

Increased Bone Formation and Bone Resorption in Patients with Hemophilic Arthropathy: A Cross-Sectional Study

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Research article

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Abstract

Background: Previous studies have revealed that hemophilia patients with severe osteoporosis have more arthropathy symptoms and that their bone metabolism is frequently abnormal. However, it is unclear how to achieve an early diagnosis of hemophilic arthropathy (HA) through abnormalities in bone metabolism.

Methods: This study includes 46 patients with HA, 48 healthy controls (HC) and 43 hemophilia without arthropathy (HWA) patients (5 patients were excluded). We measured and compared bone turnover markers (BTMs) including osteocalcin (OC), n-terminal procollagen 1 elongating pro-peptide (t-P1NP) and β -isomerized collagen I-c-terminal peptide breakdown product (β -CTx) of three groups. Receiver operating characteristic (ROC) curve was drawn to obtain the critical value of BTMs for the diagnosis of HA. One-way ANOVA was used to compare the BTMs levels in different degrees of hemophilia. Multivariate linear regression was performed to analyze the correlation between BTMs and clinical severity of HA.

Results: The t-P1NP, β -CTx and OC levels of HA and HWA patients were higher than HC, and HA patients had higher t-P1NP and β -CTx levels than HWA patients ($p < 0.001$). But there was no significant difference in OC levels between HA and HWA groups. Logistic regression analysis revealed that high β -CTx and high t-P1NP were significantly associated with HA. ROC curve showed the highest area under the curve was the t-P1NP+ β -CTx model (area under the curve was 0.686, the sensitivity was 54.5% and the specificity was 82.5%, the cut-off value was 0.578). Multivariate linear regression analysis indicated that β -CTx was positively correlated with the clinical severity of HA, and t-P1NP levels was negatively correlated with the clinical severity of HA. β -CTx levels decreased with the degree of hemophilia.

Conclusions: Increased bone resorption and bone formation were associated with the onset of HA, and the t-P1NP+ β -CTx model (β -CTx > 775.200 ng/mL and t-P1NP > 55.100 ng/mL) contributed to the diagnosis of HA.

Introduction

Haemophilia A is a recessive hereditary hemorrhagic disease with a male prevalence of 1 in 5,000 [1] that can cause a syndrome of joint manifestations such as spontaneous intra-articular haemorrhage, cartilage destruction, synovial inflammation and subchondral bone destruction, which is known as haemophilic arthropathy (HA) [2].

Osteoporosis has been recognized as one of the serious complications of hemophilia [3, 4], and bone mineral density (BMD) is the gold standard for its diagnosis [5]. Katsarou et al. [6] compared hemophilia patients with different BMD and found that low BMD in the femoral neck was associated with high clinical and radiological scores for arthropathy. In addition, Naderi et al. [7] have reported that patients at high risk for reduced BMD were those with signs of HA. Taken together, these results suggest that osteoporosis may be related to HA. However, BMD can only indicate bone mass, lagging behind changes in bone metabolism as the initiation of osteoporosis, which is closely related to the patient's dynamic bone loss and bone strength, so predicting HA by BMD is often unreliable [8]. For patients with hemophilia, there is a demand for a method to better diagnose HA at an early stage so that treatment can be adjusted to slow the progression of the disease. Based on Chopin's study [9], relevant biochemical bone turnover markers (BTMs) could provide an earlier, more accurate and realistic picture of bone resorption and bone formation in patients. Reviewing previous studies, n-terminal type 1 collagen elongating pro-peptide (t-P1NP), β -isomerized type I collagen C-melanopeptide breakdown product (β -CTx) and osteocalcin (OC) are the most commonly used markers of bone turnover markers (BTMs) in osteoporosis [10, 11]. Therefore, the present study was conducted to assess the characteristics of BTMs in hemophilia patients with and without arthropathy and to explore their association with the onset of hemophilic arthropathy (HA).

Methods

Study design and participants

This cross-sectional study included 46 HA patients, 43 hemophilia without arthropathy (HWA) patients and 48 healthy males as healthy controls (HC), aged 16–63, in Orthopedics, Hematology and Health Examination Center of Zhejiang Traditional Chinese Medicine Hospital from January 1st, 2018 to July 1st, 2020. The inclusion criteria were as follows: patients with a definite diagnosis of hemophilia A without arthropathy or HA regardless of the joint involved. Patients were excluded with one of the following conditions: concomitant diseases that could affect bone metabolism and lead to secondary osteoporosis, such as vitamin D deficiency, renal insufficiency, hypogonadism and thyroid gland dysfunction, etc; use of oral bisphosphonates, parathyroid hormone, strontium ranelate, calcitonin, or any other investigational therapy within half a year. This study was approved by the hospital ethics committee and all researchers signed informed written consent.

BTM analyses

Serum samples were collected when all participants fasted in the morning. All BTMs were performed at Clinical Laboratory using a COBAS e 601 automated analyser (Roche Diagnostics, Germany) by ECLIA according to the manufacturer's instructions.

Measurement of BMD

BMD was measured using dual-energy x-ray absorptiometry (DEXA). Both lumbar spine (LS) (L2-L4) and femoral neck (FN) sites were assessed. BMD was expressed as T-scores, which were calculated using male normative data from the Chinese population.

Assessment of the degree of haemophilia

Factor VIII (FVIII) measurement was performed with FVIII-deficient plasma as a substrate using a one-stage coagulation bioassay. The degree of hemophilia was divided into three types according to the classification standard of International Society on Thrombosis and Haemostasis (ISTH): according to FVIII in plasma, mild 5–40%, moderate 1–5%, severe < 1%.

Assessment of the clinical severity of HA

The clinical severity of HA was assessed in six major joints—knees, ankles, and elbows, using the clinical score described by the Orthopedic Advisory Council of the World Federation of Hemophilia (WFH) [12], including joint swelling, fixed flexion deformity, range of motion, crepitus, instability, axial deformity and muscle atrophy, which was scored from 0 to 12 for the knees and ankles, and from 0 to 10 for the elbows; thus, the maximum score was 68.

Statistical analysis

Data analysis was performed using SPSS 25. (IBM Corp., NY, USA.). The data are presented as mean \pm standard and percentage. Differences in baseline data were assessed by one-way ANOVA for continuous variables and chi-square test for categorical variables. Logistic regression was used to analyze the associations between BTMs and HA. Receiver operating characteristic (ROC) curve was drawn to obtain the critical value of BTMs for the diagnosis of HA. Calculate the area under the curve (AUC) and use the critical values to determine the maximum sensitivity and specificity. One-way ANOVA was used to compare the levels of BTMs in different degrees of hemophilia. Multivariate linear regression was

performed to analyze the correlation between BTMs and clinical severity of HA. The test level p value < 0.05 was considered statistically significant.

Results

Verified the 137 selected participants, 5 participants (3 cases were diagnosed with renal insufficiency, and 2 case had been taking bisphosphonates) were excluded (Fig. 1). There was no significant difference in gender, age and BMI between three groups ($p > 0.05$) (Table 1).

β -CTx levels were significantly higher in HA and HWA than in HC, and the two comparisons showed that they were significantly higher in HA than in HWA ($p < 0.001$) (HA: 707.76 ± 287.33 ng/mL; HC: 456.23 ± 205.06 ng/mL; HWA: 581.90 ± 184.70 ng/mL). t-P1NP levels were significantly higher in HA and HWA than in HC, and the two comparisons showed that they were significantly higher in HA than in HWA ($p < 0.001$) (HA: 75.22 ± 25.77 ng/mL; HC: 48.87 ± 22.29 ng/mL; HWA: 60.27 ± 24.49 ng/mL). OC levels in HA were significantly higher than HC ($p < 0.05$) (HA: 20.39 ± 8.14 ng/mL; HC: 16.24 ± 6.98 ng/mL; HWA: 17.32 ± 7.10 ng / mL), but the difference between HA and HWA was not statistically significant ($p > 0.05$). BMD in femoral neck and lumbar spine was lower in both HA and HWA than HC ($p < 0.001$), but there was no significant difference in HA and HWA ($P > 0.05$). The percentages of osteoporosis in HA and HWA were 31.8% and 40%, respectively, with no statistically significant difference between the two groups ($p > 0.05$). (Table 1).

Logistic regression analysis revealed that high β -CTx (OR: 1.002, 95% CI: 1.000–1.004, $p < 0.01$) and high t-P1NP (OR: 1.026, 95% CI: 1.006–1.047, $p < 0.05$) were significantly associated with the occurrence of HA (Table 2).

For distinguishing HA from hemophilia, ROC curve analysis was drawn, showing that the AUC of the t-P1NP + β -CTx model was the highest [t-P1NP + β -CTx: 0.686, 95% CI: 0.572–0.801; β -CTx: 0.648, 95% CI: 0.528–0.769; t-P1NP: 0.681, 95% CI: 0.563–0.799]. According to the cut-off value calculated from the ROC curve, the sensitivity of β -CTx + t-P1NP model was 54.5% and the specificity was 82.5% (the cut-off value was 0.578); the sensitivity of the β -CTx model was 47.7% and the specificity was 90%. (the cut-off value was 775.200 ng/mL); and the sensitivity of the t-P1NP model was 84.1% and the specificity was 55% (the cut-off value was 55.100 ng/mL) (Fig. 2).

Comparison of BTMs between different hemophilia degrees revealed a decreasing trend in β -CTx levels with decreasing hemophilia degree, while there was no significant difference between other BTMs and hemophilia degree, which was observed in both HA and HWA (Table 3).

Finally, according to multivariate linear regression, β -CTx was positively correlated to HA clinical severity score ($B = 0.024$, $t = 6.119$, $p < 0.001$) and t-P1NP was negatively correlated to HA clinical severity score ($B = -0.134$, $t = -3.243$, $p < 0.01$). There was no statistical statistical correlation between OC and the HA clinical severity score ($p > 0.05$) (Table 4).

Table 1
Descriptive characteristics of patients

Parameters	HA	HC	HWA	Test statistic	<i>p</i> value
n	44	48	40		
Gender (male/female)	44/0	48/0	40/0		
Age (years)	38.09 ± 10.83	42.35 ± 12.37	41.33 ± 10.73	1.719	0.183
BMI (kg/m ²)	20.27 ± 1.81	21.31 ± 2.13	21.50 ± 2.59	2.848	0.062
β-CTx (ng/mL)	707.76 ± 287.33 ^{ab}	456.23 ± 205.06	581.90 ± 184.70 ^a	13.670	0.000
t-P1NP (ng/mL)	75.22 ± 25.77 ^{ab}	48.87 ± 22.29	60.27 ± 24.49 ^a	13.687	0.000
OC (ng/mL)	20.39 ± 8.14 ^a	16.24 ± 6.98	17.32 ± 7.10	3.338	0.039
BMD (T-score)					
Femoral neck	-1.58 ± 1.52 ^a	0.38 ± 0.58	-1.61 ± 0.10 ^a	28.121	0.000
Lumbar spine	-0.71 ± 1.61 ^a	0.51 ± 0.33	-1.68 ± 1.17 ^a	21.040	0.000
Osteoporosis	31.8%	0%	40.0%		0.434

HA Hemophilic arthropathy, HC healthy control, HWA hemophilia without arthropathy, BMI body mass index, β-CTx β-isomerized type I collagen C-melanopeptide breakdown product, t-P1NP n-terminal type 1 collagen elongating pro-peptide, OC osteocalcin, BMD bone mineral density

^aStatistically significant (*p*<0.05) compared to HC group; ^bStatistically significant (*p*<0.05) compared to HWA group

Table 2
Logistic regression analysis odds ratio of β-CTx and t-P1NP

	B	S.E.	Wald	<i>p</i> value	OR	95% CI	
						Lower bound	Upper bound
β-CTx	0.002	0.001	5.322	0.021	1.002	1.000	1.004
t-P1NP	0.026	0.010	6.249	0.012	1.026	1.006	1.047

β-CTx β-isomerized type I collagen C-melanopeptide breakdown product, t-P1NP n-terminal type 1 collagen elongating pro-peptide, OR odds ratio, CI confidence interval

Table 3
Comparison of BTMs in different degrees of hemophilia

Parameters	HA			F	p value	HWA			F	p value
	Severe	Moderate	Mild			Severe	Moderate	Mild		
β -CTx (ng/mL)	866.83 ± 371.24 ^a	751.29 ± 270.30	601.8 ± 231.95	2.959	0.063	702.44 ± 269.04 ^a	598.78 ± 134.13 ^a	439.89 ± 161.01	3.391	0.044
t-P1NP (ng/mL)	83.61 ± 37.45	73.30 ± 17.05	73.39 ± 27.28	0.507	0.606	60.40 ± 26.51	59.96 ± 25.00	60.77 ± 24.40	0.023	0.977
OC (ng/mL)	20.69 ± 4.34	21.87 ± 7.89	18.93 ± 9.55	0.580	0.565	19.08 ± 5.74	16.98 ± 6.34	16.70 ± 9.49	0.147	0.864

HA Hemophilic arthropathy, HWA hemophilia without arthropathy, β -CTx β -isomerized type I collagen C-melanopeptide breakdown product, t-P1NP n-terminal type 1 collagen elongating pro-peptide, OC osteocalcin

^a Statistically significant ($p < 0.05$) compared to Mild

Table 4
Multivariate linear regression of BTMs and HA clinical severity score (WFH score)

	B	S.E.	Beta	t	p value	95%CI	
						Lower bound	Upper bound
β -CTx	0.024	0.004	0.921	6.119	0.000	0.016	0.032
t-P1NP	-0.134	0.041	-0.462	-3.243	0.002	-0.218	-0.051
OC	0.182	0.103	0.197	1.766	0.085	-0.026	0.390

β -CTx β -isomerized type I collagen C-melanopeptide breakdown product, t-P1NP n-terminal type 1 collagen elongating pro-peptide, OC osteocalcin, CI confidence interval

Discussion

The pathogenesis of HA is complex, with joint hemorrhage as the initiating factor, triggering a series of cytokine responses that culminate in synovitis, cartilage destruction, and subchondral bone destruction [13]. As an essential process of HA, bone destruction can be reflected by BTMs [14]. In this study, β -CTx was chosen to monitor bone resorption, and t-P1NP and OC were selected as markers of bone formation.

Consistent with previous findings [6], bone resorption in hemophilia was elevated in comparison to healthy individuals. In the present study, an increase in bone formation was further observed and higher levels of bone resorption as well as bone formation were noted in patients with HA than in hemophilia patients without arthropathy. Previously, bone formation was thought to be a protective factor against bone destruction [15], but logistic regression results indicated that higher levels of both resorption and formation were associated with the pathogenesis of HA. We suggest that bone resorption and bone formation are not isolated processes, which is consistent with Delaisse's view that osteoclasts always trigger osteoblasts initiation whose rate of generation tends to be congruent but osteoblasts need to reach a certain cell density for the reversal of resorption and formation to occur or permanent bone loss may occur [16]. Patients with hemophilia due to repeated bleeding and cell lossing apparently do not have the osteoblast density to meet the conditions for reversal, resulting in continuous bone loss, and in addition, in an environment of much higher bone

metabolism rate, remodeled bone that cannot keep up with the rate of resorption instead becomes increasingly fragile and more susceptible to devastating injuries such as progressive accumulation of microfractures, thus contributing to the onset of HA. Although the changes in BMD and percentage of osteoporosis were not significant, the t-P1NP + β -CTx model contributed to the early diagnosis of HA based on the ROC curves.

The present study classified hemophilia severity based on levels of coagulation factors and found that bone resorption increased with severity, regardless of the presence of arthropathy. Previous studies have shown that FVIII deficiency can cause a decrease in thrombin levels [17] and inhibit PAR-1-mediated osteoclast proliferation [18]. In addition, Recht et al [19] found that the levels of two inflammatory cytokines, IL-1 α and IL- β , which inhibit osteoclast activity, were severely reduced in FVIII-deficient mice, leading to bone resorption. Thus, coagulation factor deficiency may be a contributing factor to abnormal bone metabolism, and in addition, for HA that has already occurred, bone resorption and the clinical severity of arthropathy are positively correlated, so prophylactic coagulation factor replacement therapy may be warranted to maintain homeostasis of bone metabolism either to prevent the onset or to slow the progression of HA.

There are also some limitations in current study. First, since the prevalence of hemophilia is much lower in women than in men [20], the lack of inclusion of women with hemophilia in this study may result in selection bias. Second, individual dietary habits and physical activity intensity may be confounding factors affecting BTMs. Finally, studies with multicenter, larger study populations and larger sample sizes are still in demand.

Conclusion

In present study, we found increased bone resorption and bone formation in patients with HA. Both high bone resorption and bone formation increased the risk of onset of HA. The t-P1NP + β -CTx model (β -CTx > 775.200 ng/mL and t-P1NP > 55.100 ng/mL) assisted in HA diagnosis. Bone resorption tended to decrease with the degree of hemophilia and was associated with the clinical severity of HA, which may be instructive for the prophylactic use of coagulation factors.

Abbreviations

BTMs: Bone turnover markers; HA: Hemophilic arthropathy; HWA: hemophilia without arthropathy; HC: Healthy controls; OC: Osteocalcin; t-P1NP: n-terminal procollagen 1 elongating pro-peptide; β -CTx: β -isomerized collagen I-c-terminal peptide breakdown product; FVIII: Factor VIII; WFH: World Federation of Hemophilia; ROC: Receiver operating characteristic; AUC: Area under the curve; ISTH: International Society on Thrombosis and Haemostasis; OR: odds ratio; CI: confidence interval

Declarations

Acknowledgements

None.

Authors' contributions

QM and PJT conceived the study. QM, BJH and SYZ designed the study. QM, PJT and BJH provided study materials or patients. SYZ and ZYS collected and organize data. SYZ and HHX did data analysis and interpretation. QM and SYZ drafted the manuscript. All authors have read and approved the final manuscript.

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Availability of data and material

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The First Affiliated Hospital of Zhejiang Chinese Medical University approved all procedures (Ethical approval ID: 2018-KL-005) and written informed consent was obtained from all participants.

Consent for publication

Consent for publication in the study was obtained.

Competing interests

The authors declare that they have no competing interests

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Figures

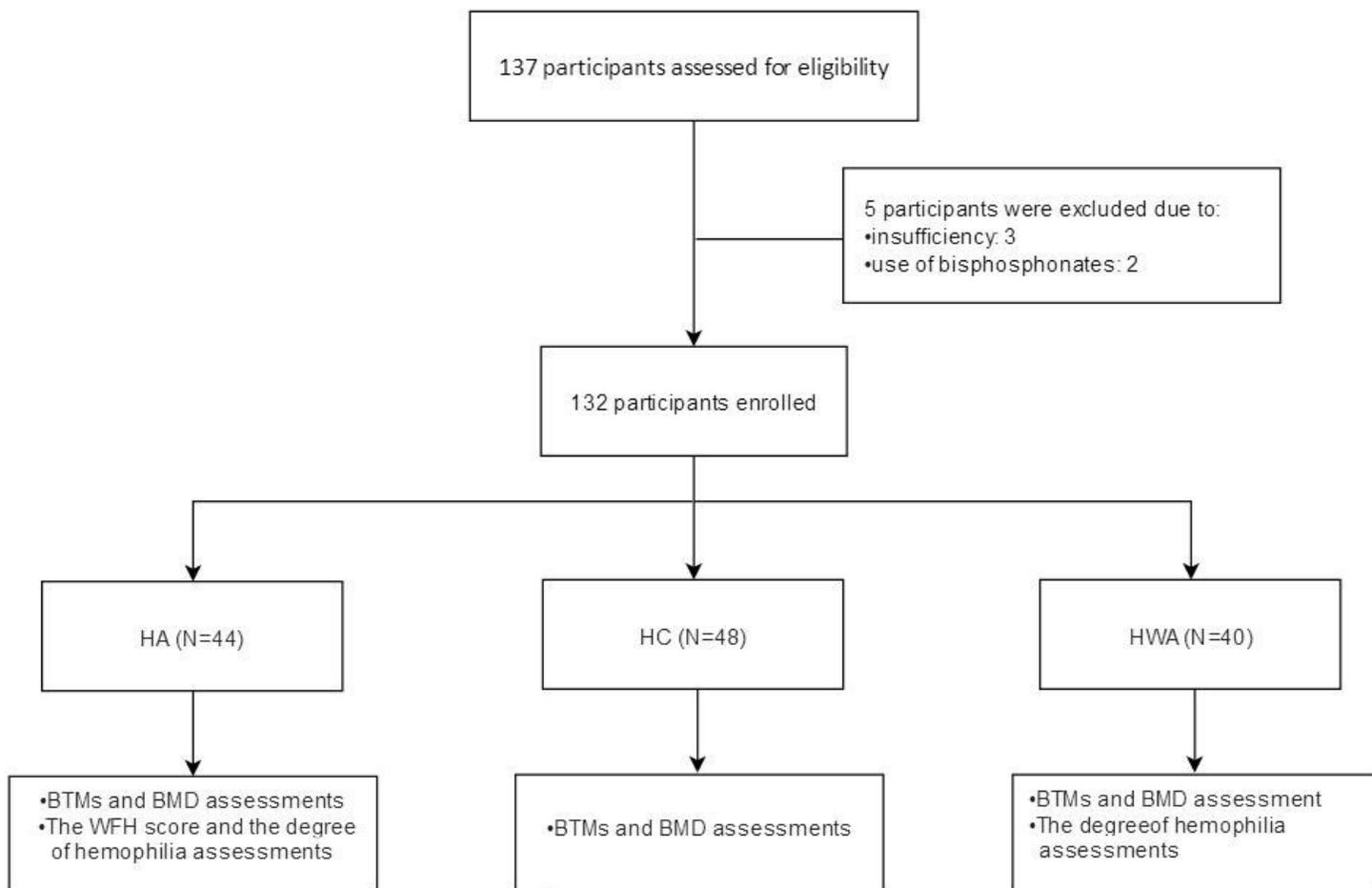


Figure 1

Study flow diagram

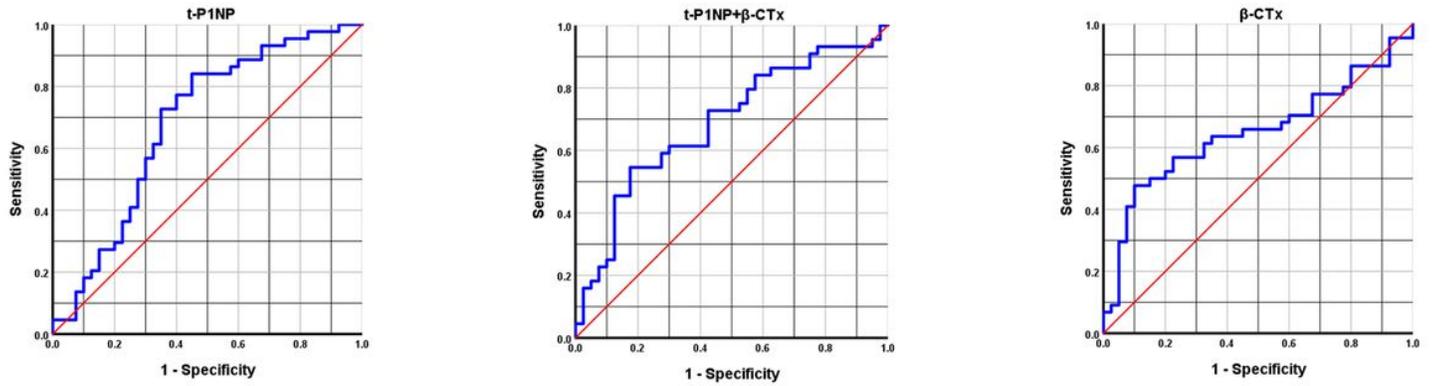


Figure 2

Receiver operating characteristic (ROC) curve analysis of β -CTx, t-P1NP and β -CTx + t-P1NP model for diagnosis of HA compared with HWA. The area under the curve (AUC) values were β -CTx + t-P1NP: 0.686; t-P1NP: 0.681; and β -CTx: 0.648.