A Novel Point-of-Care Assay for the Monitoring of Low-Molecular Weight Heparins in the Cardiac Catheterization Laboratory

Jonathan D. Marmor, MD, Manish Lakhani, MD, *Soumaya Elrouby, MD, Erdal Cavusoglu, MD

ABSTRACT: Background. We designed a study to compare the novel point-of-care assay Hemonox clotting time (Hemonox-C) with the activated clotting time (ACT) and anti-Xa activity to monitor the anticoagulation effects of enoxaparin and dalteparin during percutaneous coronary intervention (PCI). Methods. A total of 90 patients undergoing cardiac catheterization were assigned to intravenous (IV) enoxaparin 0.5 mg/kg, dalteparin 50 international units/kg or unfractionated heparin (UFH) 50 units/kg. We measured Hemonox-C, ACT and plasma anti-Xa levels after serial sampling. Results. Baseline Hemonox-C was similar in the enoxaparin (68 ± 9 sec) and dalteparin (68 ± 7 sec) groups with no detectable anti-Xa activity at baseline. Minutes after IV administration of enoxaparin and dalteparin, the mean Hemonox-C increased to 171 ± 60 sec and 214 ± 70 sec for both groups, respectively. UFH induced a higher Hemonox-C response (800 ± 243 sec). At all time points, Hemonox-C was higher than the ACT. Peak Hemonox-C was associated with a therapeutic anti-Xa activity ranging from 0.50 to 1.35 U/ml (mean 0.89 U/ml) for the enoxaparin group and 0.69 to 1.48 U/ml (mean 0.91 U/ml) for the dalteparin group. After reaching peak levels, there was a gradual and parallel decline in Hemonox-C and anti-Xa activity. The enoxaparin and dalteparin-treated patients successfully underwent PCI without major hemorrhagic complications. Conclusions. The Hemonox-C has better sensitivity to both enoxaparin and dalteparin than does the ACT. Also, Hemonox-C correlates with anti-Xa activity after IV administration. Hemonox-C may become an important tool to monitor the anticoagulation effects of low-molecular weight heparin during PCI. Additional studies are needed to determine the target Hemonox-C.

J INVASIVE CARDIOLOGY 2008;20:xxx–xxx

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Enoxaparin</th>
<th>Daltepin</th>
<th>Unfractionated Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>42</td>
<td>34</td>
<td>14</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>60 (38 – 78)</td>
<td>63 (44 – 82)</td>
<td>56 (38 – 84)</td>
</tr>
<tr>
<td>Gender M/F (n)</td>
<td>12/17</td>
<td>14/20</td>
<td>5/9</td>
</tr>
<tr>
<td>History of CAD</td>
<td>27 (64%)</td>
<td>3 (9%)</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>Prior PCI</td>
<td>12 (24%)</td>
<td>2 (6%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>7 (17%)</td>
<td>1 (3%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Prior MI</td>
<td>7 (17%)</td>
<td>13 (38%)</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29 (69%)</td>
<td>21 (62%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>10 (24%)</td>
<td>7 (21%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16 (38%)</td>
<td>11 (32%)</td>
<td>8 (57%)</td>
</tr>
</tbody>
</table>

CABG = coronary artery bypass grafting; CAD = coronary artery disease; F = female; M = male; MI = myocardial infarction; PCI = percutaneous coronary intervention

and efficacy. Although the activated clotting time (ACT) is widely available and has been proposed as a method for monitoring the anticoagulant effect of LMWH in PCI, this method has not been validated and is not widely accepted in the interventional community at large.

The Hemonox™ assay (International Technidyne Corp., Edison, New Jersey) has been developed for the purpose of monitoring the anticoagulant effects of LMWH (e.g., enoxaparin and dalteparin) in PCI. The purpose of this study is to compare this technology with the traditional measurement of the ACT and anti-Xa activity. Our data suggest that the Hemonox assay is highly sensitive to enoxaparin and dalteparin at low concentrations and that this assay may be ideal for monitoring intravenous (IV) LMWH in PCI.

Methods

Patient population. The Institutional Review Board of the State University of New York, Health Science Center at Brooklyn approved the protocol for this study. Eligible patients were men or non-pregnant women older than 18 years who were referred for cardiac catheterization and coronary angiography for a variety of indications such as stable angina pectoris, acute coronary syndrome, positive exercise or nuclear stress test or chest pain post myocardial infarction (MI). A total of 90 patients were enrolled who underwent diagnostic cardiac catheterization, and out of these, 46 patients underwent PCI. Informed consent was obtained from each patient before enrollment in this study. The demographics of the study population are shown in Table 1.

Exclusion criteria for the study included ST-segment elevation MI within 24 hours, active internal bleeding, bleeding

From the SUNY Health Sciences Center at Brooklyn, Brooklyn, New York and International Technidyne Corporation, Edison, New Jersey.
The authors report no conflicts of interest regarding the content herein.
Manuscript submitted February 22, 2008; provisional acceptance given April 16, 2008, manuscript accepted May 19, 2008.
Address correspondence to: Jonathan D. Marmor, MD, FACC, Department of Cardiology, SUNY Health Sciences Center at Brooklyn, 450 Clarkson Avenue, Box 1237, Brooklyn, NY 11203. E-mail: jonathan@marmor.com
diathesis, thrombocytopenia (< 100,000 per mm²), serum
creatinine > 2.0 mg/dl, and administration of an oral antioco-
gulant within 7 days.

Study design and medications. Enrolled patients were
consecutively assigned to three different groups to receive
intravenous (IV) enoxaparin (0.5 mg/kg), dalteparin (50
IU/kg), or unfractionated heparin (UFH) (50 Units/kg). The
treatment dose was given in 2 boluses 5 minutes apart. Bolus
doses were: (a) 0.1 mg/kg followed by 0.4 mg/kg enoxaparin;
(b) 20 IU/kg followed by 30 IU/kg dalteparin; and (c) 10
IU/kg followed by 40 U/kg UFH, respectively. Aspirin, clopi-
dogrel and glycoprotein (GP) IIb/IIIa inhibitors were given
to all patients who underwent PCI following diagnostic
angiography with the choice of which particular GP IIb/IIIa
inhibitor (epifibatide, tirofiban or abciximab) left to the dis-
cretion of the operating physician.

Blood samples. Blood samples were drawn at baseline fol-
lowing insertion of the femoral sheath 5 minutes following
each bolus dose and then 15, 30, 60, 90 and up to 120 min-
utes after the first bolus dose administration of the anticoag-
gulant medication. All the blood samples were drawn after a 5 ml
discard and were collected in duplicate in sodium citrate vacu-
tainers. Arterial access sheaths were removed when the ACT
had fallen below 160 seconds.

Standard blue-top tubes containing 3.2% sodium citrate
were used for the collection of blood samples for plasma sepa-
ration. The collection tubes were centrifuged at 3,000 g for
15 minutes at 22°C within 45 minutes for plasma separation
and immediately stored at -70°C; frozen samples were trans-
ported in dry ice for anti-Xa measurement.

Determination of the Hemonox in clinical samples from
PCI patients. The Hemonchron Jr. Signature+ Whole Blood
Microcoagulation System with software version 2.0 or higher
(International Technidyne Corp.) was used to perform the
Hemonox clotting time (Hemonox-CT) assay and the ACT tests
cuvette cat # JACT-LR). The Hemonox method uses a lipida-
ted recombinant rabbit brain tissue factor-based reagent and
formulation buffer (Pel-Freez Corp., Rogers, Arkansas). The test is used
for evaluation of anticoagulation in whole blood samples where
the expected enoxaparin or dalteparin concentration is 0–2.5
units per ml of blood. Following whole blood sample introdúc-
tion, the instrument measures 15 µl of whole blood, automatic-
ally moves it into the test channel within the Hemonox
cuvette, and the remainder of the blood sample not needed for
testing is automatically drawn into the waste channel. After
mixing with the reagent, the sample is then moved back
and forth within the test channel and monitored for clot for-
mation. The instrument recognizes that a clot endpoint has
been achieved when the movement decreases below a predeter-
mined rate. The instrument reports the whole blood clotting
time value in seconds.

Determination of plasma anti-Xa activity. Plasma anti-Xa
activity levels were determined by using a chromogenic assay at
Loyola University Medical Center, Maywood, Illinois. The
assay provides a measurement of absolute heparin/LMWH con-
centration utilizing its inhibitory effects against bovine factor Xa
and quantitation by a chromogenic substrate for factor Xa.

**In Vitro analyses of the effect of LMWH on Hemonox
and ACT coagulation tests.** Enoxaparin or dalteparin was
added at increasing concentrations to fresh whole blood sam-
ple obtained from 4 healthy donors. Testing was performed
in duplicate on the blood samples from each individual donor
and evaluation was done simultaneously for Hemonox-CT
and ACT coagulation tests. Clotting time results were com-
pared to the amount of LMWH added to the whole blood
sample. All point-of-care tests were performed using the
Hemonchron Jr. Signature+ Microcoagulation System.

Assessment of safety. A new MI was defined as an elevation
in CK-MB (or total creatine kinase in absence of CK-MB) more
than three times the upper limit of normal. Blood for CK-MB
analysis was drawn before PCI and every 8 hours twice post PCI.
Severe thrombocytopenia was defined by a platelet count below
50,000/µl. Mild thrombocytopenia was defined as a platelet count
below 100,000/µl or a count 50% of the baseline value. Minor
and major bleeding were defined according to the criteria used by
the Thrombolysis in Myocardial Infarction (TIMI) trial. Major
hemorrhage was defined as a decrease in hemoglobin > 5 g/dl
(hematocrit decrease > 15% when hemoglobin is not available) or
intracranial bleeding. Minor hemorrhage was defined as sponta-
neous and observed gross hematuria, hematemesis or observed
blood loss associated with a decrease in hemoglobin ≥ 3 g/dl or
hematocrit decrease ≥ 10% when hemoglobin is not available.

**Statistical analysis.** Results are expressed as mean± standard
deviation. Simple regression analysis and analysis of
variance (ANOVA) were used to test the association between
continuous variables; the α level was set at 0.05.

2

Figure 1 A and 1B. Time-course of Hemonox clotting time and
anti-Xa activity following intravenous administration of enoxaparin
(left) and dalteparin (right). After obtaining baseline blood samples,
ENOXAPARIN and DALTEPARIN were administered intravenously as 2
boluses 5 minutes apart.
Results

The Hemonox-CT in patients treated with enoxaparin or dalteparin during cardiac catheterization. Patients were treated with either enoxaparin (n = 42), dalteparin (n = 34) or UFH (n = 14), and the point-of-care Hemonox-CT was determined at baseline and at up to seven time points after the baseline blood sample collection for each patient. Parallel chromogenic plasma anti-Xa activity was determined for patients in the enoxaparin and dalteparin groups. Hemonox-CT was compared to anti-Xa activity at each time point in both treatment groups (Figure 1).

The mean Hemonox-CT was similar in the enoxaparin (68.9 ± 9 seconds) and dalteparin (68 ± 7 seconds) treatment groups at baseline; no detectable anti-Xa activity was observed at baseline. After the first IV bolus of 0.1 mg/kg enoxaparin or 20 IU/kg of dalteparin, the Hemonox-CT increased 0.30 ± 0.11 U/ml in the enoxaparin group and 0.61 ± 0.22 U/ml in the dalteparin group (p < 0.05) compared to baseline values for both groups. Additional boluses of 0.4 mg/kg of enoxaparin and 30 IU/kg dalteparin resulted in an increase in Hemonox-CT to 171 ± 60 seconds (range: 101–314 seconds) and 214 ± 70 seconds (range: 125–351 seconds) for the enoxaparin and dalteparin treatment groups, respectively. Peak Hemonox-CT was associated with a therapeutic anti-Xa activity level (for PCI have been reported > 0.5 U/ml) ranging from 0.50–1.35 U/ml (mean 0.89 U/ml) in the enoxaparin group and 0.69 to 1.48 U/ml (mean 0.91 U/ml) in the dalteparin group. Following the second bolus after reaching the peak levels, there was a gradual decline in Hemonox-CT and anti-Xa levels; the latter dropped to a mean value of 0.48 ± 0.15 U/ml at 60 minutes post second bolus for both LMWH-treatment groups (Figure 1).

Patients in the UFH treatment group showed Hemonox-CT baseline value similar to those observed in the LMWH treatment groups (mean 71 ± 8). After the first bolus dose of 10 U/kg of UFH, the mean Hemonox-CT rose to 118 ± 36 seconds. The second bolus dose of 40 U/kg of UFH induced a mean Hemonox response of 800 ± 243 seconds. The Hemonox-CT gradually decreased at 30–60 minutes, yielding a mean value of 110 ± 28 seconds at 120 minutes after the second dose; no anti-Xa activity level was evaluated for patients treated with UFH.

Comparison of the effect of enoxaparin and dalteparin on coagulation tests Hemonox-CT and ACT. We compared the sensitivity of the ACT and the Hemonox-CT in monitoring the anticoagulant effects of enoxaparin and dalteparin both in vitro and in clinical samples derived from patients undergoing cardiac catheterization.

In Vitro data: Enoxaparin or dalteparin was added at increasing concentrations to fresh whole blood samples obtained from healthy volunteers (n = 4). Samples were evaluated in duplicate from individual donors for Hemonox-CT and ACT coagulation tests. Clotting time results were compared to the amount of LMWH added to the whole blood sample (Figure 2). Both the Hemonox-CT and the ACT demonstrated a linear correlation of LMWH activity to clotting time. The Hemonox-CT showed a greater sensitivity to the LMWH concentration with increases of 2- to 8-fold from baseline at LMWH levels of 1 and 2 IU/ml blood compared to 1.2- to 1.8-fold for the ACT.

Clinical data: Whole blood samples from patients undergoing cardiac catheterization were obtained at baseline and at seven time points after drug administration. Hemonox-CT and ACT tests were simultaneously performed. The increases in clotting time from the baseline for both coagulation tests were calculated at each time point after drug administration (Figures 3A, B and C). All three anticoagulants induced higher clotting times for Hemonox than ACT (p < 0.05) at peak response (5 minutes after the second bolus). Enoxaparin induced a 2-fold increase (range of 1.5- to 4.1-fold increase) in Hemonox-CT and a 1.2-fold increase (with a range of 0.76 to 1.7-fold increase) in ACT from baseline. Dalteparin raised the Hemonox-CT by 2.9-fold (range of 1.0- to 4.3-fold increase) and ACT by 1.3-fold (range: 1.2- to 1.8-fold increase) from baseline. UFH induced a 7.8-fold increase in Hemonox-CT compared to 1.3-fold increase with the ACT test.

Correlation between anti-Xa activity level and Hemonox-CT and ACT coagulation tests. The correlation
between anti-Xa activity and Hemonox-CT was highly significant for individual patients (Figure 4 A), but was less significant for the entire study population (Figure 4 B). There was a correlation ($r = 0.7$) between the plasma anti-Xa level and Hemonox-CT in the enoxaparin treatment group. Similar results were obtained with the dalteparin treatment group ($r = 0.7$, not shown in Figure); anti-Xa activity level was not evaluated in the UFH treatment group. There was a poor correlation between ACT and anti-Xa level for the enoxaparin treatment group ($r = 0.45$ as seen in Figure 4 C) as well as for the dalteparin treatment group ($r = 0.4$ not shown in Figure).

Safety outcomes. PCI was successful in all patients undergoing intervention. There were no deaths, abrupt closures or

Figure 3. (A, B, C) Change in activated clotting time and Hemonox clotting time after intravenous administration of enoxaparin (left), dalteparin (middle) and unfractionated heparin (right) at different time points. The Hemonox clotting time induces better signal-to-noise ratio than does activated clotting time at all the time points. Hemonox-CT = Hemonox clotting time. ACT = activated clotting time.

Figure 4. (A, B, C) Correlation between anti-Xa activity and Hemonox-CT for individual patients (left) and for entire study population (middle) in enoxaparin group. Correlation between anti-Xa activity and ACT in enoxaparin group (right). Hemonox-CT = Hemonox clotting time. ACT = activated clotting time. Pat = patient.
urgent revascularization for the study population during the hospital course. One of the 46 PCI patients (2%) had a periprocedural non-Q-wave MI. None of the patients suffered major bleeding, and no patient received a transfusion. There were no groin complications, nor were there any episodes of thrombocytopenia.

Discussion

This is the first report of a novel LMWH monitoring device, the Hemonox-CT, for the two commonly used LMWHs, enoxaparin and dalteparin. This device has a better sensitivity and an improved signal-to-noise ratio compared to the traditional methods used to monitor anticoagulation in the cardiac catheterization laboratory. The Hemonox-CT is not affected by concurrent GP IIb/IIIa use. Furthermore, our data show a correlation between the Hemonox-CT and anti-Xa activity, which is considered the gold standard to monitor LMWH.

Although LMWH is commonly prescribed in noninvasive settings, it is not commonly used in the context of PCI. In a large randomized clinical trial, enoxaparin did not show benefit over UFH in high-risk patients undergoing an early invasive approach. In this trial, however, monitoring of the anticoagulant effect was performed for UFH, but not for enoxaparin. This raised the question that although routine monitoring of the anticoagulant activity of LMWH appears to be unnecessary in the noninvasive setting, it may be important to monitor in the setting of PCI.

Although aPTT and ACT are widely used for monitoring the anticoagulant effect of UFH in noninvasive and invasive settings, respectively, these tests are not broadly accepted as a mean to monitor LMWH during PCI. The ACT and aPTT are not significantly prolonged by enoxaparin. Anti-Xa activity monitoring of LMWH is prudent in certain clinical circumstances such as in patients with weight > 150 kg and creatine clearance < 25 ml/minute. Also, an anti-Xa level of at least 0.5 IU/ml has been recommended in patients with acute coronary syndromes treated with subcutaneous enoxaparin. However, a point-of-care anti-Xa assay is not currently available.

Hemonox-CT is sensitive to low doses of intravenously administered LMWH (Figure 1). Peak responses are reached within minutes and returned to baseline in a time course that appears favorable for current interventional practice. The anti-Xa activity paralleled this response.

The ACT is widely used for monitoring anticoagulation effects of UFH during PCI. Recent data have demonstrated the sensitivity of the ACT to the anticoagulant effect of LMWH in PCI patients. The Hemonox-CT is more sensitive and produces a greater signal-to-noise ratio than does the ACT to LMWH activity. Thus, the Hemonox-CT may be an ideal means of monitoring IV LMWH activity during PCI. The importance of LMWH monitoring may grow over time given the demonstrated benefit of enoxaparin during PCI in the STEEPELE trial. Although bivalirudin appears to be an excellent molecule for PCI, the use of IV LMWH during PCI may have several advantages including lower cost and partial reversibility with protamine in the event of complications such as coronary perforation. Furthermore, heparin-induced platelet activation is reduced with LMWH and thus may reduce the need for routine adjunctive GP IIb/IIIa inhibitors during PCI.

These theoretical and practical advantages of LMWH, along with its demonstrated benefits in the STEEPELE trial, point to the potential for a growing use of IV LMWH in PCI. Monitoring devices, such as the Hemonox-CT may therefore be of increasing importance over time.

References:

11. Marmur JD, Anand SX, Bagga RS, et al. The activated clotting time can be used to monitor the low molecular weight heparin dalteparin after intravenous administration. J Am Coll Cardiol 2003;41:494–498.

