

DISORDINI EMORRAGICI ACQUISITI: ASPETTI
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IMPLEMENTATION OF 4PL SIGMOIDAL REGRESSION FOR FACTOR VIII INHIBITOR QUANTIFICATION IN ACQUIRED HEMOPHILIA

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Background and Aims. The quantification of Factor VIII (FVIII) inhibitors is critical for managing patients with hemophilia. The standard Nijmegen-Bethesda assay relies on a theoretical linear-logarithmic relationship where 1 Bethesda Unit (BU) is defined as the amount of inhibitor neutralizing 50% of FVIII activity. While this model is effective for Type 1 inhibitors (typically alloantibodies in congenital hemophilia), it often fails to accurately represent Type 2 inhibitors (typically autoantibodies in acquired hemophilia). Type 2 inhibitors exhibit complex, non-linear kinetics and incomplete FVIII neutralization, leading to potential underestimation or overestimation of titers when using the standard formula. In this work, we evaluate the accuracy of the 4-Parameter Logistic (4PL) sigmoidal regression model compared to the traditional Bethesda method in quantifying inhibitors for patients with acquired hemophilia, across different kinetic profiles.

Methods. We analyzed longitudinal data from two clinical cases of acquired hemophilia. Inhibitor titers were initially calculated using the traditional Nijmegen-Bethesda formula based on the dilution closest to 50% residual activity (RA). These results were compared against a new calculation method using 4PL sigmoidal regression. The 4PL model creates a "best fit" curve by considering all serial dilutions, governed by specific biological constraints: Top fixed at 100%, Bottom ≥ 0 and optimized Hillslope and LogEC50. The inhibitor titer in BU was derived as $2\log EC_{50}$. Statistical accuracy was assessed by calculating the sum of squared errors (SSE) between measured and predicted RA values and by evaluating the consistency of the clinical trend.

Results. In Case 1, the inhibitor showed a more linear behavior in the 25-75% RA range. In the initial sample, the 4PL method yielded 112.3 BU compared to 124.4 BU with the traditional method (SSE = 17.9), showing relatively

good alignment. Throughout the observation period, the results of both methods remained closely aligned, indicating that the traditional calculation is reliable for linear kinetics. However, Case 2 demonstrated the limitations of the traditional assay: multiple dilutions clustered around 50% RA, making validation difficult. For the initial sample, the traditional method estimated 128.6 BU, while the 4PL model provided a more mathematically robust estimate of 105.5 BU. When examining the entire period for the second case, the traditional method showed high volatility (e.g., an inconsistent jump from 128.6 to 133.0 BU between two consecutive samples), whereas the 4PL model (moving from 105.5 to 85.1 BU) reflected a more consistent biological trend that aligned with the clinical course.

Conclusions. We demonstrate that kinetic profiles of FVIII autoantibodies frequently deviate from the ideal theoretical model of the Nijmegen-Bethesda assay. While the traditional method is simple and reliable for inhibitors following linear kinetics, it lacks accuracy when dealing with the non-linear neutralization typical of acquired hemophilia. The 4PL sigmoidal regression provides a superior fit for raw laboratory data, minimizing errors by integrating information from all dilutions rather than a single data point. Implementing 4PL regression in the clinical laboratory setting offers a more accurate, standardized, and reproducible approach to monitoring inhibitor titers, ultimately improving therapeutic decision-making in complex hemophilia cases.

