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ORIGINAL ARTICLE

Population pharmacokinetics of enoxaparin in infants, children and adolescents during secondary thromboembolic prophylaxis: a cohort study

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Summary. *Background:* Enoxaparin has been extensively studied in adults on its safety and efficacy during prevention of symptomatic thromboembolism when acute anticoagulation or secondary prevention is required as a result of venous thrombosis or stroke. In children, it is still used off-label and little is known about the pharmacokinetics in children. *Objectives:* The aim of the present study was to evaluate whether a once- or twice-daily dosing regimen would be feasible in children to achieve appropriate plasma levels of enoxaparin. *Patients/methods:* A population pharmacokinetic model was developed using anti-factor (F)Xa activity data from 126 children (median age: 5.9 years) receiving enoxaparin either as a once- or twice-daily dosing regimen. *Results:* A two-compartment model was adequate for describing the enoxaparin kinetics. Body weight proved to be the most predictive covariate for clearance and central volume of distribution: clearance 15 mL h^{-1} kg^{-1}, central volume of distribution 169 mL kg^{-1}, intercompartmental clearance 58 mL h^{-1}, peripheral volume of distribution 10 L and absorption rate 0.414 h^{-1}. Interindividual variability was found to be 54% for clearance and 42% for volume of distribution. *Conclusion:* The model is capable of describing all age groups and dosing levels of our population and predicts 12 h and 24 h enoxaparin activities sufficiently. According to our results, a once-daily enoxaparin dosing regimen with frequent monitoring is feasible. In 53.2% of the patients the median 24 h trough level was above the desired range of 0.1 IU mL^{-1} anti-FXa activity for prophylaxis therapy.

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Introduction

In pediatric patients with venous thromboembolism (VTE), appropriate patient management is seriously hampered by the lack of evidence from clinical trials, and children with vascular accidents are treated according to recommendations based on small-scale pediatric studies and guidelines adapted from adult patient protocols [1–3]. Until recently, unfractionated heparin (UFH) followed by oral anticoagulation has been considered the initial anticoagulant regimen of choice for the treatment of VTE in children [1,3]. However, low-molecular-weight-heparin (LMWH) such as enoxaparin is increasingly used in the pediatric population [3–14]. Current knowledge demonstrates that therapy with LMWH is at least equally efficacious and safe as UFH in the prevention and treatment of VTE in adult patients [15]. Clinical studies in adults have demonstrated several benefits of LMWH over UFH [16,17]. LMWHs are at least as effective as UFH. The frequency of bleeding complications and heparin-induced thrombocytopenia is significantly lower and as the pharmacokinetics of LMWH is more predictable than those of UFH, the frequency of monitoring via anti-factor (F)Xa assays is minimized [16–18]. In addition, the relatively long half-life of LMWH allows for once- (q24 h) or twice- (q12 h) daily subcutaneous application, which has been shown in adults and was also proposed for children [10,11,19,20]. Both therapeutic regimens, that is q24 h or q12 h, had no impact on safety or efficacy outcomes in the treatment of acute VTE in the patient cohorts investigated [10,19,20].

The primary aim of the present cohort study was to establish a population pharmacokinetic (PopPK) model based on the data from routinely collected blood samples during Therapeutic Drug Monitoring (TDM) for enoxaparin. The model will allow prediction of anti-FXa activities in pediatric patients receiving LMWH administered q24 h or q12 h, respectively.

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The secondary endpoints of this cohort study were the evaluation of safety and efficacy. Results from these studies will be used as a basis for future randomized controlled multicenter studies assessing safety and efficacy data of enoxaparin in the pediatric population.

Patients and methods

Study design

The present cohort study is an open-label, non-randomized, follow-up study in pediatric patients treated with enoxaparin for a first VTE. The patients were enrolled during routine TDM to establish a PopPK model and were assigned to either the q12 h or the q24 h dosing group. Based on Chest 2004 recommendations, we planned to administer standard LMWH administration twice daily in 33% (1/3) of patients and in 66% of patients (2/3) once daily according to our pilot protocol independently of thrombotic location, underlying medical condition or inherited thrombophilia status (Fig. 1). Patients were prospectively followed-up over a period of 12 months after VTE onset, efficacy study endpoints were recurrent VTE and post-thrombotic syndrome (PTS); safety endpoints were minor, major and clinically relevant non-major bleeding while on LMWH therapy. Major bleeding was defined as fatal bleeding or clinically overt bleeding associated with a decrease in hemoglobin of at least 20 g L^{-1} in 24 h, requiring transfusion of red blood cells, located retroperitoneal, intracranial, intraspinal, intraocular, or intraarticular or those bleedings which were deemed by the attending physician to necessitate permanent discontinuation of trial medication. Bleeding which results in any unusual medical or surgical interventions is defined as clinically relevant non-major bleeding. Minor bleeding are those bleedings which do not meet the criteria for major or clinically relevant non-major bleeding.

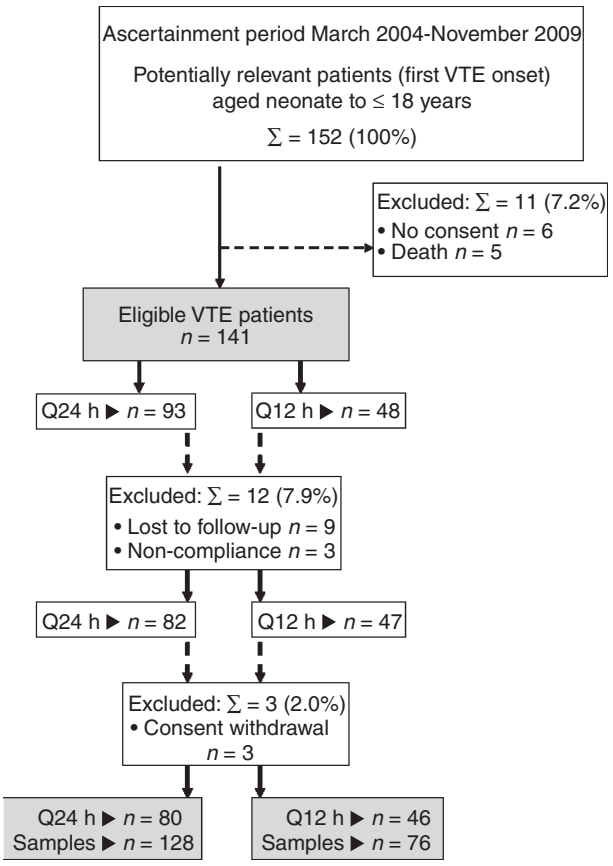

Fig. 1. Study design: enrollment, exclusions, follow-up and outcome.

Patients

One hundred and fifty-two unselected newly ascertained pediatric patients with symptomatic VTE were enrolled consecutively between March 2004 and November 2009. Out of these patients, a total of 141 patients were started on either a q24 h or a q12 h dosing regimen with the LMWH enoxaparin. After exclusion of patients owing to non-compliance, consent withdrawal and lost during follow-up a total of 126 patients (60 females and 66 males) were eligible for analysis (Fig. 1). Out of these patients eight were already reported in the previous open-label pilot study by Schobess *et al.*, in which the long-term safety and efficacy of enoxaparin were tested in children receiving enoxaparin either once daily or twice daily for venous thrombosis [10]. Inclusion criteria in this cohort study were a VTE confirmed objectively by standard imaging methods, that is compression sonography, venography, computed tomography (CT), spiral CT or magnetic resonance imaging (MRI) and perfusion lung scans, for the diagnosis of pulmonary embolism (PE). VTE/recurrent VTE in the deep veins of the leg was defined when venography performed in the acute phase of a new vascular accident showed fresh thrombotic material within a lumen of the vein, which is a new intraluminal filling defect compared with the previous tests. Children who had suffered arterial thrombosis were excluded. Further exclusion criteria were premature birth (≤ 36 gestational weeks), ongoing liver or renal diseases. The present study was performed in accordance with the ethical standards laid down in the updated relevant version of the Declaration of Helsinki: informed signed parental consent was obtained from each study participant or the patient's parent or guardians.

Anticoagulation protocol

According to the German treatment protocol, pediatric patients with a first VTE received enoxaparin twice daily during the acute thrombotic onset [10,21]. The enoxaparin dose was based on the patient's body weight. After the acute thrombotic treatment period (10–14 days), LMWH was administered as standard care q24 h or q12 h and dose adjustment was done based on anti-FXa activity. For efficacy reasons, the prophylactic 24 h anti-FXa activity trough level was targeted at 0.1 IU mL^{-1} [22,23], and the 2 or 4 h peak level was targeted at 0.5–0.8 IU mL^{-1} for safety reasons according to previous bleeding occurrences with an anti-FXa activity peak level of 1.0 IU mL^{-1} published by Dix *et al.* and Schobess *et al.* [5,10]. In the q24 h group, patients < 12 months of age

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received a starting dose of 1.5 mg kg^{-1} (150 anti-FXa IU kg^{-1}) followed by an adjusted maintenance dose not exceeding an anti-FXa activity peak level of 0.8 IU mL^{-1}. Patients > 12 months of age were started on 1 mg kg^{-1} (100 anti-FXa IU kg^{-1}) with adjustment for maintenance therapy as decribed [10,24,25]. All patients received a maintenance dose for > 3 months.

Clinical follow-up procedures

The routine diagnostic tests performed included physical examination by an experienced pediatric physician, standard imaging methods, carried out at first thrombotic onset, 6-8 weeks, 3 and 6 months later (before withdrawal of anticoagulation).

Blood sample collection When patients had reached steady state, blood samples were taken during routine TDM after the second and fourth LMWH injection either at baseline prior to the next dose or and at least one sample at 2, 4, 8 or 12 h after administration as per clinical management. The dose was adjusted if necessary. When the target range was achieved further blood sample collections were performed monthly along with partial thromboplastin time (PTT), D-Dimer and blood cell counts. Blood samples were collected by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt AG & Co., Nümbrecht, Germany) and placed immediately on melting ice. Platelet-poor plasma was prepared by centrifugation at 3000 $\times g$ for 2 × 20 min at 4 °C, aliquoted in polystyrene tubes and stored at −70 °C and thawed immediately before assay.

Laboratory analyses

PTT, antithrombin and anti-FXa activity were measured on a Dade Siemens BCS analyzer using pathromtin SL™ and BC thrombin-reagent™ from Dade Siemens and chromogenic substrates (Berichrom antithrombin™: Dade Siemens; Coamatic heparin™: Haemochrom). Intrassay-coefficient of variation (ICV) was 3.1% for antithrombin and 3.6% for heparin (0.7 IU mL^{-1}); run-to-run coefficient of variation (RCV) was 6.5% for antithrombin and 2.8% for heparin, respectively. The assay had a limit of quantification of 0.05 IU mL^{-1}, and linearity was demonstrated over the range of 0.05–1.5 IU mL^{-1}.

PopPK analysis

Enoxaparin plasma levels are described by anti-FXa activity. Anti-FXa activity-time data were analyzed using Nonlinear Mixed Effect Modeling with NONMEM (version VI; ICON Development Solutions, Ellicott City, MD, USA) [26]. Basic one and two compartment models with subroutines ADVAN 2, TRANS 2, ADVAN 4 and TRANS 4 were tested. First-order (FO), first-order conditional estimation (FOCE) and first-order conditional estimation with interaction (FOCE-I) as approximation methods were applied. Interindividual variability (IIV) was included in the model by an exponential scale on each population parameter. By assuming heteroscedasticity, the residual variability was initially modeled using a proportional error model. In addition, an additive and a combined error model for the residual variability were examined to investigate the model misspecification. PK parameters obtained through empirical Bayesian parameter estimation from the basic model were plotted against covariates such as body surface area (BSA), body weight and age [27]. Covariates showing the greatest influence on the PK parameters were stepwise integrated in the model either scaled to their median value of the population or without normalization to the median value. Different covariate models such as linear models, exponential models and allometric models were tested [28,29].

Model evaluation

The improvement of the fit of the model was assessed by a decreasing objective function value (OFV) and scatter plots of observed anti-FXa activity versus individual and population model predicted anti-FXa activity before and after addressing the covariates to the basic model. The relative standard error of the mean was graphically described by weighted residuals (WRES) over time after dose (TAD) and WRES plotted versus population model predicted anti-FXa activity. Besides these basic internal evaluation methods, a simulation-based advanced internal evaluation method was performed by Visual Predictive Check (VPC) at steady state for the q24 h and q12 h dosing regimen, respectively [30]. Simulations, based on the original dataset, were conducted using NONMEM and the median, 5% and 95% confidence intervals were calculated using the statistical analysis program SAS V9.2. VPC graphs were plotted in SigmaPlot 9.0 by displaying the observed anti-FXa activity over the simulated anti-FXa activity predictions based on the model.

Statistical analyses

Statistical analyses were performed with the StatView 5 software package (SAS Institute, Cary, NC, USA). Outcome variables, for example the number of patients with PTS, recurrent VTE, 6–12 months patency rates and number of bleeding events, in both groups, were calculated by descriptive statistics and compared by χ^2 analysis or Fisher's exact test, if necessary. For variables with a non-Gaussian frequency distribution, data were presented as medians and ranges. Evaluations and comparisons between patients of both groups were conducted using the Mann–Whitney *U*-test. The significance level was set at 0.05.

Results

Patients

The total number of patients enrolled in this cohort study was 152. Out of these patients 126 were eligible for analysis (Fig. 1).

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Patient characteristics and VTE locations are shown in Table 1. As previously described, 80% of patients had underlying medical conditions [1,2,10]. However, none of the investigated children were in renal insufficiency or had any kind of renal disease. The baseline creatinine levels were within the age-dependent reference ranges. The mean absolute individual fixed dose in our population resulted in 2000 anti-FXa IU mL^{-1} (min-max: 200–10 000). Eighty children with VTE received enoxaparin q24 h, and in the remaining 46 subjects LMWH was administered q12 h according to the study design (Fig. 1). The median (min-max) duration of enoxaparin was not different in both treatment groups [q12 h: 5 (3–10); q24 h: 6 (3.5–12) months; $P = 0.35$]. There was no statistically significant difference with respect to severity of VTE ($P = 0.19$), underlying medical conditions and outcome variables between the two groups. No symptomatic bleeding or recurrent VTE episodes were observed when patients received enoxaparin therapy during the observational follow-up of 12 months. Recurrent VTE was equally distributed between treatment groups: after cessation of anticoagulation, four children developed recurrent VTE [2/80 (2.5%) vs. 2/46 (4.3%); $P = 0.62$], and acute postthrombotic syndrome was documented in five children and adolescents, 3/80 (3.8%) in the enoxaparin q24 h group and 2/46 (4.3%) in the q12 h group ($P = 1.0$).

PopPK analysis

Anti-FXa activity measurements were obtained during routine TDM of the 126 patients during secondary enoxaparin prophylaxis for VTE. The dataset consisted of 204 observations (128 of the q24 h and 76 of the q12 h dosing regimens) in 126 subjects at various time points after achievement of maintenance dose (Fig. 1). In a first model finding step, various basic models were tested with and without IIV exponentially scaled on each population parameter. After comparing the OFV a two-compartment model with FOCE method and IIV exponentially scaled on apparent clearance (CL/F) and apparent central volume of distribution (V_1/F) described the data sufficiently. Residual variability of the model was best described with a proportional error model. The PK parameters CL/F, V_1/F, apparent peripheral volume of distribution (V_2), apparent intercompartmental clearance (Q) and the absorption rate constant (k_a) estimated through empirical Bayesian estimations from the basic model were plotted against the covariates age, body weight and BSA to assess their relationships [27].

There was an increase in CL/F with increasing body weight, whereas only a moderate influence of body weight on V_1/F was observed. None or poor influence was found between body weight and Q or V_2 and between age and BSA on the PK parameters, respectively.

Different covariate models were examined: the linear model, the exponential model and the allometric model [28]. The best covariate model with a decrease of −373 in the OFV compared with the basic model included body weight scaled to the median body weight of this patient population as a covariate for CL/F and V_1/F as a linear function. Including body weight on all PK parameters in the model led to unsuccessful minimization. The investigation of an allometric scaling model with the typical

Table 1 Patient characteristics and clinical outcome of patients

Parameters of interest (median: min–max)	Entire study cohort $n = 126$	q12 h group $n = 46$	q24 h group $n = 80$
Demographics			
Age (years)	5.9 (0.07–25)	11.8 (0.07–17.8)	3.9 (0.08–25)
Body weight (kg)	24 (2.5–97.3)	37 (2.6–97.3)	18.3 (2.5–93)
Height (cm)	125 (50–196)	145 (50–192)	107.3 (50–196)
BSA (m^2)	0.89 (0.19–2.19)	1.18 (0.19–2.19)	0.73 (0.19–2.11)
Gender (male: number)	66	26	40
Ethnicity (%)		100 white	100% white
Thrombus location (*n*; %)			
Calf	13 [10.3]	6 [13.0]	7 [8.8]
Proximal leg VTE	29 [23.0]	10 [21.7]	19 [23.7]
Leg and pelvis	24 [19.0]	8 [17.4]	16 [20.0]
Renal VTE	18 [14.3]	6 [13.0]	12 [15.0]
Cerebral VTE	16 [12.7]	6 [13.0]	10 [12.5]
Thromboembolic stroke	20 [15.9]	8 [17.4]	12 [15.0]
Pulmonary embolism	6 [4.8]	2 [4.3]	4 [5.0]
LMWH dose (mg kg^{-1} $dose^{-1}$): Secondary prophylaxis	1.04 (0.29–3.0)	1.2 (0.7–3.0)	1.0 (0.29–2.2)
12-h anti FXa (IU mL^{-1})	–	0.28 (0.05–0.74)	0.27 (0.07–4.13)
24-h anti FXa (IU mL^{-1})	–	–	0.095 (0.01–0.43)
Efficacy endpoints			
PTS	5/126	2/46	3/80
Rethrombosis	4/126	2/46	2/80
Safety endpoints			
Bleedings	–	–	–

Anti-FXa, anti-factor Xa activity; BSA, body surface area; BMI, body mass index; PTS, post-thrombotic syndrome; VTE, venous thromboembolism; LMWE, low-molecular-weight- heparin.

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value of 0.75 as a power function on body weight as a covariate for CL/F resulted in an OFV of −509 and very high standard errors for Q and V_2 (Table 2) [29]. As age showed a modest correlation on CL/F, it could be assumed that age might influence the PopPK parameters. However, the age-included models led to high standard errors, unsuccessful minimizations and an increase in the OFV. In accordance with the predictions of the model, the measured anti-FXa activity peak levels in infants ≤ 6 months of age with those > 6 months of age did not show any age-dependency [median (min-max): 0.53 IU mL^{-1} (0.35–0.64) vs. 0.53 IU mL^{-1} (0.38–0.62); $P = 0.98$].

Model evaluation

Goodness-of-fit plots were used to compare the fit of the model between the basic model and the covariate models. Figure 2 shows the comparison of the basic model (A) and the final covariate model (B) assessing the predicted versus observed anti-FXa activity in scatter plots. The final model including the entire study cohort overpredicts some observations resulting in a residual error of 31%. When comparing predicted vs. observed anti-FXa levels of individual patients (Fig. 2C) the data are regularly spread around the line of identity. The population graphs further underline the fairly high IIVs with 54% for CL/F and 42% for V_1/F. The WRES were plotted vs. TAD and vs. population model predicted anti-FXa activity (Fig. 2D,E). Both WRES plots are equally distributed without indicating an apparent bias in the final model. Based on a single anti-FXa measurement the capability of the model precision to predict anti-FXa activities of individual patients is shown for one q24 h patient in Fig. 3. The discrepancy in this figure seen between population and individual predictions further reflects the variation in IIV for CL/F and V_1/F. The estimates of the trough anti-FXa activities for q24 h and q12 h for both dosing regimens are shown in Fig. 4 with median enoxaparin trough levels of 0.095 IU mL^{-1} for the q24 h dosing regimen and 0.28 IU mL^{-1} anti-FXa activity for the q12 h dosing regimen. The 12 h enoxaparin activities for the q24 h dosing regimen were 0.27 IU mL^{-1} anti-FXa activity, similar to the activity in the q12 h dosing regimen ($P = 0.12$). In the q24 h group 53.2% of study samples were located > the median enoxaparin trough level of 0.095 IU mL^{-1}. All children with measured peak levels within the target range additionally showed predicted trough levels > 0.1 IU mL^{-1}, vice versa in those patients who did not reach the peak level, predicted trough anti-FXa activity levels were calculated below the efficacy cut-off of 0.1 IU mL^{-1}. By performance of a VPC we were able to evaluate the final model through an advanced internal evaluation method as shown in Figs 5A,B. The two dosing regimens, q24 h and q12 h, were simulated separately at steady state. Both VPCs show no distinct bias by visual inspection. The observations are regularly distributed around the median of the simulations with approximately 10% of the observations being outside the 5% and 95% confidence intervals (CI) [30].

Table 2 Population model comparison during model development, population parameter estimates

Model parameter	Basic model	Allometric model	Final model
Fixed effects			
CL/F (mL h^{-1})	292 (12%)	17 kg^{-1} (5%)	15.2 kg^{-1} (7%)
V_1/F (mL)	147 (98%)	51.6 kg^{-1} (33%)	169 kg^{-1} (18%)
Q (mL h^{-1})	864 (109%)	14.8 (264%)	58 (40%)
V_2 (l)	0.38 (31%)	0.36 (196%)	10.3 (45%)
K_a (h^{-1})	0.137 (17%)	0.11 (33%)	0.414 (30%)
Random effects			
Interindividual variability			
CL/F (%)	85 (19%)	44 (21%)	54 (26%)
V_1/F (%)	219 (111%)	70 (73%)	42 (44%)
Residual error			
Proportional (%)	76 (16%)	34 (11%)	31 (11%)
Objective function	−148	−509	−522

CL/F apparent clearance; V_1/F apparent central volume of distribution; V_2 apparent peripheral volume of distribution; Q apparent intercompartimental clearance; k_a absorption rate constant, standard errors in brackets.

Discussion

Enoxaparin, a LMWH, differs substantially from unfractionated heparin and from other LMWHs, such as tinzaparin, dalteparin and nadroparin. Its absorption rate and bioavailability patterns are consistent and predictable. In addition, a comparative study has shown that, apart from UFH, the average apparent total body clearance of enoxaparin was significantly lower (15.6 mL min^{-1}) than that of dalteparin (33 mL min^{-1}), tinzaparin (28.3 mL min^{-1}) and nadroparin (21.4 mL min^{-1}). Thus, enoxaparin is cleared more slowly from the body than dalteparin, tinzaparin and nadroparin with an extended metabolism rate and a longer apparent half-life of 4–5 h (dalteparin: 2.8 h; nadroparin 3.7 h; tinzaparin: 3–4 h) [31,32]. This longer apparent half-life of enoxaparin implies the capability of providing a sustained anticoagulant effect still present 24 h after administration [33,34], which was also documented in our q24 h cohort with observed anti-FXa activities > 0.1 IU mL^{-1} in 53.2% of samples. The former has important clinical implications, as it potentially allows for less frequent administrations [33]. The present cohort study was conducted to establish a PopPK model from anti-FXa activities derived from 126 newly enrolled neonates, infants, children and adolescents on either once- or twice-daily maintenance enoxaparin therapy after symptomatic VTE onset. Using a two-compartment model with first order absorption and an IIV on CL/F and V_1/F we were able to describe accurately the pharmacokinetic of the population investigated. It is obvious that the model is capable of predicting very similar anti-FXa activities compared with the observed anti-FXa activities. In the PopPK modeling approach we tested several covariates such as body weight, BSA and age on their influence on the PK parameters. However, the best results were obtained using body weight as a covariate for CL/F and V_1/F. As allometric modeling was used for many years

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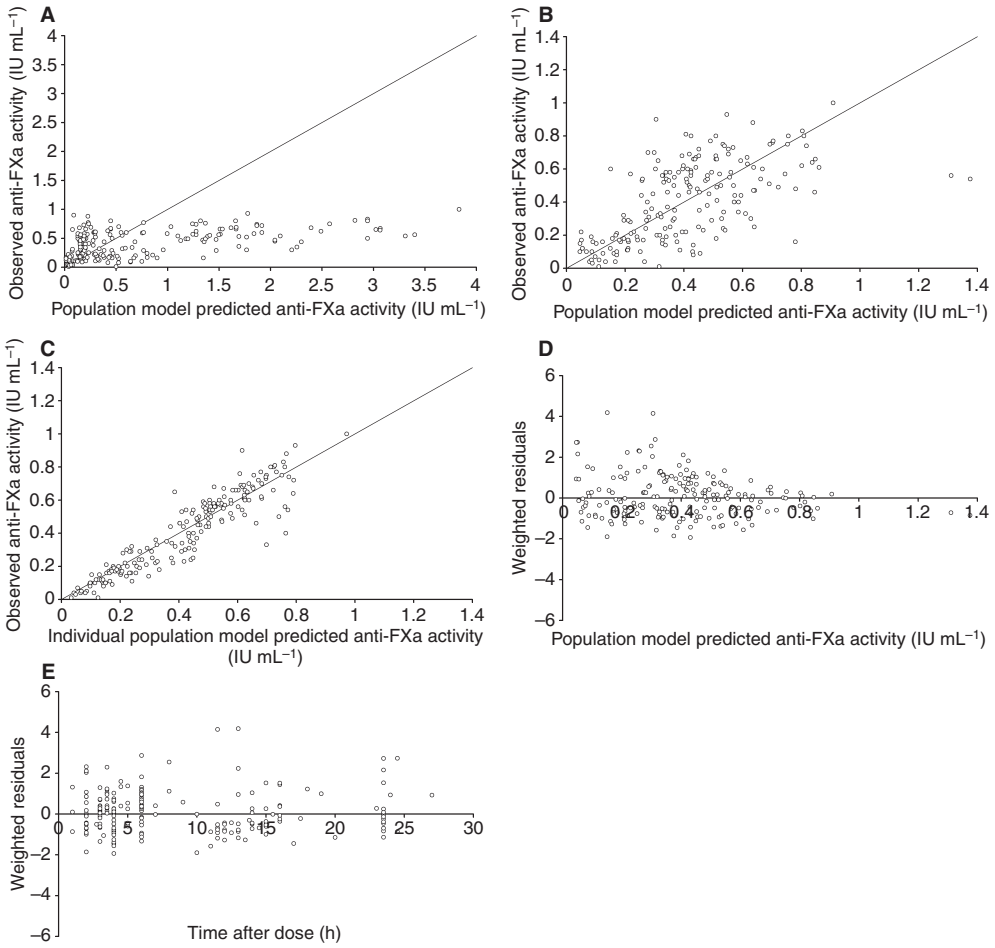

Fig. 2. Basic internal evaluation methods: Goodness-of-fit plots for the model predicted anti-FXa activity versus observed anti-FXa activity for the patient population in the basic model (A) and final covariate model with body weight included as covariate (B). (C) Individual model predicted anti-FXa activity versus observed anti-FXa activity in the final covariate model. The line of identity is shown. (D–E) Weighted residuals in the population model and weighted residuals over time after dose.

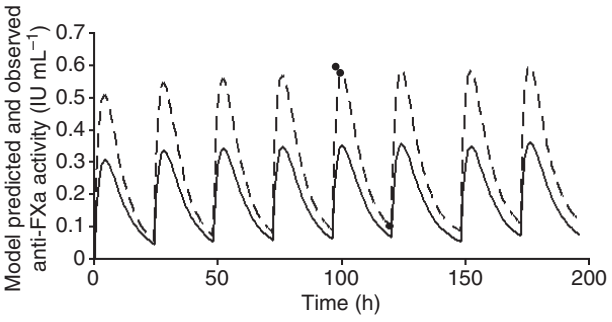

Fig. 3. Model predicted anti-FXa activity for one selected patient (dashed line = individual model predicted anti-FXa activity, solid line = population model predicted anti-FXa activity, dots = observed anti-FXa activity).

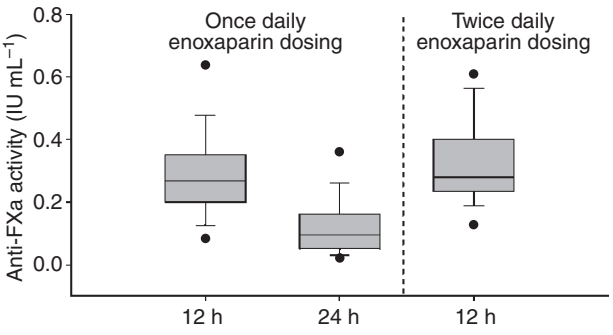

Fig. 4. Anti-FXa activity of once versus twice-daily enoxaparin dosing; once-daily dosing: 12-h median level 0.27 IU mL^{-1}; 24-h median level 0.095 IU mL^{-1}; twice-daily dosing: 12-h median level 0.28 IU mL^{-1}; plots median, 10th, 25th and 90th percentiles as vertical boxes with error bars.

to evaluate the drug dose in humans by taking body weight with a power value of 0.75 into account we further constructed an allometric scaling model [Table 2] [29]. This model, compared with our final model, demonstrated fairly high standard errors and a larger OFV. Therefore, our data do not support an allometric model as the model of choice for LMWH for the pediatric population.

Based on the longer apparent half-life as discussed above, LMWHs administered q24 h in adult patients with VTE have been found similarly efficacious and safe compared with q12 h

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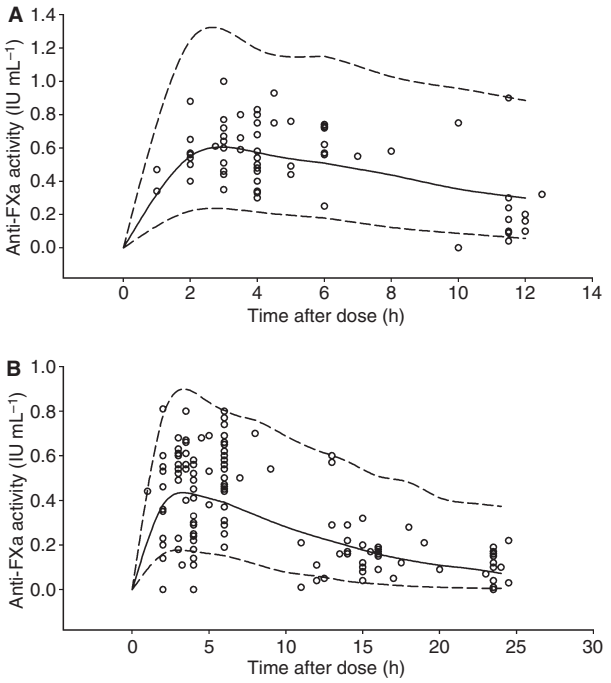

Fig. 5. Advanced internal evaluation method: Visual Predictive Checks for twice-daily enoxaparin dosing (A) and once-daily enoxaparin dosing (B). (Dashed lines = 5th and 95th confidence interval, solid line = model predicted median, dots = observed anti-FXa activity levels.)

administration [15,19,20]. Similar to the data shown in a recent Cochrane review and in agreement with data shown in a pilot study published in 2006 we found no significant difference with respect to the defined efficacy and safety endpoints [10,15]. In contrast to Dix *et al.* in 2000 who reported in a similar pediatric population a rate of major hemorrhage in 5% of children all treated q12 h according to a target anti-FXa activity between 0.5 and 1 IU mL^{-1} [5], neither major bleeding, therapy-related death nor recurrent VTE occurred in our cohort targeting an anti-FXa activity peak range of 0.5–0.8 IU mL^{-1} in both dosing regimens. Apart from preterm infants who were not included in our cohort study the higher major bleeding rate reported by Dix and coworkers (unfortunately not showing anti-FXa activity levels in children with major hemorrhage) may be explained (i) in part by accumulation of LMWH receiving a q12 h dosing regimen and (ii) by a different pediatric population with respect to more severe underlying medical conditions. In 2007 O'Brien *et al.* reported a non-compartmental enoxaparin analysis for q24 h dosing derived from 15 children during the acute therapy (after the second enoxaparin administration) [11]. The study found anti-FXa activity levels to be less than 0.5 IU mL^{-1} by hour 12 in seven out of eight cases, and defined this anti-FXa activity as sub-therapeutic without showing the individual anti-FXa activity levels. Whereas the mean anti-FXa activity level at hour 12 was observed to be 0.33 IU mL^{-1} in their pediatric population which lies within the supposed therapeutic range for enoxaparin trough levels. Unfortunately, in this study patient's anti-FXa activity levels might have not been within steady state after the second dose as steady state is assumed after day 3 or 4 in a q12 h dosing regimen. Therefore the assumption whether these anti-FXa activity levels after the second dose are subtherapeutic or not remains questionable. In our study measured anti-FXa activity peak levels reached the recommended target in > 50% of cases with no difference found in different age classes ($P = 0.97$). All patients with peak levels within the recommended range reached 12 h trough levels above the cut-off of 0.1 IU mL^{-1} [23]. Thus, by predicting the 12 and 24 h trough levels using our PopPK model (Fig. 4), we could not determine any significant differences between the 12 h anti-FXa activities. As shown in the Fig. 4, the median 24 h trough level was above 0.1 IU mL^{-1} anti-FXa activity in 53.2% of study samples. The minimum trough anti-FXa level to prevent recurrent VTE is not established yet. If a minimum required trough level is assumed at 0.1 IU mL^{-1} and targeted for at 24 h, a once-daily enoxaparin dosing regimen seems feasible in at least 50% of the population during secondary prophylaxis [22]. This is also underlined by the model predicted median shown in the VPCs over time after dose for q12 h and q24 h dosing [Fig. 5]. As demonstrated in Fig. 5, a substantial proportion of children on q24 h dosing had peak (2–4 h) anti-FXa values below target ranges. Increasing their dose based on monitoring peak levels could potentially achieve higher trough anti-FXa levels and a larger proportion of children with levels of 0.1 IU mL^{-1} or above. An alternative would be monitoring 24 h trough levels and dose adjustments to assure minimum anti-FXa levels. The PopPK model will allow us to establish whether this would result in peak anti-FXa levels considered unsafe. Potentially, a somewhat higher average enoxaparin dose for q24 h may evolve.

Our study has some limitations which have to be discussed. First, whereas the prediction of anti-FXa activities in the individual patient is congruent with the observed enoxaparin level, prediction of population predicted enoxaparin levels based on data of the entire study cohort overpredicts some observations. Thus, treating physicians at bed side should be aware of this possible anti-FXa overestimation. However, by predicting individual enoxaparin activities this overprediction can be ignored, as this bias is not observed in the individual population model predictions [Fig. 2C]. Second, in the internal evaluation cohort the CI for the q12 h dosing regimen seems to be wider compared with the CI of the q24 h dosing regimen. This might be because of less observations of the q12 h dosing regimen in the dataset and the fact that the modeling was performed with the whole dataset of q24 h and q12 h dosed patients. Third, an interesting covariate which was not tested would have been creatinine clearance as enoxaparin is cleared renally. The former was not taken into account as none of our patients was in renal insufficiency or had any kind of renal disease as described before.

Bearing in mind the study limitations, the PK model developed by us is capable of describing all age groups and dose levels of the children investigated during enoxaparin secondary prophylaxis and can be used in future randomized trials. As shown in adults, LMWH can be administered for

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secondary long-term prophylaxis in children and patients with side effects or other complications on oral anticoagulants [10,35]. The high interindividual variability in the pharmacokinetic parameters, however, underlines the need for continuous monitoring of anti-FXa activity and dose individualization in this patient group. According to the results of this cohort study, a once daily enoxaparin dosing regimen seems to be feasible for at least 50% of our study population during maintenance therapy. Besides this, monitoring anti-FXa activity levels during routine TDM is mandatory to prove if 24 h trough levels are above the predicted anti-FXa activity level of 0.1 IU mL^{-1} for prophylaxis doses. However, before recommending specific treatment algorithms for once daily LMWH administration for the general pediatric population suffering from symptomatic thromboembolism this approach should be confirmed in future pediatric cohort studies.

Addendum

Along with the principal study investigators, e.g. Mirjam Nadine Trame, Lesley Mitchell, Christoph Male and Ulrike Nowak-Göttl who act as the guarantors, all other investigators, namely Anne Krümpel and Georg Hempel had full access to the data and took part in the design, execution and data analysis, and in the writing of the report. Mirjam Nadine Trame and Ulrike Nowak-Göttl were responsible for the statistical analyses.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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