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Highlight Article

Comparison of clinical cut-points and treatment targets for urine NTX and plasma βCTX-I in osteoporosis

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ABSTRACT

Objective: We undertook to identify levels for plasma β isomerised carboxy-terminal telopeptides of type I collagen (p-βCTX-I) that are comparable to currently used urine amino-terminal telopeptides of type I collagen (u-NTX) cut-points and treatment targets in osteoporosis.

Design and methods: Fasting morning samples were collected from patients attending tertiary hospitals and clinics for investigation of metabolic bone disease. Patients with Paget's disease or <20 years of age were excluded. Second void spot urine for NTX and plasma (EDTA) samples were utilised. Urine was analysed routinely and plasma stored at −20C until analysis by enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic Systems plc), E170 (Roche Diagnostics) and IDS-iSYS (Immunodiagnostic Systems plc) methods. The relationship of u-NTX with each p-βCTX-I method's results was assessed by Passing and Bablok regression, and p-βCTX-I levels equivalent to u-NTX cut-points and targets were interpolated.

Results: One hundred and forty six patients were included. Spearman correlation coefficients ranged from 0.71 to 0.75 for the three βCTX-I assays. The equivalent βCTX-I concentrations for NTX/Cr values of 21 (fracture risk reduction target following risedronate therapy), 27 (healthy pre-menopausal women's mean value), and 38 (threshold for reduction of BMD on calcium alone) nmol BCE/mmol were 230, 312 and 462 ng/L for the automated Roche assay and 271, 395 and 624 ng/L for the automated IDS i-SYS assay respectively.

Conclusions: The p-βCTX-I equivalent to the only available fracture outcome based absolute treatment threshold of 21 nmol BCE/mmol established for u-NTX, is close to 250 ng/L but will vary between p-βCTX-I assays.

1. Introduction

Markers of bone turnover (BTM) may be useful in the management of patients with metabolic bone and metastatic disease [1–3]. In osteoporosis, treatment decisions are increasingly based on absolute fracture risk assessment which currently do not include BTM in the calculation due to lack of data [4,5]. In contrast, there is wider acceptance of the use of BTM in monitoring osteoporosis treatment [6]. Although serial bone density (BMD) measurement is used for monitoring efficacy of treatment, there are drawbacks to BMD monitoring; the change in BMD following treatment is small and slow; repeat measurement may not be useful before 12–24 months after commencement of treatment [7]. BTMs on the other hand show rapid and large changes following initiation of treatment allowing useful measurements to be undertaken 1–3 months after initiation of treatment [8].

The largest decreases in BTMs following anti-resorptive treatment are seen with u-NTX and serum/p-βCTX-I [6,9]. These two markers of bone resorption show the best association with BMD response following treatment and are the most widely used BTMs in clinical practise [6]. Serum/p-βCTX-I has recently been designated as the reference marker of bone resorption by the International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in order to consolidate accumulation of data in research studies on its potential use in clinical practise [10]. βCTX-I is more stable in EDTA plasma than in serum, and the former is the preferred sample for measuring βCTX-I in blood [10]. In addition, the National Bone Health Alliance in the US (NBHA) has embarked on efforts to establish reference intervals for serum/p-βCTX-I and serum procollagen type I

Abbreviations: p-βCTX-I, plasma β isomerised carboxy-terminal telopeptides of type I collagen; u-NTX, urine amino-terminal telopeptides of type I collagen; NTX/Cr, u-NTX/creatinine ratio; BCE, bone collagen equivalent; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; BTM, markers of bone turnover; BMD, bone density; IOF, International Osteoporosis Foundation; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NBHA, the National Bone Health Alliance; s-PINP, serum procollagen type I N propeptide.

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N propeptide, (s-PINP) and assist clinicians to gain confidence in their use to monitor osteoporosis treatment and assess future fracture risk [11]. U-NTX is widely used in North America and in some other parts of the world. The designation of serum/p-βCTX-I as the reference bone resorption marker by IOF and the efforts by NBHA to build on this decision to harmonise routine assays used in clinical laboratories and establish reference intervals for p-βCTX-I and s-PINP is expected to increase the take up of p-βCTX-I assays in laboratories and by clinicians who currently use u-NTX. This study was undertaken to identify levels for p-βCTX-I that are comparable to currently identified u-NTX thresholds and targets.

2. Participants and methods

2.1. Participants

The subjects were adult patients attending the laboratories at Fremantle and Royal Perth Hospitals for routine investigation of metabolic bone disease including osteoporosis. Plasma (EDTA) and second morning spot urine samples were collected after overnight fast. Urine was stored at 4 °C until analysis of NTX. Plasma samples for βCTX-I measurement were frozen in three aliquots at −20C after routine testing was complete. Specimens from patients <20 years of age, patients with Paget's disease, with u-NTX concentrations <20 nmol BCE/L and with NTX/creatinine ratio (NTX/Cr) > 150 nmol BCE/mmol were excluded. Approval to perform this study was granted by the Royal Perth Hospital Human Research Ethics Committee.

2.2. Assays

P-βCTX-I was measured by the three commercial assays currently available: S-CrossLaps ELISA (Immunodiagnostic Systems plc, Tyne and Wear, UK); Cobas β-Crosslaps (Roche Diagnostics Australia, Castle Hill, NSW, Australia), using an E170 immunoassay analyser (Roche Diagnostics); IDS-iSYS βCTX-I (CrossLaps) (Immunodiagnostic Systems plc, Tyne and Wear, UK). The ELISA was carried out manually, using a Labsystems Titertek Multiscan PLUS plate reader and Multicalc data reduction software. The Roche and iSYS methods are automated chemiluminescence immunoassays carried out on their respective dedicated analysers. Each method was carried out according to manufacturers' recommendations, with the exception that samples in the ELISA were analysed in singletons. For each assay, the patients' sample aliquots were kept frozen until the day of analysis. Assay performance was verified using the manufacturers' control specimens. Patient samples were analysed in 4–7 analytical runs for each system. Between-run imprecision, expressed as coefficient of variation, was 2.2% and 9.7% at 299 and 1009 ng/L for the ELISA, 6.3% and 5.5% at 329 and 707 ng/L for the Roche assay and 1.2%, 3.4% and 2.9% at 194, 814 and 2050 ng/L for the iSYS assay respectively. The maximum bias compared to the manufacturers' target values for control samples was 6.3% for the Roche assay, 13.8% for the ELISA and 12.7% for the iSYS assay. U-NTX was measured by Vitros ECi (Ortho Clinical Diagnostics, Mulgrave, Victoria, Australia) with between-run imprecision of 6.9% and 5.1% at 430 and 2280 nmol/L BCE, respectively. Plasma and urine creatinine were measured by kinetic Jaffe methods on an Architect c16000 (Abbott Diagnostics, Macquarie Park, NSW, Australia) with between-run imprecision of 1.9% and 1.3% at 70 and 570 μmol/L plasma creatinine, and 2.1% and 2.8% at 5.3 and 12.0 mmol/L urine creatinine, respectively. U-NTX results were expressed as NTX/Cr ratio, to adjust for variation in urine flow rate between patients. Glomerular filtration rate (GFR) was estimated using the CKD-EPI algorithm [12].

2.3. Statistical analysis

Analyse-it ver 2.04 (Analyse-it Software Ltd., Leeds, UK) was used. Passing and Bablok Type III regression (method conversion) was used to establish the relationships between u-NTX/Cr and the p-βCTX-I results by the three assays respectively. This is appropriate when comparing variables measured in different scales [13]. We identified the p-βCTX-I concentrations equivalent for each assay to the following u-NTX/Cr values:

- 21 nmol BCE/mmol (evidence based threshold for increased fracture risk following risedronate therapy in high-risk post-menopausal women) [14].
- 27 nmol BCE/mmol (mean of results from pre-menopausal women in the reference sample) [15]. Note this is different to the mid-point of pre-menopausal female reference interval which is 36 nmol BCE/mmol [15].
- 38 nmol BCE/mmol (u-NTX threshold for reduction of BMD on calcium alone [16]).

Correlation was assessed using Spearman rank correlation (r_s). Confidence intervals for the estimated p-βCTX-I concentrations were estimated from the graphical output.

3. Results

One hundred and forty seven patients were included in the study. Their demographic data are summarised in Table 1.

Result comparison graphs showing relationships between each p-βCTX-I assay and u-NTX/Cr and the Passing and Bablok regression parameters are shown in Fig. 1 and Table 2, respectively. The three p-βCTX-I assays had moderate correlations with u-NTX, with r_s in the range 0.73–0.75. However the regression slopes and intercepts for the p-βCTX-I assays showed noticeable differences (Table 2).

The three βCTX-I assays show good correlation to each other but also show significant inter-assay biases; these comparisons have been published previously [17].

P-βCTX-I concentrations equivalent to U-NTX/Cr thresholds or values of interest in monitoring osteoporosis therapy for each of the assays, interpolated from the regressions, along with 95% confidence intervals, are given in Table 3. Whilst the interpolated thresholds for the ELISA and iSYS assays are similar, those for the Roche assay are somewhat lower. The p-βCTX-I equivalent to the fracture outcome based absolute treatment threshold of 21 nmol BCE/mmol established for u-NTX, was 230 ng/L for the automated Roche assay, 271 ng/L for the automated IDS iSYS assay and 295 ng/L for the IDS ELISA assay. The p-βCTX-I equivalent to the premenopausal reference interval mean for u-NTX of 27 nmol BCE/mmol was 312 ng/L for the automated Roche assay, 395 ng/L for the automated IDS iSYS assay and 399 ng/L for the IDS ELISA assay.

To assess the generalizability of our observations we repeated the regression procedures on (1) female patients aged over 50 y, as these are most likely to be post-menopausal, and (2) all patients with GFR > 30 mL/min/1.73m^2, to exclude any influence of moderate renal dysfunction on the results. The regression parameters and equivalent βCTX-I concentrations are shown in Supplementary Table S1, available online at http://dx.doi.org/10.1016/j.clinbiochem.2015.12.002. There was little difference in the results obtained compared to those in Tables 2 and 3.

Table 1
Demographic characteristics of the patient sample. Data are numbers or medians [interquartile range].

	Whole sample	Females >50 y
N	147	97
Sex, M:F	40:107	0:97
Age (y)	64 [56–76]	69 [61–77]
eGFR (mL/min/1.73m^2)	80 [69–90]	78 [67–87]