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The Clinical Utility of TRACP-5b to Monitor Anti-resorptive Treatments of Osteoporosis

--Manuscript Draft--

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Full Title:	The Clinical Utility of TRACP-5b to Monitor Anti-resorptive Treatments of Osteoporosis
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Abstract:	Abstract Introduction: Bone turnover markers (BTMs) can be used to monitor response to osteoporosis treatment. However, some are affected by food intake and are not suitable to measure in a clinical setting. An assay is available which is capable of detecting the active isoform 5b of tartrate resistance acid phosphatase (TRACP-5b) and it may have minimal biological variation. Aims: Our aims were to investigate the effect of feeding on levels of TRACP-5b and compare this to CTX and PINP and then to compare the diagnostic accuracy of TRACP-5b to CTX and PINP in patients with osteoporosis given commonly used treatments. Methods: 18 patients were recruited to investigate the effect of feeding on BTMs. 97 patients (74 females and 23 males) receiving 5 mg annual intra-venous zoledronate (mean age 70) and 97 patients receiving no treatment were recruited as group-matched controls. 16 patients receiving 60 mg subcutaneous denosumab every 6 months, (mean age 76) and 16 matched controls were recruited. 76 patients were receiving oral bisphosphonates; 70 mg alendronate weekly, 35 mg risedronate and 150 mg monthly ibandronate (4%). 30 of these patients had BMD measured at the total hip and lumbar spine. An estimate of compliance was not determined. 80 patients receiving no treatment were recruited as group-matched controls. TRACP-5b (ELISA, Nittobo) and CTX and PINP were measured in serum in the non-fasting state between 0800 and 1700. Results: After feeding, there was no effect on levels TRACP-5b and significant reductions in CTX and PINP, 29% and 10%, respectively ($p < 0.001$). In the zoledronate and denosumab groups there were no differences in the areas under the curve (AUCs) between TRACP-5b, PINP and CTX. In the oral bisphosphonates group the AUCs between TRACP-5b and PINP, and TRACP-5b and CTX were significantly different, $p < 0.01$ and $p = 0.001$, respectively. TRACP-5b was negatively correlated with BMD Conclusion: TRACP-5b is not affected by food intake, unlike CTX and PINP. All three BTMs correlate with change in BMD at the lumbar spine and total hip. TRACP-5b has similar diagnostic accuracy to CTX and PINP with commonly used treatments for osteoporosis with the exception of oral bisphosphonate therapy.
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Response to Reviewers:	Reviewer #1: Although most of the comments have been addressed, concerns remain on the performances of the assay used in this study. Indeed the authors refer to technical data published by the "developers" and did not undertake a full internal validation. There is no actual data on assay specificity. It would have been more convincing to use a validated assay. Response: Thank you for the comment. We do agree and appreciate that a full validation of this assay would be useful. However, the purpose of this study was to assess the clinical utility of TRACP-5b and not its technical performance. The technical validation of the assay has previously been published as a peer-reviewed journal and we have included this reference: Ohashi, T., et al., Development of a novel fragments absorbed immunocapture enzyme assay system for tartrate resistant acid phosphatase 5b. Clin Chim Acta, 2007. 376(1-2): p. 205-12. Reviewer #2: The responses clarified the concerns. Thank you. One question remains about ethnicity/race. The authors replied that race is not known for the patients; but is this information known for the Healthy Controls? Even if not known for either group, please add this to the paper; while race would not be expected to impact the overall conclusion in this study (if you agree), mentioning this topic can apply to researchers with more diverse or different populations. Response: The race of the patient was not reported. This has now been added and highlighted in the methods section of the paper.

The Clinical Utility of TRACP-5b to Monitor Anti-resorptive Treatments of Osteoporosis

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Running Title: TRACP 5b for osteoporosis treatment monitoring.

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Declaration of interest

R Eastell receives consultancy funding from IDS, Sandoz, Nittobo, Samsung, Haoma Medica, CL Bio, Biocon, Amgen, Hindustan Unilever, Pharmacosmos, Takeda and Viking and grant funding from Nittobo, Roche, Pharmacosmos and Alexion.

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Abstract

Introduction: Bone turnover markers (BTMs) can be used to monitor response to osteoporosis treatment. However, some are affected by food intake and are not suitable to measure in a clinical setting. An assay is available which is capable of detecting the active isoform 5b of tartrate resistance acid phosphatase (TRACP-5b) and it may have minimal biological variation.

Aims: Our aims were to investigate the effect of feeding on levels of TRACP-5b and compare this to CTX and PINP and then to compare the diagnostic accuracy of TRACP-5b to CTX and PINP in patients with osteoporosis given commonly used treatments.

Methods: 18 patients were recruited to investigate the effect of feeding on BTMs. 97 patients (74 females and 23 males) receiving 5 mg annual intra-venous zoledronate (mean age 70) and 97 patients receiving no treatment were recruited as group-matched controls. 16 patients receiving 60 mg subcutaneous denosumab every 6 months, (mean age 76) and 16 matched controls were recruited. 76 patients were receiving oral bisphosphonates; 70 mg alendronate weekly, 35 mg risedronate and 150 mg monthly ibandronate (4%). 30 of these patients had BMD measured at the total hip and lumbar spine. An estimate of compliance was not determined. 80 patients receiving no treatment were recruited as group-matched controls. TRACP-5b (ELISA, Nittobo) and CTX and PINP were measured in serum in the non-fasting state between 0800 and 1700.

Results: After feeding, there was no effect on levels TRACP-5b and significant reductions in CTX and PINP, 29% and 10%, respectively ($p < 0.001$). In the zoledronate and denosumab groups there were no differences in the areas under the curve (AUCs) between TRACP-5b, PINP and CTX. In the oral bisphosphonates group the AUCs between TRACP-5b and PINP, and TRACP-5b and CTX were significantly different, $p <0.01$ and $p = 0.001$, respectively. TRACP-5b was negatively correlated with BMD

Conclusion: TRACP-5b is not affected by food intake, unlike CTX and PINP. All three BTMs correlate with change in BMD at the lumbar spine and total hip. TRACP-5b has similar diagnostic accuracy to CTX and PINP with commonly used treatments for osteoporosis with the exception of oral bisphosphonate therapy.

Keywords: TRACP-5b, bone turnover markers, osteoporosis, treatment

Summary

TRACP-5b can be used to monitor the response of treatments in osteoporosis. We investigated the effect of feeding on levels of TRACP-5b and how this markers performs in a clinical setting. After feeding, there was no effect on levels TRACP-5b. It has similar diagnostic accuracy to CTX and PINP.

Introduction

Bone turnover markers (BTMs) can be used to monitor the effect of antiresorptive treatments given for postmenopausal osteoporosis[1, 2]. The IOF/IFCC recommend that the pro-collagen I N-propeptide (PINP) and the C-telopeptide of type I collagen (CTX) (bone formation and bone resorption markers, respectively) are used as reference markers [3]. The IOF/ECTS recommend PINP and CTX for identifying poor response to treatment, particularly poor adherence [4].

CTX has a circadian rhythm and circulating levels are affected by food intake. Therefore, it has to be measured first thing in the morning with the patient in the fasting state and this is not always convenient in clinical practice [5]. Even PINP has a small circadian rhythm [6]. It would be helpful to use a bone resorption marker that has minimal circadian rhythm or the effect of feeding and correlates significantly with BMD. Tartrate resistant acid phosphatase isoform 5b (TRACP-5b) appears to meet these criteria, and in addition it has less day-to-day variability [5, 7, 8].

TRACP is an enzyme mainly expressed by the osteoclasts and also by activated macrophage in the lung alveoli [9]. It circulates in serum as two isoforms: 5a and 5b [10]. The isoforms are genetically identical and therefore protein structures are same. They differ by their carbohydrate content with isoform 5a containing sialic acid not present in isoform 5b. Isoform 5b is expressed solely by the osteoclasts whereas isoform 5a may be expressed by macrophages and dendritic cells [11]. TRACP is secreted in circulation as an active enzyme that becomes inactivated by losing its iron content. About 90% of the molecule circulates in fragments and is metabolised by the liver and kidneys. An assay is available which is capable of detecting the active isoform 5b, that utilises anti-TRACP 5b antibody [12]. Ohashi T, et al developed this assay using the monoclonal antibody TrK62 which was raised against ultra-pure TRACP-5b derived from bone. TrK62 is highly specific to TRACP-5b and not TRACP-5b. The assays lower limit of detection was 0.1 U/L, the detection range was 0.1 to 28 U/L, with a linear response and the recovery was estimated to be 92–103%, [12].

Several clinical trials have demonstrated the effects of antiresorptive treatments on levels of TRACP- 5b, in postmenopausal women with osteoporosis. In the Fracture Reduction Evaluation of Denosumab in Osteoporosis (FREEDOM) Trial 96 women received denosumab for 3 years. There was a significant 60% decrease from baseline at 1 month in TRACP-5b

compared to 90% in CTX and 70% in PINP, [13]. Nakatoh S et al, demonstrated that osteoporotic women randomised to receive either minodronate, raloxifene or eldecalcitol after teriparatide discontinuation showed a significant reduction in TRACP-5b by 50%, 30% and 25%, respectively.

It may be more useful in clinical practice to use a bone resorption marker that is affected less by food intake than CTX. We are uncertain about the size of the reduction in TRACP-5b in patients on commonly used anti-resorptive drugs for osteoporosis in clinical practice and how it compares to CTX or PINP. Our aims were: i) to investigate the effect of feeding on levels of TRAC-P 5b and to compare these to CTX and PINP, ii) to compare the diagnostic accuracy of TRACP-5b to PINP and CTX for response of patients to intravenous zoledronate, oral bisphosphonate therapy and denosumab in a clinical setting and iii) to investigate the relationship between the BTMs and the change in BMD in patients receiving oral bisphosphonates.

Subjects and Methods

Subjects

In order to investigate the effect of food intake on TRACP-5b, 18 healthy controls (10 males and 8 females) were recruited at the Clinical Research Facility, Northern General Hospital, Sheffield in 2019. The controls were given a standard breakfast of 2 slices of toast (medium size), butter (2 x 9g) and a drink of tea or coffee (with an average amount of semi-skimmed milk) which was consumed fully. The total energy intake was 308kcal. The macronutrient content was 32.7g carbohydrates, 7.2g proteins and 16.5g fats, Nutritics. (2021). Research Edition (v5.64), Computer software, (Dublin, United Kingdom). A fasting blood sample was collected between 0800 and 1000 and then again one hour after breakfast was consumed. The serum obtained after centrifugation was stored at -80°C until time of analysis in 2020.

To assess the diagnostic accuracy of TRACP-5b we recruited patients attending the Metabolic Bone Clinic at the Northern General Hospital, Sheffield, between February 2016 and February 2018. All patients had been on antiresorptive therapy for at least one year. 97 patients were receiving 5 mg annual intravenous zoledronate for osteoporosis were recruited after at least one infusion. 97 patients receiving no treatment were recruited as group-matched controls. 16 patients were receiving subcutaneous denosumab, at least their second dose of 6 monthly 60 mg. 16 patients receiving no treatment were recruited as

group-matched controls. 76 patients were receiving oral bisphosphonates; 70 mg alendronate weekly (76%) and 35 mg risedronate weekly (22%) and 150 mg monthly ibandronate (2%). 30 of these patients had BMD measured at the total hip and lumbar spine. 80 patients receiving no treatment were recruited as group-matched controls. The matching for the controls was based on age (within 3 years) and gender and they were recruited as patients referred for increased risk of fracture but not yet taking any medication. Non-fasting serum was collected on a single visit between 0800 and 1700 and stored at -80°C until time of analysis in 2018. The samples were obtained from the South Yorkshire and North Derbyshire Musculoskeletal Biobank. The BMD (g/cm2) at total hip and lumbar spine was measured by DXA using a Discovery A densitometer (Hologic Inc, Waltham, MA) in each patient and control subject. The total hip and lumbar spine coefficient of variations were 1.5% and 2.9 respectively, [14]. The race of the patients was not reported. The project was approved by the ethics committee of the South Yorkshire and North Derbyshire Musculoskeletal Biobank (REC Reference number 15/SC/0132, HTA Licence number 12182).

Laboratory methods

Bone turnover markers were measured in serum. TRACP-5b was measured using the manual ELISA from Nitto boseki Co., Ltd. (Fukushima, Japan). PINP and CTX were measured using the Cobas e411 automated immunoassay from Roche Diagnostics (High Wycombe, UK). The inter assay precisions for TRACP-5b, PINP and CTX were 5.6%, 3.4% and 4.3 %, respectively.

Statistical analysis

A Wilcoxon signed rank test was used to determine the difference in median TRACP-5b, PINP and CTX levels before and after food intake, a $p<0.05$ indicated significant differences. Receiver operating characteristic (ROC) curve analysis is a test of diagnostic accuracy was used to calculate the area under the curve (AUC) for single measurements of TRACP-5b, PINP and CTX. The AUCs were compared and the differences between them were calculated, a $p<0.05$ indicated significant differences. The Youden value for each BTM was calculated. The specificity, the percentage of controls above the Youden value and the sensitivity, the percentage of patients receiving treatment who are below were calculated. A Spearman correlation was used to determine the relationship between the change in BMD/year and BTMs, a $p<0.05$ indicated significant differences. These statistical analyses

were performed using Medcalc version 19.1 (Medcalc Statistical Software, Belgium) and GraphPad Prism version 8.2.1 (San Diego, US).

Results

Table 1 shows the demographics of the patients who were recruited on to the feeding study. The median level of TRACP-5b before feeding was 2.1 U/L. There was a 1% decrease after feeding, p>0.05. The median levels of CTX and PINP before feeding were 319 ng/L and 41.9 ug/L, respectively. There was a decrease in CTX by 29% and in PINP by 10% after feeding, p<0.001.

Table 2 shows patient characteristics and BTMs receiving zoledronate, denosumab and oral bisphosphonates with their respective matched controls. These show that the denosumab group were older and had lower BMD than the two bisphosphonate groups. The BMI values were similar among groups. The median levels of markers were similar among the three control groups. However, the median level of markers for oral bisphosphonates were higher than for zoledronate or denosumab. This was significant for levels of TRACP-5b, p<0.001, PINP levels between oral bisphosphonate and denosumab, p<0.05 and CTX levels between oral bisphosphonates and zoledronate, p<0.01 and denosumab, p<0.05.

The effect of zoledronate, oral bisphosphonates and denosumab on the levels of TRACP-5b, CTX and PINP compared to controls are shown in figure 1. Treatment with zoledronate, oral bisphosphonates and denosumab significantly decreased median levels of TRACP-5b compared to controls by 82%, 57% and 84%, P<0.001, respectively. Median levels of CTX and PINP were also significantly decreased compared to controls; by 64%, 60% and 66% and 55%, 46% and 64% respectively, P<0.001. There were some people on treatment that were below the lower limit of detection of the TRACP-5b assay: Oral bisphosphonates N=7/76, Zoledronate N=7/97 and denosumab N=4/16.

Receiver operating characteristic (ROC) curve analysis was used to compare the AUCs for TRACP 5b, PINP and CTX, Figure 2 and table 3. The Youden values, specificity and sensitivity are shown for each marker are in table 4. In the zoledronate and denosumab groups there

were no differences in the AUCs between TRACP-5b, PINP and CTX. In the oral bisphosphonates group the AUCs between TRACP 5b and PINP, and TRACP 5b and CTX were significantly different, $p<0.01$.

The changes in total hip BMD correlated significantly with TRACP-5b ($R = -0.444$, $p < 0.01$), CTX ($R = -0.487$, $p < 0.01$) and PINP ($R = -0.436$, $p < 0.05$). Similarly, the changes in lumbar spine BMD correlated significantly with TRACP-5b ($R = -0.370$, $p < 0.05$), CTX ($R = -0.453$, $p < 0.01$) and PINP ($R = -0.416$, $p < 0.05$)

Discussion

There has been previous evidence suggesting that food intake results in acute changes in levels of bone turnover markers, [15-17]. Our study has shown that feeding did not suppress levels of TRACP-5b but suppressed CTX and PINP. In comparison, another study of 20 healthy premenopausal women showed that there was a significant but small decrease of 2.4% in TRACP-5b after feeding, using a different assay [7]. Clowes J.A, et al have previously described that in twenty healthy premenopausal women there was a decrease in CTX and PINP between fed and fasting state by 18% and 4%, respectively [15]. We observed a bigger decrease in CTX and PINP (29% and 10%). These differences may be caused by the types and the quantities of foods being consumed, gender and age. Obesity can also alter levels of CTX and PINP, [18-20], and is suppressed by feeding and insulin resistance, [19, 21]. The effects on TRACP-5b has not yet been studied in these populations.

It is usual to recommend a fasting sample collection for measuring bone turnover markers. However, because there is no effect of feeding on TRACP-5b, it may therefore be a useful marker to measure in a clinical setting. In this real-world study, we have shown that TRACP-5b had similar diagnostic accuracy to PINP and CTX for treatment with zoledronate but lower accuracy with oral bisphosphonates. Diagnostic accuracy studies assume patients will be 100% compliant to the medication. This was true for zoledronate and denosumab in this study as a prior treatment given on time was an inclusion criterion, but not for oral bisphosphonates. A major challenge with oral bisphosphonates is the poor adherence and compliance compared with other treatments [22-24]. We didn't have an objective estimate of compliance in the current study. However, we did have change in BMD and this is likely to be related to compliance. We noted that all three markers related well to change in BMD at the lumbar spine and total hip. Women who adhere to oral bisphosphonates have greater reductions in BTMs and a lower fracture risk than those who do not [25]. From the systematic review previously conducted, using 89 studies, the adherence after 12 months of therapy ranged from 28 to 85% [23]. Another study has reported that less than half of the patients continue to take the medication a year after the start of treatment [26]. This may explain the onset and offset of the effect with CTX and TRACP-5b differ and so a recent period of good compliance (prior to attending the clinic) may have been sufficient to suppress CTX but not TRACP-5b. Whether this means that TRACP-5b is a more suitable marker than CTX to identify consistent compliance requires further study.

There is a similarity here to the management of diabetes where blood glucose is more responsive to recent compliance than haemoglobin A1c. Oral bisphosphonates reduce levels of CTX within one week of taking the treatment [25]. There are no detailed time course studies of TRACP-5b response to oral bisphosphonates. It may be that TRACP-5b levels are not reduced as early as CTX and this would explain the differences in the diagnostic accuracies. One previous study has shown that in patients who have undergone removal of the parathyroid adenoma, the collagen-based marker total hydroxyproline was decreased early, within 2-3 days whereas TRACP-5b level decreased slowly, within 10 days to normal levels [27].

CTX has the lowest specificity (58 to 68 %) as compared to PINP (63 to 75%) or TRACP 5b (63 to 72%) probably due to samples not being taken in the fasting state, as recommended [15, 28-30].

We used Youden value to identify a treatment target and this works by optimising sensitivity and specificity. This is a statistical and pragmatic approach and gives almost identical figures to the median (or geometric mean) of the reference interval for healthy young women, or previous target. Thus, the median values from the literature for TRACP-5b, PINP and CTX are 2, 35 and 280, and the Youden value gives 2.1, 37, and 296 [25, 31-33]. We have shown that TRACP-5b responds to anti-resorptive treatments and this is supported by others using different assays [13, 34-37].

Changes in TH and LS BMD/year showed a significant negative correlation with TRACP-5b, CTX and PINP in patients receiving oral bisphosphonates. This is supported by a previous study where 75 postmenopausal women received alendronate, showed that changes in LSMD at 12 months correlated significantly with the changes of S-TRACP5b ($r = -0.32$, $p = 0.005$) and S-CTX ($r = -0.24$, $p = 0.037$) at 3 months but not PINP [36].

Strengths:

This is the first clinical data that investigates the performance of this TRACP-5b assay. This is real world data. We used a similar approach for all markers by ROC analysis. We included the two BTM recommended by IOF, namely CTX and PINP.

Limitations

As subcutaneous denosumab injections are mainly given in primary care in the UK, only 16 patients were recruited to this cohort. The clinical patient samples were collected in a non-fasting state and feeding can effect CTX and PINP levels.

Conclusions

TRACP-5b has the advantage over CTX and PINP in that its level is not affected by feeding. The diagnostic accuracy of TRACP-5b for monitoring zoledronate and denosumab is similar to CTX and PINP, making it a useful alternative. In addition, TRACP-5b correlates significantly with the in BMD. There was less discrimination for the use of TRACP-5b in monitoring oral bisphosphonate therapy, but the reason for that needs to be explored further.

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Table 1: The demographics (Mean and SD) for the patients recruited to investigate the effect of feeding on levels of TRACP-5b (median and 95% CI for the BTMs)

Variable	**Before feeding**	**After Feeding (BTM)**
Number (M/F)	18 (10/8)	
Age (years)	41.8 (4.7)	
Height (cm)	173.7 (8.0)	
Weight (kg)	71.9 (12.1)	
BMI (kg/m^2)	23.8 (3.3)	
TRACP-5b (U/L)	2.6 (2.3-3.2)	2.6 (2.3-3.2)
CTX (ng/L)	319 (268-386)	227 (186-262)
PINP (ug/L)	41.9 (38.2-52.1)	37.7 (34.6-46.8)

Table 2: Patient characteristics (means and (standard deviations) and BTMs (medians and (25 and 75 percentiles) receiving zoledronate, denosumab and oral bisphosphonates with their respective matched controls

	Zoledronate (n=97)	Controls (n=97)	Oral Bisphosphonates (n=76)	Controls (n=80)	Denosumab (n=16)	Controls (n=16)
Age (years)	70.1 (11.1)	70.0 (11.1)	70.3 (9.4)	70.0 (9.6)	76.5 (9.9)	77.4 (9.0)
BMI (kg/m^2)	26.7 (5.5)	26.5 (5.4)	26.2 (5.6)	26.3 (5.1)	25.8 (5.4)	25.5 (5.4)
Total Hip T-score	-1.70 (1.01)	-1.80 (1.00)	-1.60 (0.91)	-1.60 (0.82)	-2.30 (0.99)	-2.40 (0.87)
Lumbar Spine T-score	-2.17 (1.25)		-2.00 (1.40)		-1.94 (1.38)	
TRACP-5b U/L	0.5 (0.2-1.2)	2.8 (1.0-4.3)	1.3 (0.3-2.8)	3.0 (1.6-4.6)	0.4 (0.1-0.8)	2.3 (0.6-4.8)
PINP ug/L	21.2(16.2-29.4)	47.0(30.5-85.0)	25.6 (17.1-34.9)	47.0 (35.9-77.9)	14.6(11.5-21.2)	40.6 (31.0-55.9)
CTX ng/L	88.5 (59.0-134.8)	245.0 (114.0-440.5)	119.0 (67.5-204.3)	291.0 (137.5-522.0)	78.5 (53.8-112.0)	233.0 (107.3-385.8)

Table 3: The AUC and 95% confidence intervals (using ROC curve analysis) for TRACP 5b, PINP and CTX for each antiresorptive treatment

	Zoledronate	Oral Bisphosphonates	Denosumab
TRACP-5b	0.80 (0.34-0.86)	0.70 (0.62-0.77)[a]	0.85 (0.68-0.95)
PINP	0.84 (0.77-0.89)	0.83 (0.76-0.88)[b]	0.82 (0.65-0.93)
CTX	0.80 (0.74-0.86)	0.78 (0.71-0.84)[b]	0.84 (0.67-0.94)

Table 4: The specificity and sensitivity of TRACP-5b, PINP and CTX for each of the treatment groups.

	Youden value	Zoledronate	Oral Bisphosphonates	Denosumab
TRACP-5b (%specificity)	2.09 U/L	65	72	63
TRACP-5b (% sensitivity)		86	64	94
PINP (% specificity)	37.3 ug/L	65	75	63
PINP (% sensitivity)		90	80	81
CTX (% specificity)	208 ng/L	58	68	56
CTX (% sensitivity)		90	80	94

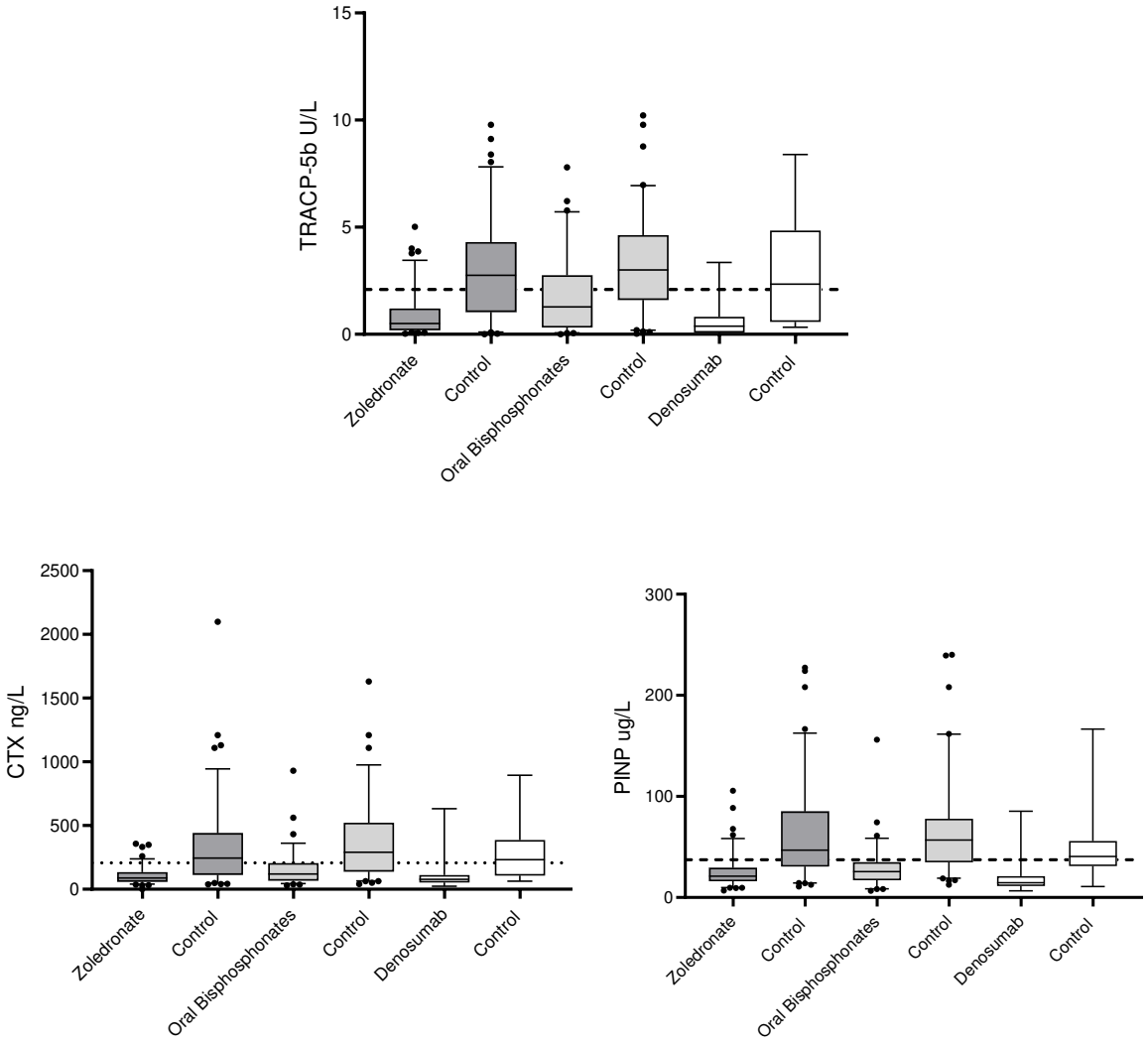

Figure 1: Box and whisker plots showing the effects of antiresorptive drugs on levels of TRACP-5b, PINP and CTX in treated patients and matched controls. The dotted lines represent the Youden value (from Table 4).

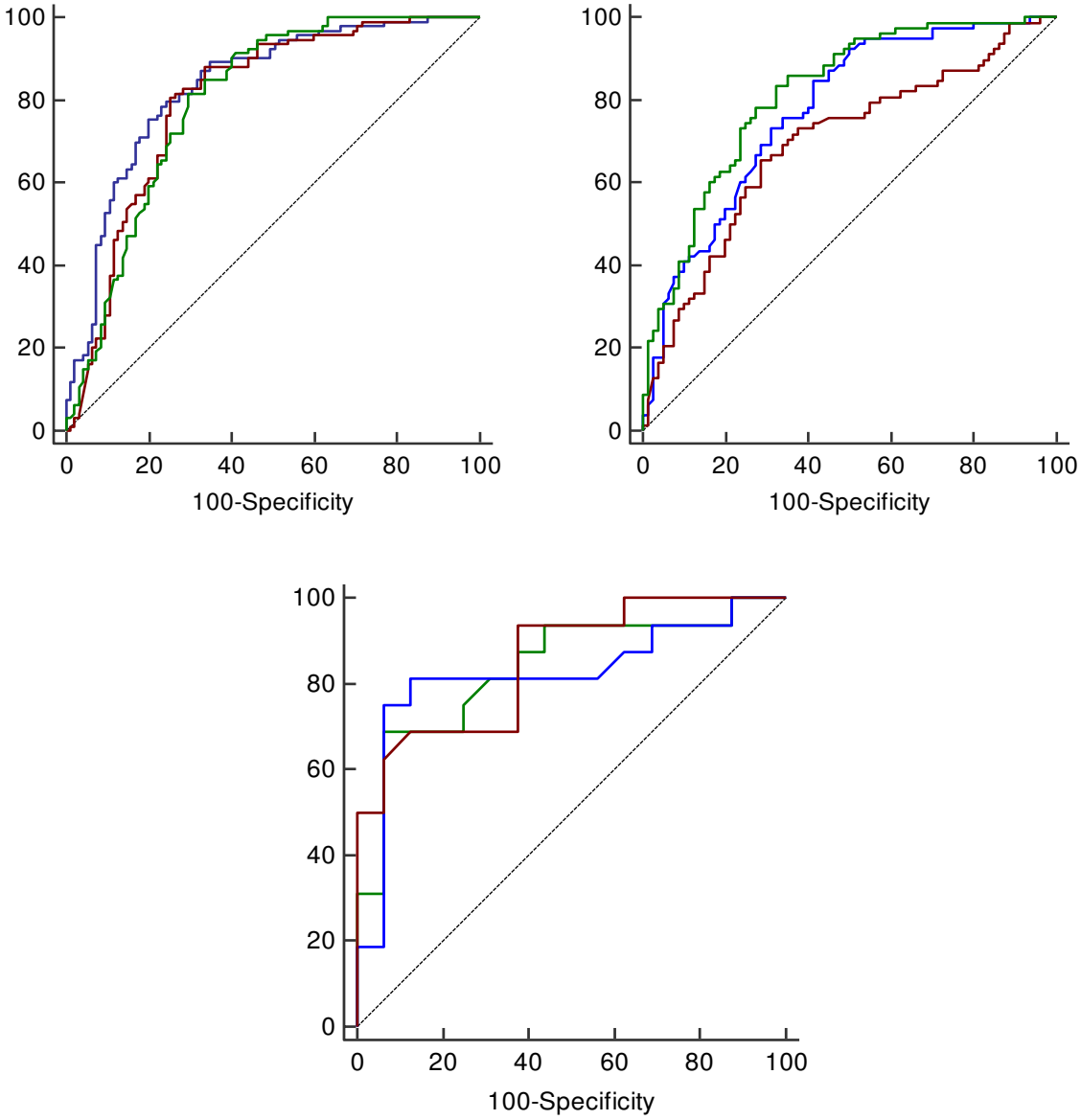

Figure 2: ROC curve analysis comparing TRACP-5b (red), PINP (blue) and CTX (green) in patients receiving zoledronate (top left), oral bisphosphonates (top right) and denosumab (bottom).