Recovery for people with hemophilia A: One stage assay or chromogenic assay

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Introduction:

Factor replacement therapy using factor VIII (FVIII) concentrates remains the standard treatment for people with hemophilia A in Tunisia and one-stage assay (OSA) is most used to measure FVIII activity. With the introduction of non-substitutive therapies such as Hemlibra (emicizumab) in Tunisia, the chromogenic assay (CSA) has been implemented.

Aim: The aim of this study was to evaluate the chromogenic assay in monitoring post administration FVIII activity of Hemophilia A people substituted with standard half life (SHL) FVIII with the advent of non-substitutive therapies.

Materials and methods: This is a comparative monocentric retrospective study that included patients from Aziza Othmana Hemophilia Treatment Center Tunis over a period of 2 years (December 2021 - December 2023). A total of 85 plasma samples were collected from patients treated with SHL FVIII.

Plasma samples were collected at T0 (before injection), 30 minutes, 6 hours, 24 hours, and 48 hours after FVIII injection, they were then kept at -80°C until assays. All analyses were

performed on an ACL TOP 350 analyzer. Each sample was analyzed simultaneously by both OSA and CSA on the same day, using the same aliquot.

The OSA employed the following reagents: SynthASil® HemosIL® and Factor VIII deficient plasma: HemosIL®. FVIII levels using the CSA were measured with the HemosIL® ELECTRACHROME Factor VIII kit.

Data were analyzed using Excel.

Differences between OSA and CSA were analyzed by two statistical methods: Pearson correlation coefficient and Bland-Altman limits of agreement (LoA) method with graphical representation.

Results:

We reported an excellent correlation between the two methods with Pearson correlation coefficients of $R = 0.952$ with a statistically significant difference ($p < 0.0001$).

Additionally, the Bland-Altman analysis involved calculating Mean differences (MD) with standard deviations (SD) which were equal to -1,2 % and 6,3% respectively.

The mean FVIII concentrations ranged from 0.05% to 84.75%.

The MD was close to zero, indicating that the two assays were in agreement.

The MD values below zero indicated an underestimation by the OSA compared to the CSA.

Most of the data points lied within the LoA, it suggested that the two measurement methods were in good agreement.

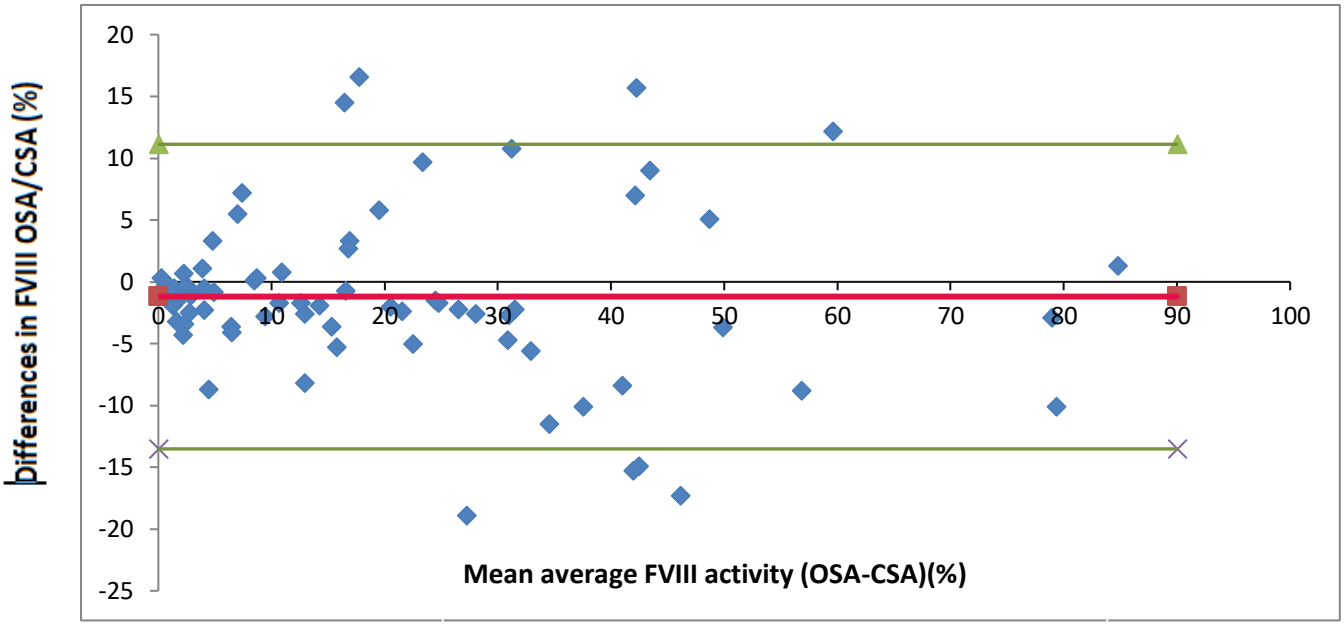

Figure 1: Bland–Altman analysis of factor VIII (FVIII) activity measured by one-stage clotting assay (OSA) and chromogenic substrate assay (CSA) in postinfusion plasma samples collected in patients treated with SHL FVIII. Mean differences and standard deviation (2SD) values are presented by red and green lines: [MD = -1,2 % ; SD = 6,3%]

Conclusion:

The interest in the CSA has been renewed, particularly with the advent of non-substitutive therapies. Both CSA and the OSA are in agreement, which is why the OSA can be utilized in low-income countries.