Variabilità biologica degli ormoni che regolano l'omeostasi ossea e minerale nel siero e plasma di individui sani

Francesca Iannone[1], Elvira Angotti[2], Fortunata Lucia[2], Luisa Martino[2], Giulio Cesare Antico[2], Francesco Galato[2], Ilenia Aversa[1], Raffaella Gallo[1], Caterina Giordano[1], Antonio Abatino[1], Serafina Mancuso[2], Lorenza Giaquinto Carinci[2], Maria Martucci[2], Consuelo Teti[2], Francesco Costanzo[1,2], Giovanni Cuda[1,2,] Camillo Palmieri[1,2]

[1] Dipartimento di Medicina Sperimentale e Clinica, Università Magna Graecia di Catanzaro,

[2] Laboratorio di Biochimica Clinica, AOU "Renato Dulbecco", Catanzaro

RIASSUNTO L'omeostasi minerale è regolata da ormoni fondamentali tra cui 25-idrossivitamina D (25OH-D), 1,25-diidrossivitamina D [1,25(OH)$_2$-D], ormone paratiroideo (PTH) e fattore di crescita dei fibroblasti 23 (FGF23). L'alterazione dell'omeostasi minerale è alla base di molte malattie, tra cui quelle ossee, renali e cardiovascolari. I dati di variabilità biologica (VB) degli analiti aiutano a distinguere tra misurazioni seriali normali e patologiche, a convalidare gli intervalli di riferimento e a stabilire le specifiche di qualità di prestazione analitica (APS). Il database contenente dati di VB per diversi analiti, aggiornato dalla Federazione Europea di Chimica Clinica e Medicina di Laboratorio (EFLM) non dispone di dati per la 1,25(OH)$_2$-D e contiene pochi studi per FGF23 e PTH 1-84. Questo studio mira a fornire, per la prima volta, dati sulla VB per 1,25(OH)$_2$-D e aggiornare le stime per FGF23 e PTH 1-84 con metodo di terza generazione. Sono stati arruolati sedici volontari sani (8 M, 8 F) dal sud Italia, tra 25 e 60 anni. Campioni di siero e plasma-EDTA sono stati raccolti per sette settimane (febbraio-marzo 2023). Le misurazioni sono state effettuate su Liaison XL (DiaSorin, Saluggia, Italia). La variabilità intra-individuale (CV$_I$) e inter-individuale (CV$_G$) è stata calcolata mediante ANOVA standard o CV-ANOVA. Sono state calcolate le APS, i valori di riferimento (RCV), l'indice di individualità e il numero di campioni necessari per raggiungere il vero set-point omeostatico, secondo il modello sostenuto dall'EFLM. I CV$_I$ con gli intervalli di confidenza al 95% ottenuti sono: 1,25(OH)$_2$-D: 22,2% (18,9–26,4); FGF23: 16,1% (13,5–19,5); PTH 1–84: 17,9% (14,8–21,8). I CV$_G$ sono: 1,25(OH)$_2$-D: 21,2% (14,2–35,1); FGF23: 21,1% (14,5–35,8); PTH 1–84: 31,1% (22,1–50,8). Pertanto, questo studio ha fornito, per la prima volta, le stime di VB per 1,25(OH)$_2$-D e ha aggiornato le stime di VB per PTH 1-84 e FGF23.

Parole chiave: Variabilità biologica; Calcitriolo; Ormone paratiroideo; FGF23; Omeostasi minerale

ABSTRACT *The biological variation of hormones regulating bone and mineral homeostasis in serum and plasma of healthy individuals. Background and aim. Mineral homeostasis is finely regulated by pivotal hormones including 25-hydroxyvitamin D (25OH-D), 1,25-dihydroxyvitamin D [(1,25(OH)$_2$-D)], parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23). Imbalanced mineral homeostasis underlies many illnesses including bone, kidney, and cardiovascular diseases. The biological variation (BV) data of the measurands helps distinguish between normal and abnormal fluctuations, interpret serial measurements, validate reference intervals, and establish analytical performance specifications (APS). The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) BV database (EFLM-BV-D, https://biologicalvariation.eu/) lacks data for 1,25(OH)$_2$-D. For FGF23, the database uses limited studies, and only a few studies focus on the bioactive form of PTH 1-84. This study seeks to provide, for the first time, BV data for 1,25(OH)$_2$-D and update BV estimates for FGF23, and PTH 1-84 using the most recent immunological assay generation.* **Materials and methods**. *Sixteen healthy volunteers (8 M and 8 F) from south of Italy, age range 25-60, without acute or chronic illnesses, pregnancy and use of vitamin D supplementations, have been enrolled for this study. Serum and plasma-EDTA were collected for seven weeks from february to march 2023. Measurements of 1,25(OH)$_2$-D, FGF23 and PTH 1-84 were carried out in duplicate in one run by the same laboratory technician with the same lot of reagents, using a fully automated chemiluminescent analyzer (Liaison XL, DiaSorin, Saluggia, Italy). The within-subject (CV$_I$) and between-subject (CV$_G$) coefficient of variation were calculated by either standard ANOVA (SD-ANOVA), or CV-ANOVA, after removal of replicates, within-subject and between-subject outliers from raw- or CV-transformed results. Normality assumption was obtained through the Shapiro-Wilk test. The steady state of subjects was verified with a linear regression on the mean of measurements from every blood draw versus the blood draw number. The analytical quality specifications (APS) were calculated for optimal, desirable and minimal quality levels according to the BV-model advocated by the EFLM. Reference change values (RCVs), index of individuality and the number of samples required to achieve the true homeostatic set-point were also calculated.* **Results.** *We found CV$_I$ estimates with 95% confidence intervals, as follows :1,25(OH)$_2$-D: 22.2% (18.9–26.4); FGF23: 16.1% (13.5–19.5); and PTH 1–84: 17.9% (14.8–21.8). The CV$_G$ estimates were: 1,25(OH)$_2$-D: 21.2% (14.2–35.1); FGF23: 21.1% (14.5–35.8) and PTH 1–84: 31.1% (22.1–50.8).* **Discussion and conclusions.** *The present study has been conceived and conducted in adherence to the standards provided by the Biological Variation Data Critical Appraisal Checklist (BIVAC). We provided, for the first time, BV estimates with associated APS and RCVs for 1,25(OH)$_2$D and we refined the BV estimates of PTH 1-84, and FGF23 already found in EFLM-BV database, using advanced immunometric techniques.*

Keywords: Biological variability; Calcitriol; Parathyroid hormone; FGF23; Mineral homeostasis