Impact of characteristic inhibition kinetics of different FVIII inhibitors on the inhibitor titer quantification in the modified Nijmegen-Bethesda assay

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INTRODUCTION

- The development of inhibitors to infused factor VIII (FVIII) in congenital hemophilia A (HA) patients displays a serious complication. Moreover, FVIII inhibitors can occur due to an autoimmune disorder (acquired HA).
- The inhibitors are classified as type I and II according to their different inhibition kinetics.[1]
- A reliable FVIII inhibitor titer quantification is important to provide optimal care for HA patients with FVIII inhibitors.
- Routinely the inhibitor titer is quantified using the modified Nijmegen-Bethesda assay (MNBA).[2] The resulting titers are often difficult to compare due to the different criteria to select the dilutions used for titer calculation (see Table 1).[3,4,5]

AIM

- To analyze the impact of different FVIII inhibitor kinetics on the titer calculation in the MNBA applying different criteria.

METHODS

- Two type I and type II FVIII antibodies were analyzed (concentrations: 0.25-4 µg/mL) with the MNBA using the cryocheck™ Factor VIII Inhibitor Kit (Precision BioLogic).

Inhibitor	Type	FVIII domain
4A4†	Type I	A2
BO2C11‡	Type I	C2
2-54†	Type II	A2
ESH-8§	Type II	C2

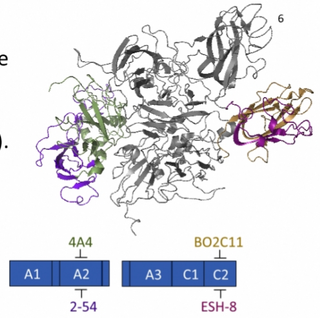

Figure 1. *Overview of different FVIII inhibitors and their binding domains.*
† Green Mountain Antibodies; ‡ Creative Biolabs; § ImmBioMed

- FVIII residual activities (RAs) were measured in duplicates with a FVIII One-Stage Clotting Assay (FVIII assay kit; Roche Diagnostics) using a **cobas t** 511/711 analyzer.
- The RAs were plotted against inhibitor dilution in a semi-log(x)-plot.

RESULTS

- The kinetics of four different FVIII antibodies were compared to the theoretical kinetic model in the MNBA (black lines), where every RA would lead to the same calculated titer result (**Figure 2**). This model uses a defined slope and curve shape assuming full inhibition.
- Only the inhibition kinetic of one analyzed antibody 4A4 (A) was perfectly represented by the theoretical kinetic while the other antibody kinetics showed significant deviations from the used model.
- These deviating inhibition kinetics (B and C/D) can have a significant impact on titer calculation when using different criteria (**Table 1**).

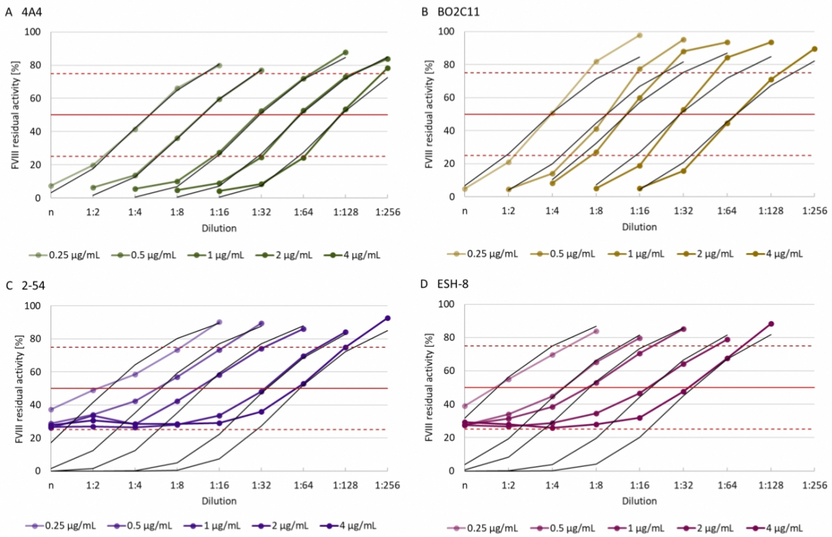

Figure 2. *Inhibition kinetics of FVIII inhibitors in relation to the theoretical kinetic (black curves).*

Table 1. *Overview and limitations of different criteria for titer quantification.*

Criteria 1-3 describe different strategies to select dilutions with RAs which can be used for a reliable inhibitor titer calculation. Criteria 4+5 determine the theoretical dilution that gives 50% RA in different ways. Its reciprocal value is equal to the inhibitor titer.

Criteria		Limitations for inhibitors with deviating kinetics*
Number	Inhibitor titer calculation	
1[3]	first dilution with RA > 25%	B: selected dilution with RA close to 25% ↑ C D: long plateau phase (> 25% RA) ↓ selected dilution with RA close to 25% ↓
2[4]	mean value of all titers with RAs between 25 and 75%	B: uneven distribution of values ↓↑ C D: long plateau phase (> 25% RA) ↓ uneven distribution of values ↓↑
3[4]	dilution closest to 50% RA	B, C D: RAs far from 50% ↓↑
4[5]	semi-log(y)-plot: RAs (25-75%) vs. dilution dilution that correlates with 50% RA	B: no calculation possible when only one value is between 25-75% RA
5	semi-log(x)-plot: all RAs vs. dilution dilution that gives 50% RA by sigmoidal regression $\text{RA}\,[\%] = \text{bottom} + \frac{100-\text{bottom}}{1+10^{(\text{LogEC50}-x)\cdot\text{HillSlope}}}$	

* A – D corresponding to the generated kinetics as presented in figure 2. Showing either a kinetic profile with a higher slope (e.g. B) or a lower slope (e.g. C+D) at 50% RA. ↓↑ indicate a resulting over- or underestimation of the calculated titer depending on the criteria in combination with an outlined deviation of the kinetic profile.

CONCLUSIONS

- **Inhibition kinetic of both type I and II inhibitors can deviate from the theoretical kinetic model in the MNBA. These discrepancies can lead to differences in the calculated titers depending on the different criteria used for titer calculation.**
- **For inhibitors with deviating kinetics, it is particularly important to achieve a value close to 50% RA either by measurement/re-measurement or theoretical calculation.**

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