HHS Public Access

Author manuscript

Osteoporos Int. Author manuscript; available in PMC 2021 March 01.

Published in final edited form as:

Osteoporos Int. 2020 March ; 31(3): 409–412. doi:10.1007/s00198-020-05310-6.

Harmonization of commercial assays for PINP; the way forward

S. D. Vasikaran[1], **H. P. Bhattoa**[2], **R. Eastell**[3], **A. C. Heijboer**[4], **N. R. Jørgensen**[5,6], **K. Makris**[7,8], **C. Ulmer**[9], **J. A. Kanis**[10], **C. Cooper**[11], **S. Silverman**[12], **E. Cavalier**[13]

[1]PathWest Laboratory Medicine, Fiona Stanley Hospital, Murdoch, WA, Australia [2]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary [3]Mellanby Centre for Bone Research, University of Sheffield, Sheffield, UK [4]Department of Clinical Chemistry, Endocrine Laboratory, Amsterdam Gastroenterology & Metabolism, Vrije Universiteit Amsterdam and University of Amsterdam, Amsterdam UMC, Amsterdam, Netherlands [5]Department of Clinical Biochemistry, Rigshospitalet, DK-2600 Glostrup, Denmark [6]OPEN, Odense Patient data Explorative Network, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, DK-8000 Odense, Denmark [7]Clinical Biochemistry Department, KAT General Hospital, 14561 Athens, Greece [8]Laboratory for Research of the Musculoskeletal System “Th. Garofalidis”, Medical School, University of Athens, Athens, Greece [9]Clinical Chemistry Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Buford, Atlanta, GA, USA [10]Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK [11]The MRC Epidemiology Resource Centre, Southampton General Hospital, University of Southampton, Southampton, UK [12]Cedars-Sinai Medical Center and UCLA School of Medicine, Beverly Hills, CA, USA [13]Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, Domaine du Sart-Tilman, B-4000 Liège, Belgium

Abstract

Introduction—In order to examine the agreement between current commercial assays, a multi-center study was performed for PINP in serum and plasma.

Methods—The automated methods for PINP (Roche Cobas and IDS iSYS) gave similar results. A significant proportional bias was observed between the two automated assays and the Orion radioimmunoassay (RIA) for PINP.

Results—Results from other published studies comparing PINP values among these three assays broadly support our findings. Taken together, these results confirm that harmonized PINP measurements exist between the two automated assays (Roche Cobas and IDS iSYS) when the eGFR is > 30 mL/min/1.73m^2, but a significant bias exists between the Orion RIA and the two automated assays.

S. D. Vasikaran, samuel.vasikaran@health.wa.gov.au.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, and the US Department of Health and Human Services.

Conflicts of interest None.

Conclusion—Therefore, in subjects with normal renal function, PINP results reported by the Roche Cobas and IDS iSYS assays are similar and may be used interchangeably, and similar reference intervals and treatment targets could be applied for the two automated assays. Harmonization between the automated assays and the RIA is potentially possible with the use of common calibrators and the development of a reference method for PINP. This should also help ensure that any new commercial assay developed in the future will attain similar results. IOF and IFCC are committed to working together towards this goal with the cooperation of the reagent manufacturing industry.

Summary

International Federation of Clinical Chemistry and Laboratory Medicine and The International Osteoporosis Foundation Joint Committee on Bone Metabolism believes that the harmonization of PINP assays is an achievable and practical goal.

Keywords

Bone resorption; Bone turnover markers; Harmonization; PINP; Procollagen type I N-propeptide

Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to an increased risk of fracture with associated morbidity and mortality [1]. Its prevalence is increasing in part due to an aging population, resulting in a major public health burden globally [2]. When subjects with a high fracture risk are identified and the appropriate treatment is instituted and adhered to, the fracture risk can be significantly reduced [3]. Biochemical markers of bone turnover may be useful in monitoring the response to treatment and as a potential adjunct to improving adherence to treatment, which has to be long-term [4]. The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Joint Working Group on Bone Marker Standards (WG-BMS) recommended one bone formation marker, namely the procollagen type I N-propeptide (PINP), and one bone resorption marker, the C-terminal telopeptide of type I collagen (CTX), be used as reference markers for clinical research studies [5]. They further recommended that standardization or harmonization of commercial assays be achieved in order to establish internationally agreed decision limits and target values for these markers in the management of osteoporosis. This perspective addresses issues related to the harmonization of commercial PINP assays.

PINP was first isolated from amniotic fluid as “fetal antigen 2”, and amino acid sequencing identified the high-molecular weight peptide (intact form, MW 35,000) as a heterotrimer of two 14,250 MW proα1-chains and a 5500 MW proα2-chain [6]. However, its molecular structure has not been accurately characterized. Currently, there are three commercially-available immunoassays for the measurement of PINP in blood, two of which are available on automated platforms: Immunodiagnostic Systems plc on the iSYS automated analyzer (IDS, Boldon, UK) and Roche Diagnostics (Mannheim, Germany) instruments. Both use an electrochemiluminescence immunoassay (ECLIA) technology. The third manual radioimmunoassay (RIA) is produced by Orion Diagnostica (UniQ PINP RIA, Orion Diagnostica, Espoo, Finland). For obvious reasons, automated assays are less labor intensive, with a higher throughput and quicker turnaround times. Unlike RIAs, automated

assays are not hampered by drawbacks associated with the use of radioactive reagents such as the necessity for dedicated facilities and specially trained staff, which have led to major reductions in the use of RIA assays and the facilities for performing such assays globally. However, only the Orion Diagnostica PINP assay is currently approved by the FDA and therefore the only method available in the USA for clinical use. The automated assays are widely used in clinical laboratories elsewhere worldwide.

The PINP calibrator used in the Orion RIA is purified from human ascitic fluid and characterized by electrophoresis. The value assignment of the first purified antigen stock standard was performed by amino acid quantitative analysis (Orion PINP RIA kit insert). The IDS assay calibrator, similar to the Orion assay calibrator, is a purified trimeric PINP, and the assay is standardized against the manufacturer's master curve [7]. The Roche Diagnostics assays uses a synthetic amino procollagen peptide made from pre-procollagen $\alpha_1$1 as the standard. The assay is calibrated against the precisely-defined standard by weighing native P1NP into an analyte-free human serum matrix [7]). The IDS iSYS assay and the Orion Diagnostica assay are specific to the trimeric (intact PINP) molecule and do not cross react with the monomer or fragments of the PINP molecule, which accumulate in circulation in patients with chronic kidney disease stages 4 and 5 (i.e., when the glomerular filtration rate decreases to approximately less than 30 ml/min/1.73 m^2). The Roche PINP assay, on the other hand, cross reacts with the monomeric fragments in addition to recognizing the intact molecule (total PINP) [7].

A lack of knowledge of the molecular structure of PINP and the different peptides measured by the intact and total PINP assays pose problems in the standardization of these assays. Therefore, the IFCC/IOF Joint Committee on Bone Metabolism believes that the harmonization of PINP assays is the more practical goal. In order to examine the agreement between current commercial assays, a multi-center study was performed for PINP in serum and plasma among four laboratories in Europe [8]. PINP was measured in serum and EDTA plasma samples from 796 patients with normal renal function (eGFR > 30 mL/min/1.73m^2) present in osteoporosis clinics. All assays gave equivalent results for both serum and EDTA plasma, indicating that both matrices are acceptable and may be used interchangeably [8].

The automated methods for PINP (Roche Cobas and IDS iSYS) gave similar results (Fig. 1a) [8]. On the other hand, a significant proportional bias was observed between the Orion RIA and the two automated assays (i.e., a correlation was observed, but agreement was not shown between the automated methods and the RIA Fig.1b) [8]. Results from other published studies comparing PINP values among these three assays broadly support our findings [9, 10]. Taken together, these results confirm that harmonized PINP measurements exist between the two automated assays (Roche Cobas and IDS iSYS) when the eGFR is > 30 mL/min/1.73m^2, but a significant bias exists between the Orion RIA and the two automated assays. The good news is that in subjects with normal renal function, PINP results reported by the Roche Cobas and IDS iSYS assays are similar and may be used interchangeably. The perception that the two automated assays are used by the vast majority of laboratories worldwide outside of the USA (based on data from external quality assurance providers) leads us to conclude that similar reference intervals and treatment targets could be applied in those instances. This agreement does not extend to the Orion RIA, but the use of a

single assay for PINP within the USA should ensure harmonized results in routine service for clinical practice within that country. However, if the universal harmonization of PINP assays is to be achieved, and this is crucial for international multicentre trials as well as for the development of clinical guidelines with universally applicable reference intervals and treatment targets, then further work will be required in order to harmonize all three assays for PINP. Since there is an excellent correlation between the RIA and the automated assays, harmonization between the automated assays and the RIA is potentially possible with the use of common calibrators and the development of a reference method for PINP. This should also help ensure that any new commercial assay developed in the future will attain similar results and would be a step forward in the use of P1NP as a biomarker in the management of osteoporosis. The International Federation of Clinical Chemistry and Laboratory Medicine and The International Osteoporosis Foundation are committed to working together towards this goal with the cooperation of the reagent manufacturing industry.

Funding information

RE receives consultancy funding from IDS, Roche Diagnostics, GSK Nutrition, FNIH, Mereo, Lilly, Sandoz, Nittobo, Abbvie, Samsung, Haoma Medica, CL Bio, and Viking and grant funding from Nittobo, IDS, Roche. Amgen, and Alexion. NRJ he has received free reagents for research use from Roche Diagnostics and IDS. JAK reports grant support from Amgen, Lilly, and Radius Health. CC has received lecture fees and honoraria from Amgen, Danone, Eli Lilly, GSK, Kyowa Kirin, Medtronic, Merck, Nestlé, Novartis, Pfizer, Roche, Servier, Shire, Takeda, and UCB outside of the submitted work. SS is a consultant for Radius, Lilly/Pfizer, speaker for Radius, Amgen, and on the advisory boards of Radius, Amgen. EC is a consultant for Diasorin and IDS.

References

1. Consensus Development Conference (1993) Diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 94:646–650 [PubMed: 8506892]
2. Cheng SY, Levy AR, Lefaivre KA, Guy P, Kuramoto L, Sobolev B (2011) Geographic trends in incidence of hip fractures: a comprehensive literature review. Osteoporos Int 22:2575–2586 [PubMed: 21484361]
3. Khosla S, Hofbauer LC (2017) Osteoporosis treatment: recent developments and ongoing challenges. Lancet Diabetes Endocrinol 5: 898–907 [PubMed: 28689769]
4. Eastell R, Pigott T, Gossiel F, Naylor KE, Walsh JS, Peel NF (2018) Diagnosis of endocrine disease: bone turnover markers: are they clinically useful? Eur J Endocrinol 178:R19–R31 [PubMed: 29046326]
5. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, McClung M, Morris HA, Silverman S, Trenti T, Wahl DA, Cooper C, Kanis JA, IOF-IFCC Bone Marker Standards Working Group (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 22:391–420 [PubMed: 21184054]
6. Linkhart SG, Linkhart TA, Taylor AK, Wergedal JE, Bettica P, Baylink DJ (1993) Synthetic peptide-based immunoassay for amino-terminal propeptide of type I procollagen: application for evaluation of bone formation. Clin Chem 39:2254–2258 [PubMed: 8222218]
7. Koivula MK, Risteli L, Risteli J (2012) Measurement of aminoterminal propeptide of type I procollagen (PINP) in serum. Clin Biochem 45:920–927 [PubMed: 22480789]
8. Cavalier E, Eastell R, Rye Jørgensen N, Makris K, Tournis S, Vasikaran S, Kanis JA, Cooper C, Pottel H, Morris HA, IFCC-IOF Joint Committee for Bone Metabolism (C-BM), IFCC-IOF Joint Committee for Bone Metabolism (C-BM) (2019) A multicenter study to evaluate harmonization of assays for N-terminal propeptide of type I procollagen (PINP): a report from the IFCC-IOF joint committee for bone metabolism. Clin Chem Lab Med 57: 1546–1555 [PubMed: 31085740]

9. Koivula MK, Richardson J, Leino A, Valleala H, Griffiths K, Barnes A et al. (2010) Validation of an automated intact N-terminal propeptide of type I procollagen (PINP) assay. Clin Biochem 43:1453–1457 [PubMed: 20923676]

10. Jørgensen NR, Møllehave LT, Hansen YBL, Quardon N, Lylloff L, Linneberg A (2017) Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population. Osteoporos Int 28:2103–2113 [PubMed: 28455749]

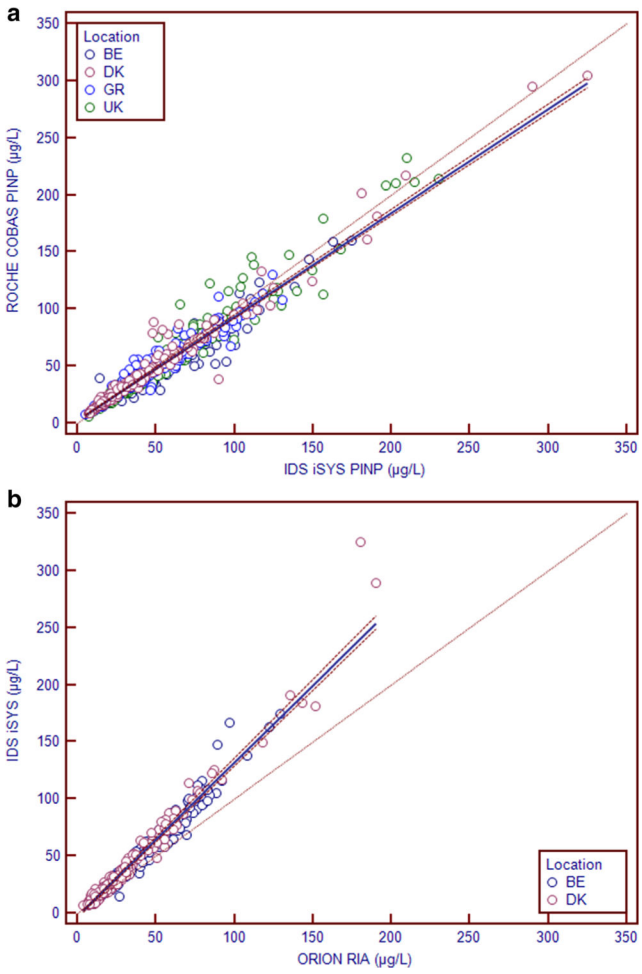

Fig. 1.

a Passing-Bablock regression plot of PINP values observed on IDS iSYS vs. Roche Cobas which show good agreement (Cobas = 0.91x iSYS+2.6). (BE Belgium, DK Denmark, GR Greece, UK United Kingdom). **b** Passing-Bablock regression plot of PINP values observed on IDS iSYS vs. Orion RIAwhich show a significant proportional difference iSYS = 1.35xOrion RIA−3.2). (BE Belgium, DK Denmark).