A novel methodology for low range heparin detection

A single-centre prospective diagnostic study

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Editor,

Unfractionated heparin is used in multiple clinical settings. Its over- and underdosing and residual effect after suboptimal reversal must be adequately recognised. This mandates precise monitoring of low-range activity. Although applicable bedside and widely used, monitoring with activated clotting times (ACT) is imprecise.(1) Anti-Xa activity assays are recognised as laboratory standard, but are not point-of-care tests (POCT).(2) Viscoelastic tests (VET) provide a broad bedside view of coagulation.(3) ROTEM proposes its HEPTEM/INTEM clotting time (CT) ratio, and Sonoclot, another VET, proposes glass bead ACT (gbACT) for heparin detection.(4,5) In a previous observational study, we identified a novel potential Sonoclot parameter: the Slope-45.(6) We compared the performance of these parameters' ability to identify isolated low-range heparin.

For this single-centre prospective observational study, institutional board review was obtained (Ghent Ethical Committee, BC-09735, 20/4/2021). In elective adult cardiac surgery patients (April - July 2021), after written informed consent, blood sampling took place before and five minutes after administration of a fraction of the heparin (Leo, Copenhagen, Denmark) required for extracorporeal circulation: 5, 10 or 15 iu.kg^{-1}, randomised upon inclusion. Other clinically relevant changes were unlikely in this time frame. Further patient management was conducted as per institutional guidelines. Data were collected and blinded to the analyser. A priori power analysis (G*Power v3.1.9.4) based on earlier observational data(6) suggested the inclusion of ten patients in each dosage group (power 0.8, alpha 0.05, two-tailed and expected effect size of 1).

Sonoclot raw data were imported into custom-written R code (R Foundation, Vienna, Austria, v4.0.3). The slope between the reported gbACT-point (onset of clot formation) and the point on the curve 45 seconds later was automatically calculated and termed the Slope-45 (figure 1). We performed ROC and classification performance analysis to compare the Sonoclot slope-based parameters with anti-Xa activity, iSTAT and Sonoclot ACT and ROTEM's HEPTEM/INTEM CT ratio. Our trial subjects represented a representative cohort of cardiac surgery patients in our centre. In six patients baseline anti-Xa levels were mildly elevated, although all anticoagulant therapy was timely stopped according to guidelines.(7) These patients were excluded from the analysis (low molecular weight heparin, 3; rivaroxaban, 2; fondaparinux, 1). The resulting study population consisted of respectively 9, 8 and 7 patients in the low to highest dosage group.

All parameters tended to display a dose-dependent response to heparin. Comparing means using Wilcoxon, strongest statistical significance was achieved for anti-Xa activity and the Sonoclot slope-based tests, especially in the lowest dosage range (p values for 5 iu.kg^{-1}: anti-Xa: 0.00057, iSTAT: 0.73, ROTEM ratio: 0.024, gbACT: 0.01, gbCR: 0.0046, Slope-45: 0.0033 and their ratio: 0.0028). These results

were reflected in the ROC curves (figure 2). While all tests provided a fair detection of the higher range of heparin, this was not the case in the lower range. iSTAT ACT provided the least discriminatory potential, while the Sonoclot slope-based parameters performed best of the POCT, especially over the low range. Classification performance was assessed with sensitivity, specificity, accuracy, positive and negative likelihood ratio (LR). We used cut-offs based on ROC Youden Index, as well as available upper normal values as reported (table 1).(4,6) For anti-Xa 0 $u.mL^{-1}$ was used as a cut-off for any heparin detection. Anti-Xa performed well over the whole range of examined heparin dosages, with a minimal accuracy of 0.94. iSTAT ACT performed poorly for 5 and 10 $iu.kg^{-1}$ (accuracy <0.69), and only differentiated the 15 $iu.kg^{-1}$ dose with moderate accuracy. ROTEM's ratio performed suboptimally when using the proposed 0.8 and 0.9 (maximal accuracy 0.86). Using a 0.96 ratio (from Youden), resulted in moderate accuracies of 0.69-0.78 for the lowest investigated doses, and good accuracy for 15 $iu.kg^{-1}$ (0.93). Sonoclot gbACT performed only moderately well, whereas Clot Rate showed good results (accuracy >0.88). The novel Slope-45 performed well best in the whole range of investigated low-dose heparin (accuracy >0.89). Its ratio to the gbCR likewise performed well (accuracy >0.81).

We conclude that in a setting of isolated heparin, the Sonoclot slope-based parameters outperformed other investigated POCT (iSTAT ACT, ROTEM CT ratio and Sonoclot glass bead ACT) in terms of detection performance of isolated low dose heparin. These results may help to improve strategies for the detection of heparin under- and overdosing, rebound, and aid in the correct antagonisation of heparin. We hypothesise that this better performance results from the blood pre-treatment requirements of the devices. Mild glass bead activation (vs strong kaolin activation, iSTAT) allows the initiation as well as the ensuing clot formation rate to be investigated, the latter being a reflection of ongoing thrombin generation. Additionally, the use of citrated and recalcified blood in the ROTEM analyser may decrease its sensitivity to low range heparin: its manufacturer acknowledges that in an INTEM test up to 0.3 $u.ml^{-1}$ of heparin can go unrecognised. The calculation of the slope-45 is straightforward (beta version of picture based calculation app is available on https://michael-vandenheuvel.shinyapps.io/Slope45/), and an additional advantage of the Sonoclot device is that its operating cost is roughly comparable to iSTAT ACT measurement, whereas ROTEM is ten times as expensive.(6)

While the heparin doses investigated in this setup are deliberately low range, heparin reversal aims to have no residual effect at all in most clinical settings. Here, the mean anti-Xa activity after heparin administration was 0.03 ± 0.03, 0.11 ± 0.053 and 0.18 ± 0.09 $u.mL^{-1}$ at 5, 10 and 15 $iu.kg^{-1}$ of heparin, respectively (overall mean 0.10 ± 0.08 $u.mL^{-1}$ of anti-Xa). Our low range reference value for trough level heparin is 0.2 $u.mL^{-1}$ – thus, the investigated residual activities are considered clinically important.

We investigated the isolated effect of low range heparin. These results cannot be readily extrapolated to the post-extracorporeal circulation setting, where hemodilution or acquired coagulopathy play a major role. However, it provides a basis to investigate the classification performance of the proposed parameters in the setting of low-range heparin activity detection. Further research is warranted to evaluate the effects of other coagulation factors, the interaction of protamine and heparin as well as the effects of extracorporeal circulation on these findings.

Figures and Table Legend

Figure 1 - Determination of the Slope-45 Sonoclot parameter. *Black lines connect the ACT point and the point on the curve 45 seconds later. The black angle indicates the inclination of the black line, i.e. the Slope-45. Dotted curve: pre heparin, full curve: low range heparin.*

Figure 2 – ROC curves for detection of low range heparin (5, 10 and 15 iu.kg^{-1}) *AUC: area under the curve*

Table 1 – Test characteristics for detection of low range heparin (5, 10 and 15 iu.kg^{-1}) *Se: sensitivity, Sp: specificity, Acc: accuracy, LR^{+}: positive likelihood ratio, LR^{-}: negative likelihood ratio, inf: infinity. †: based on published reference range. ††: based on ROC Youden for heparin 5 iu.kg^{-1}.*

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