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Diabetic Foot Osteomyelitis: Bone Markers and Treatment Outcomes

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Abstract

Aims—Novel bone turnover markers could help with the diagnosis and monitoring of osteomyelitis patients. We compared levels of two bone turnover markers, serum amino-terminal telopeptides (NTx) and bone alkaline phosphatase (BAP), in diabetic patients with and without osteomyelitis.

Methods—Matched case-control study was conducted with diabetic patients with and without osteomyelitis. Cases not undergoing immediate amputation were followed with repeat measurements after osteomyelitis treatment and for outcome determination.

Results—Analysis included 54 subjects, 27 cases and 27 controls. Median BAP levels were similar between cases and controls at enrollment ($p=.55$) as were median NTx levels ($p=.43$). Cases with follow-up data ($n = 18$) had similar bone marker levels at enrollment and 6 weeks. No significant differences in BAP or NTx levels at enrollment or follow-up were seen between cases with poor versus favorable outcomes.

Conclusions—No differences in NTx or BAP levels were seen between cases and controls. Cases with follow-up data had similar levels at enrollment and 6 weeks. Lack of difference may be due to small sample size, small areas of bone involved in foot osteomyelitis, or limitations of these specific markers. More research is needed.

Keywords

Osteomyelitis; diabetic foot ulcers; bone markers; treatment outcomes

INTRODUCTION

Foot infections and osteomyelitis are frequent causes of hospitalization in diabetic patients, as more than 15% of the 16 million diabetic patients in the US develop foot ulcers in their

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Conflict of Interest The authors declare that they have no conflict of interest.

lifetime [1]. Diabetic foot infections, including osteomyelitis, are the leading cause of hospitalization for diabetic patients and are the most common precipitant of lower extremity amputation. [2,3] Thus, a major goal of Healthy People 2020 is to reduce lower extremity amputations in diabetics [4]. In general, osteomyelitis treatment requires four to six weeks of antibiotic therapy and usually surgical debridement. Patients with impaired bone health may be at higher risk for bone infection and may have worse outcomes, due to impairment of both mechanical bone remodeling [5] and osteoblast mediated innate immunity in bone [6–7], but this has not been studied.

Ideal bone remodeling is an ongoing cyclical process, characterized by the coupling of osteoblastic bone formation with osteoclastic bone resorption and interaction between these two events. Biochemical markers of bone metabolism are being increasingly used in clinical research and practice to identify and monitor high bone turnover states [8–10]. Different bone markers are associated with resorption and formation [8, 11–12]. Osteoclast activity releases amino-terminal telopeptides of type I collagen, NTx, a marker of bone resorption. Studies in multiple populations have reported elevated NTx levels in patients with osteoporosis [10], metastatic bone disease [13], osteoarthritis [14], Paget's disease [15], and renal osteodystrophy [9]. Furthermore, NTx levels decrease in osteoporosis patients on bisphosphonate therapy and are used to monitor treatment response [8]. Markers of bone formation, such as bone alkaline phosphatase (BAP), are released during osteoblast activity. Several studies have reported elevated BAP levels in patients with metastatic bone disease [13, 16–17]. These markers have been used to define high matched turnover states and to define mismatched turnover, with increased resorption compared to formation, in various disease states [8,11–12].

Osteomyelitis could cause increased bone turnover as bone is destroyed by infection and then rebuilt during healing. There are limited data about these markers in osteomyelitis. Philopov *et al* and Southwood *et al* found increased turnover markers in experimentally induced osteomyelitis in animals [18–19]. Springer *et al* found elevated urine bone resorption marker levels of lysylpyridnoline and hydroxyllysylpyridinoline in human patients with acute mandibular osteomyelitis [20]. Levels decreased in patients with a good response to treatment but remained elevated in patients with ongoing disease. Persistently elevated turnover markers despite antibiotic treatment may identify patients at risk for poor outcomes, even without net bone loss.

Validated biological markers to monitor treatment response and predict treatment outcomes are extremely limited for diabetic foot infections. Currently, patients with poor clinical outcomes are identified by signs or symptoms of persistent or relapsing infection [2,3]. Earlier identification could prompt prolonging antibiotic treatment, more aggressive surgical debridement and possibly the use of adjunctive therapies to limit osteoblast dysfunction and improve bone density. Changes in treatment based on knowledge of bone biomarkers might decrease rates of disease progression and amputation among diabetic patients and ultimately even be used to prevent the development of osteomyelitis in high risk patients. We investigated the association between abnormal bone metabolism and osteomyelitis by comparing novel biomarkers of bone turnover in diabetic patients with and without osteomyelitis and assessed the relationship between bone marker levels and clinical outcomes in diabetic patients with osteomyelitis.

SUBJECTS, MATERIALS AND METHODS

A matched case-control study with a nested cohort study of cases was conducted with adult diabetic inpatients and outpatients who presented to Barnes-Jewish Hospital from July 29, 2009 to September 30, 2010. Barnes-Jewish Hospital is a 1250-bed tertiary care hospital in

St. Louis, MO. Potential subjects were identified by two methods. Admitting diagnoses of all patients admitted to medicine, orthopedic and vascular surgery services were screened daily for any diagnoses suggesting diabetes, wound infections, or foot ulcers. Physicians on those services were also asked to refer potential subjects to us. Medical records of potential subjects were reviewed for evidence of osteomyelitis as diagnosed through routine medical care. Although a combination of histologic and microbiologic findings are usually considered the gold standard for the diagnosis of osteomyelitis, these procedures may not be routinely performed. Therefore, clinical, laboratory and imaging findings are often used as surrogates for diagnosing osteomyelitis.[2] For this study, osteomyelitis cases were defined by the presence of either visible/probe-able bone underlying an ulcer of any age, consistent pathological findings on biopsy, radiological evidence of osteomyelitis, or intraoperative findings of osteomyelitis. Controls were diabetic patients without evidence of a current foot ulcer or osteomyelitis, and were matched 1:1 to cases on gender, race, age (± 5 years), season, dialysis status, and inpatient/outpatient status. Patients were excluded if they were not diabetic, had osteomyelitis at other sites, had osteomyelitis due to recent trauma, were on medications that altered bone metabolism, or if they had hardware in the involved foot. Written informed consent was obtained and a fasting blood draw was collected for serum levels of BAP and NTx.

Data collection and analysis

A brief questionnaire was administered and the patient's medical records were reviewed to determine relevant demographics, diabetes related factors, and comorbidities. Infection related factors such as microbiology results were also collected. All cases not undergoing immediate amputation were followed with repeat lab measurements after six weeks of osteomyelitis treatment and with follow-up phone calls for six months to determine if patients had recent surgery and other problems (n=18). From the original cohort, nine cases had no follow-up data due to either amputation at the site of osteomyelitis (n = 7), refusal to complete the six week interview (n=1), or death (n=1) (Figure 1). All patients had type 2 diabetes. Poor outcome was defined as undergoing further surgery on the affected limb or readmission for osteomyelitis in the same limb within the first 6 months of follow-up. All other patients were classified as having a favorable outcome. This study was approved by the Washington University Human Research Protection Office.

Conditional logistic regression was performed to compare bone marker levels between cases and controls. Change in BAP and NTx were compared with a paired t-test. Comparisons between cases with poor and favorable outcomes and among cases at enrollment versus follow-up were done with simple t-tests. A *p*-value of .05 was considered significant. Statistical analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC).

Laboratory methods

Serum BAP was measured by enzyme-linked immunosorbent assay (ELISA, MicroVue BAP EIA Kit, Quidel Corporation, San Diego, CA). The results were reported as IU/L. Per manufacturer, the intra- and inter-assay coefficients of variation were 3.9% and 7.6% at 35 IU/L. Serum NTx was measured by competitive-inhibition enzyme-linked immunosorbent assay (ELISA, Osteomark NTx Serum, Wampole Laboratories, Princeton, NJ). The results were reported as nanomole bone collagen equivalents (nM BCE). The intra- and interassay coefficients of variation were 4.6% and 6.9 %, respectively.

RESULTS

Sixty-seven adults were approached for enrollment. Fifty-four patients (80%), twenty-seven cases and twenty-seven controls, consented to administration of a questionnaire and fasting

blood draw the following morning. Thirty subjects were white (56%) and thirty-eight were male (70%), with a median age of fifty-seven years. Differences between the twenty-seven case-control pairs are displayed in Table 1.

Overall, twenty of twenty-seven cases had any cultures done (bone, wound, or blood) and a wide variety of organisms were identified, predominantly gram-positive organisms. Four of twenty-seven cases had only blood cultures done, while seven of twenty-seven cases had no cultures done at all. Sixteen of twenty-seven cases had either bone or wound cultures done. Eight of twenty-seven cases had bone cultures done; of all bone cultures performed (n=8), four were polymicrobial and two had no growth.

No significant differences in bone marker levels were seen between cases and controls. On univariate analysis, BAP levels were similar between cases and controls at enrollment ($p=.55$) as were NTx levels ($p=.43$) (Figure 2, Table 2). Cases with follow-up data (n= 18) had slightly increased BAP levels at follow-up compared to enrollment ($p=.07$) (Table 3). NTx levels were slightly increased at follow-up compared to enrollment, which was statistically significant ($p=.0065$) (Figure 2, Table 2). Multivariate analysis showed no differences in BAP or NTx levels. Similar results were obtained after excluding dialysis patients.

No significant differences in BAP or NTx levels at enrollment or follow-up were seen between cases with poor versus favorable outcomes. Among cases who had favorable outcomes (n=5), all bone marker levels were slightly elevated at follow-up versus enrollment, although this was not statistically significant (Figure 3). Median BAP levels were 29 IU/L at follow-up versus 23 IU/L at enrollment ($p=.23$). Median NTx levels were 36 nM BCE at follow-up versus 20 nM BCE at enrollment ($p=.14$). Similar results were found among cases with poor outcomes (n=13), with smaller differences between bone marker levels at follow-up versus enrollment (Figure 3). Median BAP levels were 25 IU/L at follow-up versus 23 IU/L at enrollment ($p=.06$). Median NTx levels were 24 nM BCE at follow-up versus 14 nM BCE at enrollment ($p=.03$).

Markedly elevated bone marker levels were seen in six subjects, three cases and three controls. Three of these patients (1 case, 2 controls) were on dialysis and one control had peripheral vascular disease. One case had chronic kidney disease. The remaining two patients had none of these comorbidities.

DISCUSSION

This is the first study to evaluate bone turnover markers in diabetic patients with osteomyelitis. We hypothesized osteomyelitis would result in increased bone turnover that would be detected by changes in BAP and NTx. However, no differences were seen in BAP or NTx levels between cases and controls at enrollment. Among cases, serum NTx levels increased slightly at follow-up; this change was not seen with BAP.

When comparing bone, wound, and blood culture results, a small number of patients had bone cultures done, with a wide variety of identified organisms. Bone and wound culture results were not well correlated. In addition, more wound cultures were performed than bone cultures, an indication of treatment practices at our institution. Even though bone biopsies are the gold standard for osteomyelitis confirmation, they may not be routinely performed in clinical practice due to cost, invasiveness, and concern for possible adverse events [2, 21]. In our study, all bone cultures were obtained during surgery.

This study found no differences in BAP or NTx levels between cases and controls at enrollment. The overall lack of differences may be due to a small sample size; great variability in marker levels among controls, limiting our ability to detect subtle differences;

small areas of bone involved in osteomyelitis of the foot; or limitations of these specific markers. Overall, cases with follow-up data had similar levels at enrollment and six weeks, though serum NTx follow-up levels were slightly elevated. Despite smaller differences between follow-up and enrollment levels among cases with poor outcomes versus favorable outcomes, statistical significance was achieved due to larger number of cases. Increased osteoclast activity due to diabetes impairs lower extremity healing in preclinical models [22]. Thus, the elevated NTx levels seen among osteomyelitis cases with poor outcomes may similarly reflect diabetes-dependent alterations in osteoclastic function that impair skeletal wound healing. Longer follow-up may be needed to detect bone marker differences, with testing for resorption markers first, since they respond more quickly compared to formation markers [23]. Alternatively, diabetic patients both with and without osteomyelitis may have bigger baseline differences in bone metabolism compared to non-diabetic patients, which we could not detect as both cases and controls had diabetes mellitus. In addition, osteomyelitis is a challenging diagnosis to make in diabetic foot infection patients [2,3] and the use of a composite diagnostic strategy in this study, while clinically relevant, may allow inclusion of more variable clinical syndromes.

There are limitations to the use of bone markers for treatment purposes. Even though bone biochemistry research has been ongoing for many years, the clinical measurement of bone biomarkers is still relatively new [23]. Methods to detect and test for these markers are still being developed, with limited options available to clinicians. Furthermore, bone markers are subject to variability, such as inter-individual variability, day-to-day variability, diurnal variability, and analytic variability [23]. Therefore, bone biomarker results need to be interpreted with caution.

It is also possible that bone biomarkers may be less useful for certain patient populations. Diabetes mellitus can affect bone metabolism [24]. In addition, prolonged hemodialysis has been shown to alter bone metabolism [24]. Furthermore, most diabetic patients have poor glucose control, which can also affect bone metabolism. Lower bone turnover rates in diabetic patients are seen with good control of hyperglycemia, even after initiation of hemodialysis [24].

Despite these limitations, the potential utility of bone biomarkers is promising. Bone biomarkers are increasingly being used to manage patients across a wide range of medical specialties [25]. Pathological changes in bone are slow to present symptomatically or become evident on imaging. Bone biomarker changes may reflect these underlying changes well in advance and allow clinicians to respond quickly and effectively, allowing bone biomarkers to be used in conjunction with imaging methods. Further study of other bone turnover markers in different populations with larger sample size would be helpful.

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Figure 1.
Flow of participants through study.

A. Comparison of bone marker levels at enrollment between cases and controls.

B. Comparison of bone marker levels at enrollment and follow up among cases.

Figure 2.
BAP and NTx levels for cases and controls.

A. Comparison of bone marker levels at enrollment for cases with poor versus favorable outcomes.

B. Comparison of bone marker levels at follow up for cases with poor versus favorable outcomes.

Figure 3.
BAP and NTx levels among cases (n=18) with poor versus favorable outcomes.

Table 1

Study demographics (n=54).

Male *	19 (70%)	19 (70%)
Race *		
African-American	12 (44%)	12 (44%)
Caucasian	15 (56%)	15 (56%)
Median age *	57	58
Median household income		
\$ 35,000	15 (56%)	9 (33%)
\$ 35,000	12 (44%)	18 (67%)
Complications		
Neuropathy	26 (96%)	15 (55%)
Retinopathy	2 (7%)	1 (4%)
Peripheral vascular disease	7 (26%)	5 (18%)
Coronary artery disease	8 (30%)	8 (30%)
End stage renal disease *	2 (7%)	2 (7%)
Current smoker	5 (18%)	7 (26%)

* matching characteristics

Table 2

Comparison of bone marker results among cases and controls (n=54).

BAP (enrollment)	23, 24 ± 5	22, 26 ± 16	0.985 (p=.55, 0.937 – 1.036)
BAP (6 week follow-up)	25, 28 ± 13	-----	-----
NTx (enrollment)	16, 21 ± 19	14, 27 ± 47	0.990 (p=.43, 0.965 – 1.015)
NTx (6 week follow -up)	27, 35 ± 36	-----	-----