The Journal of Rheumatology 2020;47:12
doi:10.3899/jrheum.191098
First Release October 1 2020

The Journal of Rheumatology

Letter

Urinary N-telopeptide as a Biomarker of Disease Activity in Patients with Chronic Nonbacterial Osteomyelitis Who Have Not Received Bisphosphonates

To the Editor:

Chronic nonbacterial osteomyelitis (CNO) is a rare autoinflammatory disease that causes bone destruction, soft tissue swelling, and bone pain[1]. Diagnosis and treatment are hindered by a lack of a reliable laboratory test to assess disease activity. Bone inflammation in CNO is associated with increased osteoclastic activity and bone resorption, causing focal accelerated breakdown of bone collagen. This may be measured through elevated urinary N-terminal telopeptide (NTx). Miettunen, *et al* reported rapid decline of urinary NTx in children with CNO after pamidronate treatment and subsequent rise of NTx was correlated with disease flare[2]. The initial NTx from patients with CNO was not different from that of healthy children. Correlation of NTx with disease activity in patients with CNO not treated with pamidronate has not been investigated. We sought to determine if NTx values correlate with CNO disease activity in children treated without a bisphosphonate.

Children with a CNO diagnosis made by a pediatric rheumatologist as well as their nonadult healthy siblings were recruited from the rheumatology clinics after written informed consent was obtained (approved by Seattle Children's Hospital, IRB 14426, and University of Iowa, IRB 200308051). Inclusion criteria consisted of a clinical diagnosis of CNO, and age under 18 years. Patients treated with bisphosphonates prior to or during the study were excluded. Due to the difficulty of collecting second morning void urine consistently, only random urine samples were collected.

Our study included 3 cohorts. In the first, 11 children with active CNO were paired with age- and sex-matched healthy controls. Our second cohort consisted of a group of 6 children with active CNO who were tracked until their disease became inactive, as confirmed by magnetic resonance imaging (MRI). NTx values were measured in active versus inactive disease. Our final cohort was made up of 2 patient groups, followed over time while disease remained either active (n = 9) or inactive (n = 6). Disease activity in all cohorts was determined based on clinical symptoms and MRI findings[1]. NTx was analyzed using the Osteomark NTx Urine assay[3]. Assay values were normalized to urine creatinine. A paired *t* test or Wilcoxon test was performed based on the data distribution.

Demographic and clinical characteristics were shown in Table 1. There was no significant difference in NTx between CNO and healthy groups (P = 0.09), seen in Figure 1A. Normal values for 9-year-old children in the literature were comparable to values in the healthy group (range 185–1241 for females and 167–1275 for males)[4,5]. Patients with active disease had a median NTx value of 335 (range 204–460), compared to 306 (range 231–383) nM bone collagen equivalents (BCE)/mM creatinine at follow-up visit (inactive disease), shown in Figure 1B (P = 0.64). In patients with stable disease status, the median NTx value at 2 different timepoints was not significant (persistent active disease, P = 0.50; persistent inactive

Table 1. Demographic and clinical characteristics.

	Cohort 1		Cohort 2A		Cohort 2B			
	CNO Group, n = 11	Healthy Group, n = 11	Active Disease, n = 6	Inactive Disease, n = 6	Persistent Active Disease, Timepoint 1, n = 9	Persistent Active Disease, Timepoint 2, n = 9	Persistent Inactive Disease, Timepoint 1, n = 6	Persistent Inactive Disease, Timepoint 2, n = 6
Age, yrs at sample collection, median (range)	9.7 (5.7–11.5)	9.8 (4.7–11.3)	9.2 (6.0–12.8)	9.9 (6.9–13.6)	8.5 (5.7–15.2)	8.9 (6.5–14.6)	13.3 (4.2–14.8)	13.2 (4.6–15.7)
Female, n (%)	6 (55)	6 (55)	3 (50)	3 (50)	2 (22)	2 (22)	1 (17)	1 (17)
Median no. active bone lesions	2	NA	3	NA	2	2.5	NA	NA
Lesion distribution, n (%)								
Lower extremities	8 (73)	NA	4 (67)	NA	8 (89)	9 (100)	NA	NA
Head and/or face	1 (9)	NA	0 (0)	NA	0 (0)	0 (0)	NA	NA
Clavicles	2 (18)	NA	0 (0)	NA	0 (0)	0 (0)	NA	NA
Pelvis	2 (18)	NA	4 (67)	NA	2 (22)	0 (0)	NA	NA
Spine	0 (0)	NA	0 (0)	NA	0 (0)	0 (0)	NA	NA
Average interval between visits, mos	NA	NA	NA	7.7	NA	7.2	NA	8.0
Treatment, n (%)								
NSAID	6 (55)	NA	5 (83)	4 (67)	8 (89)	8 (89)	2 (33)	2 (33)
DMARD	5 (45)	NA	4 (67)	4 (67)	4 (44)	6 (67)	1 (17)	2 (33)
Glucocorticoids	2 (18)	NA	1 (17)	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)
Anti-TNF	1 (9)	NA	1 (17)	2 (33)	1 (11)	4 (44)	4 (67)	4 (67)
NTX values, nM BCE/mM Cr, median (range)	353 (204–839)	388 (184–685)	335 (204–460)	306 (231–383)	303 (139–411)	323 (183–839)	370 (159–1819)	248 (148–362)

BCE/mM Cr: bone collagen equivalents/mM creatinine; CNO: chronic nonbacterial osteomyelitis; NSAID: nonsteroidal antiinflammatory drug; DMARD: disease-modifying antirheumatic drug; TNF: tumor necrosis factor; NA: not applicable; NTx: N-terminal telopeptide.

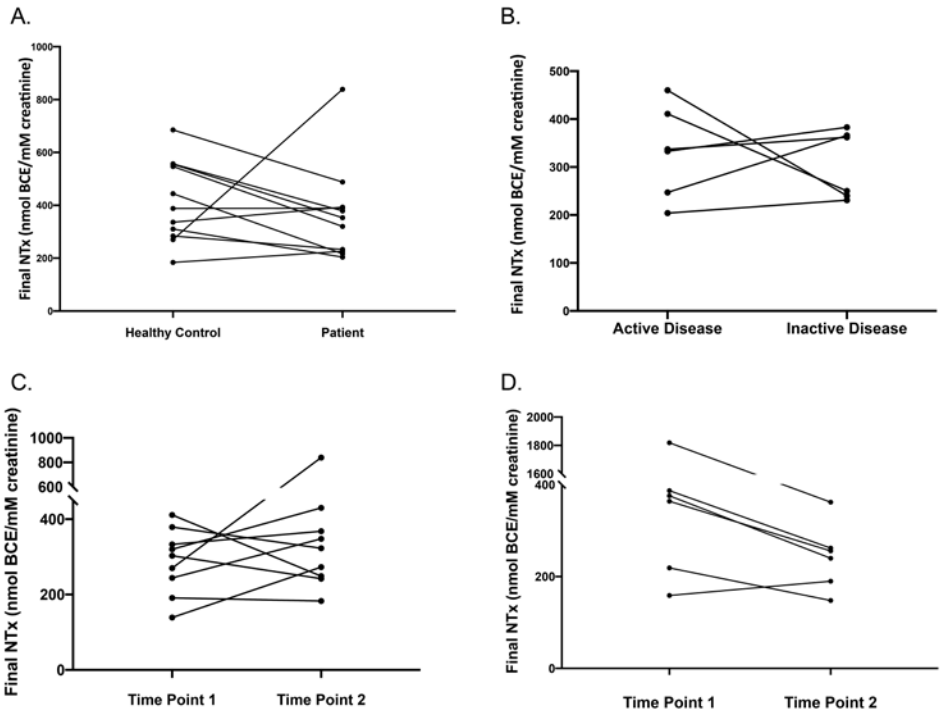

Figure 1. (A) Urinary NTx values of healthy controls vs patients with CNO, in pairs matched based on sex and age. (B) Urinary NTx values in longitudinal samples collected from patient in active vs inactive disease. (C). Urinary NTx values in patients with stable persistent active disease status. (D) Urinary NTx in patients with stable persistent inactive disease. BCE: bone collagen equivalents; CNO: chronic nonbacterial osteomyelitis; NTx: N-terminal telopeptide.

disease, $P = 0.06$; Figure 1C–D). Six patients had a medication change, with a median NTx change of 34% (range –80 to 96%).

To our knowledge, this is the first pilot study to investigate the value of NTx as a biomarker to monitor disease activity in patients with CNO not on bisphosphonate therapy. We did not find a statistically significant difference in NTx values between children with active CNO lesions and healthy children, nor did we find a significant association between NTx values and disease activity. Variation within same individuals during persistent inactive disease was almost significant. Our results are consistent with previous reports that at initial diagnosis, the NTx values of patients with CNO was not significantly higher than that of healthy controls[2]. Thus, it is not suitable as a screening tool for CNO diagnosis based on available data.

Patients in this study were on medications including, but not limited to, nonsteroidal antiinflammatory drugs, disease-modifying antirheumatic drugs, glucocorticosteroids, and anti–tumor necrosis factor biologics. It is likely that the increased collagen breakdown from affected CNO sites is not significant enough to raise urinary NTx compared to the overall turnover rate within bones in children. NTx values are much higher in young children and normal range is very broad[4,5]. Thus, the difference between healthy children and those with CNO is trivial as a small portion of intersubject variation.

There is a known diurnal variation of NTx, and a previous study established methods for NTx assayed in second morning void samples, because the diurnal variation was 37% as compared to the 23% day-to-day variation of NTx[6]. Unfortunately, only random urine samples were collected in our study. Within the CNO group, NTx was not correlated with disease activity. Thus, current data do not support urinary NTx as a sensitive marker for CNO, but it may still have value in tracking disease flares after bisphosphonate treatment. Other biomarkers using proteomic or metabolomic techniques, such as differentially expressed protein analysis or the analysis of urine metabolic profiles, may prove useful[7,8].

Alexandra Perkins[1], MD, MPH
Anne M. Stevens[2], MD, PhD
Polly J. Ferguson[3], MD
Yongdong Zhao[4], MD, PhD
[1]University of Washington School of Medicine, Seattle, Washington;
[2]Seattle Children's Hospital, Seattle, Washington, and Janssen Research & Development, LLC, Spring House, Pennsylvania;
[3]University of Iowa Carver College of Medicine, Iowa City, Iowa;
[4]Seattle Children's Hospital and Clinical and Translational Research Center, Seattle, Washington, USA.

This study is supported by The Childhood Arthritis and Rheumatology Research Alliance (CARRA), the Arthritis Foundation Small Grants program, and the Chronic Recurrent Multifocal Osteomyelitis (CRMO) Research Fund at the University of Iowa. PJF is supported by R01 AR059703 and by the Marjorie K. Lamb Professorship. YZ received research support from CARRA and Bristol-Myers Squibb.

Address correspondence to Dr. Y. Zhao[4], 4800 Sand Point Way NE, Seattle, WA 98105, USA. Email: yongdong.zhao@seattlechildrens.org.

ACKNOWLEDGMENT

The authors would like to thank all the families who generously participated in this study, those who donated to the University of Iowa CRMO Research Fund, and assistance from Lucas Reichley and Ching Hung.

REFERENCES

1. Zhao Y, Ferguson P. Chronic nonbacterial osteomyelitis and chronic recurrent multifocal osteomyelitis in children. Pediatr Clin North Am 2018;65:783–800.
2. Miettunen P, Wei X, Kaura D, Reslan W, Aguirre A, Kellner J. Dramatic pain relief and resolution of bone inflammation following pamidronate in 9 pediatric patients with persistent Chronic Recurrent Multifocal Osteomyelitis (CRMO). Pediatr Rheumatol Online J 2009;7:2.
3. Hanson DA, Weis MA, Bollen AM, Maslan SL, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-Telopeptides in urine. J Bone Miner Res 1992;7:1251-8.
4. Bollen AM, Eyre DR. Bone rates in children monitored by the urinary assay of collagen type I cross-linked peptides. Bone 1994;15:31-4.
5. Sato J, Hasegawa K, Tanaka H, Morishima T. Urinary N-telopeptides of type I collagen in healthy children. Pediatri Int 2010;52:398-401.
6. Ju HS, Leung S, Brown B, Stringer MA, Leigh S, Scherrer C, et al. Comparison of analytical performance and biological variability of three bone resorption assays. Clin Chem 1997;43:1570-6.
7. Hu HM, Du HW, Cui JW, Feng DQ, Du ZD. New biomarkers of Kawasaki disease identified by urine proteomic analysis. FEBS Open Bio 2019;9:265-75.
8. Thomaidou A, Chatziioannou AC, Deda O, Benaki D, Gika H, Mikros E, et al. A pilot case-control study of urine metabolomics in preterm neonates with necrotizing enterocolitis. J Chromatogr B Analyt Technol Biomed Life Sci 2019;1117:10-21.