**FIGURES AND TABLES**

**Table 1. Patient characteristics at baseline**

|  |  |  |
| --- | --- | --- |
|  | | **Number (n, %) or median [IQR] (range)** |
| **Patients in total** | | |
| Number | | 37 |
| Severe haemophilia (FIX<1 IU/dL) | | 32 (86%) |
| Baseline FIX non-severe patients (IU/dL) | | 1.00 (1-2) |
| Age (years) | | 15.8 [11-30] (2.3-71.0) |
| Body weight (kg) | | 65.4 [32.8-76.7] (11.9-103.0) |
| Height\* (cm) | | 169 [140-180] (85-192) |
| BMI\* (kg/m2) | | 22.02 [17.8-25.1] (13.2-32.4) |
| Lean body mass (kg)  Fat free mass (kg) | | 51.74 [29.51-58.94] (11.27-73.59)  51.7 [29.5-61.1] (11.2-73.6) |
| Fat free mass (kg) | | 51.12 [26.30-58.91] (9.92-73.58) |
| **Paediatric patients** | | |
| Number of paediatric patients# | |  |
|  | < 18 years (% of total patients) | 19 (51%) |
|  | < 12 years | 14 (38%) |
|  | < 6 years | 7 (19%) |
| Age (years) | | 11.0 [3.6-12.3] (2.3-15.8) |
| Body weight (kg)  BMI (kg/m2)\*\* | | 32.8 [16.3-32.4] (11.90-51.74)  17.67 [16.7-19.0] (13.2-29.9) |
| Fat free mass (kg) | | 29.5 [14.83-43.8] (11.2-51.7) |
| **Treatment** | | |
| Dose (IU/kg) | | 36 (10-132) |
| Blood samples at PK profiling^  Total PK samples | | 5 (3-7)  7 median [6-10] (4-12) |

\* No data available in two patients.

# Percentages reflect proportions of the total population (n=37)

^ No first PK profile assessment in one patient.

**Table 2. rFIX-Fc population pharmacokinetic parameters from the published and novel model**

|  |  |  |  |
| --- | --- | --- | --- |
| **Population PK model** | **Published model version**  **Original Model** | **Novel model** | |
| **Parameters** | **Estimate** | **Estimate (RSE %) [Shr.]** | **Bootstrap estimate (95% CI\*)** |
| CL (dL/h) | 2.39 | 1.41 (5) | 1.41 (1.27-1.57) |
| Body weight exponent on CL | 0.436 | 0.75 |  |
| Age exponent on CL |  | 0.0047 (11) | 0.0050 (0.001-0.010) |
| V1 (dL) | 71.4 | 73.1 (5) | 72.5 (64.9-80.4) |
| Body weight exponent on V1 | 0.396 | 1.00 |  |
| Q2 (dL/h) | 1.67 | 2.77 (14) | 2.87 (2.06-6.22) |
| Body weight exponent on Q2 |  | 0.75 |  |
| V2 (dL) | 87.0 | 80.1 (11) | 80.6 (65.6-99.8) |
| Body weight exponent on V2 |  | 1.00 |  |
| Q3 (dL/h) | 39.3 |  |  |
| V3 (dL) | 39.9 |  |  |
| **Interindividual variability (IIV)** | | | |
| IIV# on CL (%) | 17.7 | 23.6 (20) [14] | 22.0 (13.9-32.1) |
| IIV on V1 (%) | 21.7 | 31.6 (16) [8] | 29.8 (20.5-40.0) |
| Correlation^ IIV CL and V1 (%) | 75.6 | 44.0 | 42.0 (-9.4-62.4) |
| IIV on Q2 (%) | 35.8 |  |  |
| IIV on V2 (%) | 46.2 | 41.2 (17) [39] | 38.1 (19.0-53.2) |
| IIV on V3 (%) | 37.7 |  |  |
| **Interoccasion variability (IOV)** | | | |
| IOV# on CL (%) | 15.1 | 19.8 (22) [36] | 19.8 (8.4-27.2) |
| IOV on V1 (%) | 17.4 |  |  |
| **Residual variability** | | | |
| Proportional error (%) | 10.6 | 16.3 (14) | 15.7 (8.7-21.8) |
| Additive error (IU/dL) | 0.24 | 1.04 (21) | 1.08 (0.25-1.52) |

CL and V1 and V2 were scaled and normalized for an average patient with a body weight of 73kg.

CL, clearance; V1, central volume of distribution; V2, volume of compartment 2; V3, Volume of compartment 3; Q2, intercompartmental clearance between compartments 1 and 2; Q3, inter-compartmental clearance between compartments 1 and 3; RSE, relative standard error; Shr, shrinkage.

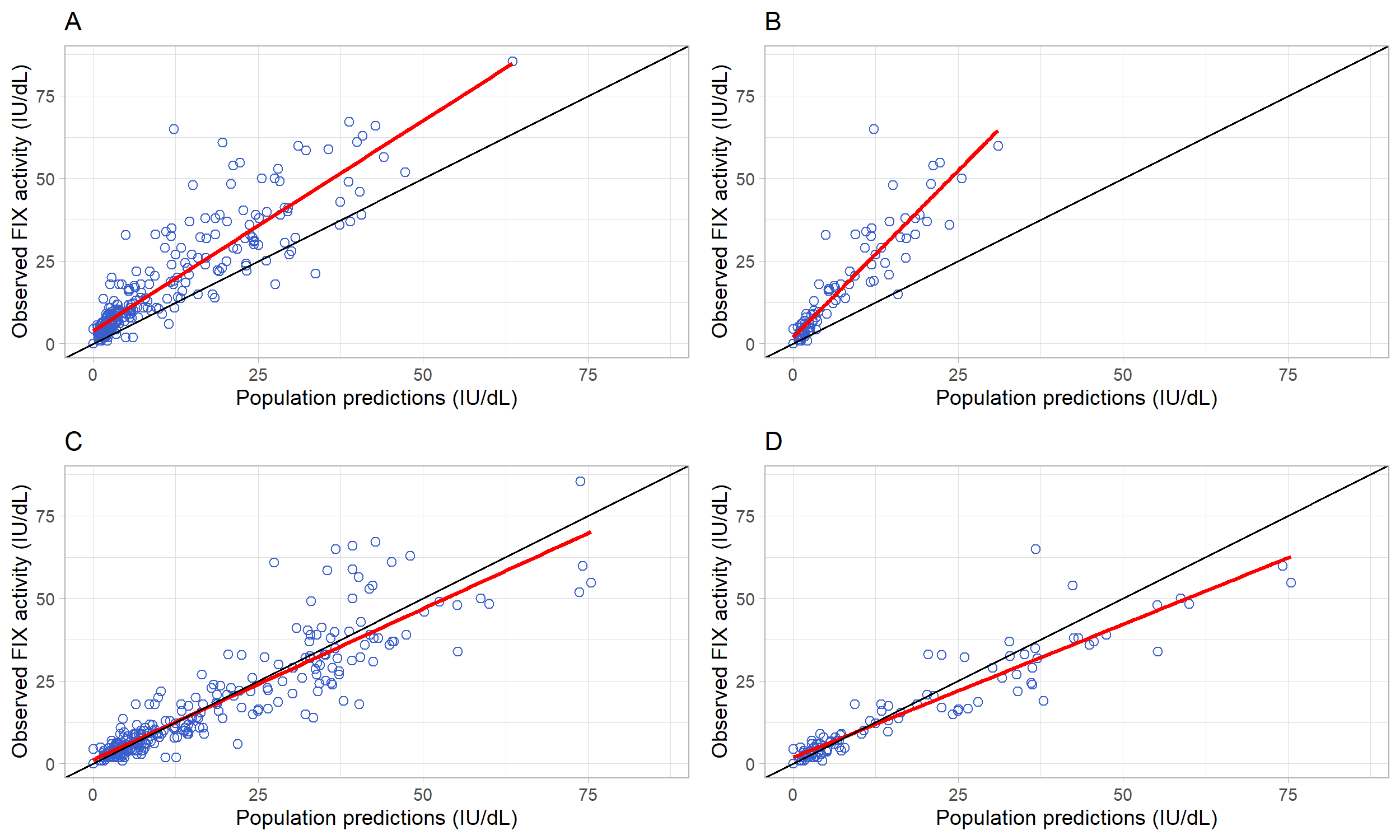
\* 95% CI, non-parametric 95% confidence interval from bootstrap results with 2000 datasets

# IIV and IOV coefficient of variation calculated as: √(variance) \*100%

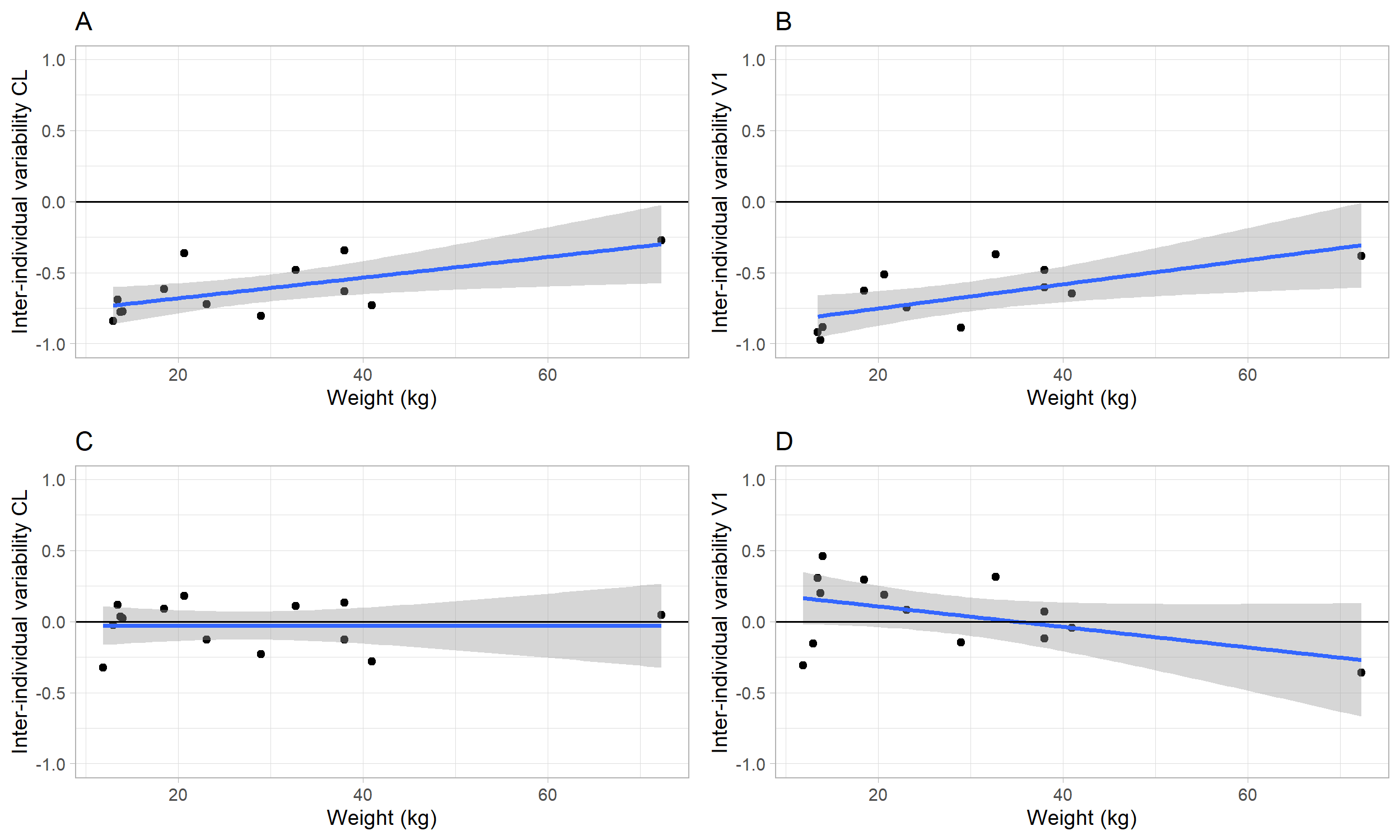
^ Correlation calculated as: covariance/(√(variance1)\* √(variance2)) \*100%

Published model:

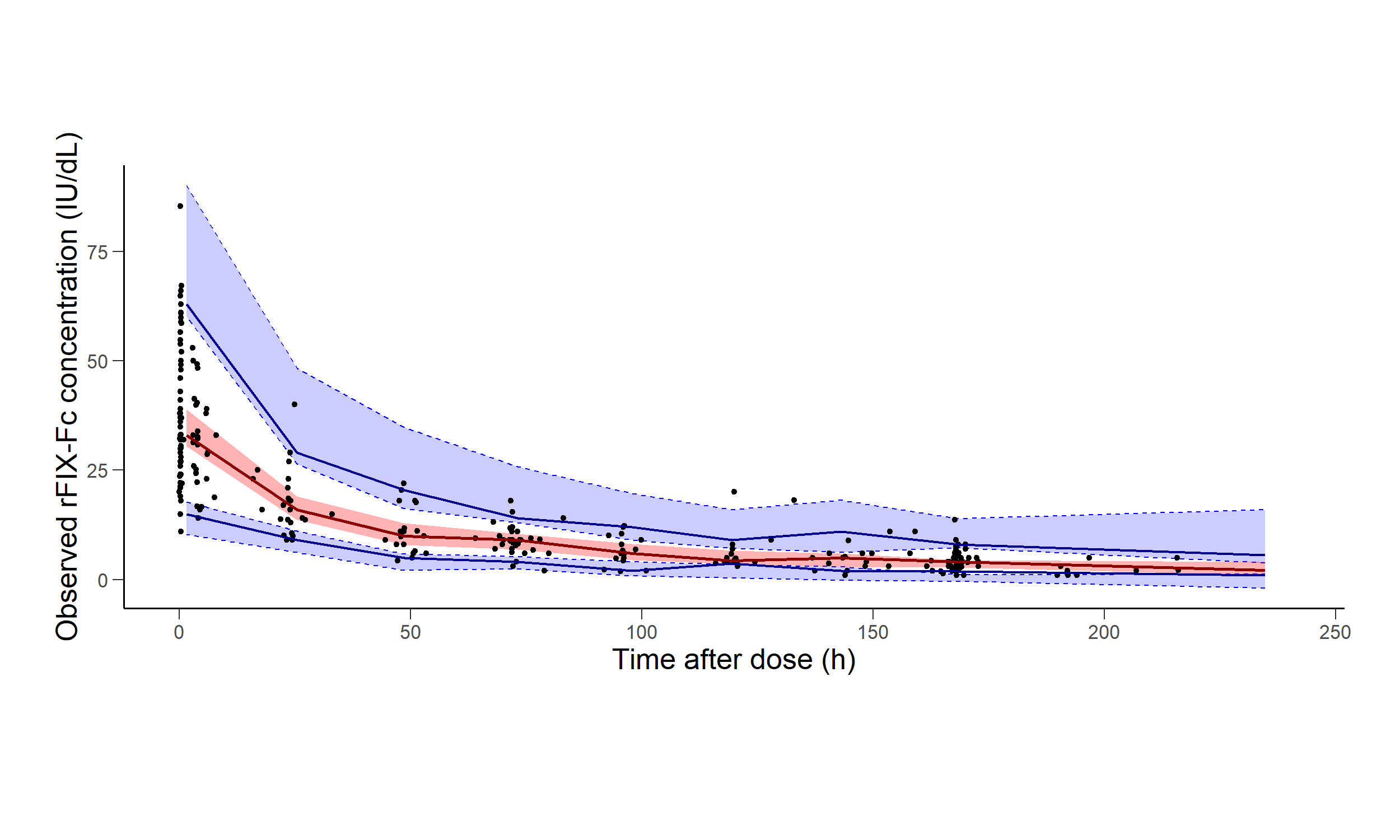
Novel model:

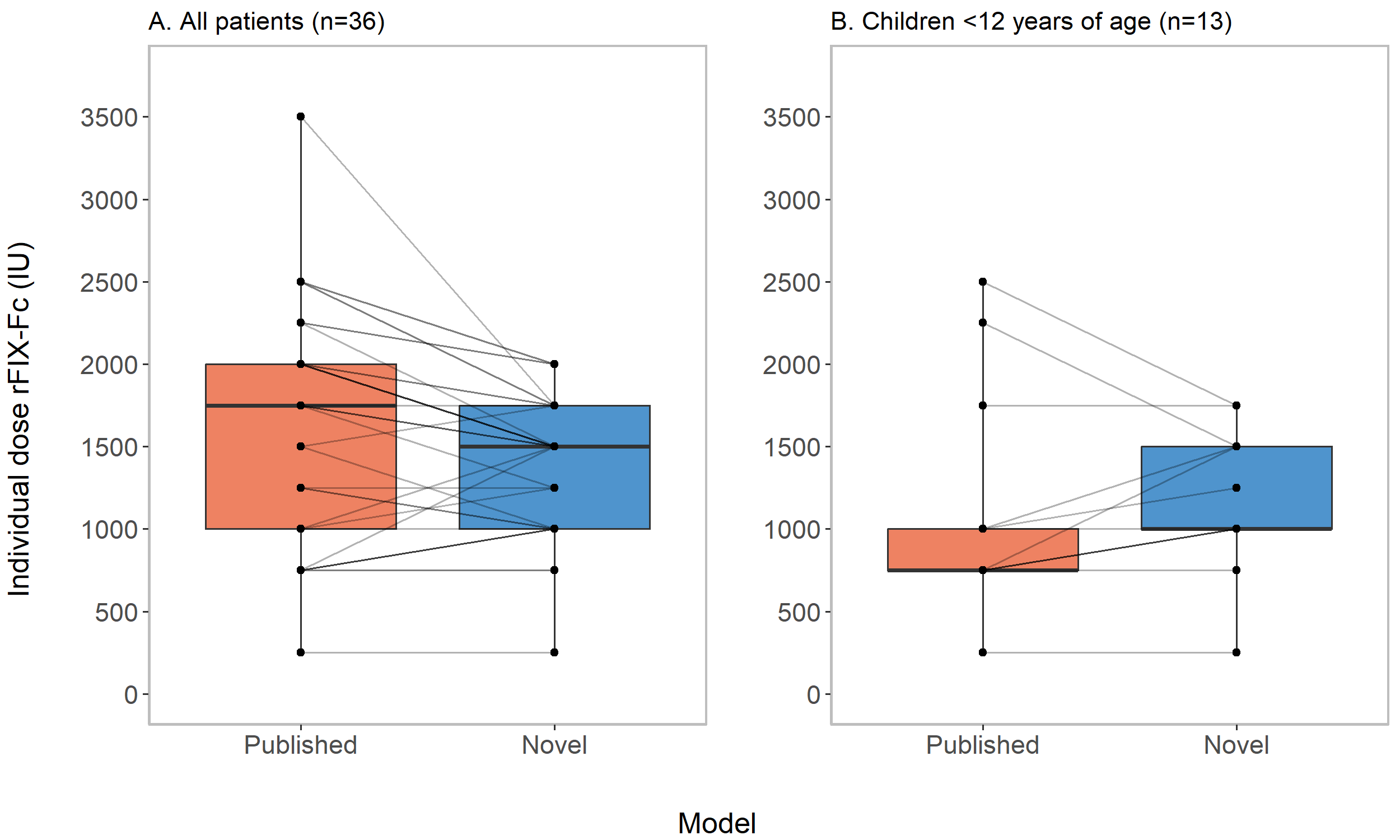


**Figure 1. Population goodness of fit (GOF) plots of both the published model (A, B) and novel model (C, D) using real world clinical data**, **including children <12 years of age.** Observed FIX activity levels are plotted against the predicted FIX activity levels for all patients (A, C) and children <12 years of age (B, D). The trend line (red line) combines all individual data points (blue circles) and should approximate the line of identity (black line). The novel model shows an improved fit compared to the published model for all patients – but especially for children <12 years – as the trend lines better approximate the line of identity.



**Figure 2. Interindividual variability of CL and V1 for the published (A, B) and novel (C, D) model exclusively for children <12 years of age.** Individual data (black dots) is visualized as a trend line (blue line) approximating the line of identity (black line). For an adequate population model, interindividual variability should be randomly distributed around the axis y=0 with no apparent trend. The novel model shows an improved fit compared to the published model for the IIV of CL, as the trend line is closer to the line of identity.

**Figure 3. Visual predictive check of the novel rFIX-Fc model.** The median (red line) and 95% confidence interval (blue lines) of the observed data (black dots) are plotted against the simulated data (n=1000) indicated as highlighted areas: the red area represents the median and the blue area the 90% prediction interval. A model predicts the factor concentrations adequately when the red and blue lines run through the corresponding areas.

****

**Figure 4. Individual dose calculations for both models to maintain a FIX level >3 IU/dL 168h after infusion of rFIX-Fc**. Calculations for all patients (A) and calculations for children <12 years of age (B) are presented separately. Calculations are made based on a situation in which three clinically relevant pharmacokinetic profile measurements were used for dose calculation. The boxes of the boxplots present the median (middle line) and interquartile range (IQR) with whiskers extending to Q1 or Q3 + 1.5 IQR. The lines represent individual patients, and darker lines indicate multiple patients. For all patients (A) a significant difference was found (p<0.01). For children <12 years of age, individual dose advices seemed higher with the novel model, but no significant differences were found (p=0.63).

**SUPPLEMENTARY FILES**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Center** | **Assay** | **Machine** | **Reagent** | **Calibrator** | **Deficient plasma** |
| **United-Kingdom Extended Half-Life Outcome Registry** | | | | | |
| **Royal Free Hospital** | OSA | ACL TOP (Werfen-IL) | IL HemosIL Synthasil | CRYOcheck | HemosIL FIX deficient plasma |
| CSA | Hyphen Biophen FIX chromogenic | Hyphen Biomed – Biophen FIX kit |
| **Glasgow Royal Infirmary** | OSA | ACL TOP | IL HemosIL Synthasil | HemosIL | HemosIL FIX deficient plasma |
| **Oxford University Hospital** | OSA | Sysmex  OSCA | Actin FS Siemens | Siemens | Technoclone |
| **Great Ormond Street Hospital** | OSA | Sysmex CS2000i | Actin FS Siemens | Standard Human Plasma | Precision Biological FIX |
| CSA | Rossix Factor IX Human FXIa in kit | Biophen Calibrator | Precision Biological FIX |
| **Sheffield Children’s Hospital** | OSA | Werfen ACL TOP 550 | IL HemosIL Synthasil | IL Hemosil Calibration Plasma | IL Factor IX deficient plasma |
| **University Hospital of Wales** | OSA | IL HemosIL Synthasil | IL Calibrator Plasma | IL Factor IX deficient plasma |
| **OPTI-CLOT TARGET study**1 | | | | | |
| **Erasmus University Medical Center** | OSA | Sysmex CS5100 | Actin FS Siemens | SHP Siemens | FIX deficient plasma Siemens |
| CSA | Rox FIX chromogenic kit. | Rox FIX chromogenic kit |
| **Amsterdam University Medical Centers** | OSA | Siemens  CS-2500 | Actin FS Siemens | SHP Siemens | FIX deficient plasma Siemens |
| CSA | Rox FIX chromogenic kit. | Biohyphen plasma calibrator | Rox FIX chromogenic kit |

**Supplementary Table 1. Laboratory specifications of factor IX (FIX) measurements.**

**Supplementary Table 2. Predictive performance of the published and novel model using the predictive error (PE) and root mean square error (RMSE).** **A**: Bias is represented throughout median PE for all patients and children <12 years of age. **B**: Differences of predictions between the published and novel model is presented by showing the RMSE of peak (time after dose 0-2 h), mid (time after dose 2-120 h) and trough (time after dose 120-300 h) FIX activity levels.

**A**

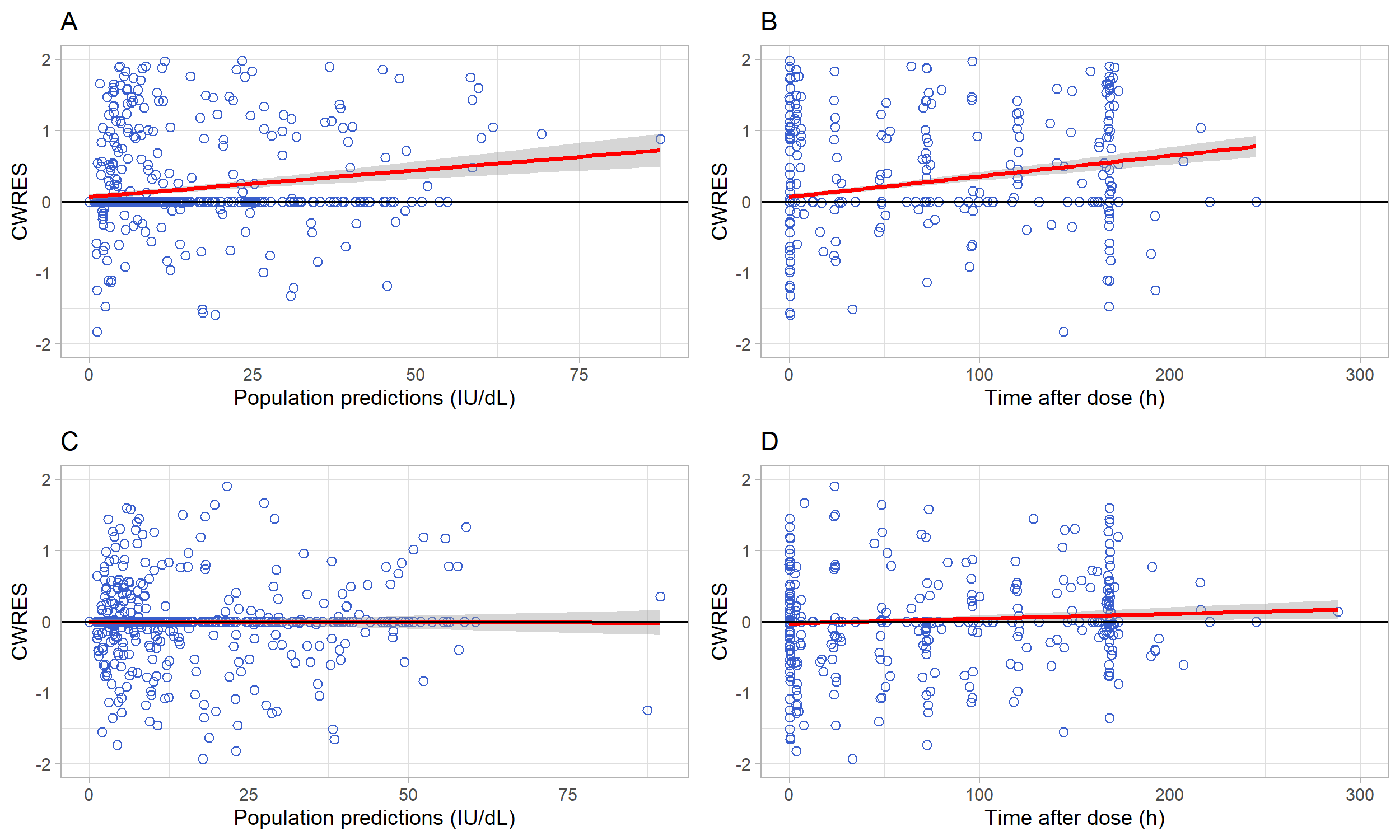
|  |  |  |
| --- | --- | --- |
|  | **Published model** | **Novel model** |
| **All patients** | | |
| PE (%) (median; IQR) | -48.8 (-29.9 – -63.9) | 3.4 (-22.2 – 25.8) |
| **Children <12 years** | | |
| PE (%) (median; IQR) | -54.1 (-43.3 – -65.8) | 4.9 (-20.8 – 27.5) |

**B**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Published model** | | | **Novel model** | | |
| **All patients** | *Peak* | *Mid* | *Trough* | *Peak* | *Mid* | *Trough* |
| IPRED RMSE + SD (IU/dL) | 3.0 (2.8) | 1.7 (1.2) | 0.5 (0.5) | 3.8 (4.3) | 2.1 (2.0) | 0.6 (0.5) |
| **Children <12 years** | *Peak* | *Mid* | *Trough* | *Peak* | *Mid* | *Trough* |
| IPRED RMSE + SD (IU/dL) | 3.0 (2.9) | 1.9 (1.5) | 0.3 (0.3) | 4.0 (6.0) | 2.8 (3.0) | 0.6 (0.4) |

**Supplementary Table 3. Calculated post hoc terminal half-life (t1/2) for the published and novel model.**

|  |  |  |
| --- | --- | --- |
|  | **Published model** | **Novel model** |
| **Adults ≥ 18 years** | | |
| Half-life (t1/2; h) (median; range) | 101 (60 – 192) | 88 (67 – 166) |
| **Adolescents ≥12 and <18 years** | | |
| Half-life (t1/2; h) (median; range) | 99 (67 – 114) | 76 (66 – 82) |
| **Children <12 years** | | |
| Half-life (t1/2; h) (median; range) | 88 (47 – 223) | 70 (51 – 103) |



**Supplementary Figure 1. Conditional weighted residual (CWRES) plots of the published model (A, B) and novel model (C, D) using real world clinical data, including children <12 years of age.** CWRES is plotted against the population predictions (IU/dL) and time after dose (h) for all patients (A, C) and children <12 years of age (B, D). The trend line (red line) combines all individually weighted data points (blue circles) and should approximate the line of identity (black line). The enriched model shows an improved fit compared to the published model, as the trend lines better approximate the identity lines and an increased number of values of CWRES are concentrated between -2 and 2.

**References**

1. Goedhart, T. M. H. J. *et al.* Design of a Prospective Study on Pharmacokinetic-Guided Dosing of Prophylactic Factor Replacement in Hemophilia A and B (OPTI-CLOT TARGET Study). *TH Open* **06**, e60–e69 (2022).