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Reference intervals for serum concentrations of three bone turnover markers for men and women

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ABSTRACT

Objective: Bone turnover markers (BTMs) reflect the metabolic activity of bone tissue and can be used to monitor osteoporosis therapy. To adequately interpret BTMs, method-specific reference intervals are needed. We aimed to determine reference intervals for serum concentrations of intact amino-terminal propeptide of type I procollagen (PINP), bone-specific alkaline phosphatase (BAP) and carboxy-terminal telopeptide of type I collagen (CTX).
Material and methods: We established a healthy reference population of 1107 men as well as 382 pre- and 450 postmenopausal women, who participated in the first follow-up of the Study of Health in Pomerania. Serum PINP, BAP and CTX concentrations were measured on the IDS-iSYS Automated System (Immunodiagnostic Systems, Frankfurt am Main, Germany). The reference interval was defined as the central 95% range. We determined age-specific reference intervals for PINP, BAP, and CTX for men by quantile regression. Reference intervals for women were age-independent.
Results: Reference intervals for men for PINP and CTX decreased with age (25–29 year-old men: PINP 31.1–95.9 ng/mL, CTX 0.12–0.83 ng/mL; 75–79 year-old men: PINP 15.7–68.1 ng/mL, CTX 0.05–0.58 ng/mL). The reference interval for men for BAP did not significantly change with age (25–29 year-old men: 7.4–27.7 ng/mL; 75–79 year-old men: 7.6–24.4 ng/mL). The reference intervals for 30–54 year-old premenopausal women were: PINP 19.3–76.3 ng/mL, BAP 6.0–22.7 ng/mL, and CTX 0.05–0.67 ng/mL. The reference intervals for 50–79 year-old postmenopausal women were: PINP 18.2–102.3 ng/mL, BAP 8.1–31.6 ng/mL, and CTX 0.09–1.05 ng/mL.
Conclusion: An intensively characterized, large reference population free of bone-related diseases allowed us to determine robust reference intervals for serum concentrations of PINP, BAP and CTX. Our normative data may aid to interpret bone turnover in adult men and pre- and postmenopausal women.

Introduction

The population in the industrialized countries is ageing [1]. The rise in mean age is accompanied by an increasing prevalence of osteoporosis and osteoporotic fractures [2]. Osteoporotic fractures, including hip and vertebral fractures, cause substantial pain, have detrimental effects on quality of life, are associated with disability and mortality and produce high costs [3,4]. Thus, the main target of any osteoporosis therapy is to prevent fractures [3,4].

In clinical routine, the efficacy of osteoporosis therapy is usually assessed by measuring BMD[1] [3]. However, BMD changes slowly, often over several months or even years [5]. Earlier effects of osteoporosis therapy can be monitored by the determination of BTMs,[2] e.g. two or three months after initiation of oral bisphosphonate therapy [6–9]. BTMs reflect the metabolic activity of bone tissue [6], can be measured in serum or urine samples, and allow for the assessment of bone formation and bone resorption processes [7].

Various bone formation and bone resorption markers have been suggested for clinical use [5,10], e.g. intact PINP,[3] BAP,[4] or CTX.[5] PINP

Abbreviations: ATC, Anatomical Therapeutic Chemical Classification System; BAP, bone-specific alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; BTM, bone turnover marker; CTX, carboxy-terminal telopeptide of type I collagen; PINP, amino-terminal propeptide of type I procollagen; PTH, parathyroid hormone; SHIP, Study of Health in Pomerania.

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[1] BMD, bone mineral density.
[2] BTM, bone turnover marker.
[3] PINP, amino-terminal propeptide of type I procollagen.
[4] BAP, bone-specific alkaline phosphatase.
[5] CTX, carboxy-terminal telopeptide of type I collagen.

is cleaved from procollagen and is released into the circulation during bone formation, while CTX is a degradation product of type I collagen and is released into the circulation during bone resorption [5]. BAP is a glycoprotein involved in bone mineralization whose serum concentration is strongly determined by osteoblast activity [5]. PINP and CTX were recommended as the most informative BTMs for monitoring of osteoporosis in the current European guidance for the diagnosis and management of osteoporosis in postmenopausal women [3].

To interpret BTM concentrations, population-, sex-, and method-specific reference intervals are needed [6,9,11]. Currently, reference intervals for serum PINP, BAP, and CTX concentrations have neither been established for the German population nor for measurement with the IDS-iSYS Multi Discipline Automated Analyser. For males, PINP and CTX reference intervals have been established in Spanish men older than 50 years of age [12]. BAP reference intervals have not yet been established for men at all. For females, previous studies reported large differences between pre- and postmenopausal serum BTM concentrations [11,13]. BTMs increase during the menopausal transition due to increased osteoclastic activity which in turn is due to decreasing estrogen levels [14]. Accordingly, the intake of sex hormones for menopausal hormone therapy or contraception may alter BTM concentrations [11,15,16]. The majority of previous studies that determined reference intervals for serum PINP [15–19], BAP [16,18,19] or CTX [15–19] concentrations, were limited to premenopausal women. There is only one study [20] that reported reference intervals for PINP and CTX for postmenopausal women. BAP reference intervals for postmenopausal women have not yet been established.

We aimed to determine sex- and age-specific reference intervals for serum PINP, BAP and CTX concentrations for adult healthy Caucasian men as well as for pre- and postmenopausal women living in northeast Germany. Additionally, we aimed to analyze the impact of sex hormone therapy on serum BTM concentrations.

Material and methods

The Study of Health in Pomerania (SHIP)

The present study is based on data from the first follow-up of the SHIP.[6] SHIP is a population-based cohort study in northeast Germany. In the baseline study (SHIP-0), which was conducted between October 1997 and May 2001, 4308 men and women from a representative sample of 7008 subjects were examined. All subjects were invited to participate in the first follow-up (SHIP-1), which took place between March 2003 and July 2005 with 3300 subjects being re-examined. All SHIP participants gave written informed consent. Further details on study design and sampling have been previously reported [21]. The study was reviewed by an external scientific review board and conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the Board of Physicians Mecklenburg-West Pomerania at the University of Greifswald.

All SHIP participants underwent standardized medical examinations including blood sampling and an extensive computer-aided personal interview. Data on socio-demographic characteristics and medical histories was collected. Intake of medication was recorded and classified using the ATC[7] code. All women were divided according to menopausal status in pre- or postmenopausal. Women younger than 40 years of age and women between 40 and 60 years of age who reported menstrual cycling were defined as premenopausal, all other women were defined as postmenopausal. It was not possible to define perimenopause as the respective information was not collected.

Blood sampling was performed between 8.00 a.m. and 8.00 p.m. More than half of all blood samples (58.2%) were taken before 12 a.m., another third (31.0%) between 12:00 a.m. and 2:59 p.m., and one-tenth (10.8%) after 3:00 p.m. Blood samples were taken from the cubital vein of mostly non-fasting participants in the supine position. Serum aliquots were stored at −80 °C. Serum PINP, BAP, and CTX concentrations were determined on the IDS-iSYS Multi-Discipline Automated Analyser (Immunodiagnostic Systems Limited, Frankfurt am Main, Germany). For each analyte three concentrations of control material were measured by skilled technical personnel. Serum PINP concentrations were measured with the IDS-iSYS Intact PINP assay. During the course of the study the coefficients of variation were 7.33% at low concentrations, 5.67% at medium concentrations, and 7.88% at high concentrations, respectively, of control material. The assay manufacturer reports good linearity in the range of 5.8–203.8 ng/mL, with variations between −3% and +3% of predicted and measured PINP concentrations. Serum BAP concentrations were measured with the IDS-iSYS Ostase + BAP assay. During the course of the study the coefficients of variation were 12.28% at low concentrations, 13.47% at medium concentrations, and 13.23% at high concentrations, respectively, of control material. The assay manufacturer reports good linearity in the range of 9.7–71.0 ng/mL, with variations between −2% and +7% of predicted and measured BAP concentrations. The assay demonstrates a comparable cross-reactivity to liver isoenzyme as the Tandem R Ostase assay [22], as it was developed using the same monoclonal antibody. The assay provides a result of 6.9 ng/mL BAP for each 100 U/L liver enzyme. Serum CTX concentrations were measured with the IDS-iSYS CTX-I (CrossLaps) assay. During the course of the study, the coefficients of variation were 12.23% at low concentrations, 10.40% at medium concentrations, and 11.40% at high concentrations, respectively, of control material. The assay manufacturer reports good linearity in the range of 0.245–5.293 ng/mL, with variations between −6% and +3% of predicted and measured CTX concentrations.

Reference population

To generate a healthy reference population we excluded all subjects with missing data on serum PINP, BAP, or CTX concentrations ($n = 39$), or with any of the following conditions: renal disease defined as estimated glomerular filtration rate (Cockcroft-Gault) <30 mL/min ($n = 30$), hyperparathyroidism defined as serum parathyroid hormone concentration >120 pg/mL ($n = 24$), hyperthyroidism defined according to local reference intervals [23] as serum thyroid-stimulating hormone concentration <0.25 mU/L and serum free thyroxine concentration >18.9 pmol/L ($n = 70$), or a self-reported history of cancer ($n = 189$), a self-reported physician's diagnosis of osteoporosis ($n = 207$), or a self-reported history of liver disease ($n = 41$). We further excluded all pregnant women ($n = 12$), all subjects with serum 25-hydroxy vitamin D concentration <10 ng/mL ($n = 342$), and all subjects ($n = 85$) who reported intake of any of the following medication: bisphosphonates, strontium ranelate, vitamin D, calcium, parathyroid hormone, calcitonin, steroids, selective estrogen receptor modulators, testosterone, anticonvulsants, heparin, antiandrogens, or aromatase inhibitors. Moreover, we excluded all premenopausal women using oral contraceptives ($n = 126$) and all postmenopausal women using hormone replacement therapy ($n = 55$). Furthermore, we excluded subjects with a serum osteocalcin concentration >100 ng/mL ($n = 2$) and, due to small numbers in the respective age groups, all subjects older than 79 years of age ($n = 58$), all premenopausal women younger than 30 years of age ($n = 36$) or older than 54 years of age ($n = 3$), and all postmenopausal women younger than 50 years of age ($n = 44$). This resulted in a reference population of 1939 subjects, including 1107 men as well as 382 premenopausal women (aged 30–54 years) and 450 postmenopausal women (aged 50–79 years).

Statistical analyses

Continuous data are expressed as median (1st-3rd quartile) and nominal data are expressed as percentage. We report median instead

[6] SHIP, Study of Health in Pomerania.
[7] ATC, Anatomical Therapeutic Chemical Classification System.