COMPARISON BETWEEN TWO METHODS FOR ROUTINE TESTING OF HEMATOLOGIC PARAMETERS
POROVNANIE DVOCH METÓD PRE RUTINNÉ TESTOVANIE HEMATOLOGICKÝCH PARAMETROV

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ABSTRACT

Objective: In addition to methodological variability in biomedical laboratory parameters, their own innate nature and interrelations are also important factors to be considered in routine examination. In our study, we verified the degree of agreement between the results of routine haematology parameters obtained from two different laboratory analysers. With the aid of the Passing-Bablok regression method we tested the results of activated partial thromboplastin time (APTT) and APTT-ratio obtained from 22 samples parallel analysed on two devices (CA1500 and Coag XL).

Results: We found out systematic and proportional differences between the results from two devices that suggest such a non-compliance rate, which does not allow us to accept the interchangeability of methods. In addition, in the case of intrinsically related laboratory parameters there can be different characteristics inherent due to the nature of primary data. For these reasons, the reciprocal substitutability of the two tested devices and interchangeability of their results has not been possible.

Conclusions: We can conclude that not only methodological variability, but also the intrinsic nature of the tested biomedical laboratory parameters can significantly affect the resulting data.

Key words: Interpretation of laboratory results. Interchangeability of laboratory methods. Passing-Bablok regression. APTT. APTT-ratio.

ABSTRAKT

Úvod: Okrem metodickej variability biochemických laboratórnych parametrov sú pri rutinnom vyšetrení dôležitými faktormi aj ich samotná povaha a vzájomné vzťahy.

Cieľ: V našej štúdii sme overili mieru zhody medzi výsledkami rutinných hematologických parametrov získaných z dvoch rôznych laboratórnych analyzátorov. Pomocou Passing-Bablokovej regresnej metódy sme testovali výsledky aktivovaného parciálneho tromboplastínového času (APTT) a APTT-pomeru získané z 22 vzoriek paralelne analyzovaných na dvoch zariadeniach (CA1500 a Coag XL).

Výsledky: Zistili sme systematické a proporcionálne rozdiely medzi výsledkami z dvoch zariadení, ktoré naznačujú takú mieru nesúladu, ktorá nám neumožňuje akceptovať zameniteľnosť metód. Okrem toho v prípade vnútorne príbuzných laboratórnych parametrov môžu existovať rôzne charakteristické znaky vzhľadom na povahu primárnych údajov. Z týchto dôvodov nie je možná vzájomná zameniteľnosť dvoch testovaných zariadení a zameniteľnosť ich výsledkov.

Závery: Môžeme konštatovať, že nielen metodická variabilita, ale aj vnútorná povaha testovaných biomedicínskych laboratórnych paramctrov môžu významnc ovplyvniť výslednć údajc.

Kľúčové slová: Interpretácia laboratórnych výsledkov. Zameniteľnosť laboratórnych metód. Passing-Bablokova regresia. APTT. Pomer APTT.

INTRODUCTION

Laboratory examinations in healthcare and biomedicine are an example of the practical implementation of knowledge about the chemical and biological basis of human metabolism in order to achieve efficient and effective therapy of diseases. As a result of a wide range of methodological options available for each laboratory parameter, there is an increasing demand for verification of their compliance [1-3]. The assessing agreement between two methods in the routine testing of identical parameters is of crucial importance for the modernization and renovation of laboratory instrumentation [4]. Each routine laboratory should be able to carry out the modernization of the instrumentation so as not to alter the continuity of the results of the laboratory parameters in long-term monitoring of patients [5].

Appropriate statistical tools for this testing appears to be the Passing-Bablok regression model due to robustness to outliers [6,7]. “Classical” parametric or non-parametric paired tests (such a Wilcoxon test or paired t-test for example) are not appropriate for the testing of two methods in regard to the substitutability of the two different devices and interchangeability of different methods. Those tests give us an information about the accordance in medium values and the distributions of tested variables, but say nothing about the extent of compliance. In that case both methods can be used only in a parallel and independent way, following criteria and recommendations by authorities (e.g. International Federation of Clinical Chemistry and Laboratory Medicine, IFCC; International Committee for Standardisation in Haematology, ICSH) [8, 9].

Most laboratory parameters investigated in biomedicine have the character of primary data, which refers to a numerical value obtained by testing sam-

ples directly without further adjustments and calculations. However, they are an important laboratory parameters, whose resultant value is not the result of "simple" obtaining numerical data from the analyser, but is a result of the conversion in a defined formula. The most commonly used parameters of this type include, for example Creatinine clearance which is calculated as following: the calculation formula NH4 + excretion (UAG), atherogenic index of plasma (AIP), activated partial thromboplastin time ratio, based on the parameters of a standard plasma (APTT-R) as well as several others [10, 11]. The latter parameter (APTT-R, is given in percentages) is important for assessing the activity of intrinsic coagulation system and the monitoring of heparin anticoagulant therapy [12]. Compared to the direct determination of the activated partial thromboplastin time (APTT in seconds) it is given by calculating the proportion of the patient's coagulation time (tP) and the time of coagulation of control plasma (tK): APTT-R (%) = tP / tK. Clinical laboratories issued on their result from both parameters together. In terms of clinical interpretation the preferable parameter is APTT-R because it is more objective and results of the various laboratory are in this case better corresponding [13,14].

The aim of our work was conformity testing of laboratory determination of APTT and APTT-ratio in two laboratory analysers. The aim of the test was to verify the consistency of the results of two differrent laboratory analysers in the specific case of closely related testing laboratory parameters, one of which has a character of primary data and the second parameter is obtained by calculation.

MATERIALS AND METHODS

Devices: Both parameters – APTT and APTT-R were tested by the two devices: Reference instrument was coagulation analyser Sysmex CA1500 (Sysmex, Japan) commonly used in routine laboratory diagnostics with the laboratory reagents for the determination of APTT (Siemens Dade® Actin® FS Activated PTT Reagent ACTIN FS Item number: B4218-100, batch number: 538507, expiry date: 08.31.2017). This reference analyser is covered by monitoring parameters within the external quality control provided by the suppliers SEKK and RIQAS. As a tested analyser was used the coagulation analyser COAG XL (Diagonal, Hungary) with reagents for APTT (Dia-PTT Liquid, catalogue number: 72024, lot number: 960130, expiry date: 01/2017).

Patients: Laboratory parameters were determined in samples of 22 individuals. The group consisted of ambulatory patients examined during the June 2016, the collections of samples had been done consecutively without any restrictive selection criterion.

Ethics: All individuals were treated in accordance with the Declaration of Helsinky requirements and all physicians are obliged to obtain patient's informed consent before treatment. The laboratory did not perform any tests beyond the parameters requested by physicians.

Statistical analysis: Verification of compliance rate of the two devices were tested using the method of regression by Passing-Bablok with the CUSUM linearity test. Next, for the comparison we used paired *t*-test and nonparametric Wilcoxon test. Normality of data distribution was verified using the Kolmogorov-Smirnov test. For statistical processing we used MS Excel 2013 with post-module Analyse-It (ver. 4.65.2, Analyse-it Software, Ltd. US trial version) and statistical software Instat (ver. 2.3, GraphPad Software, Inc., USA). If the *p*-value of the test result fell below 0.05 ($p < 0.05$) differrences in mean and distribution of files, respectively, we considered to be non-random and statistically significant.

RESULTS AND DISCUSSION

The results of the Passing-Bablok regression are shown in the Table 1. Given data also indicates that, in the case of the APTT, the 95% confidence inter-

Table 1 Testing the agreement of laboratory examinations of APTT and APTT-R with the Passing-Bablok regression method

Parameter (units)	Instruments	*n*	Regression	*p*	*CI* (intercept)		*CI* (slope)	
					-95%	+95%	-95%	+95%
APTT (sec.)	CA1500	22	y=0.8435x+4.8425	0.76	0.0921	10.9375	0.6389	0.9841
	COAG XL	22						
APTT–R (ratio)	CA1500	22	y=0.8537x+0.1024	0.41	-0.0927	0.3611	0.6194	1.0208
	COAG XL	22						

Legend: n – number of samples, *CI* – confidence interval, *p*- value of CUSUM linearity test

Table 2 Basic statistics of APTT and APTT-R and results of paired statistical tests

Parameter (units)	Instruments	*n*	$\bar{x}$	*sd*	x_m	*min.*	*max.*	Wilcoxon test	*t*-test	
								p	*d.f.*	*p*
APTT (sec.)	CA1500	22	32.12	6.70	31.00	23.60	49.10	0.425	21	0.490
	COAG XL	22	31.77	5.57	30.10	23.50	44.10			
APTT–R (ratio)	CA1500	22	1.14	0.22	1.11	0.82	1.69	**0.001**	21	**0.001**
	COAG XL	22	1.08	0.20	1.03	0.80	1.55			

Legend: n – number of samples, $\bar{x}$ – arithmetic mean, *sd* – standard deviation, x_m – median, *min.* – minimal observed value, *max.* – maximal observed value, *p* – values of test criterion of both paired tests, *d.f.* – degrees of freedom of the paired *t*-test

val (CI) of the intercept does not include zero, thus suggesting the presence of systematic differences between the two test methods. The 95% CI for slope does not include the number 1, which suggests the presence of a proportional component of bias. In the case of the Passing-Bablok analysis of the APTT-R, the 95% CI for the intercept includes number 0 (-0.0927; 0.3611) as well as the 95% CI for the slope includes the number 1 (0.6194; 1.0208).

Therefore, there can be admitted the methodological consensus of both tested laboratory methods. For both parameters APTT and APTT-R we did not reject the assumption of linearity (CUSUM test, $p > 0.05$), which entitles us to accept the application of Passing-Bablok model.

From the results shown in the Table 2 it can be concluded that the differences between the arithmetic averages and distributions between the results of the two tested devices were not statistically significant in the case of APTT parameter ($p = 0.49$ paired *t*-test; $p = 0.425$ Wilcoxon test). On the other hand, the derived parameter APTT-R showed for both used tests statistically significant differences ($p < 0.001$). Before using both *t*-test and the Wilcoxon tests, the normality of distribution had been tested by Kolmogorov-Smirnov test. In all cases we have accepted the presence assumption of normality ($p > 0.10$).

By the interpretation of obtained results there is at first sight a collision of bio-statistical interpretation with an analytical and diagnostic approaches. Passing-Bablok model does not imply the acceptance of conformity compared to two methods for the direct determination of parameters of APTT, but does not discount it in the case of APTT-R, which is given by calculation.

Moreover, the two paired test (Wilcoxon test and the paired *t*-test) confirmed the conformity of the averages and the distribution of both methods in the case of directly measured data of APTT ($p > 0.05$), but not the case of the calculated data of APTT-R ($p < 0.001$). From the view of interpretation and requirements of biomedical diagnostics both tests are unhelpful in contrast to the Passing-Bablok regression.

The reason is that in the cases of the APTT there are "pure" primary numerical data obtained directly from laboratory analysis, but this cannot be said for the parameter APTT-R. From the bio-statistical point of view the data of APTT-R are "modified" by the calculation of the ratio of the coagulation time of patient's sample and the coagulation time of the control plasma, which also probably affected the used regression model (Tab. 1). The same way were affected both used paired tests, and that naturally results from the method of their calculation.

The problem of interpretation of our results consists at two levels:

- *The level of laboratory diagnostics:* The practical application of the interchangeability of any two comparted devices is rational only in the case of consistence in a whole range of parameters [15]. Otherwise, the testing loses practical significance both from the point of economic efficiency as well as from the perspective of the diagnostic validity. In our study, instead of testing independent parameters clinically, very similar and closely related laboratory parameters had been tested. The test results appear different, apparently due to the very nature of the calculation of APTT-R, different. In clinical practice, these parameters are issued on the score sheet always together, and therefore we cannot accept interchangeability of both devices.
- *The level of the clinical trials and interpretations:* In large clinical trials, the results of the tested laboratory parameters are often an important part, which may have a crucial influence on the next steps e.g. in the case of the development of new diagnostic methods or drugs [16, 17]. The presence of related variables such as e.g. APTT and APTT-R or another derived parameters can significantly affect the results of multivariate statistical analysis with the consequences to interpretation in

biomedical context and reproducibility of the obtained data.

CONCLUSION

Currently in the field of biomedicine there is available enormous amount of laboratory diagnostic methods which are able to deliver high-quality primary data with the required accuracy and precision. A persistent problem remains in the determination of the degree of compliance and acceptability of interchangeability of the methodologically different laboratory analyses of the same parameter. The results of our study also revealed that apart from methodological variability, the inherent, intrinsic nature of the tested parameter (i.e. whether there are primary numerical data or derived numbers) has a significant impact on the conformity of the compared laboratory methods.

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REFERENCES

[1] MELUŠ V., KIRICOVÁ G., ŠIMEK M. Laboratory testing of hereditary thrombophilia: Previous data in the face of verification. *Bratisl Lek Listy.* 2009; 110 (1): 18-20.

[2] ANTONELLI G., PADOAN A., AITA A. et al. Verification of examination procedures in clinical laboratory for imprecision, trueness and diagnostic accuracy according to ISO 15189:2012: a pragmatic approach. *Clin Chem Lab Med.* 2017; 55 (10): 1501-1508.

[3] GEENS T., VERTESSEN F., MALFAIT R. et al. Validation of the Sysmex CS5100 coagulation analyzer and comparison to the Stago STA-R analyzer for routine coagulation parameters. *Int J Lab Hematol.* 2015; 37: 372-381.

[4] MCFARLANE A., ASLAN B., RABY A. et al. Internal Quality Control Practices in Coagulation Laboratories: recommendations based on a patterns-of-practice survey. *Int J Lab Hematol*. 2015; 37: 729-738.

[5] PASSING H., BABLOK W.J. Comparison of several regression procedures for method comparison studies and determination of sample sizes. Application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. *Clin Chem Clin Biochem.* 1983; 21: 709-720.

[6] BILIČ-ZULLE L. Comparison of methods: Passing and Bablok regression. *Biochem Med.* 2011; 21: 49-52.

[7] OZARDA Y. Reference intervals: current status, recent developments and future considerations. *Biochem Med.* 2016; 26: 5-11.

[8] PANTEGHINI M. Traceability, Reference Systems and Result Comparability. *Clin Biochem Rev.* 2007; 28: 97-104.

[9] EBNER M., BIRSCHMANN I., PETER A. et al. Emergency Coagulation Assessment During Treatment With Direct Oral Anticoagulants: Limitations and Solutions. Stroke. 2017; 48: 2457-2463.

[10] DE LUCA R., FONTANA P., PONCET A. et al. Evaluation of the GEM®PCL Plus point-of-care device for neonatal coagulation assessment: an observational study on cord blood. *Thromb Res.* 2014;134:474-478.

[11] LEDNICKÝ P., NETRIOVÁ J. Koagulačný analyzátor Xprecia – pomoc pri monitorovaní INR priamo v ambulancii. *Zdravotnícke listy.* 2019; 7 (1): 17-23.

[12] LAWRIE A.S., KITCHEN S., EFTHYMIOU M. et al. Determination of APTT factor sensitivity – the misguiding guideline. *Int Jnl Lab Hem.* 2014; 35: 652-657.

[13] MA Y., HUH H.J., KIM S.H. et al. Low intra-individual variability of activated partial thromboplastin time revealed in population of 10,487 control individuals. *Blood Coagul Fibrinolysis.* 2013; 24: 746-748.

[14] MILOS M., HERAK D.C., ZADRO R. Discrepancies between APTT results determined with different evaluation modes on automated coagulation analyzers. *Int J Lab Hematol*. 2010; 32: 33-39.

[15] GUY S., KITCHEN S., MACLEAN R. et al. Limitation of the activated partial thromboplastin time as a monitoring method of the direct thrombin inhibitor argatroban. *Int J Lab Hematol.* 2015; 37: 834-843.

[16] VAN COTT E.M., ROBERTS A.J., DAGER W.E. Laboratory Monitoring of Parenteral Direct Thrombin Inhibitors. *Semin Thromb Haemost.* 2017; 43: 270-276.

[17] SOLANO C., ZERAFA P., BIRD R. A study of atypical APTT derivative curves on the ACL TOP coagulation analyser. *Int J Lab Hematol.* 2011; 33: 67-78.