Clinica Chimica Acta 509 (2020) 264–267

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Analytical performances and biological variation of PIVKA-II (des-y-carboxy-prothrombin) in European healthy adults

Jean-Louis Bayart*, Antoine Mairesse, Damien Gruson, Marie-Astrid van Dievoet

Département des laboratoires cliniques, Cliniques Universitaires St-Luc – Université Catholique de Louvain, Brussels, Belgium

ARTICLE INFO

Keywords:
Biological variation
PIVKA
Des-y-carboxy-prothrombin
Carcinoma

ABSTRACT

Background: PIVKA-II (DCP) is increasingly used for the diagnosis and the surveillance of hepatocellular carcinoma (HCC) in at-risk populations. However, to date, few data are available concerning the intra- and inter-individual variability of this marker, which makes the interpretation of serial measurements difficult in the context of monitoring.

Methods: 19 European healthy volunteers (HVs) were taken each week during five consecutive weeks. Samples were analyzed in duplicate on the Lumipulse® analyzer (Fujirebio, Gent, Belgium). Analytical variation (CV_A), within-subject biological variation (CV_I) and between-subject biological variation (CV_G) were calculated using nested ANOVA following normality assessment, outlier exclusion, and homogeneity of variance analysis.

Results: No significant difference was observed for the mean values ($p = 0.23$) between men (mean: 30.66 mAU/mL) and women (mean: 33.90 mAU/mL) subgroups. CV_A was 2.82% while sex-independent CV_I and CV_G were 13.35% and 16.05%, respectively. Taking these values, the calculated reference change value (RCV) and index of individuality were 37.70% and 0.85, respectively.

Conclusion: We reported for the first-time biological variation data for PIVKA-II in a cohort of European HVs. We believe that our results can be used to set analytical specification goals as well as to improve the interpretation of serial measurements of PIVKA-II for monitoring purposes.

1. Introduction

Tumor markers are useful for the diagnosis and the follow-up of disease progression in cancer-related patients. Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and the prognosis of this type of cancer remains still unsatisfying due to a lack of early detecting methods. Indeed, hepatic ultrasound and alpha-fetoprotein (AFP) testing have been for a long time the only available methods for the diagnosis of HCC [1]. First reported in 1984, Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), also called Des-y-Carboxy-Prothrombin (DCP), is a marker that shows good performances for the diagnosis and surveillance of HCC in at-risk populations [2–4]. While being increasingly used worldwide, data concerning the within and between-subject biological variation of this marker are still scarce. Indeed, biological variation (BV) is of substantial interest for tumor markers since changes over times in their related levels can be clinically relevant but sometimes difficult to assess [5]. These variations in serial measurements can be due to pre-analytical sources of variation, analytical imprecision or within-individual fluctuation around the own homeostatic set point [6]. Taking these sources of variation into consideration, the aim of this study was to assess the analytical performances of the Lumipulse® G600II PIVKA-II assay (Fujirebio, Ghent, Belgium) as well as the within- and between-individual variation in healthy volunteers (HV).

2. Material & methods

2.1. Study participants and samples collection

19 European HVs were recruited, comprising 8 males (25–59 years, median age: 33.5 years ± 10,8 years) and 11 females (22–60 years, median age: 39 years ± 11,4 years). None of the participants consumed substantial (> 10 g/day) quantities of alcohol or were smokers. All participants signed an informed consent form and study protocol was approved by the Ethics Committee of our hospital (Cliniques Universitaires Saint-Luc, Brussels, Belgium) (Ref number: 2019/04SEP/388). A total of five venous samples were collected from each subject, on the same day of the week for 5 consecutive weeks. Venous blood

* Corresponding author at: Département des laboratoires cliniques, Université catholique de Louvain, Tour Franklin, 10 Avenue Hippocrate, B-1200 Brussels, Belgium.
E-mail address: jean-louis.bayart@uclouvain.be (J.-L. Bayart).

https://doi.org/10.1016/j.cca.2020.06.035
Received 2 June 2020; Received in revised form 20 June 2020; Accepted 22 June 2020
Available online 24 June 2020

samples were systematically drawn by the same skilled phlebotomist between 8.00 and 11.00 AM in serum-tubes (S Monovette® 7.5 Ml tubes, Sarstedt, Nümbrecht, Germany). Samples were then spun for 10 min at 3500 rpm, immediately aliquoted in cryovials and stored at −80 °C until analysis.

2.2. Analytical evaluation of the Lumipulse G600II analyzer

Before aiming to determine BV, a brief analytical evaluation of the Lumipulse G600II analyzer (Fujirebio Europe NV, Gent, Belgium) was performed. Repeatability, with-in and between laboratory coefficients of variation (CV) were determined according to CLSI EP15-A2 guidelines. For linearity assessment, a serum sample with high PIVKA-II concentration was serially diluted with manufacturer's diluent.

2.3. Measurements of PIVKA-II in HV

Specimens issued from volunteers were thawed at room temperature for 30 min, mixed and analyzed in a single run in duplicate on the Lumipulse® G600II analyzer. Internal quality controls (IQC) were measured before and after the run. The limit of detection of the assay is 1.37 mAU/mL.

2.4. Statistical analysis

Statistical analyses were conducted with Prism 6 (GraphPad Software, San Diego, CA) and JMP Pro (JMP®, version 14.3.0, SAS Institute Inc, Cary, NC). Outlier identification was performed using Cochran's test among observations between duplicate measurements and between within-subject total variance (S^2_{I+A}) to see if any individual's dispersion was smaller or larger compared to the whole group. Reed's criterion was also applied on mean concentration values of the HVs. Normality was assessed with the Shapiro-Wilk test both on the set of each individual results and on the mean concentration values of the whole cohort. If the hypothesis of normality was rejected, alternative normality test (Kolmogorov-Smirnov) was applied. Mean and S^2_{I+A} values of the subgroups (males and females) were compared using Student's *t*-test and *F*-test, respectively.

Sources of variability were calculated using a nested model with three factors (individuals, sampling occasions and replicates) with JMP® Pro. In this way, within-individual, between-individual and analytical variances were obtained to calculate within-individual coefficient of variation (CV_I), between-individual coefficient of variation (CV_G) and analytical variation (CV_A), respectively. The index of individuality (II) was obtained from CV_I and CV_G while the index of heterogeneity (IH) was calculated as the observed CV of the set of individual variances to the theoretical CV, which is $[2/(n-1)]^{1/2}$ (where "n" is the average number of samples collected per subject). The SD of the difference between this ratio and its expected value of unity is $1/(2n)^{1/2}$. A significant heterogeneity is present if the ratio differs from unity by at least twice the SD. Therefore, assessing five samples for each subject, an IH < 0.63 suggests that the within-subject variations are homogeneous. Reference change value (RCV) was determined using the formula $2^{1/2} \times Z \times (CV_A{}^2 + CV_I{}^2)^{1/2}$, where Z value of 1.96 represents probability of 95%. Finally, desirable analytical goal for imprecision, total error and bias was calculated. Further details of all these calculations and statistical tests can be found elsewhere [7].

3. Results

Results of EP15-A2 imprecision study gave satisfactory results, with all CVs being < 5%. Mean concentrations were 30.9 and 138.0 mAU/mL. With-in CV ranged from 2.2% (low concentration) to 2.9% (high concentration). Between-run variability was 3.7% (low concentration) and 2.3% (high concentration). These levels of imprecision were comparable with values reported by the manufacturer. The linear equation of linearity was y = 0,985x + 148,3 with $r^2 = 0.99$.

For the BV study, all results were above the LOD of the assay. Shapiro-Wilk test accepted the hypothesis of normality for the distribution of mean concentration values while the intra-subject data distribution normality was accepted in 17 out of the 19 patients. However, the 2 concerned patients passed normality test according to Kolmogorov-Smirnov. Cochran's statistical test (for $p = 0.01$) among duplicate measurements revealed an outlier for subject 11. This subject was therefore excluded for analysis. Following this exclusion, no outlier values were identified for both Cochran's test among observations (derived from duplicate measurements) and S^2_{I+A} values. Finally, Reed's criterion applied on the mean concentration values of all subjects was successfully passed.

No significant differences were observed for the mean values ($p = 0.23$) nor for S^2_{I+A} values ($p = 0.29$) between the men (mean: 30.66 mAU/mL) and women (mean: 33.90 mAU/mL) subgroups; this allowing us the derivation of sex-independent CV_I and CV_G values.

Fig. 1 illustrates intra-indivual means and absolute range of values after removing patient 11. Analytical variation was 2.82% and the CV_I was slightly lower than the CV_G (13.35% vs 16.05%, respectively). Taking CV_I and CV_A values together, the calculated RCV was 37.70%. Calculated II and IH were 0.85 and 0.52, respectively (Table 1).

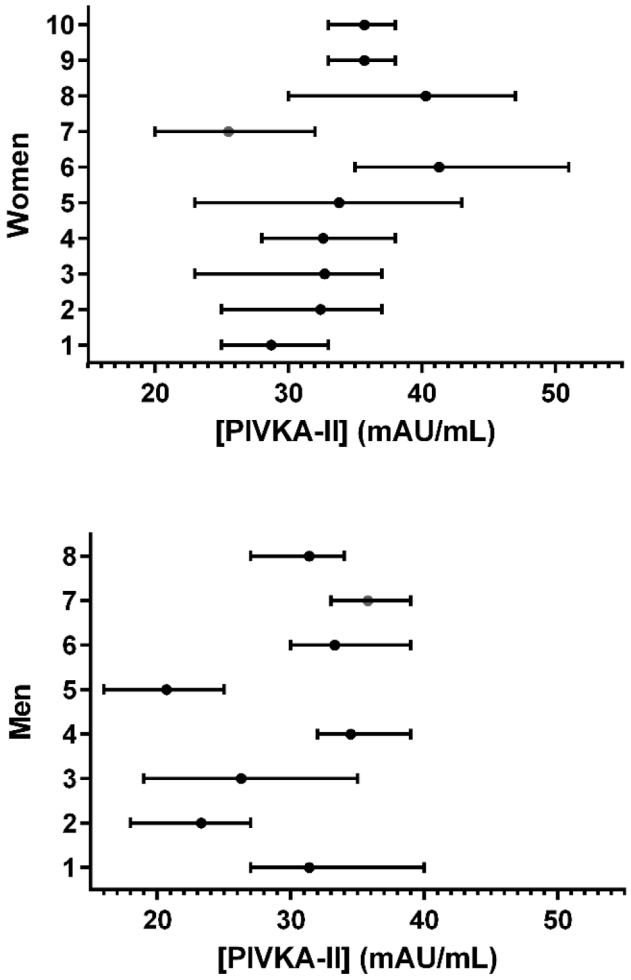

Fig. 1. Serum PIVKA-II intra-individual means and absolute range of values in women and men HVs.

Table 1
Mean values, analytical (CV_A), intra-individual (CV_I) and inter-individual (CV_G) variations, and derived analytical goals (desirable specifications). II: index of individuality; IH: Index of heterogeneity.

Parameter	Group (n)	Mean (mAU/mL) (IC_{95})	CV_A% (IC_{95})	CV_I% (IC_{95})	CV_G% (IC_{95})	II	IH	RCV (%)	Desirable specifications		
									Imprecision (%)	Bias (%)	Total error (%)
PIVKA-II	All (18)	32.0 (31.0–33.0)	2.82 (2.46–3.31)	13.35 (11.44–16.01)	16.05 (11.64–25.85)	0.85	0.52	37.70	≤6.7	≤5.2	≤16.2

4. Discussion

Largely used in Asia for decades, PIVKA-II is a noninvasive biomarker that has clearly gained interest over the last years in Western countries. While this marker is mainly used for HCC diagnosis purposes and as an independent predictive factor of microvascular invasion, it is also increasingly used for the evaluation of tumor response and disease recurrence [3,8–10]. However, to accurately estimate longitudinal changes in patients, the notion of BV is critical to assess if the changes in serial values are clinically relevant or not. Recently, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) established a new web-based BV Database which is destined to replace the former 2014 BV database hosted on the Westgard website. However, there is currently no data for PIVKA-II in the new EFLM database.

In this study we used the Lumipulse® analyzer to measure PIVKA-II levels in both men and women subgroups. In line with a previous multicenter study aiming to set reference values in a healthy population, no difference in mean concentrations was observed between men and women [4]. The calculated CV_A was in line with the with-in run CV issued from the EP15 experiment and lower than ½ CV_I, therefore indicating that BV can be used to set desirable performance specifications for imprecision [6]. Our results reveal that PIVKA-II shows a CV_I (13.35%) nearly as high as the CV_G (16.05%), leading to a calculated RCV of 37.7%. This means that when this marker is used for monitoring purpose, variations in the successive results need to be higher to this latter value before being considered as a clinically relevant change. However, clinicians should be aware that RCVs derived from BV studies are probably oversensitive when applied in some clinical applications where the collection and processing of the samples are less controlled as in this kind of studies.

Our CV_I and CV_G values are lower than the only former study on BV for PIVKA-II, where reported CV_I and CV_G were 24.6% and 40.4%, respectively [11]. However, this study was performed on a shorter timeframe (14 days) with only 10 patients (5 men and 5 women) having cirrhosis due to HCV infection. Unfortunately, the generated data didn't show a normal distribution and no logarithmic transformation was applied to generate their BV data. Moreover, the inclusion of patients suffering from an underlying hepatic disease raises some questions as these patients may have elevated PIVKA-II levels. As pointed out by current guidelines, the enrolled subjects in a BV study should be healthy due to the fact that the presence of a disease, even if well controlled, may amplify the fluctuation in the measured biomarker [7,12].

Our data suggest that PIVKA-II shows a relatively low CV_G compared to other tumor markers previously studied. As a matter of fact, AFP, the other commonly used marker for monitoring patients suffering from HCC, showed a CV_I of 26.7% and a CV_G of 43.7% [13]. The low difference observed between the CV_I and the CV_G for PIVKA-II results in an II of 0.85. While higher than most other tumor markers, this value still suggests that the use of classical population reference intervals is of less relevance when deciding whether variations observed in an individual are clinically significant [7,13]. Further, our calculated IH fulfilled the homogeneity condition, therefore indicating that the mean of the observed within-subject variances can be used for calculating a reference difference between two successive measurements, which is applicable in different individuals.

This study presents several minor limitations. Firstly, this experiment was only performed over 5 consecutive weeks. Therefore, further studies should be needed to assess if this marker could be differently affected by seasonal variation. Secondly, we were not able to assess the circulating Vitamin K concentrations of our HVs in this study. Indeed, there are growing evidences that PIVKA-II can be used as a sensitive surrogate marker of Vitamin K status, as supplementation with Vitamin K_2 induces a dose and time-dependent decrease in circulating PIVKA-II concentrations [14]. Therefore, while all our HVs had a regular diet during the extent of the study, without dietary restrictions, we can't firmly exclude that some part of the intra- and inter-subject's variation are linked to variation in daily or weekly food intake. Finally, the present study included only European HVs. As reported in previous studies, PIVKA-II levels seem to show significant ethnic differences between Asian and European people, with higher levels in the latter population [4,15]. This important difference leads most manufacturers to mention distinct reference intervals between these two populations in their insert kit [15]. Whether this is also the case for biological variation data is unknown. Therefore, pending further studies on these ethnic differences, our data should be used cautiously when used for non-European subjects.

5. Conclusion

In this study we used the most recent recommendations concerning pre-analytical aspects, measuring procedures and statistical analysis to generate reliable BV data for PIVKA-II in 18 European HVs [12]. We believe that these results are valuable for laboratories aiming to set quality specification goals and could also be useful for the clinicians which are increasingly using this marker for the follow-up of their patients suffering from HCC.

CRediT authorship contribution statement

Jean-Louis Bayart: Conceptualization, Methodology, Investigation, Writing - original draft. **Antoine Mairesse:** Conceptualization, Investigation. **Damien Gruson:** Supervision. **Marie-Astrid Dievoet:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision.

Acknowledgements

We thank all our HV who provided their blood several times to perform this experiment. We also thank Fujirebio (Ghent, Belgium) for graciously providing the reagents to conduct this study.

References

[1] P. Tabrizian, S. Roayaie, M.E. Schwartz, Current management of hepatocellular carcinoma, World J. Gastroentero 20 (30) (2014) 10223.
[2] H.A. Liebman, B.C. Furie, M.J. Tong, et al., Des-γ-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma, N. Engl. J. Med. 310 (22) (1984) 1427–1431.
[3] A. Payancé, B.M. Dioguardi, K. Peoc'h, et al., Biological response under treatment and prognostic value of protein induced by vitamin K absence or antagonist-II in a French cohort of patients with hepatocellular carcinoma, Eur. J. Gastroenterol. Hepatol. (2019).

[4] X. Qin, G. Tang, R. Gao, et al., A multicenter study on PIVKA reference interval of healthy population and establishment of PIVKA cutoff value for hepatocellular carcinoma diagnosis in China, Int. J. Lab. Hematol. 39 (4) (2017) 392–401.
[5] C.G. Fraser, Biological variation: a still maturing aspect of laboratory medicine, Adv. Lab. Med. (2020) (ahead-of-print).
[6] G.G. Fraser, E.K. Harris, Generation and application of data on biological variation in clinical chemistry, Crit. Rev. Clin. Lab. Sci. 27 (5) (1989) 409–437.
[7] F. Braga, M. Panteghini, Generation of data on within-subject biological variation in laboratory medicine: an update, Crit. Rev. Clin. Lab. Sci. 53 (5) (2016) 313–325.
[8] N. Poté, F. Cauchy, M. Albuquerque, et al., Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion, J. Hepatol. 62 (4) (2015) 848–854.
[9] M. Yang, X. Zhang, J. Liu, Prognostic value of des-γ-carboxy prothrombin in patients with hepatocellular carcinoma treated with transarterial chemotherapy: a systematic review and meta-analysis, PLoS ONE 14 (11) (2019).
[10] R. Yu, Z. Tan, X. Xiang, et al., Effectiveness of PIVKA-II in the detection of hepatocellular carcinoma based on real-world clinical data, BMC Cancer 17 (1) (2017) 608.
[11] F. Kanke, T. Kumada, H. Toyoda, et al., Reference change values for lens culinaris agglutinin-reactive α-fetoprotein and des-γ-carboxy prothrombin in patients with chronic hepatitis C, Clin. Chem. Lab. Med. 50 (5) (2012) 957–960.
[12] A.K. Aarsand, T. Røraas, P. Fernandez-Calle, et al., The biological variation data critical appraisal checklist: a standard for evaluating studies on biological variation, Clin. Chem. 64 (3) (2018) 501–514.
[13] G. Erden, A. Barazi, G. Tezcan, et al., Biological variation and reference change values of CA 19–9, CEA, AFP in serum of healthy individuals, Scand. J. Clin. Lab. Invest. 68 (3) (2008) 212–218.
[14] R. Westenfeld, T. Krueger, G. Schlieper, et al., Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial, Am. J. Kidney Dis. 59 (2) (2012) 186–195.
[15] C. Yan, J. Hu, J. Yang, et al., Serum ARCHITECT PIVKA-II reference interval in healthy Chinese adults: sub-analysis from a prospective multicenter study, Clin. Biochem. 54 (2018) 32–36.