Data interpretation of the ETP-based APC resistance assay and the concept of nAPCsr10, an improved nAPCsr

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DATA INTERPRETATION OF THE ETP-BASED APC RESISTANCE ASSAY AND THE CONCEPT OF nAPCsr10, AN IMPROVED nAPCsr

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BACKGROUND

- The evaluation of the activated protein C resistance assay based on the endogenous thrombin potential (ETP-based APCr assay) is requested during the development of steroid contraceptives.[1]
- Results are usually expressed as "normalized APC sensitivity ratio" (nAPCsr) which is the ratio of the patient's sample on a reference plasma regarding the inhibitory sensitivity of the ETP towards exogenous APC.[2,3]
- The reference plasma should achieve near 10% residual ETP in the presence of exogenous APC.
- However, the use of homemade reference plasma makes the nAPCsr difficult to compare between studies and the impossibility to obtain exactly 10% residual ETP, because of the inter-assay variability, can significantly affect the theoretical 0 to 10 scale of nAPCsr.

AIM

To compare the nAPCsr with this new method for calculation of APC resistance: the nAPCsr10.

METHOD

- 790 individual plasmas (issued from two sponsored clinical trials) were analyzed to compared their nAPCsr and nAPCsr10
- Measurements were performed following our validated protocol of the ETP-based APCr assay.
- A commercially available reference plasma and three levels of quality controls (QCs) were used to validate each experiment.
- Results were expressed as nAPCsr [▶ equation 1] and as nAPCsr10 [▶ equation 2]

$$\text{nAPCsr} = \frac{Sample\ ETP\ (+APC) / Sample\ ETP\ (-APC)}{Reference\ plasma\ ETP\ (+APC) / Reference\ plasma\ ETP\ (-APC)} \quad \text{[equation 1]}$$

$$\text{nAPCsr}_{10} = \frac{Sample\ residual\ ETP\ (\%)}{10\%} \quad \text{[equation 2]}$$

$$\text{with residual ETP \%} = \frac{Sample\ ETP\ (+APC)}{Sample\ ETP\ (-APC)}$$

RESULTS

A. Test performances

- Over 86 measurements (38 with batch 1 and 48 with batch 2), no statistically significant difference (p-value>0.05) was observed between the two batches of reference plasma.
- QCs fitted into their acceptability ranges regardless the batch used but nAPCsr was less reproducible (higher SD) than nAPCsr10. [▶Table 1]

		nAPCsr			nAPCsr$_{10}$		
		Mean	SD	95% CI	Mean	SD	95% CI
QC high	Batch 1	8.80	0.75	[7.32-10.28]	9.37	0.27	[8.85-9.90]
	Batch 2	8.88	0.90	[7.11-10.64]	9.39	0.23	[8.94-9.84]
QC interm	Batch 1	7.51	0.49	[6.55-8.47]	8.01	0.29	[7.44-8.59]
	Batch 2	6.46	0.49	[5.50-7.42]	6.86	0.40	[6.07-7.65]
QC low	Batch 1	0.00	/	/	0.00	/	/
	Batch 2	0.00	/	/	0.00	/	/

TABLE 1: Range values of the nAPCsr and the nAPCsr10 among the two batches of reference plasma on QC high, QC intermediate (QC int.) and QC low. The mean, standard deviation (SD) and 95% confidence interval (=1.96*SD) for each batch on the three levels of QC are shown.

- Mean inhibition % of the reference plasma (both batches mixed) was 89.30% (SD: 1.03%, 95% CI: [87.29%-91.31%]) and expressed in ratio, it equalled 0.107 (95% CI: 0.087-0.127].
- Despite a weak variability of the reference plasma, the nAPCsr scale could vary widely.
- In the case of inhibition % of the reference plasma equalled to 91.8 (maximal tolerated limit in our study [mean inhibition % + 2.5%]), residual ETP ratio would be 0.082. Therefore, nAPCsr scale could go up to 12.2 instead of the maximal value of 10 that is theoretically proposed.

B. Correlation between nAPCsr and nAPCsr10

- Wilcoxon signed-rang paired showed a statistically significant difference between nAPCsr and nAPCsr10.
- Nonetheless, a correlation between nAPCsr and nAPCsr10 was demonstrated with a Spearman correlation (rs) coefficient of 0.99 (95%CI: 0.9880-0.9910; p-value<0.0001).
- Linear regression showed a slope of 0.93 which denoted 7% downward systematic deviation of nAPCsr compared to nAPCsr10. [▶ Figure 1]

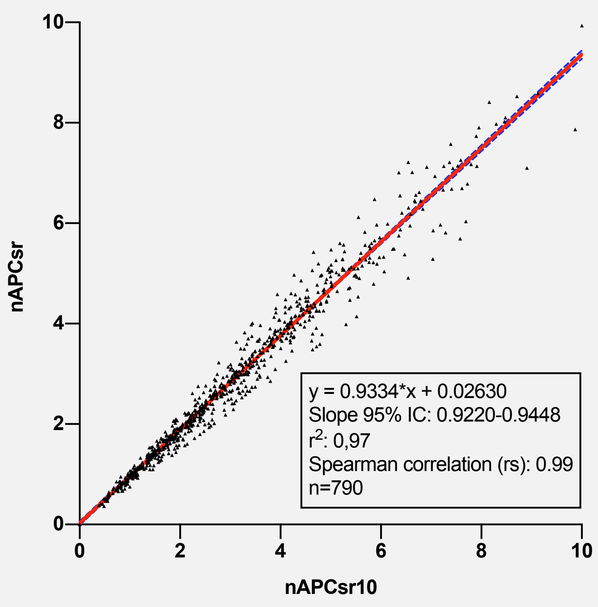

FIGURE 1: Correlation between nAPCsr and nAPCsr10. Spearman correlation coefficient (rs) [95% CI] of 0.9896 [0.9880-0.9910]; p-value<0.0001; r^2 for linear regression = 0.9704. Linear regression (red straight line) with a slope [95% CI] of 0.9334 [0.9220-0.9448] (blue dotted lines) and Y-intercept of 0.02630.

- Bland-Altman analysis confirmed our statement that nAPCsr underestimated APC resistance compared to nAPCsr10 with a mean difference (nAPCsr10-nAPCsr) of 5.64% compared to nAPCsr10. [▶ Figure 2]
- As mean residual ETP ratio of the refence plasma was greater than 0.1 (0.107), when used at the denominator, it gave smaller values than if we had 0.1 at the denominator as for the nAPCsr10.

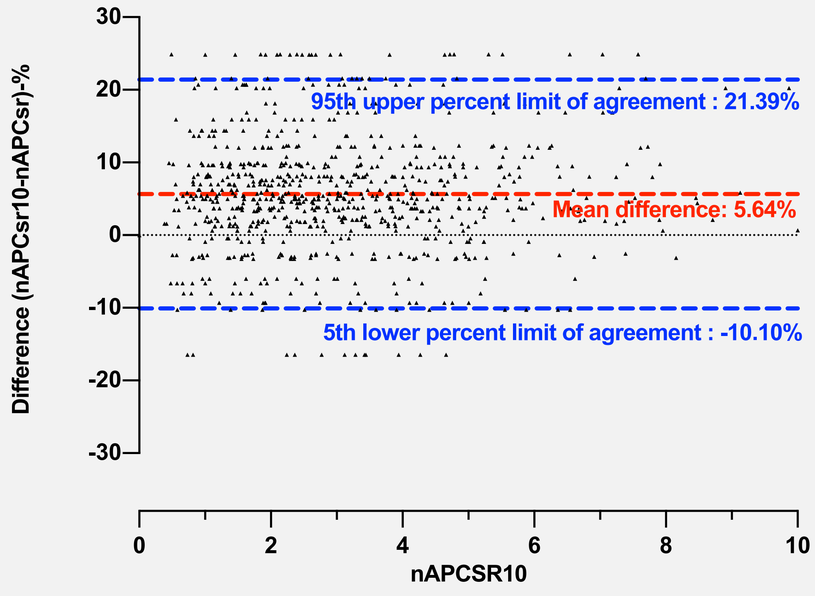

FIGURE 2: Derived Bland-Altman analysis between nAPCsr and nAPCsr10 for the measurement of APC resistance in plasma samples. Differences (nAPCsr10-nAPCsr) are expressed as a percentage of nAPCsr10. The red dotted line represents the mean difference and blues dotted lines represent 95% IC [=1.96*SD].

CONCLUSION

This is the first study presenting a new scale for the harmonization and normalization of the nAPCsr.

Results revealed a better reproducibility with the nAPCsr10. It avoids the additional variability and the unharmonized scale brought by the use of a reference plasma. Instead, this reference plasma can serve as an additional level of control targeting the desired 10% of residual activity.

This new method and result expression will provide the pharmaceutical industry, the regulatory bodies and the physician, with the opportunity of better reproducibility and harmonization. This will definitively help in study-to-study comparison.

This standardized expression could ease the establishment of thresholds to assess patient's thrombotic state according to his resistance to APC.

Conflict of Interest :

Jonathan Douxfils reports personal fees from Daiichi Sankyo, Diagnostica Stago, Roche and Roche Diagnostics outside the submitted work. Jonathan Douxfils is CEO and founder of QUALIblood s.a.

References

[1] Guideline on clinical investigation of steroid contraceptives in women - EMEA/CPMP/EWP/519/98 Rev 1.

[2] Nicolaes GA, Thomassen MC, Tans G, Rosing J, Hemker HC. Effect of activated protein C on thrombin generation and on the thrombin potential in plasma of normal and APC-resistant individuals. *Blood Coagul Fibrinolysis*. 1997; **8**: 28-38

[3] Tripodi A, Chantarangkul V, Negri B, Mannucci PM. Standardization of the APC Resistance Test. Effects of Normalization of Results by Means of Pooled Normal Plasma. *Thromb Haemost*. 1998; **79**: 564-6.

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