thrombosis. Expert Rev Hematol. 2023;16:593–605.
3. Barnes GD, Ageno W, Castellucci LA, Chiasakul T, Eslick R, Ferreiro JL, et al. Recommendation on the nomenclature for anticoagulants: updated communication from the International Society on Thrombosis and Haemostasis Scientific and Standardization Commitee on the Control of Anticoagulation. J Thromb Haemost. 2023;21:1381–4.
4. Exner T, Michalopoulos N, Pearce J, Xavier R, Ahuja M. Simple method for removing DOACs from plasma samples. Thromb Res. 2018;163:117–22.
5. Frans G, Meeus P, Bailleul E. Resolving DOAC interference on aptt, Pt, and lupus anticoagulant testing by the use of activated carbon. J Thromb Haemost. 2019;17:1354–62.
6. Sevenet PO, Cucini V, Herve T, Depasse F, Carlo A, Contant G, et al. Evaluation of DOAC filter, a new device to remove direct oral anticoagulants from plasma samples. Int J Lab Hematol. 2020;42:636–42.
7. Exner T, Ahuja M, Ellwood L. Effect of an activated charcoal product (DOAC stop) intended for extracting DOACs on various other APTT-prolonging anticoagulants. Clin Chem Lab Med. 2019;57:690–6.
8. Slavik L, Jacova J, Friedecky D, Ulehlova J, Tauber Z, Prochazkova J, et al. Evaluation of the DOAC-stop procedure by LC–MS/MS assays for determining the residual activity of dabigatran, rivaroxaban, and Apixaban. Clin Appl Thromb Hemost. 2019;25:1–6.
9. Cox-Morton S, Macdonald S, Thomas W. A diagnostic solution for haemostasis laboratories for patients taking direct oral anticoagulants using DOAC-remove. Br J Haematol. 2019;187:377–85.
10. Kopatz WF, Brinkman HJM, Meijers JCM. Use of DOAC stop for elimination of anticoagulants in the thrombin generation assay. Thromb Res. 2018;170:97–101.
11. Platton S, Hunt C. Influence of DOAC stop on coagulation assays in samples from patients on rivaroxaban or apixaban. Int J Lab Hematol. 2019;41:227–33.
12. de Kesel PMM, Devreese KMJ. The effect of unfractionated heparin, enoxaparin, and danaparoid on lupus anticoagulant testing: can activated carbon eliminate false-positive results? Res Pract Thromb Haemost. 2020;4:161–8.
13. Hirsh J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. Chest. 2001;119:64S–94S.
14. Young E, Cosmi B, Weitz J, Hirsh J. Comparison of the non-specific binding of unfractionated heparin and low molecular weight heparin (enoxaparin) to plasma proteins. Thromb Haemost. 1993;70:625–30.
15. Brodin E, Appelbom H, Osterud B, Hilden I, Petersen LC, Hansen JB. Regulation of thrombin generation by TFPI in plasma without and with heparin. Transl Res. 2009;153:124–31.
16. Ray MJ. An artefact related to the ratio of sample volume to the blood collection vial size which effects the APTTs of specimens taken to monitor heparin therapy. Thromb Haemost. 1991;66:387–8.
17. Ray MJ, Carroll PA, Just SJ, Hawson GA. A low volume specimen container suitable for monitoring the aPTT of heparinized patients. Blood Coagul Fibrinolysis. 1993;4:805–7.
18. Adcock D, Kressin D, Marlar RA. The effect of time and temperature variables on routine coagulation tests. Blood Coagul Fibrinolysis. 1998;9:463–70.
19. Baker P, Platton S, Gibson C, Gray E, Jennings I, Murphy P, et al. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Br J Haematol. 2020;191:347–62.
20. Toulon P, Appert-Flory A, Fischer F, Buvat S, Jambou D, Mahagne MH. Monitoring unfractionated heparin therapy. 4 hour-stability of anti-Xa activity in unspun citrated tubes. Thromb Res. 2020;186:7–12.
21. Gremillet M, Talon L, Lebreton A, Sinegre T. Monitoring heparin therapy: stability of two different anti-Xa assays using blood samples collected in citrate-containing and CTAD tubes. Thromb J. 2023;21:21.
22. Contant G, Gouault-Heilmann M, Martinoli JL. Heparin inactivation during blood storage: its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole-C.T.A.D. mixture. Thromb Res. 1983;31:365–74.
23. van den Besselaar AM, Meeuwisse-Braun J, Jansen-Gruter R, Bertina RM. Monitoring heparin therapy by the activated partial thromboplastin time—the effect of pre-analytical conditions. Thromb Haemost. 1987;57:226–31.
24. Arachchillage DRJ, Kamani F, Deplano S, Banya W, Laffan M. Should we abandon the APTT for monitoring unfractionated heparin? Thromb Res. 2017;157:157–61.
25. White D, Macdonald S, Bull T, Hayman M, de Monteverde-Robb R, Sapsford D, et al. Heparin resistance in COVID-19 patients in the intensive care unit. J Thromb Thrombolysis. 2020;50:287–91.
26. Kamphuisen PW, Eikenboom JC, Vos HL, Pablo R, Sturk A, Bertina RM, et al. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. Thromb Haemost. 1999;81:680–3.
27. Levine MN, Hirsh J, Gent M, Turpie AG, Cruickshank M, Weitz J, et al. A randomized trial comparing activated thromboplastin time with heparin assay in patients with acute venous

thromboembolism requiring large daily doses of heparin. Arch Intern Med. 1994;154:49–56.
28. Basu D, Gallus A, Hirsh J, Cade J. A prospective study of the value of monitoring heparin treatment with the activated partial thromboplastin time. N Engl J Med. 1972;287:324–7.
29. Brill-Edwards P, Ginsberg JS, Johnston M, Hirsh J. Establishing a therapeutic range for heparin therapy. Ann Intern Med. 1993;119:104–9.
30. Kitchen S, Preston FE. The therapeutic range for heparin therapy: relationship between six activated partial thromboplastin time reagents and two heparin assays. Thromb Haemost. 1996;75:734–9.
31. D'angelo A, Seveso MP, D'angelo SV, Gilardoni F, Dettori AG, Bonini P. Effect of clot-detection methods and reagents on activated partial thromboplastin time (APTT). Implications in heparin monitoring by APTT. Am J Clin Pathol. 1990;94:297–306.
32. Hirsh J, Bauer KA, Donati MB, Gould M, Samama MM, Weitz JI. Parenteral anticoagulants: American College of Chest Physicians evidence-based clinical practice guidelines (8th edition). Chest. 2008;133:141S–59S.
33. Cuker A, Ptashkin B, Konkle BA, Pipe SW, Whinna HC, Zheng XL, et al. Interlaboratory agreement in the monitoring of unfractionated heparin using the anti-factor Xa-correlated activated partial thromboplastin time. J Thromb Haemost. 2009;7:80–6.
34. Smythe MA, Priziola J, Dobesh PP, Wirth D, Cuker A, Wittkowsky AK. Guidance for the practical management of the heparin anticoagulants in the treatment of venous thromboembolism. J Thromb Thrombolysis. 2016;41:165–86.
35. Amiral J, Amiral C, Dunois C. Optimization of heparin monitoring with anti-FXa assays and the impact of dextran sulfate for measuring all drug activity. Biomedicine. 2021;9:700–16.
36. Hollestelle MJ, van der Meer FJM, Meijer P. Quality performance for indirect Xa inhibitor monitoring in patients using international external quality data. Clin Chem Lab Med. 2020;58:1921–30.
37. Mouton C, Calderon J, Janvier G, Vergnes MC. Dextran sulfate included in factor Xa assay reagent overestimates heparin activity in patients after heparin reversal by protamine. Thromb Res. 2003;111:273–9.
38. Hardy M, Cabo J, Deliege A, Douxfils J, Gouin-Thibault I, Lecompte T, et al. Reassessment of dextran sulfate in anti-Xa assay for unfractionated heparin laboratory monitoring. Res Pract Thromb Haemost. 2023;7:102257.
39. Guervil DJ, Rosenberg AF, Winterstein AG, Harris NS, Johns TE, Zumberg MS. Activated partial thromboplastin time versus antifactor Xa heparin assay in monitoring unfractionated heparin by continuous intravenous infusion. Ann Pharmacother. 2011;45:861–8.
40. Whitman-Purves E, Coons JC, Miller T, Dinella JV, Althouse A, Schmidhofer M, et al. Performance of anti-factor Xa versus activated partial thromboplastin time for heparin monitoring using multiple nomograms. Clin Appl Thromb Hemost. 2018;24:310–6.
41. Belk KW, Laposata M, Craver C. A comparison of red blood cell transfusion utilization between anti-activated factor X and activated partial thromboplastin monitoring in patients receiving unfractionated heparin. J Thromb Haemost. 2016;14:2148–57.
42. Swayngim R, Preslaski C, Burlew CC, Beyer J. Comparison of clinical outcomes using activated partial thromboplastin time versus antifactor-Xa for monitoring therapeutic unfractionated heparin: a systematic review and meta-analysis. Thromb Res. 2021;208:18–25.
43. Zhu E, Yuriditsky E, Raco V, Katz A, Papadopoulos J, Horowitz J, et al. Anti-factor Xa as the preferred assay to monitor heparin for the treatment of pulmonary embolism. Int J Lab Hematol. 2024;46:354–61.
44. Andrassy K, Eschenfelder V. Are the pharmacokinetic parameters of low molecular weight heparins predictive of their clinical efficacy? Thromb Res. 1996;81:S29–S38.
45. Kjaergaard AB, Fuglsang J, Hvas AM. Anti-Xa monitoring of low-molecular-weight heparin during pregnancy: a systematic review. Semin Thromb Hemost. 2021;47:824–42.
46. Lester W, Walker N, Bhatia K, Ciantar E, Banerjee A, Trinder J, et al. British Society for Haematology guideline for anticoagulant management of pregnant individuals with mechanical heart valves. Br J Haematol. 2023;202:465–78.
47. Chalmers E, Ganesen V, Liesner R, Maroo S, Nokes T, Saunders D, et al. Guideline on the investigation, management and prevention of venous thrombosis in children. Br J Haematol. 2011;154:196–207.
48. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, et al. Antithrombotic therapy in neonates and children: antithrombotic therapy and prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141:e737S–801S.
49. Bara L, Samama M. Pharmacokinetics of low molecular weight heparins. Acta Chir Scand Suppl. 1988;543:65–72.
50. Harenberg J. Pharmacology of low molecular weight heparins. Semin Thromb Hemost. 1990;16(Suppl):12–8.
51. Wu T, Xia X, Chen W, Fu J, Zhang J. The effect of anti-Xa monitoring on the safety and efficacy of low-molecular-weight heparin anticoagulation therapy: a systematic review and meta-analysis. J Clin Pharm Ther. 2020;45:602–8.
52. Nutescu EA, Spinler SA, Wittkowsky A, Dager WE. Low-molecular-weight heparins in renal impairment and obesity: available evidence and clinical practice recommendations across medical and surgical settings. Ann Pharmacother. 2009;43:1064–83.
53. Jennings I, Kitchen D, Kitchen S, Woods T, Walker I. The importance of commutability in material used for quality control purposes. Int J Lab Hematol. 2019;41:39–45.
54. Ip BK, Thomson AR, Moriarty HT. A comparison of the sensitivity of APTT reagents to the effects of enoxaparin, a low-molecular weight heparin. Pathology. 2001;33:347–52.
55. Arachchillage DJ, Platton S, Hickey K, Chu J, Pickering M, Sommerville P, et al. Guidelines on the investigation and management of antiphospholipid syndrome. Br J Haematol. 2024:1–26.
56. Arachchillage DRJ, Gomez K, Alikhan R, Anderson JAM, Lester W, Laffan M, et al. Addendum to British Society for Haematology guidelines on investigation and Management of Antiphospholipid syndrome, 2012 (Br. J. Haematol. 2012; 157: 47–58): use of direct acting oral anticoagulants. Br J Haematol. 2020;189:212–5.
57. Gerotziafas GT, Petropoulou AD, Verdy E, Samama MM, Elalamy I. Effect of the anti-factor Xa and anti-factor IIa activities of low-molecular-weight heparins upon the phases of thrombin generation. J Thromb Haemost. 2007;5:955–62.
58. Thomas O, Lybeck E, Strandberg K, Tynngard N, Schott U. Monitoring low molecular weight heparins at therapeutic levels: dose-responses of, and correlations and differences between aptt, anti-factor Xa and thrombin generation assays. PLoS One. 2015;10:e0116835.
59. Pavoni V, Gianesello L, Conti D, Ballo P, Dattolo P, Prisco D, et al. "In less than no time": feasibility of rotational Thromboelastometry to detect anticoagulant drugs activity and to guide reversal therapy. J Clin Med. 2022;11:1407.
60. Depasse F, Gilbert M, Goret V, Rolland N, Samama MM. Anti-Xa monitoring: inter-assay variability. Thromb Haemost. 2000;84:1122–3.
61. Kitchen S, Iampietro R, Woolley AM, Preston FE. Anti Xa monitoring during treatment with low molecular weight heparin or danaparoid: inter-assay variability. Thromb Haemost. 1999;82:1289–93.
62. Smogorzewska A, Brandt JT, Chandler WL, Cunningham MT, Hayes TE, Olson JD, et al. Effect of fondaparinux on coagulation assays: results of College of American Pathologists proficiency testing. Arch Pathol Lab Med. 2006;130:1605–11.
63. Donat F, Duret JP, Santoni A, Cariou R, Necciari J, Magnani H, et al. The pharmacokinetics of fondaparinux sodium in healthy volunteers. Clin Pharmacokinet. 2002;41(Suppl 2):1–9.
64. Gerotziafas GT, Depasse F, Chakroun T, van Dreden P, Samama MM, Elalamy I. Comparison of the effect of fondaparinux and enoxaparin on thrombin generation during in-vitro clotting of whole blood and platelet-rich plasma. Blood Coagul Fibrinolysis. 2004;15:149–56.

65. Danhof M, de Boer A, Magnani HN, Stiekema JC. Pharmacokinetic considerations on Orgaran (org 10172) therapy. Haemostasis. 1992;22:73–84.
66. Stiekema JC, Wijnand HP, van Dinther TG, Moelker HC, Dawes J, Vinchenzo A, et al. Safety and pharmacokinetics of the low molecular weight heparinoid org 10172 administered to healthy elderly volunteers. Br J Clin Pharmacol. 1989;27:39–48.
67. Wilde MI, Markham A. Danaparoid: a review of its pharmacology and clinical use in the management of heparin-induced thrombocytopenia. Drugs. 1997;54:903–24.
68. Coppell JA, Thalheimer U, Zambruni A, Triantos CK, Riddell AF, Burroughs AK, et al. The effects of unfractionated heparin, low molecular weight heparin and danaparoid on the thromboelastogram (TEG): an in-vitro comparison of standard and heparinase-modified TEGs with conventional coagulation assays. Blood Coagul Fibrinolysis. 2006;17:97–104.
69. Tardy-Poncet B, Combe M, Piot M, Chapelle C, Akrour M, Tardy B. Effects of argatroban, danaparoid, and fondaparinux on trombin generation in heparin-induced thrombocytopenia. Thromb Haemost. 2013;109:504–9.
70. Gosselin RC, Adcock DM, Bates SM, Douxfils J, Favaloro EJ, Gouin-Thibault I, et al. International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost. 2018;118:437–50.
71. Ng JW, Mohd Tahir NA, Chin PKL, Makmor-Bakry M, Mohd Saffian S. A systematic review and meta-analysis of dabigatran peak and trough concentration in adults. Br J Clin Pharmacol. 2022;88:4443–59.
72. NICE. Dabigatran etexilate. 2008 [Online]. https://bnf.nice.org.uk/drugs/dabigatran-etexilate/. Accessed 15 July 2024
73. Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, et al. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY trial (randomized evaluation of long-term anticoagulation therapy). J Am Coll Cardiol. 2014;63:321–8.
74. Steffel J, Verhamme P, Potpara TS, Albaladejo P, Antz M, Desteghe L, et al. The 2018 European heart rhythm association practical guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation: executive summary. Europace. 2018;20:1231–42.
75. Halton JML, Albisetti M, Biss B, Bomgaars L, Brueckmann M, Gropper S, et al. Phase IIa study of dabigatran etexilate in children with venous thrombosis: pharmacokinetics, safety, and tolerability. J Thromb Haemost. 2017a;15:2147–57.
76. Akpan IJ, Cuker A. Laboratory assessment of the direct oral anticoagulants: who can benefit? Kardiol Pol. 2021;79:622–30.
77. Levy JH, Ageno W, Chan NC, Crowther M, Verhamme P, Weitz JI, et al. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. J Thromb Haemost. 2016;14:623–7.
78. Seiffge DJ, Meinel T, Purrucker JC, Kaesmacher J, Fischer U, Wilson D, et al. Recanalisation therapies for acute ischaemic stroke in patients on direct oral anticoagulants. J Neurol Neurosurg Psychiatry. 2021;92:534–41.
79. Hawes EM, Deal AM, Funk-Adcock D, Gosselin R, Jeanneret C, Cook AM, et al. Performance of coagulation tests in patients on therapeutic doses of dabigatran: a cross-sectional pharmacodynamic study based on peak and trough plasma levels. J Thromb Haemost. 2013;11:1493–502.
80. Halton JML, Picard AC, Harper R, Huang F, Brueckmann M, Gropper S, et al. Pharmacokinetics, pharmacodynamics, safety and tolerability of dabigatran etexilate oral liquid formulation in infants with venous thromboembolism. Thromb Haemost. 2017b;117:2168–75.
81. van Ryn J, Stangier J, Haertter S, Liesenfeld KH, Wienen W, Feuring M, et al. Dabigatran etexilate—a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. Thromb Haemost. 2010;103:1116–27.
82. Henskens YMC, Gulpen AJW, van Oerle R, Wetzels R, Verhezen P, Spronk H, et al. Detecting clinically relevant rivaroxaban or dabigatran levels by routine coagulation tests or thromboelastography in a cohort of patients with atrial fibrillation. Thromb J. 2018;16:3.
83. Pollack CV Jr, Reilly PA, Bernstein R, Dubiel R, Eikelboom J, Glund S, et al. Design and rationale for RE-VERSE AD: a phase 3 study of idarucizumab, a specific reversal agent for dabigatran. Thromb Haemost. 2015;114:198–205.
84. Pollack CV Jr, Reilly PA, van Ryn J, Eikelboom JW, Glund S, Bernstein RA, et al. Idarucizumab for dabigatran reversal—full cohort analysis. N Engl J Med. 2017;377:431–41.
85. Walenga JM. An overview of the direct thrombin inhibitor argatroban. Pathophysiol Haemost Thromb. 2002;32(Suppl 3):9–14.
86. EMC. Summary of product characteristics (SmPC) for argatroban. 2012 [Online]. https://www.medicines.org.uk/emc/product/2012/smpc/print. Accessed 23 August 2023
87. Lewis BE, Wallis DE, Berkowitz SD, Matthai WH, Fareed J, Walenga JM, et al. Argatroban anticoagulant therapy in patients with heparin-induced thrombocytopenia. Circulation. 2001;103:1838–43.
88. Guy S, Kitchen S, Maclean R, van Veen JJ. Limitation of the activated partial thromboplastin time as a monitoring method of the direct thrombin inhibitor argatroban. Int J Lab Hematol. 2015;37:834–43.
89. Tardy-Poncet B, Nguyen P, Thiranos JC, Morange PE, Biron-Andreani C, Gruel Y, et al. Argatroban in the management of heparin-induced thrombocytopenia: a multicenter clinical trial. Crit Care. 2015;19:396.
90. Beiderlinden M, Werner P, Bahlmann A, Kemper J, Brezina T, Schafer M, et al. Monitoring of argatroban and lepirudin anticoagulation in critically ill patients by conventional laboratory parameters and rotational thromboelastometry—a prospectively controlled randomized double-blind clinical trial. BMC Anesthesiol. 2018;18:18–33.
91. Guy S, Kitchen S, Hopkins B, Chunara Z, Stephenson-Brown A, van Veen JJ. Laboratory methods for monitoring argatroban in heparin-induced thrombocytopenia. Int J Lab Hematol. 2022;44:399–406.
92. Guy S, Kitchen S, Makris MRMM, Saccullo G, Vanveen JJ. Caution in using the activated partial thromboplastin time to monitor Argatroban in COVID-19 and vaccine-induced immune thrombocytopenia and Thrombosis (VITT). Clin Appl Thromb Hemost. 2021;27:10760296211066945.
93. Kennedy DM, Alaniz C. Apparent argatroban resistance in a patient with elevated factor VIII levels. Ann Pharmacother. 2013;47:e29.
94. Poyant JO, Gleason AM. Early identification of argatroban resistance and the consideration of factor VIII. J Pharm Pract. 2021;34:329–31.
95. Seidel H, Kolde HJ. Monitoring of Argatroban and Lepirudin: what is the input of laboratory values in "real life"? Clin Appl Thromb Hemost. 2018;24:287–94.
96. Alberio L, Angelillo-Scherrer A, Asmis L, Casini A, Fontana P, Graf L, et al. Recommendations on the use of anticoagulants for the treatment of patients with heparin-induced thrombocytopenia in Switzerland. Swiss Med Wkly. 2020;150:w20210.
97. Gruel Y, Vayne C, Rollin J, Weber P, Faille D, Bauters A, et al. Comparative analysis of a French prospective series of 144 patients with heparin-induced thrombocytopenia (FRIGTIH) and the literature. Thromb Haemost. 2020;120:1096–107.
98. Vu N, Jaynes E, Chan C, Dorsch M, Pipe S, Alaniz C. Argatroban monitoring: aPTT versus chromogenic assay. Am J Hematol. 2016;91:E303–E304.
99. Al-Qawzai Z, Dale C, Dave M, Yartey N, Platton S. Effect of DOAC-remove on coagulation screening assays in samples from patients receiving oral or parenteral anticoagulation. Int J Lab Hematol. 2022;44:e95–e99.
100. Baker SA, Jin J, Pfaffroth C, Vu T, Zehnder JL. DOAC-stop in lupus anticoagulant testing: direct oral anticoagulant interference removed in most samples. Res Pract Thromb Haemost. 2021;5:314–25.

101. Warkentin TE. Bivalent direct thrombin inhibitors: hirudin and bivalirudin. Best Pract Res Clin Haematol. 2004;17:105–25.
102. EMA. Efient: EPAR—Product information. 2009 [Online]. https://www.ema.europa.eu/en/documents/product-information/efient-epar-product-information_en.pdf. Accessed 22 Aug 2023
103. Hohlfelder B, Deicicchi D, Sylvester KW, Connors JM. Development of a predictive nomogram for the change in PT/INR upon discontinuation of bivalirudin as a bridge to warfarin. Clin Appl Thromb Hemost. 2017;23:487–93.
104. Jennings I, Lester W, Gray E, Reilly-Stitt C, Gomez K, Williams S, et al. Effect of direct thrombin inhibitors on laboratory measurement of fibrinogen: potential for errors in clinical decision-making. Int J Lab Hematol. 2023;45:599–602.
105. Platton S, Hill C, Lester W, Yartey N, Maccallum P. Effect of argatroban on laboratory measurement of fibrinogen activity in ex vivo samples—potential for errors in clinical decision-making. Int J Lab Hematol. 2023;45:781–3.
106. NICE. Anticoagulation—oral. 2023 [Online]. https://cks.nice.org.uk/topics/anticoagulation-oral/. Accessed 26 Jul 2023
107. Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, et al. Association between edoxaban dose, concentration, anti-factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind engage AF-TIMI 48 trial. Lancet. 2015;385:2288–95.
108. Connolly SJ, Eikelboom J, Joyner C, Diener HC, Hart R, Golitsyn S, et al. Apixaban in patients with atrial fibrillation. N Engl J Med. 2011;364:806–17.
109. Testa S, Paoletti O, Legnani C, Dellanoce C, Antonucci E, Cosmi B, et al. Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. J Thromb Haemost. 2018;16:842–8.
110. Wada S, Toyoda K, Sato S, Matsuki T, Okata T, Kumamoto M, et al. Anti-Xa activity and event risk in patients with direct factor Xa inhibitors initiated early after stroke. Circ J. 2018;82:2872–9.
111. Rottenstreich A, Zacks N, Kleinstern G, Raccah BH, Roth B, Da'as N, et al. Direct-acting oral anticoagulant drug level monitoring in clinical patient management. J Thromb Thrombolysis. 2018;45:543–9.
112. Douxfils J, Chatelain B, Chatelain C, Dogne JM, Mullier F. Edoxaban: impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost. 2016;115:368–81.
113. Hillarp A, Baghaei F, Fagerberg Blixter I, Gustafsson KM, Stigendal L, Sten-Linder M, et al. Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. J Thromb Haemost. 2011;9:133–9.
114. Hillarp A, Gustafsson KM, Faxalv L, Strandberg K, Baghaei F, Fagerberg Blixter I, et al. Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. J Thromb Haemost. 2014;12:1545–53.
115. Mani H, Rohde G, Stratmann G, Hesse C, Herth N, Schwers S, et al. Accurate determination of rivaroxaban levels requires different calibrator sets but not addition of antithrombin. Thromb Haemost. 2012;108:191–8.
116. Smith AR, Dager WE, Gulseth MP. Transitioning hospitalized patients from rivaroxaban or apixaban to a continuous unfractionated heparin infusion: a retrospective review. Am J Health Syst Pharm. 2020;77:S59–S65.
117. NICE. Andexanet alfa for reversing anticoagulation from apixaban or rivaroxaban. 2021 [Online]. https://www.nice.org.uk/guidance/ta697. Accessed 1 Aug 2023
118. DOHSC. Prevention of future death reports [Online]. Courts and Tribunal Judiciary. 2020. https://www.judiciary.uk/prevention-of-future-death-reports/maureen-waterfall/. Accessed 04 Dec 2023
119. Bourdin M, Perrotin D, Mathieu O, Herve T, Depasse F, Lu G, et al. Measuring residual anti-Xa activity of direct factor Xa inhibitors after reversal with andexanet alfa. Int J Lab Hematol. 2021;43:795–801.
120. EMA. Ondexxya (andexanet alfa): commercial anti-FXa activity assays are unsuitable for measuring anti-FXa activity following administration of andexanet alfa. 2020 [Online]. https://www.ema.europa.eu/en/documents/dhpc/direct-healthcare-professional-communication-dhpc-ondexxya-andexanet-alfa-commercial-anti-fxa_en.pdf. Accessed 01 Aug 2023
121. Ansell JE, Bakhru SH, Laulicht BE, Steiner SS, Grosso M, Brown K, et al. Use of PER977 to reverse the anticoagulant effect of edoxaban. N Engl J Med. 2014;371:2141–2.
122. Parng C, Bolt M, Pittman DD, Caiazzo T, Dyleski L, Gorovits B, et al. Induction and impact of anti-drug responses elicited by a human recombinant coagulation factor FXa(I16L) in preclinical species. AAPS J. 2019;21:52.
123. Verhoef D, Visscher KM, Vosmeer CR, Cheung KL, Reitsma PH, Geerke DP, et al. Engineered factor Xa variants retain procoagulant activity independent of direct factor Xa inhibitors. Nat Commun. 2017;8:528.
124. von Drygalski A, Bhat V, Gale AJ, Averell PM, Cramer TJ, Elias DJ, et al. An engineered factor Va prevents bleeding induced by direct-acting oral anticoagulants by different mechanisms. Blood Adv. 2020;4:3716–27.
125. Shaw JR, Castellucci LA, Siegal D, Carrier M. DOAC-associated bleeding, hemostatic strategies, and thrombin generation assays—a review of the literature. J Thromb Haemost. 2023;21:433–52.
126. Metze M, Kloter T, Stobe S, Rechenberger B, Siegemund R, Siegemund T, et al. Plasma levels do not predict thrombin generation in patients taking direct oral anticoagulants. Int J Lab Hematol. 2021;43:1539–48.
127. Siddiqui F, Tafur A, Ramacciotti LS, Jeske W, Hoppensteadt D, Ramacciotti E, et al. Reversal of factor Xa inhibitors by andexanet alfa may increase thrombogenesis compared to pretreatment values. Clin Appl Thromb Hemost. 2019;25:1–7.
128. Connolly SJ, Crowther M, Eikelboom JW, Gibson CM, Curnutte JT, Lawrence JH, et al. Full study report of Andexanet Alfa for bleeding associated with factor Xa inhibitors. N Engl J Med. 2019;380:1326–35.
129. Sahli SD, Castellucci C, Roche TR, Rossler J, Spahn DR, Kaserer A. The impact of direct oral anticoagulants on viscoelastic testing—a systematic review. Front Cardiovasc Med. 2022;9:991675.
130. Artang R, Anderson M, Nielsen JD. Fully automated thromboelastograph TEG 6s to measure anticoagulant effects of direct oral anticoagulants in healthy male volunteers. Res Pract Thromb Haemost. 2019;3:391–6.
131. Dias JD, Lopez-Espina CG, Ippolito J, Hsiao LH, Zaman F, Muresan AA, et al. Rapid point-of-care detection and classification of direct-acting oral anticoagulants with the TEG 6s: implications for trauma and acute care surgery. J Trauma Acute Care Surg. 2019;87:364–70.
132. Ebner M, Birschmann I, Peter A, Spencer C, Hartig F, Kuhn J, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants. Crit Care. 2017;21:32.
133. Hartig F, Birschmann I, Peter A, Horber S, Ebner M, Sonnleitner M, et al. Point-of-care testing of coagulation in patients treated with edoxaban. J Thromb Thrombolysis. 2020;50:632–9.
134. Hartig F, Birschmann I, Peter A, Horber S, Ebner M, Sonnleitner M, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants including edoxaban. Neurol Res Pract. 2021;3:9.
135. Harenberg J, Beyer-Westendorf J, Crowther M, Douxfils J, Elalamy I, Verhamme P, et al. Accuracy of a rapid diagnostic test for the presence of direct oral factor Xa or thrombin inhibitors in urine-a multicenter trial. Thromb Haemost. 2020;120:132–40.
136. Harenberg J, Gosselin RC, Cuker A, Becattini C, Pabinger I, Poli S, et al. Algorithm for rapid exclusion of clinically relevant plasma levels of direct Oral anticoagulants in patients using the

DOAC dipstick: an expert consensus paper. Thromb Haemost. 2024;124:770–7.
137. Papageorgiou L, Hetjens S, Fareed J, Auge S, Tredler L, Harenberg J, et al. Comparison of the DOAC dipstick test on urine samples with chromogenic substrate methods on plasma samples in outpatients treated with direct oral anticoagulants. Clin Appl Thromb Hemost. 2023;29:1–7.
138. Bonar R, Favaloro EJ, Mohammed S, Ahuja M, Pasalic L, Sioufi J, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology. 2016;48:60–71.
139. Douxfils J, Chatelain C, Chatelain B, Dogne JM, Mullier F. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost. 2013;110:283–94.
140. Gosselin RC, Adcock DM, Douxfils J. An update on laboratory assessment for direct oral anticoagulants (DOACs). Int J Lab Hematol. 2019;41(Suppl 1):33–9.
141. Nehaj F, Sokol J, Ivankova J, Mokan M, Mokan M, Stasko J. Edoxaban affects TRAP-dependent platelet aggregation. J Thromb Thrombolysis. 2020;49:578–83.
142. Sokol J, Nehaj F, Ivankova J, Mokan M, Mokan M. First evidence: rivaroxaban and apixaban reduce thrombin-dependent platelet aggregation. J Thromb Thrombolysis. 2018;46:393–8.
143. Carter RLR, Talbot K, Hur WS, Meixner SC, van der Gugten JG, Holmes DT, et al. Rivaroxaban and apixaban induce clotting factor Xa fibrinolytic activity. J Thromb Haemost. 2018;16:2276–88.
144. Dirienzo L, Vitulli A, Mancazzo F, Ammollo CT, Dellanoce C, Paoletti O, et al. Differential effect of direct oral anticoagulants on thrombin generation and fibrinolysis in patients with atrial fibrillation and venous thromboembolism. Blood Transfus. 2022;20:505–15.
145. Gosselin RC, Adcock D, Dorgalaleh A, Favaloro EJ, Lippi G, Pego JM, et al. International Council for Standardization in Haematology recommendations for hemostasis critical values, tests, and reporting. Semin Thromb Hemost. 2020;46:398–409.
146. Fanikos J, Tawfik Y, Almheiri D, Sylvester K, Buckley LF, Dew C, et al. Anticoagulation-associated adverse drug events in hospitalized patients across two time periods. Am J Med. 2023;136:927–36.e3.

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APPENDIX A

A.1 | Search criteria

Meta-analysis, randomised controlled trial, clinical trial + review, systematic review (2014 onwards) (Direct thrombin inhibitors OR DTI OR Direct Xa inhibitors OR Apixaban OR Argatroban OR Bivalirudin OR Dabigatran OR Fondaparinux OR Rivaroxaban OR Tinzaparin OR Enoxaparin OR Dalteparin OR Danaparoid OR Edoxaban OR Low molecular weight heparin OR LMWH OR Unfractionated heparin OR UFH) AND (Measurement OR Monitor/Monitoring) AND (Coagulation assays OR Haemostasis assays OR Laboratory).