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ORIGINAL ARTICLE

European Biological Variation Study (EuBIVAS): within- and between-subject biological variation estimates of β-isomerized C-terminal telopeptide of type I collagen (β-CTX), N-terminal propeptide of type I collagen (PINP), osteocalcin, intact fibroblast growth factor 23 and uncarboxylated-unphosphorylated matrix-Gla protein—a cooperation between the EFLM Working Group on Biological Variation and the International Osteoporosis Foundation-International Federation of Clinical Chemistry Committee on Bone Metabolism

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Abstract

Summary We have calculated the biological variation (BV) of different bone metabolism biomarkers on a large, well-described cohort of subjects. BV is important to calculate reference change value (or least significant change) which allows evaluating if the difference observed between two consecutive measurements in a patient is biologically significant or not.

Introduction Within-subject (CV_I) and between-subject (CV_G) biological variation (BV) estimates are essential in determining both analytical performance specifications (APS) and reference change values (RCV). Previously published estimates of BV for bone metabolism biomarkers are generally not compliant with the most up-to-date quality criteria for BV studies. We calculated

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the BV and RCV for different bone metabolism markers, namely β-isomerized C-terminal telopeptide of type I collagen (β-CTX), N-terminal propeptide of type I collagen (PINP), osteocalcin (OC), intact fibroblast growth factor 23 (iFGF-23), and uncarboxylated-unphosphorylated Matrix-Gla Protein (uCuP-MGP) using samples from the European Biological Variation Study (EuBIVAS).

Methods In the EuBIVAS, 91 subjects were recruited from six European laboratories. Fasting blood samples were obtained weekly for ten consecutive weeks. The samples were run in duplicate on IDS iSYS or DiaSorin Liaison instruments. The results were subjected to outlier and variance homogeneity analysis before CV-ANOVA was used to obtain the BV estimates.

Results We found no effect of gender upon the CV_I estimates. The following CV_I estimates with 95% confidence intervals (95% CI) were obtained: β-CTX 15.1% (14.4–16.0%), PINP 8.8% (8.4–9.3%), OC 8.9% (8.5–9.4%), iFGF23 13.9% (13.2–14.7%), and uCuP-MGP 6.9% (6.1–7.3%).

Conclusions The EuBIVAS has provided updated BV estimates for bone markers, including iFGF23, which have not been previously published, facilitating the improved follow-up of patients being treated for metabolic bone disease.

Keywords Biological variation · Bone markers · CTX · FGF23 · MGP · Osteocalcin · PINP · Reference change value

Introduction

Biological variation (BV) is an essential concept in laboratory medicine. BV can be separated into within-subject (CV_I) and between-subject (CV_G) variations. CV_I is defined as the physiological fluctuation of a biomarker around its «natural» set-point in a healthy individual under steady-state conditions whereas CV_G is the variation observed between the homeostatic set-points between different individuals [1]. CV_I and CV_G have important implications, both from analytical and post-analytical perspectives. For analytical applications, they aid in setting the analytical performance specifications (APS), that are used to monitor the performance of analytical methods [2]. From a post-analytical perspective, they can be used to calculate reference change values (RCVs) [3], (also known as least significant change (LSC) by metabolic bone disease doctors, even if RCV is now the recommended appellation [4]), which may be used to assess the difference between two consecutive biomarker measurements when monitoring an individual. In clinical practice, RCVs are frequently applied in the follow-up of patients after the introduction of a pharmacological treatment (to verify compliance or adequate response) or for the monitoring of biomarkers associated with chronic pathologies. RCVs are derived from estimates of analytical variation (CV_A) and CV_I [2]. So far, CV_I estimates for most routine biomarkers have been most readily available in the historical 2014 BV database hosted on the Westgard website [5]. However, the data there are outdated and the quality of some of the included studies has been questioned [6, 7]. Accordingly, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on BV (WG-BV) and the Task Group for the BV Database (TG-BVD) developed the Biological Variation Data Critical Appraisal Checklist (BIVAC), which is used to evaluate the quality of published BV papers [6], and established a new BV Database, the recently launched web-based EFLM BV Database [8]. With regard to BIVAC, publications are assessed for 14 Quality Items (QI) that focus on the study design, applied measurement procedure, and statistical handling of data on CV_I estimates. This assessment results in a final BIVAC grade, ranging from A to D (A represents full compliance with all 14 QIs, B or C is assigned if the lowest QI score was either B or C, respectively; D is given when QIs considered essential were not fulfilled). The WG-BV and TG-BVD have also performed systematic reviews using the BIVAC and a meta-analysis of BV studies for different measurands [9, 10]. The results of these reviews are included in the EFLM BV Database.

Bone turnover markers are protein or protein derivative biomarkers released during bone remodeling by osteoblasts or osteoclasts, which provide an estimate of bone resorption and bone formation. In the EFLM BV Database [7], data by June 2019 had been included for β-isomerized C-terminal telopeptide of type I collagen (β-CTX), N-terminal propeptide of type I collagen (PINP), and osteocalcin (OC). Six papers were included for β-CTX [11–16], three for PINP [11, 12, 15] and five for OC [12, 14, 17–19], all having been given a BIVAC grade "C," indicating the lack of compliance with one or more QIs. For intact Fibroblast Growth Factor 23 (iFGF23), a single paper had been included, given an "A" grade [20].

Considering the general lack of robust and updated BV data for many measurands, the EFLM WG-BV has designed the European Biological Variation Study (EuBIVAS), a large-scale study aiming to establish CV_I estimates for a wide range of biochemical biomarkers. In the EuBIVAS, a high number of participants from five different European countries were included, and the study was performed according to the best methodological practices with application of contemporary analytical methods [6, 21–25]. In conjunction with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on Bone Markers (C-BM), it was decided that samples from the EuBIVAS study

will be used to establish robust CV_I estimates for important biochemical markers of bone metabolism and vascular calcification, namely OC, PINP, β-CTX, iFGF23, and uncarboxylated-unphosphorylated Matrix-Gla Protein (uCuP-MGP).

Materials and methods

The EuBIVAS

The EuBIVAS has been described in detail elsewhere [21]. Briefly, six European laboratories from Italy, Norway, Spain, the Netherlands, and Turkey were involved, resulting in the recruitment of 91 healthy volunteers (38 males and 53 females; age range 21–69 years). The participants completed an enrollment questionnaire to provide information on their lifestyle and health status, which was further verified by a set of laboratory tests during each collection. All laboratories followed the same protocol for the pre-analytical phase. Fasting blood samples were drawn weekly for ten consecutive weeks (April–June 2015) on a set day (Tuesday to Friday), and at the same hour between 08.00 and 10.00 at each weekly visit from the same phlebotomist at most visits further minimizing variation. Seventy-seven participants completed all ten collections, ten participants completed nine collections, two participants completed eight collections, and two participants completed seven collections. The serum and EDTA plasma samples collected by each laboratory were sent frozen on dry ice to San Raffaele Hospital in Milan, Italy. The samples were stored in a − 80 °C freezer until they were shipped on dry ice to the CHU de Liège (Belgium), where the bone markers were analyzed in 2018.

Analytical methods

EDTA plasma was the only remaining sample type from the EuBIVAS study. OC, PINP, β-CTX, and uCuP-MGP were measured on the IDS iSYS platform (Boldon, UK) and iFGF-23 on the DiaSorin Liaison XL instrument (Saluggia, Italy) according to the manufacturers' instructions in the ISO 15189 accredited clinical chemistry laboratory of the CHU de Liège. The manufacturers' internal quality controls (two levels) were measured at the beginning and end of each testing series, following the validation of the calibration curves by the instrument, and each test series was accepted if the results fell within the manufacturers' expected ranges. Samples donated from the same individual were measured in duplicate within the same instrument run for a single day. All the analyses were performed by the same, experienced and well-trained, technician during January and February 2019.

Data analysis and statistics

The data analysis was performed as previously described [24].

Briefly, the CV_I was estimated using CV-ANOVA for males and females, as well as for women above and below the age of 50 years [26]. The outlier assessment was performed for replicates and samples on CV-transformed data. The homogeneity of the CV_A and CV_I was examined by the Bartlett [27] and Cochran [28] tests, respectively. Larger individual systematic changes were identified by the homogeneity test of the CV_I (Cochran test). A linear regression of the mean of all the values of each blood drawing (1, 2,. .. 10) vs. the blood drawing number was performed for each measurand. The subjects were considered to be in a steady state if the 95% confidence interval (CI) of the slope included the zero. When a trend was identified, all data were corrected accordingly prior to the estimation of CV_I. The CV_G estimates were calculated from natural log-transformed data after assessment for outliers between individuals (Dixon criterion [29]). The normality assumption was verified by the Shapiro–Wilk test [24].

95% CIs for the BV estimates were also calculated [30], and the lack of overlap in the 95% CI for the individual CV_I and CV_G estimates was used to indicate significant differences between subgroups.

If mean concentrations between the men and women were significantly different, the lower of the two CV_G was applied in the APS [24].

The APS for the analytical imprecision (CV_{APS}) and analytical bias (B_{APS}) were calculated according to:

$$CV_{APS} = 1/2\ CV_I$$

$$B_{APS} = 0.25\ \sqrt{CV_I^2 + CV_G^2}$$

The RCVs were calculated using the log-normal approach, which generated asymmetric values for the rise and fall at the 95% probability level for significant unidirectional changes and applied CV_A estimates based on duplicate measurements for all study samples [24].

To evaluate differences in mean concentrations between participants from the various countries, data were visually inspected (data not shown). Data analyses were performed using Excel 2010 and IBM SPSS statistics, version 23.

Results

The median number of participants per center was 15 (range 12–19). The participants were generally physically active and approximately 3% were regular smokers (detailed characteristics are available in previous publications [21, 24]). Their

median BMI was 22.5 kg/m^2 (range 17.6–32.5 kg/m^2) and none of them suffered from renal impairment.

For uCuPMGP, six subjects were excluded because some of the obtained results were below the limit of quantification of the instrument.

Overall, 2.5%, 0.3%, 1.9%, 4.3%, and 2.1% were excluded for OC, uCuP-MGP, PINP, β-CTX, and iFGF23, respectively, as they were identified as outliers or excluded to obtain variance homogeneity analyses of the results (Supplemental Data Table 01).

Outliers were identified with the Dixon q-test and excluded for CV_G calculations (see Supplemental Table 01). One postmenopausal participant was excluded from the PINP and β-CTX calculation, and another individual from the same subgroup was also excluded from the β-CTX calculation. For OC, one man was identified as an outlier, and for uCuP-MGP 4 other subjects (one in the male subgroup, two in the pre-menopausal, and one in the post-menopausal women's subgroup) were also identified as outliers and excluded. For PINP and β-CTX, a trend was identified and corrected prior to analysis.

Differences in mean concentrations between men and women were found for all measurands except uCuP-MGP (Table 1). No differences in the CV_I estimates between males and females for any of the bone markers were observed (Table 1). Median values and range concentrations for each individual included in the study after exclusion of outliers, ordered by gender and age, are represented in Figs. 1, 2, 3, and 4 and in Supplemental Data Fig. 01. The CV_I, CV_G, and CV_A and mean values observed in this study are presented in Table 1 and the APS for imprecision (CV_{APS}) and bias (B_{APS}) as well as the RCVs based on the BV estimates are reported in Table 2.

Discussion

Biomarkers of bone formation and bone resorption are frequently used for monitoring patients' responses to therapies and compliance. Indeed, adherence to oral bisphosphonates is known to be low, specifically during the first year of treatment, which hampers its anti-fracture effect. The IOF, the European Calcified Tissue Society (ECTS) and the European Society on Clinical and Economic Aspects of Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases (ESCEO) have thus proposed an algorithm based on the response of biochemical markers of bone turnover after 3 months of therapy to detect the non-responders or the non-compliant [31, 32]. Hence, in this context, the RCV serves as an important tool used to correctly interpret the biological significance of a change observed between two consecutive biomarker measurements. If the changes observed are lower than the RCV, then the compliance or any other issue (malabsorption, interferences with concomitant absorption of milk,…) should be assessed and/or treatment should be changed or adapted.

The EuBIVAS, a large and methodologically-sound European BV study, provides together with meta-analysis based review of published BV studies, updated BV results for a large number of measurands. In the present study, we established updated BV estimates with associated APS and RCVs for five different biomarkers implicated in bone metabolism and vascular calcification, namely OC, PINP, β-CTX, iFGF23, and uCuP-MGP. It is, however, important to point out that the results of this study were derived from samples that had been stored up to 3 years at − 80 °C. Therefore, some degradation could have occurred. However, since the samples of a single subject were obtained and stored for an equally long time period, this perhaps ensures consistency in our findings. Also, we used EDTA-plasma, which is the recommended matrix for an optimal storage of β-CTX [33].

OC, also known as bone Gla protein, is generally considered as a marker of bone remodeling [34–37]. It is a vitamin K– and vitamin D–dependent protein produced by osteoblasts and is the most abundant of the non-collagenous proteins in bone [38]. OC is not very stable in vitro and is rapidly cleaved. Most immunoassays, like the one used in this study, detect the total "intact" form as well as the N-terminal mid-fragment, which results from the cleavage of OC and shows greater apparent stability. The historical 2014 BV database hosted on the Westgard website, gives CV_I and CV_G estimates at 6.35 and 30.9%, respectively, based on the median of five studies [17–19]. Two additional papers [12, 39] were, as of June 2019, included in the EFLM Biological Variation Database [8]. The results in the paper from Hannon et al. [18] were obtained in a population of 11 early postmenopausal women and the reported CV_I estimate of 7.2% was the same as that which we found in our population of postmenopausal women (Table 2). The other studies included in the historical database were all performed in healthy subjects and the CV_I estimates ranged from 5.3% [17] to 13% [39]. None of these CV_I estimates were included in the 95% CI we found because they were either too low or too high.

PINP and β-CTX reflect type I collagen metabolism and even if they are not fully bone-specific, they are recommended by the International Osteoporosis Foundation (IOF) and the IFCC as the markers of choice for monitoring bone turnover in clinical studies [33]. PINP is a trimer constituted by three non-covalently linked type I collagen subunit chains (2 pro-α1 and 1 pro- α2), synthesized by the osteoblasts and deposited in the resorption cavity during the bone formation phase. In human serum, PINP is present in two major forms, a trimeric and a monomeric form, the latter being elevated in patients suffering from chronic renal failure. Assays available on the market either measure (1) the "Total" form and thus recognize both the trimeric and the monomeric fragments (Roche Elecsys)

Table 1 Within-subject (CV_I) and between-subject (CV_G) biological variation (BV) estimates for osteocalcin (OC), uncarboxylated-unphosphorylated Matrix-Gla Protein (uCuP-MGP), N-terminal propeptide of type I collagen (PINP), C-terminal telopeptide of type I collagen (β-CTX), and intact fibroblast growth factor 23 (iFGF-23) with 95% confidence interval (CI)[a]

	Number of subjects	Total number of results	Mean number of samples/individual	Mean number of replicates/sample	Mean value (95% CI)	CV_A % (95% CI)[b]	CV_I % (95% CI)	CV_G% (95% CI)
OC, μg/L								
All subjects	91	1738	9.56	2.00	22.5 (22.1–23.0)	2.3 (2.2–2.4)	**8.9** (8.5–9.4)	
Males	38	727	9.58	1.99	24.4 (23.7–25.1)		8.6 (8.0–9.4)	**32.6** (26.7–43.9)
Females < 50	43	816	9.49	2.00	20.4 (19.8–21.0)		9.3 (8.6–10.0)	36.5 (29.9–47.7)
Females > 50	10	187	9.40	1.98	26.4 (24.6–28.2)		7.3 (6.2–8.7)	45.2 (29.5–89.9)
uCuP-MGP, pmol/L								
All subjects	85	1464	9.21	1.77	397 (393–401)	6.9 (6.6–7.3)	**6.9** (6.1–7.3)	**13.9**[c] (12.5–17.1)
Males	36	642	9.42	1.81	394 (389–399)		6.7 (5.8–7.5)	10.6 (8.80–14.7)
Females < 50	40	659	8.93	1.74	388 (382–394)		6.7 (5.5–7.4)	12.5[c] (10.4–17.0)
Females > 50	9	159	9.44	1.78	367 (358–375)		6.7 (5.2–8.7)	7.4 (4.4–15.8)
PINP, μg/L								
All subjects	91	1748	9.60	2.00	63.7 (62.3–65.0)	3.7 (3.6–3.9)	**8.8** (8.4–9.3)	
Males	38	726	9.55	2.00	70.9 (68.6–73.3)		8.5 (7.8–9.2)	35.6 (28.5–46.9)
Females < 50	43	820	9.53	2.00	54.9 (53.3–56.4)		9.2 (8.6–10.0)	**35.2** (29.0–46.1)
Females > 50	10	200	10.0	2.00	78.2 (75.2–81.3)		8.4 (7.2–9.9)	23.8 (15.8–47.7)
β-CTX, ng/L								
All subjects	91	1704	9.41	1.98	514.3 (499.5–529.1)	5.0 (4.8–5.3)	**15.1** (14.4–16.0)	
Males	38	721	9.55	1.97	597.0 (571.5–622.6)		14.4 (13.3–15.6)	48.0 (38.5–64.8)
Females < 50	43	784	9.16	1.98	407.3 (392.1–422.5)		16.5 (15.3–17.8)	**45.9** (38.1–62.2)
Females > 50	10	198	9.90	2.00	738.7 (706.0–771.4)		12.2 (10.3–14.3)	21.0 (13.4–45.0)
iFGF23, ng/L								
All subjects	91	1694	9.31	2.00	35.3 (34.7–35.9)	4.4 (4.2–4.6)	**13.9** (13.2–14.7)	
Males	38	712	9.37	2.00	37.2 (36.3–38.1)		12.7 (11.8–13.8)	**26.4** (21.6–35.3)
Females < 50	43	792	9.21	2.00	32.9 (32.1–33.8)		14.8 (13.7–16.0)	34.5[c] (28.7–46.0)
Females > 50	10	190	9.50	2.00	38.1 (36.1–40.2)		14.4 (12.5–17.1)	35.8 (24.5–72.8)

[a] Results were divided in males, females < 50, and females > 50 subgroups

[b] Analytical variation (CV_A) estimates were based on CV-ANOVA of duplicate analysis of all study samples

[c] Normality assumption was not satisfied based on the Shapiro-Wilk test

Results in bold were used to estimate APSs and RCVs (see Table 2)

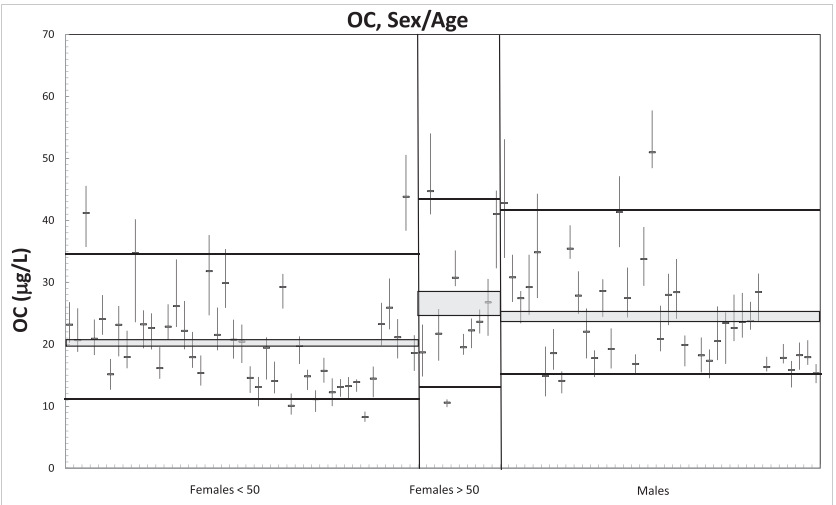

Fig. 1 Median values (dots) and range of osteocalcin (OC) concentrations (vertical error bars) for each individual included in the study after exclusion of outliers, ordered by gender and age. Continuous horizontal lines indicate the 95% confidence interval (CI) of the mean and the fifth and 95th percentiles for females < 50 years old, females > 50 years old, and males

and are influenced by renal function or (2) the "Intact" trimeric PINP only (IDS iSYS and Orion Diagnostica) [40]. The automated methods (Roche Cobas and IDS iSYS) give similar results and even if there is a correlation between automated methods and Orion radioimmunoassay, this latter shows a significant proportional bias compared with Roche and IDS [41, 42]. The 2014 historical database presents CV_I estimates of 7.4% and a CV_G of 57.3%, based on two papers [16, 18] and an abstract. In the EFLM Biological Variation Database, the critical appraisal of studies for bone markers was as of June 2019 ongoing and for PINP, three out of six studies (lvarez [11], Clowes [12] and Nguyen [15]) had been appraised and published. The Nguyen study [17] involved a large number of healthy participants (> 1000) and was graded as a BIVAC C study [15]. The reported CV_I estimate (14.2%) was much higher compared to our results, possibly related to a different population (young (22.2 ± 5.1 years old) elite athletes). Of note, intensive physical exercise and age have an important impact on PINP [43–45] which renders the interpretation of these results difficult. The results of the two other studies (also graded as a BIVAC "C" studies) were obtained from much smaller cohorts of healthy subjects or diseased patients who presented a lower (6.2%) or higher (10.6%) CV_I compared to our results. The Hannon study reported a CV_I estimate of 7.4% which is comprised in the 95% CI of the CV_I of postmenopausal women we found in our study (7.2–9.9%).

The β-CTX assays measure collagen type I fragments derived from the C-terminal telopeptide region of the protein and the antibodies are specific for the isomerized form of the sequence EKAHD-β-GGR from the α_1 chain. β-CTX measurements are frequently requested to monitor the efficacy of antiresorptive therapy. Two automated methods (Roche Cobas and IDS iSYS) and one ELISA (IDS) are available on the market but, unfortunately, these methods are not harmonized yet. The historical 2014 database presents a CV_I of 10.85% and a CV_G of 30.6% based on three papers, namely the already mentioned Hannon paper [18] and two other

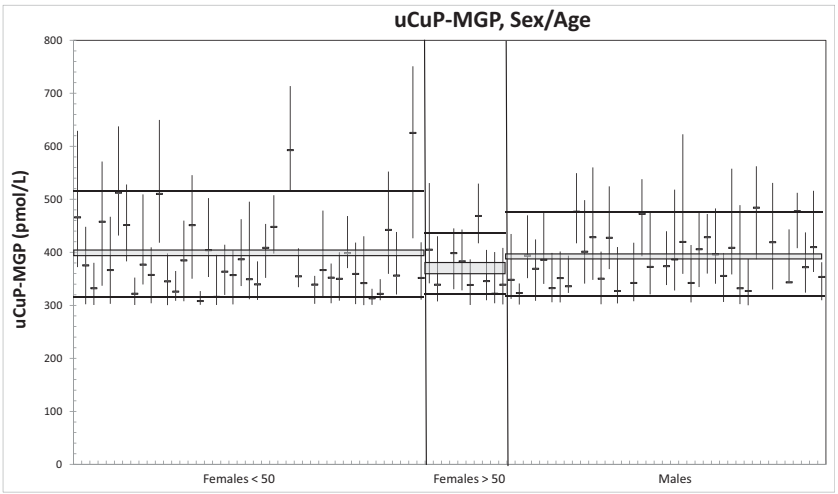

Fig. 2 Median values (dots) and range of uncarboxylated-unphosphorylated matrix-Gla protein (uCuP-MGP) concentrations (vertical error bars) for each individual included in the study after exclusion of outliers, ordered by gender and age. Continuous horizontal lines indicate the 95% confidence interval (CI) of the mean and the fifth and 95th percentiles for females < 50 years old, females > 50 years old, and males

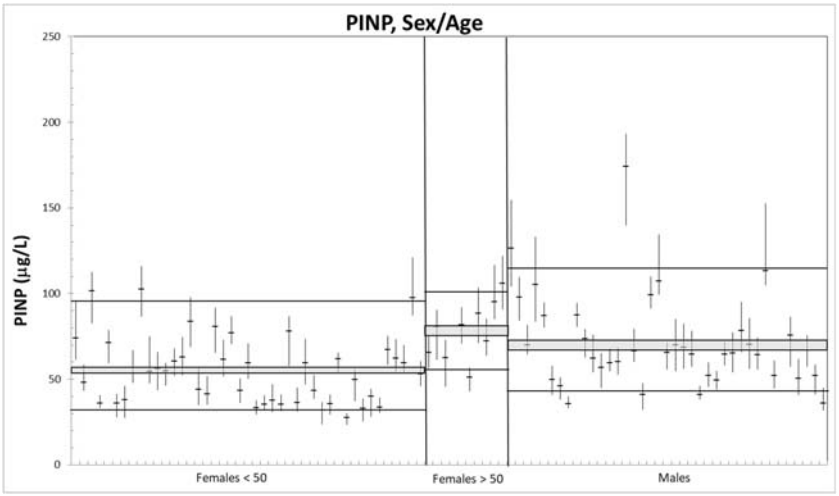

Fig. 3 Median values (dots) and range of N-terminal propeptide of type I collagen (PINP) concentrations (vertical error bars) for each individual included in the study after exclusion of outliers, ordered by gender and age. Continuous horizontal lines indicate the 95% confidence interval (CI) of the mean and the fifth and 95th percentiles for females < 50 years old, females > 50 years old, and males

papers [16, 46]. The EFLM Biological Variation Database had as of June 2019 included four other papers in the review, i.e., Alvarez et al. [11], Clowes et al. [12], Garnero et al. [39], and Nguyen et al. [15]. Again, we noticed significant differences between the CV_I we found in this study and those from the literature which were either lower [11, 16] or higher [12]. The CV_I observed in the Nguyen study [15] was close to the values observed in the post-menopausal women, which seemed contraintuitive, but the samples collected in this study were obtained in non-fasting subjects, at all times of the day, which is known to severely impact β-CTX values.

The use of the RCV to monitor patients' response and adherence to pharmacological treatment is a frequent approach in osteoporosis management. It can be assumed that a change in β-CTX and PINP from baseline greater than the RCV shows the efficiency of the treatment. The RCV should theoretically be calculated by each laboratory according to its own CV_A that is preferably obtained using quality controls for a long period of time. In this study, the CV_A derived from the duplicate measurements was taken into consideration. We calculated that the RCV for β-CTX and PINP (− 30.8% and − 19.9%, respectively) was very similar to that provided by Hannon et al. (− 27.6 and − 21%, respectively) and close to that provided by Clowes et al. (− 45 and − 25%, respectively) or Rogers et al. (− 28 and − 25%, respectively) [12, 18, 47] but were different from those proposed in the recommendations for the screening of adherence to oral bisphosphonates [31] by the Adherence Working Group of the International Osteoporosis Foundation and the European Calcified Tissue Society (− 56 and − 38%, respectively, based on [48]), calculated as the within-person variability for measurements a week apart from women on bisphosphonate treatment.

FGF23 is a phosphaturic hormone mainly produced by the osteocytes and osteoblasts that acts on the Klotho-FGFR complex present in renal tubules to increase phosphate excretion and decrease vitamin D activation. Intact FGF23 is cleaved by a protease into inactive amino-terminal and carboxy-terminal fragments. FGF23 needs to be O-glycosylated by GALNT3 in order to be protected against proteolysis. Two different kinds of FGF assays are available on the market and (1) measure the

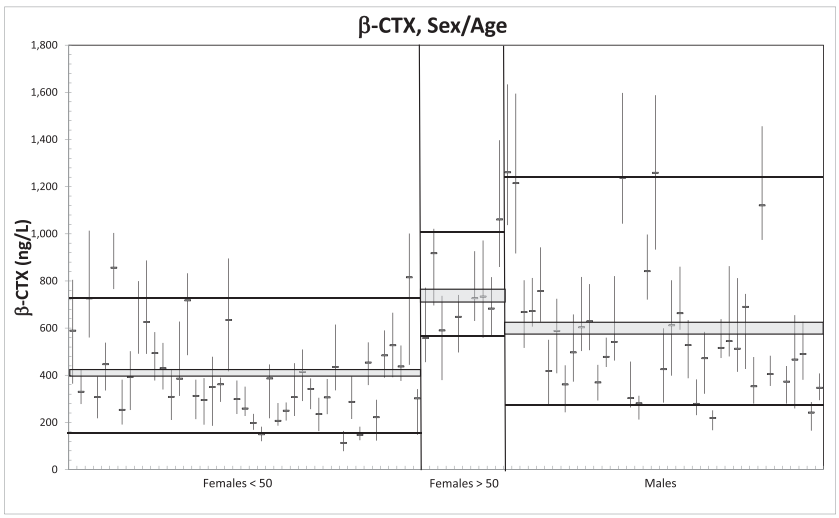

Fig. 4 Median values (dots) and range of C-terminal telopeptide of type I collagen (β-CTX) concentrations (vertical error bars) for each individual included in the study after exclusion of outliers, ordered by gender and age. Continuous horizontal lines indicate the 95% confidence interval (CI) of the mean and the fifth and 95th percentiles for females < 50 years old, females > 50 years old, and males

Table 2 Analytical performance specification (APS) for imprecision (CV_{APS}) and bias (B_{APS}) and reference change values (RCV) based on the biological variation (BV) estimates as reported in Table 1

	CV_{APS} (%)[a]	B_{APS} (%)[b]	RCV (%)[c] decrease; increase
OC	4.5	8.4	− 19.2; 23.8
MGP	3.5	3.9	− 20.3; 25.4
PINP	4.4	9.2	− 19.9; 24.8
β-CTX	7.6	12.6	− 30.8; 44.5
FGF23	7.0	7.5	− 28.7; 40.2

[a] $CV_{APS} = ½\ CV_I$;

[b] $B_{APS} = 0.25(CV_I^2 + CV_G^2)^{05}$

[c] RCV were calculated as described in the text delivering asymmetric values for rise and fall at the probability level of 95% for significant unidirectional change, applying CV_A estimates based on duplicate measurement of all study samples

intact form and the C-terminal fragments, or (2) the intact form only. The DiaSorin Liaison iFGF23 is the only available automated method (outside Japan) allowing measurement of iFGF23 and has been extensively validated [49]. Using the Immutopics ELISA to measure iFGF23, Smith et al. showed a CV_I estimate of 18.3% and a RCV of 54%, in a population of eight healthy volunteers (five males and three females) followed weekly for 6 weeks [50]. Very recently, Jabor et al. showed, with the same analytical method as ours, a CV_I and RCV of 14.2 and 40.5%, respectively, for iFGF23 assessed in a population of 14 healthy subjects in a 6-week study [20]. These results were very close to what we observed in this study (CV_I of 13.9% and RCV of − 28.7; 40.2%).

uCuP-MGP is a marker of vascular calcification and reflects the vitamin K status of patients and healthy subjects [51, 52]. Poor vitamin K status, as defined by higher levels of uCuP-MGP, has been shown to be associated with lower bone mineral density and increased fracture risk in patients with end-stage renal disease [53]. To the best of our knowledge, no studies have evaluated the BV of this promising new biomarker. Our results show that the CV_I of uCuP-MGP is rather low, compared to the bone markers included in our study. These results will be useful when monitoring the response to vitamin K treatment in hemodialyzed patients [54, 55].

Two points, however, regarding the data delivered by our study deserve some attention. First, the RCVs calculated in this study are based on CV_A estimates derived from the duplicate analysis of the EuBIVAS study samples and are therefore not applicable sensu stricto to other laboratories. Indeed, each laboratory should calculate its own RCVs with the analytical CV of the instrument/method used and take into account the appropriate time intervals between patient controls. Second, the "measurands" (i.e., what is captured by the antibodies of the immunoassays and measured) of this study are not necessarily always well-defined and it remains to be shown that the results obtained by the use of the assays available to us, are transposable to other assays. From a very practical point of view, this means that the values obtained for intact PINP cannot literally be transposed to total PINP, especially in patients suffering from decreased renal function. Finally, these results have been obtained in EDTA plasma and the transposition to serum has not been evaluated.

In conclusion, we have provided in this study CV_I values and RCVs for different bone markers. These values have been obtained with the most up-to-date methodologies for estimating BV and should replace the ones obtained in smaller, lower-quality studies.

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Compliance with ethical standards

Conflicts of interest Etienne Cavalier is consultant for IDS and DiaSorin. Pierre Lukas, Michela Bottani, Aasne Arsand, Ferruccio Cerriotti, Abdurrahman Coskun, Jorge Diaz-Garzon, Pilar Fernandez-Calle, Elena Guerra, Massimo Locatelli, Sverre Sandberg, and Anna Carobene declare that they have no conflict of interest.

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