blood

Prepublished online December 4, 2008;
doi:10.1182/blood-2008-07-162271

Dosing lepirudin in patients with heparin-induced thrombocytopenia and normal or impaired renal function: A single-centre experience with 68 patients

Martina Tschudi, Bernhard Lammle and Lorenzo Alberio

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Blood First Edition Paper, prepublished online December 4, 2008; DOI 10.1182/blood-2008-07-162271

Dosing lepirudin in patients with heparin-induced thrombocytopenia and normal or impaired renal function: A single-centre experience with 68 patients

Martina TSCHUDI, Bernhard LÄMMLE, Lorenzo ALBERIO

Department of Hematology and Central Hematology Laboratory,
Inselspital, Bern University Hospital and University of Bern, Switzerland

Running title: Dosing Lepirudin

Correspondence: Lorenzo Alberio, MD
Dpt. of Hematology and Central Hematology Laboratory
University Hospital Inselspital
Freiburgstrasse 10
CH – 3010 Bern, Switzerland

Phone: +41.31.632 3513
FAX: +41.31.632 3406
E-Mail: lorenzo.alberio@insel.ch

Abstract

The officially recommended dose (i.v.-bolus 0.4 mg kg^{-1} followed by 0.15 mg kg^{-1} h^{-1}) of lepirudin, a direct thrombin inhibitor licensed for treatment of heparin-induced thrombocytopenia (HIT), is too high. Starting in 2001 we omitted the bolus and reduced maintenance-dose by at least 1/3. Analysing 53 HIT-patients treated at our institution between 1.2001 and 2.2007 we observed that patients with therapeutic anticoagulation intensity already at first monitoring (4 hours after lepirudin start) had been treated with following initial lepirudin doses (median): 0.078 mg kg^{-1} h^{-1} in those with creatinine clearance (CrCl) >60 ml/min, 0.040 mg kg^{-1} h^{-1} (CrCl 30–60 ml/min), and 0.013 mg kg^{-1} h^{-1} (CrCl <30 ml/min). The efficacy of this treatment was documented by increasing platelet counts, decreasing D-dimer values and fewer thrombotic or bleeding episodes. Based on this experience we derived an in-house lepirudin dosing-regimen, which was prospectively evaluated treating 15 HIT-patients between 3.2007 and 2.2008. We show that omitting the initial lepirudin bolus dose and administering 0.08 mg kg^{-1} h^{-1} in patients with CrCl >60 ml/min, 0.04 mg kg^{-1} h^{-1} in patients with CrCl 30–60 ml/min, and 0.01-0.02 mg kg^{-1} h^{-1} in those with CrCl <30 ml/min is efficacious and safe, as documented by increasing platelet counts, decreasing D-dimer levels, and rare thrombotic (n=1/46) and major bleeding (n=4/46) complications.

Introduction

Heparin-induced thrombocytopenia (HIT) is a drug-induced antibody-mediated condition characterised by a highly prothrombotic state.[1,2] HIT is caused by IgG antibodies directed against heparin-bound platelet factor 4 (PF4). Macromolecular ternary complexes (HIT-antibody/PF4/heparin) are able to activate platelets, endothelial cells and monocytes, leading to excessive in vivo thrombin generation.[3] The treatment of HIT requires not only the immediate discontinuation of all heparin but also the introduction of alternative non-heparin anticoagulation in therapeutic doses, in order to counterbalance the strong procoagulant state.[1,2] Drugs approved for HIT treatment are the direct thrombin inhibitors lepirudin and argatroban, and the heparinoid danaparoid.

Lepirudin (Refludan; Celgene International Sàrl, Boudry, Switzerland), a recombinant hirudin, is an irreversible, specific, direct thrombin inhibitor that is renally excreted and has a half-life of 60-90 min.[4] The problems related to its usage are: the difficulty of its laboratory monitoring,[5] the high bleeding risk,[6] and fluctuations of its half-life, which can be dramatically increased by renal dysfunction or antibodies delaying its elimination.[7] As approved by the European Medical Evaluation Agency (www.emea.europa.eu/humandocs/Humans/EPAR/refludan/refludan.htm) and the U.S. Food and Drug Administration (www.fda.gov/cder/consumerinfo/druginfo/refludan.HTM), the manufacturer recommends in the summary of product characteristics to start lepirudin treatment with a 0.4 mg kg^{-1} bolus dose followed by a continuous intravenous infusion of 0.15 mg kg^{-1} h^{-1}, adjusted to maintain an aPTT 1.5 – 2.5 times the baseline value. Lower dosages are recommended for patients with impaired renal function: 50% of the standard dose for patients with a creatinine clearance (CrCl) between 45-60 ml/min, 30% for CrCl 30-44

ml/min, 15% for CrCl 15-29 ml/min; patients with a CrCl < 15 ml/min should receive no more than a bolus of 0.1 mg kg^{-1} every second day.

Late in 2000 we observed that HIT patients receiving the recommended dose of lepirudin were systematically found to be over-anticoagulated at first laboratory monitoring and always required a dose reduction. Starting in 2001 we therefore reduced the dosing regimen of lepirudin as follows: initially by omitting the bolus and by decreasing the recommended maintenance dose by 1/3, taking into account the renal function for further adjustments; over time we realized that this was not yet adequate and successively further reduced starting-doses. Three recent publications reporting that lower lepirudin doses than those officially recommended may be sufficient are in agreement with our observation; however, no experimentally established dosing regimen was proposed.[6,8,9]

The aim of the present two-phase study (see Methods section) was to define an adequate in-house lepirudin dosing-scheme for HIT patients with normal and variably decreased renal function. To the best of our knowledge this is the first study to propose lepirudin dosing regimens for HIT patients with normal, moderately and severely impaired renal function, thus providing evidence for reduced lepirudin doses, as recently recommended by the ACCP-panel.[2]

Methods

Patients. This was a single-centre two-phase study conducted between January 2001 and February 2008 at the University Hospital Inselspital, Bern, Switzerland. In a first, retrospective investigational study we collected clinical and laboratory data on 53 HIT patients treated with lepirudin between January 2001 and February 2007. The data allowed us to establish an in-house dosing scheme for lepirudin according to the degree of renal function.[10] In a second, prospective study between March 2007 and February 2008 we treated 15 HIT patients with the in-house lepirudin dosage (see Results section) in order to verify the validity of our approach. The study was conducted in accordance with institutional guidelines for observational studies at our University Hospital and in accordance with principle 32 of the Declaration of Helsinki. Noteworthy, at our institution we evaluate all patients with suspected HIT by assessing the pre-test clinical probability according to the 4T score[11] and by combining it with the antibody titre detected by a rapid particle gel immunoassay, ID-HPF4-PaGIA,[12] which has a turnaround-time of less than 1 hour. Therefore, lepirudin treatment can be started – after informed patient consent – without delay, usually within a few hours after HIT has been suspected.[13]

Data collection. Retrospective investigational study: We reviewed the clinical files of all patients with a laboratory test for HIT, identifying those treated with lepirudin. We collected information about personal data (age, gender, body weight), work-up for suspected HIT (date of evaluation, 4T pre-test clinical probability[11] assessed by the consulting hematologist [for HIT-patients treated with lepirudin between 2001 and 2003 the 4T score was retrospectively calculated by two authors, MT and LA], detection of anti-heparin/PF4-antibodies by ID-HPF4-PaGIA and by ELISA [GTI-PF4]), lepirudin treatment (initial and final lepirudin dosage, number of dose adjustments [increments, reductions, and

interruptions lasting ≥ 2 hours], duration of therapy), laboratory data (hemoglobin, hematocrit, leucocyte and platelet count, aPTT, thrombin time, D-dimers, serum creatinine), complications occurring during lepirudin treatment (symptomatic thrombotic and bleeding events), and blood products transfused (packed red blood cells, fresh frozen plasma, platelet concentrates [confirming that no patient received platelet concentrates during acute HIT]). Major bleeding was defined as fatal or life-threatening and/or associated with a 20 g/L or greater decrease of hemoglobin level and/or requiring transfusion of > 2 units packed RBC. Prospective evaluation study: The above-mentioned data were prospectively collected. Day 0 is the day of evaluation for suspected HIT and start of lepirudin treatment.

Laboratory methods. Blood was drawn into 10 ml plastic syringes (Monovette®, Sarstedt, Nümbrecht, Germany) containing 1 ml 0.106 mol/l trisodium citrate. Plasma was prepared within 1 hour by twice centrifuging at 1500 x g for 10 min each at room temperature. Aliquots were stored in polypropylene tubes at -70ºC. Anti-PF4/heparin-antibodies were detected by ELISA (GTI-PF4 Enhanced, Genetic Testing Institute, Waukesha, WI, USA) measured at 492 nm with a microtiter plate reader (Anthos ht III, Hemotec, Gelterkinden, Switzerland), and by a rapid particle-gel-immunoassay (ID-HPF4-PaGIA)[14] according to the manufacturer's instructions (DiaMed SA, Cressier sur Morat, Switzerland). In case of a positive test result with undiluted plasma, the antibody titre was determined as previously described.[12] Briefly, for a 1:2 dilution, 50 µl of plasma were mixed with 50 µl of Diluent II (DiaMed SA), and subsequent dilutions were obtained by mixing 50 µl of the preceding one with 50 µl of Diluent II. The reported titre is the last positive detection followed by either borderline or negative results. Coagulation assays were performed on a Behring Coagulation System (BCS) automated analyzer (Dade Behring, Marburg, Germany). Activated partial thromboplastin time (aPTT) was measured with Pathromtin SL (Dade

Behring), the results were the average of duplicate measurements.[15] Thrombin time (TT) was measured in duplicate with Thrombin-Reagent (Dade Behring), with final thrombin concentrations of 1.5 U/ml ($TT_{1.5}$) and 5 U/ml (TT_5). D-dimers were measured by an automated quantitative immunoassay, according to the manufacturer's instructions (VIDAS D-dimer New, bioMérieux, Marcy l'Etoile, France).

Lepirudin monitoring. The in-house therapeutic range had been previously defined by measuring both aPTT and thrombin times (TT) in several samples of pooled normal plasma spiked with increasing concentrations of lepirudin (Table 1). The lepirudin summary of product characteristics published in the official Swiss pharmacopoea (Arzneimittel-Kompendium der Schweiz, Documed AG, Basel, Switzerland 2008, www.kompendium.ch) states that "in order to define specific and exact aPTT reference values the in-house reagent/coagulometer system can be calibrated by measuring standardized human plasma spiked with 0.15 µg/ml lepirudin (lower limit) and 1.5 µg/ml lepirudin (upper limit)". When treating HIT patients with lepirudin we aim for an unclottable $TT_{1.5}$ and a clottable TT_5; in case of an unclottable TT_5 we allow for a maximum aPTT prolongation of 2.5 times the patient's baseline value (Table 1); in case of $TT_{1.5}$ and TT_5 in target range and a > 2.5x prolongation of the aPTT, we dismiss the latter result because in our hands TT assays are robust and many variables besides lepirudin can affect the aPTT. The therapeutic target we aim for is narrower than that stated above and is in line with the lepirudin concentration of 0.6 – 1.0 µg/ml reported by Greinacher and Warkentin.[4] Laboratory monitoring is performed at 4 hour intervals after starting lepirudin infusion or any dosage adjustment; after steady state has been reached (defined as two consecutive TT/aPTT values in the target range) laboratory monitoring is performed once daily.[2]

Renal function. Creatinine clearance (CrCl) was either measured and/or calculated according to the Cockroft-Gault formula:[16] (1.04 for ♀ / 1.23 for ♂) x (140 – age, years) x body weight (kg) / serum creatinine (µmol/L). Patients were divided in three groups, according to the degree of renal function impairment: normal (CrCl >60 ml/min, n=34), moderately decreased (CrCl 30-60 ml/min, n=20) and severely decreased (CrCl <30 ml/min, n=14).

Statistical analysis.

Quantitative data are expressed as median and interquartile range (IQR) or range. Comparison of categorical data was performed by Fisher's exact test or Chi-square test as appropriate. Comparison between two non-paired groups was performed by Mann-Whitney rank sum test. Comparison between more than two groups was performed by non-parametric ANOVA. Significance was set at the 5% level. Data were analysed by SigmaStat software (version 3.1). Platelet values on figures 2A and 3A are reported as mean ± 1SD; D-dimers on figures 2B and 3B are depicted by box plots visualizing the median value (horizontal line within the box), the 25th and 75th percentiles (lower and upper borders of the box), the 10th and 90th percentiles (lower and upper whiskers), and each outlier outside the 10th and 90th percentiles (black dots).

Results

Patient population.

Overall, we treated 68 HIT patients with lepirudin (median age 69.3 years, range 26.5 – 92.2). The population of the first, retrospective investigational study (January 2002 – February 2007) consisted of 21 women (median age 73.0 years, range 50.6 – 88.7) and 32 men (median age 69.1 years, range 26.5 – 87.0). During the second, prospective validation study (March 2007 – February 2008), additional 15 consecutive HIT patients (6 women and 9 men; median age 66.5 and 69.2 years, range 28.4 – 83.8 and 30.3 – 92.2, respectively) were treated with the in-house lepirudin dosage scheme derived from the results of the first study (see below). Table 2 summarizes relevant personal data, renal function and results of HIT work-up of both patient cohorts.

Retrospective investigational study

Lepirudin starting dose and anticoagulation intensity at first monitoring.

Among the 29 patients with a normal renal function, median lepirudin starting dose was 0.087 (interquartile range: 0.048 – 0.096) mg kg^{-1} h^{-1}. However, a starting dose > 0.11 mg kg^{-1} h^{-1} always led to over-treatment (Figure 1A). Seventeen out of 29 patients (58.6 %) were within therapeutic range (see Methods section) already at first laboratory monitoring 4 hours after initiation of lepirudin infusion, showing a median aPTT prolongation of 2.03 (IQR: 1.79 – 2.83) times the baseline value and a clottable TT_5 (median 21.9 sec; IQR: 17.9 – 54.3 sec) in 15 out of 17 instances. For these 17 patients the median starting dose was 0.078 (IQR: 0.049 – 0.093) mg kg^{-1} h^{-1}. Eight patients (27.6 %) who were over-anticoagulated at first monitoring, had received a median starting lepirudin dose of 0.098 (IQR: 0.081 – 0.124) mg kg^{-1} h^{-1}. Four patients (13.8 %) who were under-treated had received a median dose of 0.022 (IQR: 0.014 – 0.039) mg kg^{-1} h^{-1} (Table 3).

Among the 15 patients with a moderately decreased renal function (CrCl 30 – 60 ml/min) median lepirudin starting dose was 0.050 (IQR: 0.025 – 0.091) mg kg^{-1} h^{-1}. Starting with lepirudin doses over 0.090 mg kg^{-1} h^{-1} always led to over- and doses below 0.010 mg kg^{-1} h^{-1} to under-treatment (Figure 1B). Seven out of 15 patients (46.7 %) were within therapeutic range already 4 hours after initiation of lepirudin infusion, showing a median aPTT prolongation of 2.09 (IQR: 1.81 – 2.75) and a clottable TT_5 (median 28.3 sec; IQR: 9.4 – 52.7 sec) in 7/7 cases. These seven patients had received a median starting dose of 0.040 (IQR: 0.025 – 0.050) mg kg^{-1} h^{-1}. Six patients (40 %) who were over-anticoagulated at first monitoring, had received a median starting dose of 0.098 (IQR: 0.086 – 0.102) mg kg^{-1} h^{-1}. Two patients (13.3 %) who were under-treated had received a starting dose of 0.009 and 0.011 mg kg^{-1} h^{-1}, respectively (Table 3).

In 9 patients with severely impaired renal function (CrCl < 30 ml/min) median lepirudin starting dose was 0.012 (IQR: 0.007 – 0.020) mg kg^{-1} h^{-1}. Seven out of 9 patients (77.8 %) were therapeutically anticoagulated at first monitoring, showing a median aPTT-prolongation of 2.11 (IQR: 1.98 – 2.26) and a clottable TT_5 (median 8.4 sec; IQR: 7.9 – 9.3 sec) in 7/7 cases. They had received a median starting dose of 0.013 (IQR: 0.008 – 0.026) mg kg^{-1} h^{-1}. Two patients (22 %) who were under-treated had received a starting dose of 0.005 and 0.012 mg kg^{-1} h^{-1}, respectively (Table 3).

The median lepirudin starting doses resulting in therapeutic anticoagulation intensity at first monitoring (i.e. 0.078, 0.040 and 0.013 mg kg^{-1} h^{-1}) significantly differed among the three groups of HIT patients with normal, moderately or severely impaired renal function ($p<0.001$).

Duration and dose adjustments of lepirudin treatment.

The median duration of all lepirudin treatments was 7 (IQR: 4 – 14; range: 1 – 75) days. During the whole treatment period we registered a median of 4 (IQR: 2 – 8; range: 0 – 16) dose adjustments per patient, 61 % of which were dose reductions. While treatment length was similar among all HIT patients independently from the initial anticoagulation intensity, the number of dose adjustments was lower among patients who were within therapeutic limits already at first monitoring (median 3, IQR: 1 – 6, range: 0 - 11) compared to those who were not (median 7, IQR: 3 – 12, range: 0 – 16; p=0.003). Dose adjustments where downwards in 60 % of patients within therapeutic range already at first monitoring, in 75 % of those who were initially over-anticoagulated and in 35 % of those with insufficient anticoagulation 4 hours after starting lepirudin (p<0.001). Table 3 displays initial and final lepirudin dosages among the different patient categories.

Efficacy of lepirudin starting dose.

The biological efficacy of omitting a bolus and using a reduced lepirudin starting dose was assessed by the course of platelet count and D-dimer values after initiation of the alternative anticoagulation. Figure 2A shows that the platelet count increased and normalised among the 31 patients who were within therapeutic range already at first monitoring despite reduced starting lepirudin dose. Moreover, D-dimer levels decreased in all 31 patients from a median of 4170 µg/l at HIT diagnosis to 1892 µg/l at follow-up, between day 2 and 5 (Figure 2B). For comparison, during the same time frame D-dimers decreased from median 5668 µg/l to 3322 µg/l in patients initially over-anticoagulated, while remained stable in those with initial sub-therapeutic anticoagulation (1888 µg/l versus 1612 µg/l).

Of note, among the 31 patients with therapeutic anticoagulation already at first monitoring, only one thromboembolic complication occurred during lepirudin treatment (Table 4, patient D). In this patient therapeutic anticoagulation with lepirudin was interrupted for several hours on two occasions: on day 5, for the placement of an intravenous catheter (complication diagnosed after this interruption: distal deep vein thrombosis of the left leg) and on day 7, for a suspected (but eventually not confirmed) intra-abdominal hemorrhage (complication diagnosed after the second interruption: massive pulmonary embolism).

We observed 3 additional thromboembolic complications while on lepirudin (Table 4): thrombosis of muscle-flap of the distal left lower leg after polytrauma leading to below-knee amputation on day 7; occlusion of a great saphenous vein by-pass in the context of a chronic peripheral arterial occlusive disease leading to amputation of the right leg on day 6; re-thrombosis of the right superficial femoral artery and truncus tibio-fibularis leading to below-knee amputation on day 4. All three patients were initially over-anticoagulated and experienced frequent dose reductions, including a total of 5 interruptions (each lasting at least 2 hours) among two of them; all three patients were still on lepirudin at time of amputation. Interestingly, no thromboembolic complications were observed among the 8 patients with an insufficient anticoagulation intensity at first control.

Bleeding during lepirudin treatment.

Overall, no fatal bleedings were observed. Seventeen out of 53 patients (32.1 %) required transfusion of packed red blood cells (RBC) during lepirudin treatment (Table 5): 6 among the 14 patients with a too high starting dose (43.6 %), 10 among the 31 patients with an adequate starting dose (32.6 %) and 1 among the 8 patients with insufficient starting dose (12.5 %). The incidence of severe bleeding (defined as fatal or life-threatening and/or associated with a hemoglobin decrease of $\geq$ 20 g/l or requirement of > 2 packed RBC),

was higher among those patients that were initially over-anticoagulated (28.6 %) compared to those with adequate starting dose (9.7 %). This is also reflected by a higher median number of packed RBC units administered per transfused patient (Table 5).

Prospective evaluation cohort

Based on the results of the retrospective investigation, in March 2007 we started to treat HIT patients with following dosing schedule: 0.08 mg kg^{-1} h^{-1} (without initial bolus) for patients with normal renal function (n=5), 0.04 mg kg^{-1} h^{-1} for those with a CrCl between 30-60 ml/min (n=5), and 0.01-0.02 mg kg^{-1} h^{-1} for those with a severely impaired renal function (0.02 mg kg^{-1} h^{-1} for 3 patients with a CrCl close to 30 ml/min and 0.01 mg kg^{-1} h^{-1} for two patients with a CrCl below 15 ml/min). Patient characteristics are summarized in table 2.

All 15 patients were within therapeutic ranges already at first control, showing a clottable TT_5 (median 14.9 sec; IQR: 11.6 – 26.9 sec) in 14/15 cases and a median aPTT-prolongation of 2.02 (IQR: 1.84 – 2.21) times the baseline value. The median duration of lepirudin treatment was 8 (IQR: 5 – 18; range: 3 – 98) days, with a median of 1 (IQR: 0 – 2; range: 1-3) dose-adjustments (62 % of which were dose reductions). Final lepirudin doses were 0.073 mg kg^{-1} h^{-1} (median; range 0.067 – 0.080) in patients with normal renal function, 0.030 mg kg^{-1} h^{-1} (median; range 0.023 – 0.050) and 0.013 mg kg^{-1} h^{-1} (median; range 0.010 – 0.023) among patients with moderate and severe renal impairment, respectively.

The biological efficacy of our in-house lepirudin dosage scheme is demonstrated on figure 3, showing that the platelet count normalised in all three patient groups (Figure 3A) and that D-dimer levels decreased in all 15 patients from a median of 4874 µg/l at HIT

diagnosis to 2111 µg/l at follow-up, between days 2 and 5 (Figure 3B). No thromboembolic complications occurred during lepirudin treatment.

No fatal bleedings were observed. Five out of 15 patients required transfusion of packed RBC during lepirudin treatment, with a median of 2 units packed RBC administered per transfused patient (Table 5). The incidence of severe bleeding was 6.7 %, comparable to that observed among the 31 patients of the investigational cohort with adequate anticoagulation intensity already at the first control after lepirudin start (Table 5).

Discussion

The EMEA- and FDA-approved lepirudin dose for HIT patients (e.g., for patients with normal renal function administration of an 0.4 mg kg^{-1} bolus followed by a continuous intravenous infusion of 0.15 mg kg^{-1} h^{-1}, adjusted to maintain an aPTT 1.5 – 2.5 times the baseline value) leads to over-anticoagulation. Recent publications have reported that lower doses may be sufficient.[6,8,9]

In the present work we show that omission of the initial bolus and a reduced lepirudin starting dose (0.08 mg kg^{-1} h^{-1} for patients with normal renal function, 0.04 mg kg^{-1} h^{-1} for patients with CrCl 30 – 60 ml/min and 0.01 – 0.02 mg kg^{-1} h^{-1} for those with CrCl < 30 ml/min) is efficacious and safe. This in-house dosing scheme was derived from the retrospective analysis of 53 HIT patients[10] and prospectively evaluated treating additional 15 consecutive HIT patients.

Overall, among the 46 patients (retrospective cohort, n=31; prospective cohort, n=15) within therapeutic range already at first monitoring the aPTT was about twice the baseline value (median 2.08; IQR: 1.82 – 2.55); the platelet count increased and normalised within 10 days (Figures 2A and 3A); the D-dimer value decreased within 2-5 days (Figures 2B and 3B), demonstrating a dampening of the in vivo thrombin generation characteristic of acute HIT.[3] The rate of thrombotic complications observed during lepirudin treatment in the whole patient population is 5.9 % (4/68) and compares very well with the values of 11.2 % observed in the HAT-3 study[6] and of 13.8 % reported by the GEHT-HIT study group.[9] Of note, three out of the 4 thromboembolic complications occurred among patients who were initially overanticoagulated and required frequent dose-reductions (Table 4). Among the 31 patients of the retrospective cohort with a therapeutic

anticoagulation intensity already at first monitoring we observed only one thromboembolic complication and none among the 15 patients of the prospective evaluation cohort, giving an overall value of 2.2 % (1/46) among HIT patients with early adequate anticoagulation intensity. Taken together, these data strongly suggest a good efficacy of our reduced dosing schedule.

The occurrence of progressive thromboses among 3 of the 14 patients who were initially overanticoagulated is noteworthy (Table 4). As suggested by Greinacher and Warkentin,[4] premature discontinuation of lepirudin may lead to “rebound hypercoagulability” due to persisting in vivo thrombin generation. The three patients reported here experienced many more dose reductions than increases (21 versus 6), among two of them (patients A and B of table 4) lepirudin administration was interrupted a total of 5 times for periods of at least 2 hours each, and final lepirudin doses were much lower than expected for the degree of renal function. These observations suggest that excessive lepirudin dose reductions and in particular prolonged interruptions of lepirudin infusion may be responsible for the failure of controlling the severe procoagulant state of acute HIT. This concept is further illustrated by patient D of table 4: despite therapeutic anticoagulation with lepirudin from the very beginning, two interruptions over several hours led to severe and ultimately fatal thromboembolic complications.

Concerning safety, we did not register any fatal bleeding. Overall 22 out of 68 patients (32.4 %) required transfusion of packed RBC (Table 5). We observed major bleedings (defined as fatal or life-threatening and/or associated with a 20 g/L or greater decrease of hemoglobin level and/or requiring transfusion of > 2 units packed RBC) in 10/68 patients (14.7 %), which is in the range (14.0 – 21.7 %) reported by a combined analysis of the 3 HAT lepirudin studies.[6] In the present study, 4 of the 46 patients within therapeutic range

already at first monitoring (8.7 %) required > 2 units packed RBC compared to 4/14 (28.6 %) of those who were initially over-anticoagulated (p=0.077) and median units of packed RBC administered per transfused patient were 2 compared to 5 (p=0.072, Table 5). In sum, among the HIT patients reported in this study, those with a therapeutic anticoagulation already at first monitoring appear to have less severe bleeding. This observation is in line with the data reported by the GEHT-HIT study group.[9] However, in countries were argatroban is available, this drug may represent a safer treatment option for HIT patients with severe renal failure because its elimination is mainly hepatobiliary.[2]

Of note, among all 46 patients (retrospective cohort, n=31; prospective cohort, n=15) with therapeutic anticoagulation intensity already at first monitoring we observed a doubling of the baseline aPTT (see above) and 93.5 % of them (43/46) had a clottable TT_5 (median 17.2 sec; range: 9.4 – 45.7 sec). A comparison of these data with the in vitro calibration studies (Table 1) suggests that a plasma lepirudin concentration close to 0.50 µg/ml is sufficient to achieve an adequate anticoagulation in HIT patients. This is about 1/3 of the upper limit of the therapeutic range (0.15 – 1.50 µg/ml) reported in the lepirudin product information leaflet and at the lower limit of the therapeutic concentration (0.6 – 1.0 µg/ml) reported by Greinacher and Warkentin.[4]

Among the investigational patient cohort, there was a significant dose reduction from a median starting dose of 0.078 mg kg^{-1} h^{-1} to a final one of 0.034 mg kg^{-1} h^{-1} in patients with normal renal function who were within therapeutic ranges since first monitoring Table 3). This might be explained by the development of antibodies decreasing lepirudin clearance[7] or by a reduced lepirudin requirement consecutive to the attenuation of the procoagulant state characteristic of acute HIT.[3] This observation suggests that even lower lepirudin

dosages may be required in the course of HIT treatment and that lepirudin dose should be assessed daily by clotting assays and course of platelets and D-dimers.

In conclusion, we show that omission of the initial bolus and a starting lepirudin dose of 0.08 mg kg^{-1} h^{-1} for HIT patients with normal renal function (CrCl > 60 ml/min), 0.04 mg kg^{-1} h^{-1} for those with moderately impaired renal function (CrCl 30 – 60 ml/min) and 0.01 – 0.02 mg kg^{-1} h^{-1} for those with CrCl < 30 ml/min is an efficacious treatment of the prothrombotic state of acute HIT and appears to be associated with a less severe bleeding tendency as compared to that induced by the officially recommended lepirudin dosage. Our proposal is in line with the recently published ACCP-guideline;[2] however, with some differences: the ACCP-panel stratifies renal function by serum creatinine only and for patients with normal and moderately reduced renal function recommends slightly higher lepirudin doses as we report. Full validation of our proposed regimen will require appropriately designed multi-center studies.

Acknowledgments

We would like to thank all the physicians of our Department of Hematology who were involved in care of HIT patients over the years, the technicians of the Central Hematology Laboratory who perform routine diagnostic HIT assays, Irmela Sulzer for performing the GTI-ELISA and Therese Jost for administrative assistance.

Lorenzo Alberio is supported by a grant from the Swiss National Science Foundation (Grant Nr. 3200-065337.01)

Authorship and Conflict of Interest Statements

M. Tschudi collected and analysed the data, and wrote the first draft of the manuscript.

B. Lämmle helped designing research and writing the manuscript.

L. Alberio conceived the study, collected and analysed the data and wrote the manuscript.

The authors declare no competing financial interests.

References

1. Keeling D, Davidson S, Watson H. The management of heparin-induced thrombocytopenia. Br J Haematol. 2006;133:259-269
2. Warkentin TE, Greinacher A, Koster A, Lincoff AM. Treatment and prevention of heparin-induced thrombocytopenia: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133:340S-380S
3. Chilver-Stainer L, Lämmle B, Alberio L. Titre of anti-heparin/PF4-antibodies and extent of in vivo activation of the coagulation and fibrinolytic systems. Thromb Haemost. 2004;91:276-282
4. Greinacher A, Warkentin TE. The direct thrombin inhibitor hirudin. Thromb Haemost. 2008;99:819-829
5. Gray E, Harenberg J. Collaborative study on monitoring methods to determine direct thrombin inhibitors lepirudin and argatroban. J Thromb Haemost. 2005;3:2096-2097
6. Lubenow N, Eichler P, Lietz T, Greinacher A. Lepirudin in patients with heparin-induced thrombocytopenia - results of the third prospective study (HAT-3) and a combined analysis of HAT-1, HAT-2, and HAT-3. J Thromb Haemost. 2005;3:2428-2436
7. Eichler P, Friesen HJ, Lubenow N, Jaeger B, Greinacher A. Antihirudin antibodies in patients with heparin-induced thrombocytopenia treated with lepirudin: incidence, effects on aPTT, and clinical relevance. Blood. 2000;96:2373-2378
8. Hacquard M, de Maistre E, Lecompte T. Lepirudin: is the approved dosing schedule too high? J Thromb Haemost. 2005;3:2593-2596
9. Tardy B, Lecompte T, Boelhen F, Tardy-Poncet B, Elalamy I, Morange P, Gruel Y, Wolf M, Francois D, Racadot E, Camarasa P, Blouch MT, Nguyen F, Doubine S, Dutrillaux F, Alhenc-Gelas M, Martin-Toutain I, Bauters A, Ffrench P, de Maistre E, Grunebaum L, Mouton C, Huisse MG, Gouault-Heilmann M, Lucke V. Predictive factors for thrombosis and major bleeding in an observational study in 181 patients with heparin-induced thrombocytopenia treated with lepirudin. Blood. 2006;108:1492-1496
10. Tschudi M, Lämmle B, Alberio L. Dosing lepirudin in patients with heparin-induced thrombocytopenia and various degress of renal function impairment. J Thromb Haemost. 2007;5 Supplement 2:P-S-673 (Abstract)

11. Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. Br J Haematol. 2003;121:535-555
12. Alberio L, Kimmerle S, Baumann A, Mansouri Taleghani B, Demarmels Biasiutti F, Lämmle B. Rapid determination of anti-heparin/platelet factor 4 antibody titers in the diagnosis of heparin-induced thrombocytopenia. Am J Med. 2003;114:528-536
13. Alberio L. Heparin-induced thrombocytopenia: some working hypotheses on pathogenesis, diagnostic strategies and treatment. Curr Opin Hematol. 2008;15:456-464
14. Meyer O, Salama A, Pittet N, Schwind P. Rapid detection of heparin-induced platelet antibodies with particle gel immunoassay (ID-HPF4). Lancet. 1999;354:1525-1526
15. Zürcher M, Sulzer I, Barizzi G, Lämmle B, Alberio L. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. Thromb Haemost. 2008;99:416-426
16. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16:31-41

Table 1. Defining target ranges for lepirudin

Lepirudin	**$TT_{1.5}$**	**TT_5**	**aPTT**	
µg/ml	sec	sec	sec	ratio
	min-max	min-max	**median** [IQR]	**median** [IQR]
0.00	13.2 – 16.2	---	**29.0** [28.8 – 30.2]	**1**
0.10	21.4 – 29.6	---	**39.0** [38.8 – 41.0]	**1.35** [1.34 – 1.38]
0.15	34.4 – 46.5	---	**42.1** [41.2 – 45.5]	**1.45** [1.42 – 1.53]
0.25	no clot *	8.5 – 9.4	**49.4** [46.9 – 52.0]	**1.71** [1.62 – 1.75]
0.50	no clot	16.1 – 38.4	**59.8** [58.4 – 63.8]	**2.06** [2.02 – 2.15]
0.75	no clot	50.6 – no clot	**69.0** [66.2 – 73.2]	**2.39** [2.33 – 2.43]
1.00	no clot	91.3 – no clot	**72.9** [70.8 – 81.2]	**2.56** [2.46 – 2.69]
1.25	no clot	no clot	**79.8** [77.3 – 86.5]	**2.81** [2.68 – 2.87]
1.50	no clot	no clot	**85.1** [82.1 – 92.3]	**3.01** [2.84 – 3.06]
1.75	no clot	no clot	**90.9** [86.3 – 98.3]	**3.15** [3.03 – 3.25]
2.00	no clot	no clot	**95.8** [92.0 – 102.7]	**3.29** [3.18 – 3.47]

Pooled normal plasma was spiked with increasing concentrations of lepirudin. Thrombin time (TT) and aPTT were measured with different reagent lots (n = 7). See Methods section for details.

* = no clot signifies TT > 120 sec.

Table 2. Patient characteristics

		Investigation cohort (N=53)		Evaluation cohort (N=15)	
Personal data					
Age, years	median (IQR)	69.4	(58.9 – 74.0)	69.2	(61.2 – 74.7)
Gender, females	n (%)	21	(40)	6	(40)
Body weight, kg	median (IQR)	79.7	(70.7 – 87.7)	77.5	(71.0 – 80.0)
HIT work-up					
Platelet count at day 0, 10^9/L	median (IQR)	53	(37 – 88)	53	(28 – 66)
Thrombosis at day 0 (HIT-T)	n (%)	27	(51)	7	(47)
D-dimer at day 0, µg/L	median (IQR)	5'510	(3'401 – 6'129)	4'874	(3'277 – 10'688)
Pre-test score ("4T")	median (range)	4	(4 – 7)	4	(4 – 6)
Titer (ID-HPF4-PaGIA)	median (range)	8	(2 – 128)	4	(2 – 256)
O.D. (GTI-PF4)	median (IQR)	2.801	(1.822 – 3.000)	2.401	(1.300 – 2.868)
Renal function					
Creatinine, µmol/l	median (IQR)	114	(75 – 150)	130	(81 – 198)
CrCl >60 ml/min	n (%)	29	(55)	5	(33.3)
CrCl 30-60 ml/min	n (%)	15	(28)	5	(33.3)
CrCl <30 ml/min	n (%)	9	(17)	5	(33.3)

IQR = interquartile range; Day 0 = day of clinical and laboratory evaluation for suspected HIT; HIT-T = HIT with thrombosis;
O.D. = optical density; CrCl = creatinine clearance

Table 3. Renal function, anticoagulation intensity at first control and lepirudin dose (investigation cohort)

CrCl (ml/min)	N	Anticoagulation intensity *	n	Lepirudin dose (mg kg^{-1} h^{-1})				p-value	
				Median [interquartile range]					
				start		end		paired	
> 60	29	over	8	**0.098**	[0.081 – 0.124]	**0.033**	[0.014 – 0.055]	0.007	
		target	17	**0.078**	[0.049 – 0.093]	**0.034**	[0.027 – 0.058]	0.001	
		under	4	**0.022**	[0.014 – 0.039]	**0.036**	[0.030 – 0.042]	0.235	
				0.001		*0.867*			ANOVA
30 - 60	15	over	6	**0.098**	[0.086 – 0.102]	**0.015**	[0.011 – 0.029]	0.005	
		target	7	**0.040**	[0.025 – 0.050]	**0.037**	[0.017 – 0.047]	0.294	
		under	2	**0.010**	[0.009 – 0.011]	**0.011**	[0.007 – 0.016]	0.737	
				< 0.001		*0.313*			ANOVA
< 30	9	over	0	---		---			
		target	7	**0.013**	[0.008 – 0.026]	**0.007**	[0.003 – 0.018]	0.182	
		under	2	**0.008**	[0.006 – 0.009]	**0.005**		N/A	
				0.316		*N/A*			ANOVA

* = “over”, TT/aPTT above therapeutic ranges; “target” TT/aPTT within therapeutic ranges; “under”, TT/aPTT below therapeutic ranges 4h after lepirudin start

Table 4. Thrombotic complications during lepirudin treatment

Patient	Gender	Age	HIT-T	anti-PF4/H antibodies		CrCl	Lepirudin treatment			Anticoagulation	Dose adjustments			Event	
							Dose		Length	at first monitoring	↑	↓	Stop *	Localization	Day
		yrs		titer	O.D.	ml/min	mg kg^{-1} h^{-1}		days		n	n	n		
							start	end							
A	Male	36.0	arterial	32	2.426	64	0.100	0.014	7	over	3	5	2	Amp., distal	7
B	Female	73.5	ven.bp	16	2.129	35	0.150	0.003	17	over	3	13	3	Amp., prox.	6
C	Male	50.2	arterial	64	2.801	95	0.100	0.070	5	over	0	3	0	Amp., distal	4
D	Female	88.7	arterial	16	2.260	24	0.020	0.020	7	target	0	0	2	DVT; PE	5; 7

Amp. = Limb amputation (distal = below-knee, proximal = above-knee); DVT = Deep vein thrombosis; PE = Pulmonary embolism; HIT-T = HIT with thrombosis; CrCl = creatinine clearance; O.D. = optical density; “over” = TT/aPTT above therapeutic ranges; “target” = TT/aPTT within therapeutic ranges; ven.bp = occlusion of a greater saphenous vein by-pass in the context of chronic peripheral arterial occlusive disease.

* Stop = Instances where lepirudin infusion was temporarily interrupted for 2 hours or longer

Table 5. Bleeding complications during lepirudin treatment

		Investigation cohort			Evaluation cohort
Anticoagulation intensity *		**over** (N=14)	**target** (N=31)	**under** (N=8)	**target** (N=15)
Life-threatening bleeding	n	1	0	0	0
Hb-decrease, g/L	median (IQR)	11 (9-15)	10 (3-14)	10 (6-17)	10 (3-14)
Hb-decrease ≥ 20 g/L	n/N (%)	3/14 (21.4)	3/31 (9.7)	2/8 (25.0)	1/15 (6.7)
Patients requiring PRBC	n/N (%)	6/14 (43.6)	10/31 (32.6)	1/8 (12.5)	5/15 (33.3)
Patients requiring > 2 PRBC	n/N (%)	4/14 (28.6)	3/31 (9.7)	1/8 (12.5)	1/15 (6.7)
Units PRBC / transfused patient	median	5	2	6	2

PRBC = packed red blood cells

* = “over”, TT/aPTT above therapeutic ranges; “target”, TT/aPTT within therapeutic ranges; “under”, TT/aPTT below therapeutic ranges at initial monitoring, 4 hours after starting lepirudin infusion

Figure legends

Figure 1. Anticoagulation intensity at first laboratory monitoring 4 hours after starting lepirudin infusion (investigation cohort). Panel A: patients with normal renal function (CrCl > 60 ml/min, n=29) classified according to lepirudin starting dose: > 0.11 mg kg^{-1} h^{-1} (n=2), 0.09 – 0.11 kg^{-1} h^{-1} (n=11), 0.03 – 0.089 kg^{-1} h^{-1} (n=11), and 0.01 – 0.029 kg^{-1} h^{-1} (n=5). **Panel B**: patients with moderately impaired renal function (CrCl 30 – 60 ml/min, n=15) classified according to lepirudin starting dose: > 0.09 mg kg^{-1} h^{-1} (n=4), 0.07 – 0.089 mg kg^{-1} h^{-1} (n=3), 0.03 – 0.069 mg kg^{-1} h^{-1} (n=4), 0.01 – 0.029 mg kg^{-1} h^{-1} (n=4), and < 0.01 mg kg^{-1} h^{-1} (n=1). **Panel C**: patients with severely impaired renal function (CrCl < 30 ml/min, n=9) classified according to lepirudin starting dose: 0.03 – 0.069 mg kg^{-1} h^{-1} (n=1), 0.01 – 0.029 mg kg^{-1} h^{-1} (n=4), and < 0.01 mg kg^{-1} h^{-1} (n=4). Black columns represent patients with clotting times over, dashed bars represent patients with clotting times within and white bars patients with clotting times below therapeutic ranges (see Methods section for details).

Figure 2. Course of platelet count and D-dimer among the 31 patients of the investigation cohort who were within therapeutic ranges already at first monitoring after starting lepirudin. Panel A: Platelet count; mean values ± 1SD. Black circles represent patients with normal renal function (CrCl > 60 ml/min; n=17), open circles those with moderately impaired renal function (CrCl 30 – 60 ml/min; n=7), and black triangles those with severely impaired renal function (CrCl < 30 ml/min; n=7). **Panel B:** Box plot of

D-dimers in all 31 patients depicting the median value (horizontal line within the box), the 25th and 75th percentiles (lower and upper borders of the box), the 10th and 90th percentiles (lower and upper whiskers), and outliers outside the 10th and 90th percentiles (black dots). Day 0 is the day of clinical and laboratory evaluation for suspected HIT and start of lepirudin treatment.

Figure 3. Course of platelet count and D-dimer among the 15 patients of the evaluation cohort. Panel A: Platelet count; mean values ± 1SD. Black circles represent patients with normal renal function (CrCl > 60 ml/min; n=5), open circles those with moderately impaired renal function (CrCl 30 – 60 ml/min; n=5), and black triangles those with severely impaired renal function (CrCl < 30 ml/min; n=5). **Panel B:** Box plot of D-dimers in all 15 patients depicting the median value (horizontal line within the box), the 25th and 75th percentiles (lower and upper borders of the box), the 10th and 90th percentiles (lower and upper whiskers), and outliers outside the 10th and 90th percentiles (black dots). Day 0 is the day of clinical and laboratory evaluation for suspected HIT and start of lepirudin treatment.

Figure 1A

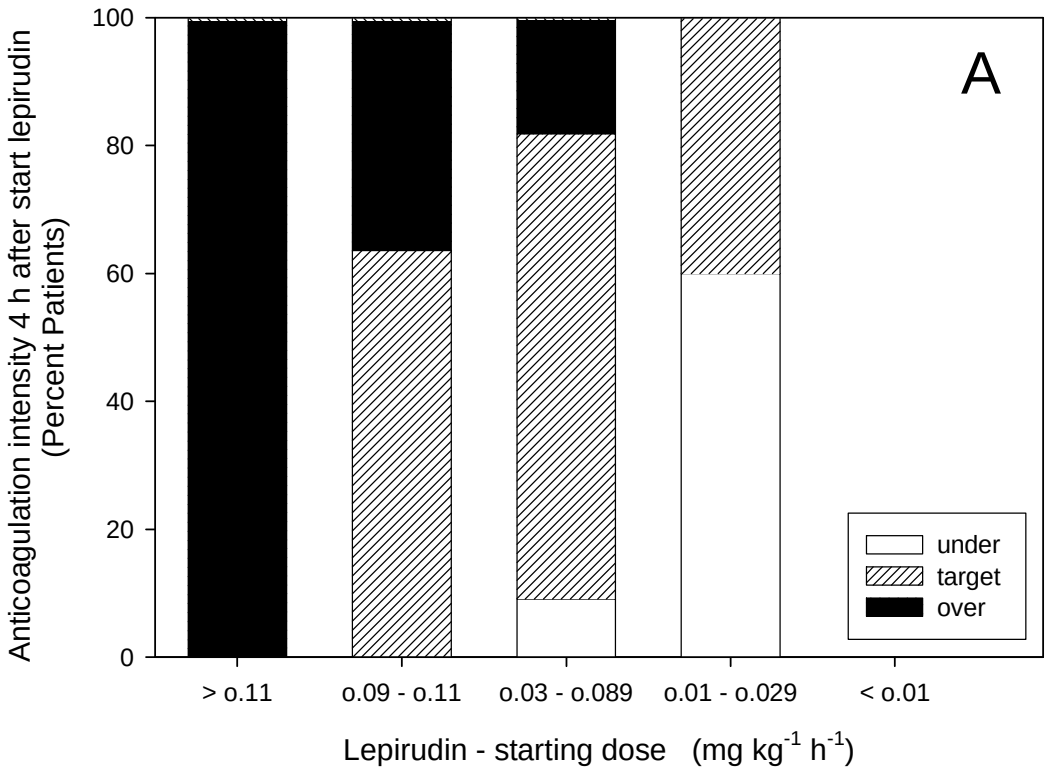

Figure 1B

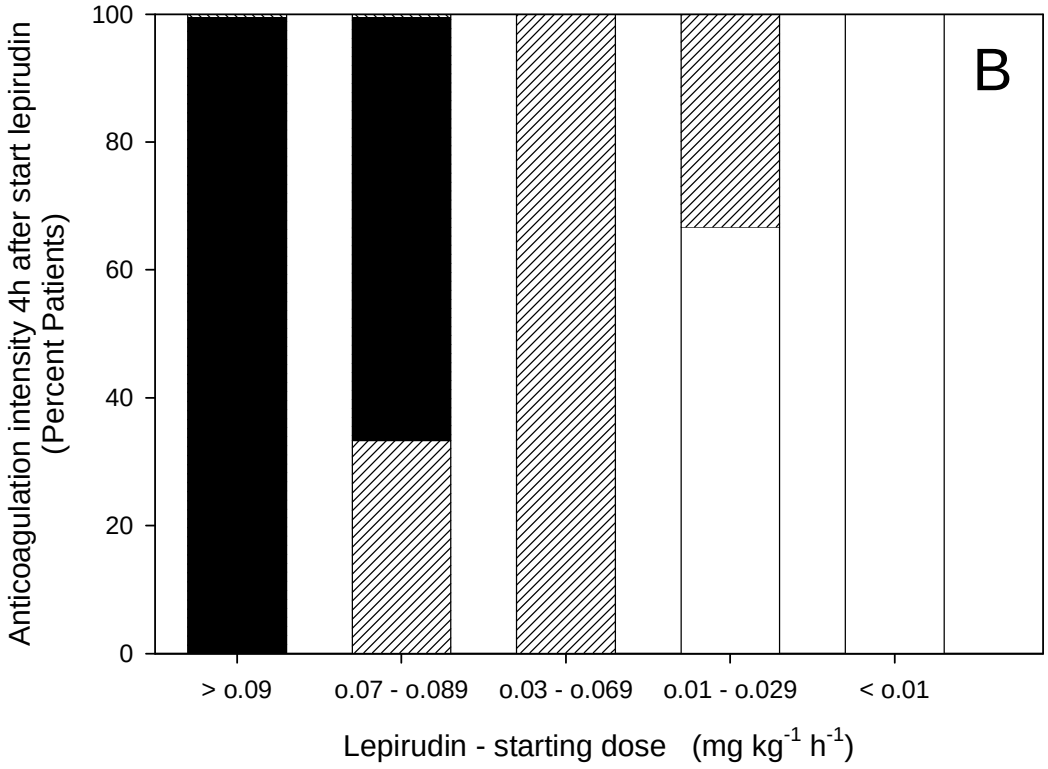

Figure 1C

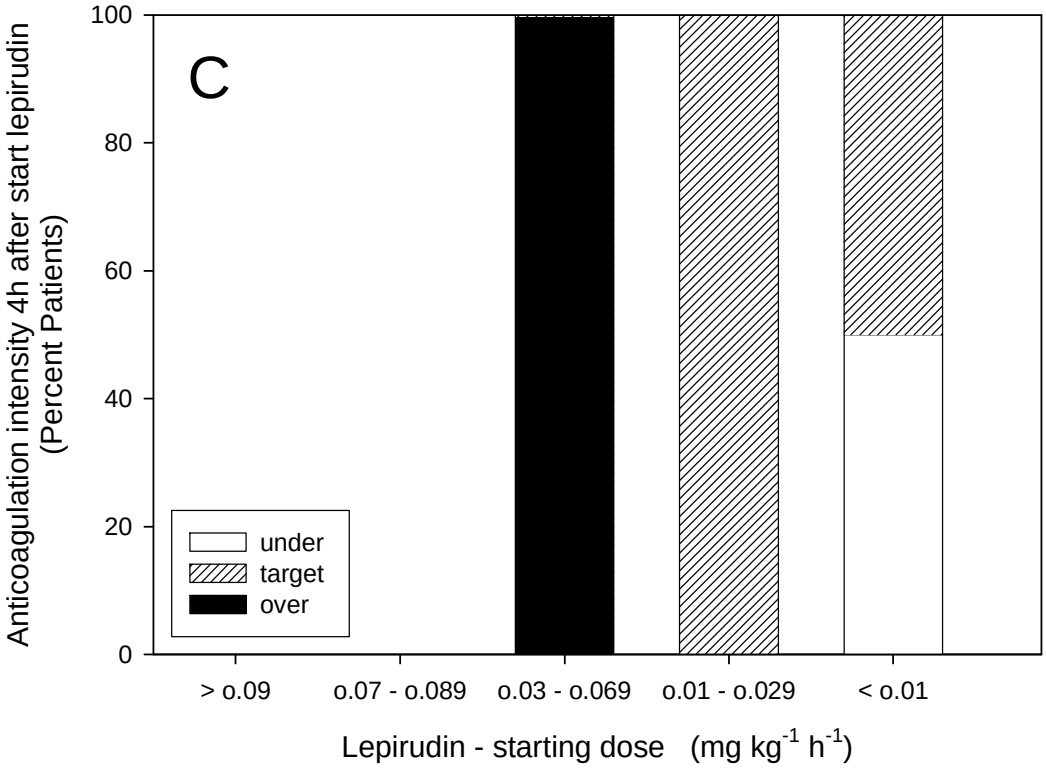

Figure 2A

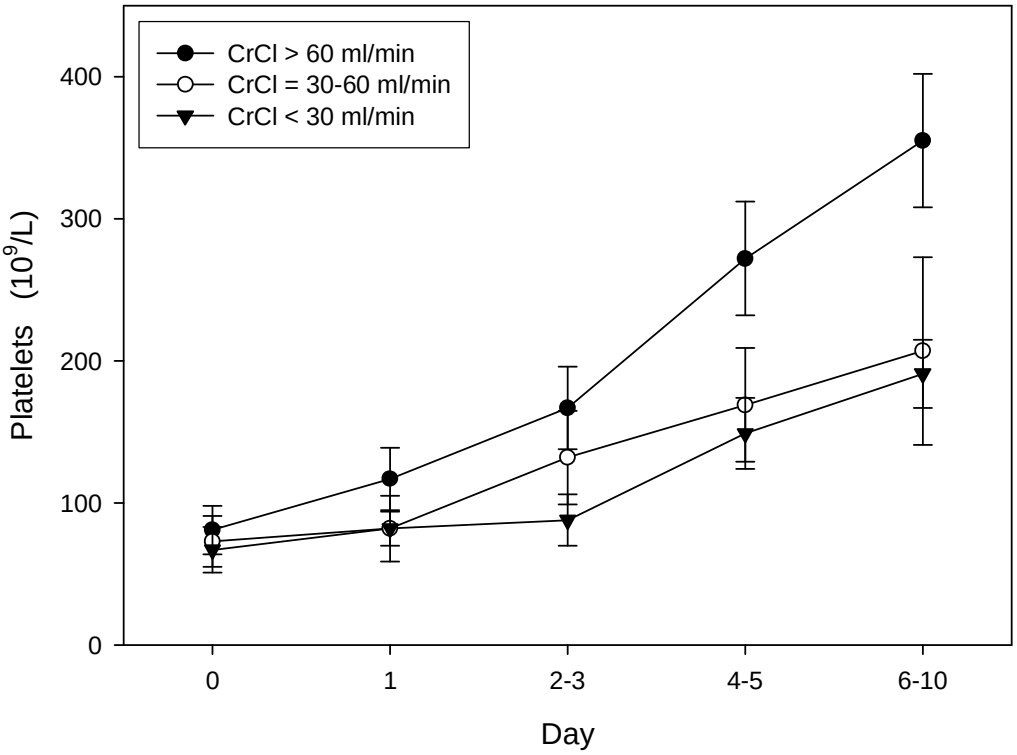

Figure 2B

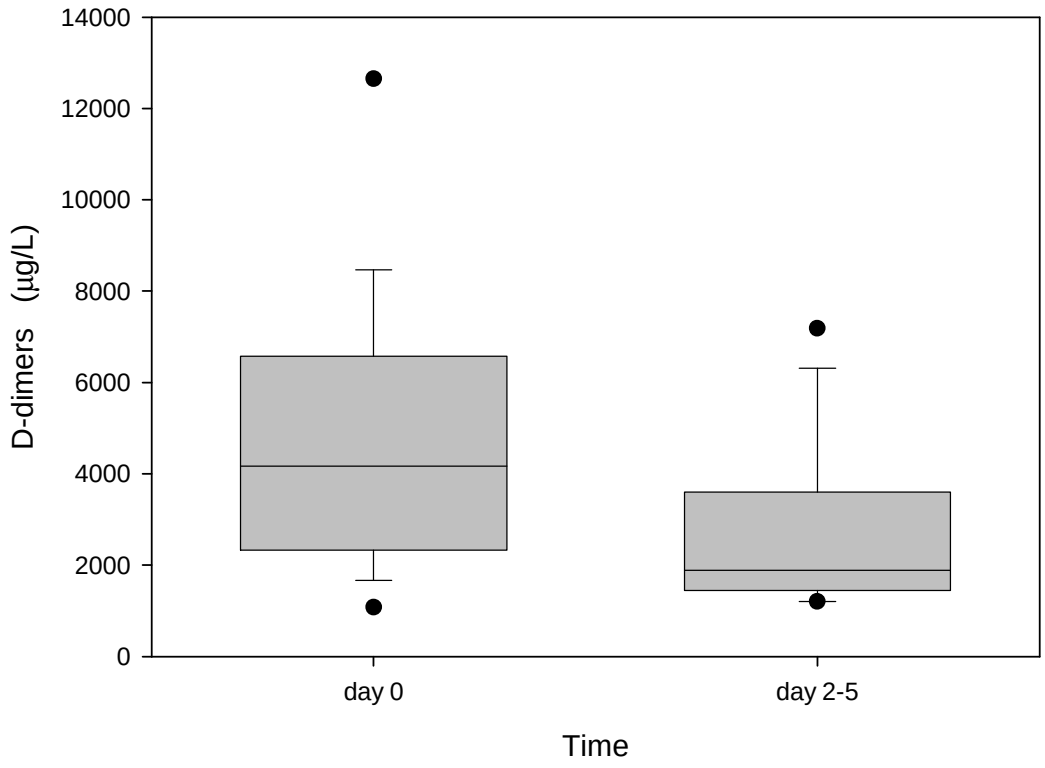

Figure 3A

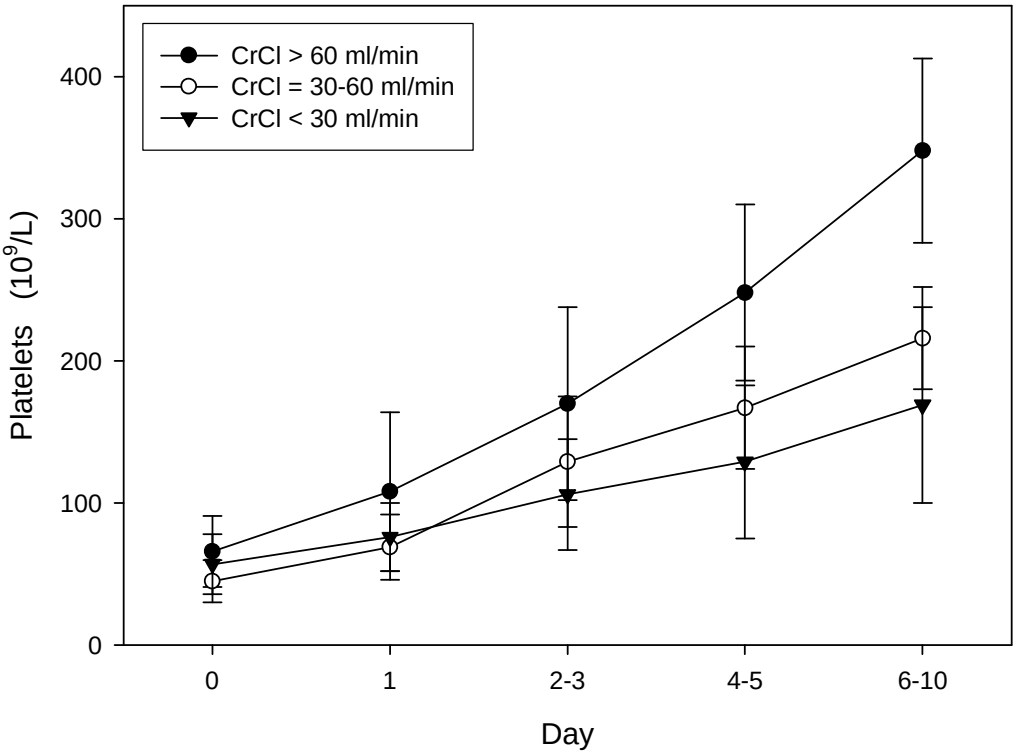

Figure 3B