C. Back and H. Knauß performed the research, W.D. Ludwig contributed leukaemic cells, M. Schrappe provided clinical data, K.M. Debatin contributed to data interpretation and C. Beltinger designed the research and wrote the paper. We thank Peter Seibel, BBZ, Universität Leipzig for rho-cells and stimulating discussions.

Christina Back[1]
Helgard Knauß[1]
Wolf-Dieter Ludwig[2]
Martin Schrappe[3]
Klaus-Michael Debatin[1]
Christian Beltinger[1]

[1]*Department of Paediatrics and Adolescent Medicine, University Medical Centre Ulm, Ulm,* [2]*Department of Haematology and Oncology, Robert-Roessle-Clinic at HELIOS Klinikum Berlin, Charité-Campus Buch, and* [3]*Department of Paediatrics, University Hospital Schleswig-Holstein, Kiel, Germany.*
E-mail: christian.beltinger@uniklinik-ulm.de

Keywords: acute lymphoblastic leukaemia, mitochondrial genome, apoptosis, chemoresistance, doxorubicin.

First published online 25 May 2011
doi: 10.1111/j.1365-2141.2011.08743.x

Supporting information

Additional Supporting information may be found in the online version of this article:

Fig S1. L196 and surrounding amino acids of *MT-CO1* are evolutionary conserved.

Table SI. Non-remitting paediatric ALL blasts harbour variants in the mt genome.

Appendix S1. Materials and methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J., Staden, R. & Young, I.G. (1981) Sequence and organization of the human mitochondrial genome. *Nature*, **290,** 457–465.

Brandon, M., Baldi, P. & Wallace, D.C. (2006) Mitochondrial mutations in cancer. *Oncogene*, **25,** 4647–4662.

Carew, J.S., Zhou, Y., Albitar, M., Carew, J.D., Keating, M.J. & Huang, P. (2003) Mitochondrial DNA mutations in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. *Leukemia*, **17,** 1437–1447.

Doroshow, J.H. (1983) Anthracycline antibiotic-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. *Cancer Research*, **43,** 4543–4551.

Grist, S.A., Lu, X.J. & Morley, A.A. (2004) Mitochondrial mutations in acute leukaemia. *Leukemia*, **18,** 1313–1316.

Holleman, A., den Boer, M.L., de Menezes, R.X., Cheok, M.H., Cheng, C., Kazemier, K.M., Janka-Schaub, G.E., Gobel, U., Graubner, U.B., Evans, W.E. & Pieters, R. (2006) The expression of 70 apoptosis genes in relation to lineage, genetic subtype, cellular drug resistance, and outcome in childhood acute lymphoblastic leukemia. *Blood*, **107,** 769–776.

Petros, J.A., Baumann, A.K., Ruiz-Pesini, E., Amin, M.B., Sun, C.Q., Hall, J., Lim, S., Issa, M.M., Flanders, W.D., Hosseini, S.H., Marshall, F.F. & Wallace, D.C. (2005) mtDNA mutations increase tumorigenicity in prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America*, **102,** 719–724.

Shidara, Y., Yamagata, K., Kanamori, T., Nakano, K., Kwong, J.Q., Manfredi, G., Oda, H. & Ohta, S. (2005) Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Research*, **65,** 1655–1663.

Silverman, L.B., Gelber, R.D., Young, M.L., Dalton, V.K., Barr, R.D. & Sallan, S.E. (1999) Induction failure in acute lymphoblastic leukemia of childhood. *Cancer*, **85,** 1395–1404.

Suresh, A., Guedez, L., Moreb, J. & Zucali, J. (2003) Overexpression of manganese superoxide dismutase promotes survival in cell lines after doxorubicin treatment. *British Journal of Haematology*, **120,** 457–463.

Stability of low molecular weight heparin anti-factor Xa activity in citrated whole blood and plasma

Low molecular weight heparins (LMWH) are administered in fixed, weight-adjusted doses without the need of routine laboratory monitoring. However, measurement of anti-factor Xa (anti-FXa) activity is suggested in obese patients and in those with renal insufficiency (Samama & Poller, 1995; Kessler, 1997; Abbate *et al*, 1998) and recommended in pregnant women treated with therapeutic doses of LMWH (Hirsh *et al*, 2008). To the best of our knowledge there are no data regarding the influence of various sample processing and storage methods on LMWH anti-FXa activity.

The present study aimed to evaluate stability of LMWH anti-FXa activity in citrated whole blood (WB) and citrated

platelet-poor-plasma (PPP) stored at room temperature (RT), and in plasma samples after a freezing/thawing cycle.

Anti-FXa activity was investigated in 60 patients treated with different LMWH compounds at the University Hospital Inselspital, Bern, Switzerland, for whom monitoring of anti-FXa levels was required. Thirty patients (21 women; median age: 37·2 years, range: 11·2–77·5) received nadroparin (Fraxiparine/Fraxiforte, GlaxoSmithKline, Münchenbuchsee, Switzerland). In 19 of them with therapeutic treatment (nine pregnant women, seven individuals with cancer and three with renal insufficiency) blood sampling was performed either 4 h after dosing (n = 12) or immediately before the next administration (n = 7). Additionally, in 11 patients with prophylactic nadroparin treatment (six pregnant women, four patients with renal insufficiency and one with cancer) trough levels of anti-FXa activity were requested in order to exclude LMWH accumulation. Twenty patients (14 women; median age: 58·2 years, range 32·9–92·4) received enoxaparin (Clexane, Sanofi Aventis, Genève, Switzerland) in therapeutic (eight pregnant women, five individuals with renal insufficiency and one with cancer) or prophylactic (five pregnant women, one with renal insufficiency) dosage. Blood sampling was performed either 4 h after dosing (n = 14) or immediately before the next administration (n = 6). Finally, 10 patients (two males aged 55·5 and 75·5 years with cancer and renal insufficiency, and eight male children aged between 4·5 and 15·6 years with cancer) received dalteparin (Fragmin, Pfizer, Zürich, Switzerland) in therapeutic (n = 7; blood sampling 4 h after dosing) or prophylactic (n = 3; blood sampling immediately before next application) dosage.

Blood was collected by venipuncture into 0·106 mol/l tri-sodium citrate (9:1 vol/vol) in 5 ml plastic syringes (Monovette, Sarstedt, Nümbrecht, Germany) and sent to the laboratory by pneumatic tube mail. WB (2 ml) was centrifuged twice at 1500 ***g*** for 10 min each at 20°C. Anti-FXa activity was measured immediately (PPP fresh) and 600 μl of the remaining plasma was stored in a 750 μl capped polypropylene vial at RT for 4–6 and 24 h, when anti-FXa activity was measured again (PPP 4–6 h, PPP 24 h). An aliquot of 400 μl plasma was snap-frozen, stored in a 750 μl capped polypropylene vial at −20°C for 24 h, and subsequently assessed (PPP thawed). The remainder of WB (3 ml) were kept in the original collecting tube on a shelf rotator for 4–6 and 24 h, when 1 ml WB was centrifuged and immediately assessed (WB 4–6 h, WB 24 h). Anti-FXa activity was measured in 60 μl plasma by a chromogenic method (Biophen Heparin 6; Hyphen BioMed, Neuville-sur-Oise, France) according to the manufacturer's instruction on a BCS-XP coagulometer (Siemens Healthcare Diagnostics, Eschborn, Germany). The standard curve was prepared with commercial calibrators (Biophen LMWH Calibrator; Hyphen BioMed). The coefficient of variation (CV) between assays was 6·4% and 14·6% for anti-FXa levels close to 0·50 and 0·20 IU anti-FXa/ml, respectively. The assay does not provide excess antithrombin, and relies on the antithrombin activity of the sample. In order to assess the impact of the freeze-thawing process on antithrombin, we assessed its activity (Coamatic LR Antithrombin; Chromogenix – Instrumentation Laboratory, Milano, Italy) in 10 samples, without noticing a statistically significant difference between fresh (median 106%; interquartile range, IQR: 88–118) and thawed (median 101%; IQR:

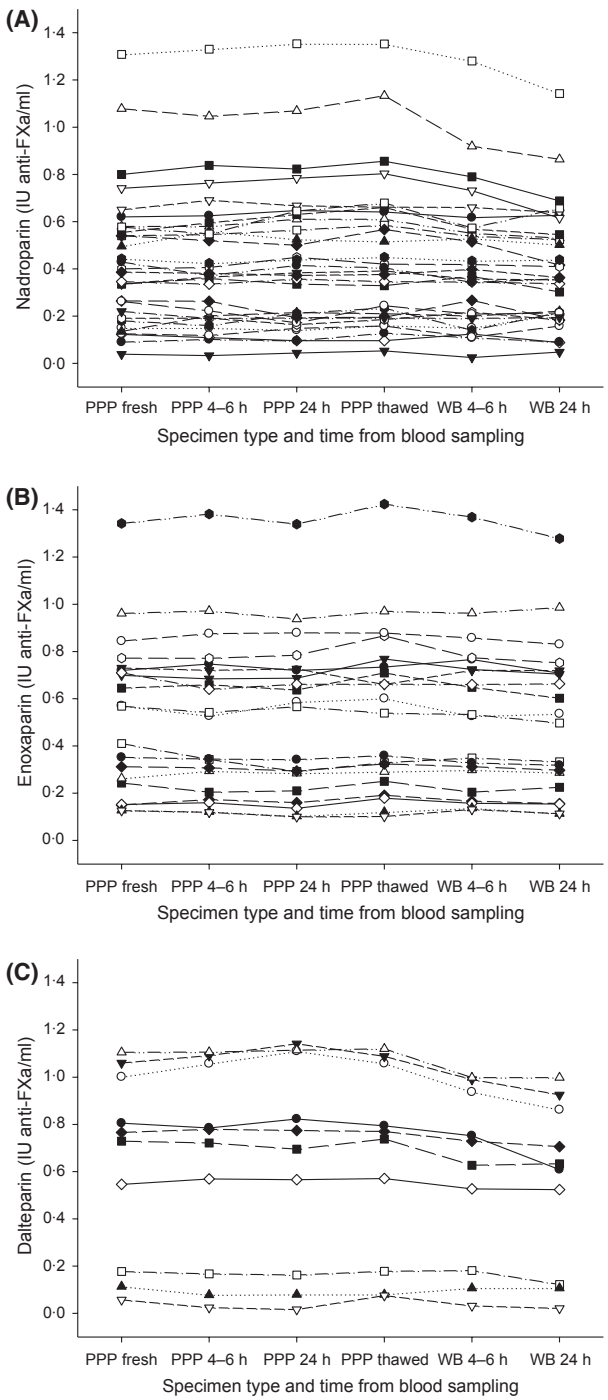

Fig 1. Preanalytical stability of anti-factor Xa activity. (A) Nadroparin (n = 30), (B) Enoxaparin (n = 20), (C) Dalteparin (n = 10). PPP, platelet poor plasma; WB, citrated whole blood; IU, international units.

81–109) plasma samples ($P = 0{\cdot}105$, Wilcoxon signed rank test, SigmaStat v3.1; Systat Software Inc., San Jose, CA, USA).

Friedman repeated measures analysis of variance on ranks showed statistically significant differences in all three treatment groups (nadroparin: $P = 0{\cdot}047$, Fig 1A; enoxaparin: $P = 0{\cdot}004$, Fig 1B; dalteparin: $P = 0{\cdot}002$, Fig 1C). Multiple comparisons versus anti-FXa activity in the fresh plasma samples demonstrated statistically significant lower values in WB 24 h samples ($P < 0{\cdot}05$; Dunn's method), with following median differences: −2·6% for nadroparin (IQR: −13·5 to 2·0), −4·8% for enoxaparin (IQR: −8·0 to −1·0), and −13% for dalteparin (IQR: −14·2 to −8·3). Anti-FXa activity measured in PPP 24 h and PPP thawed samples did not differ from the initial value. Of note, anti-FXa activity in WB 4–6 h samples also did not statistically differ from the initial value ($P > 0{\cdot}05$; Dunn's method).

It has been shown that platelet factor 4 (PF4) binding to heparin can lead to underestimation of heparin activity (Levine *et al*, 1984) but that this interaction is weaker with LMWH than unfractionated heparin (Denton *et al*, 1983). The present data indicate that the amount of PF4 released from platelets in citrated WB stored at RT for up to 24 h on a shelf rotator does not exert a clinically relevant inhibition of LMWH anti-FXa activity. In fact, observed median percentage changes were comparable to the method's CV and anti-FXa values measured in WB 24 h samples would not have led to different clinical decisions compared to fresh PPP results (for example, for nadroparin, the pair with the highest percentage decline: 0·183 vs. 0·264 IU anti-FXa/ml, or the sample with the second-highest anti-FXa activity: 0·864 vs. 1·078 IU anti-FXa/ml).

Our work shows that the LMWH compounds nadroparin, enoxaparin and dalteparin are stable in specimens stored at RT, either as fresh plasma for at least 24 h or as WB up to 4–6 h, and it also demonstrates that anti-FXa activity in WB stored at RT for 24 h shows a statistically significant, but clinically not relevant decrease (Fig 1). These data suggest that shipping plasma or WB specimens to a central laboratory does not significantly affect anti-FXa activity measurements. Of note, shipping blood in the original collection tube would enable the laboratory to control several preanalytical variables, such as anticoagulant type, tube filling, and centrifugation (Zürcher *et al*, 2008). Finally, this study also indicates that assessment of anti-FXa activity in frozen/thawed plasma samples is reliable for all three LMWH compounds investigated.

In conclusion, anti-FXa activity in citrated plasma and WB samples from patients treated with nadroparin, enoxaparin or dalteparin is sufficiently stable for 24 h after blood drawing in order to allow shipping to a central laboratory. This information is practically relevant for outpatients requiring monitoring of anti-FXa activity.

Authors contributions

N.B., D.B., T.C., A.H., K.W., M.P., M.R. and I.S.: performed research; G.B. and L.A. designed the research study, analyzed the data and wrote the paper.

Nicole Birri
Daniela Baumgartner
Tiziana Conte
Anh Huynh
Katja Weller
Marko Pavicic
Marianne Reusser
Ilse Siegenthaler
Gabriela Barizzi
Lorenzo Alberio

Department of Haematology and Central Haematology Laboratory, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland.
E-mail: lorenzo.alberio@insel.ch

Keywords: anticoagulation, heparin, laboratory haematology.

First published online 25 May 2011
doi: 10.1111/j.1365-2141.2011.08745.x

References

Abbate, R., Gori, A.M., Farsi, A., Attanasio, M. & Pepe, G. (1998) Monitoring of low-molecular-weight heparins in cardiovascular disease. *American Journal of Cardiology*, **82,** 33L–36L.

Denton, J., Lane, D.A., Thunberg, L., Slater, A.M. & Lindahl, U. (1983) Binding of platelet factor 4 to heparin oligosaccharides. *Biochemical Journal*, **209,** 455–460.

Hirsh, J., Bauer, K.A., Donati, M.B., Gould, M., Samama, M.M. & Weitz, J.I. (2008) Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*, **133,** 141S–159S.

Kessler, C.M. (1997) Low molecular weight heparins: practical considerations. *Seminars in Hematology*, **34,** 35–42.

Levine, S.P., Sorenson, R.R., Harris, M.A. & Knieriem, L.K. (1984) The effect of platelet factor 4 (PF4) on assays of plasma heparin. *British Journal of Haematology*, **57,** 585–596.

Samama, M.M. & Poller, L. (1995) Contemporary laboratory monitoring of low molecular weight heparins. *Clinics in Laboratory Medicine*, **15,** 119–123.

Zürcher, M., Sulzer, I., Barizzi, G., Lämmle, B. & Alberio, L. (2008) Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. *Thrombosis and Haemostasis*, **99,** 416–426.