Joint Diseases and Related Surgery

ORIGINAL ARTICLE

Plasma *RANKL* level is not a reliable marker to monitor the bone destruction in mice model of osteomyelitis

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Bone destruction is the hallmark pathological feature of osteomyelitis.[1] Despite advanced antimicrobial treatments, osteomyelitis often leads to severe morbidity, mortality and healthcare expenditure as a consequence of difficult to treat, long-standing infections and frequent relapses. Early initiation of antibiotics has been shown to limit the progression of infection and lower the amount of bone destruction.[1,2] However, debridement of necrotic and/or infected tissue is almost always required in subjects with chronic osteomyelitis.

The diagnosis of osteomyelitis is generally based on clinical signs, imaging studies and also blood tests measuring markers of inflammation, including erythrocyte sedimentation rate (ESR), leukocyte count, C-reactive protein (CRP), and procalcitonin levels.[3] However, these blood tests are neither sensitive nor specific for osteomyelitis, since they can be elevated in any kind of infectious or non-infectious activation of inflammation. Moreover, these blood tests reflect

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ABSTRACT

Objectives: In this experimental study, we aimed to investigate the specific value of receptor activator of nuclear factor kappa-B ligand (RANKL) plasma level in osteomyelitis to show the bone destruction and to determine its correlation with classical markers of infection in mice model of osteomyelitis.

Materials and methods: Sixty Balb/c female mice (30 to 40 g weight, 3.5 to 4 month-old) were divided into two groups: Controls (n=15) and study group (n=45). All mice underwent tibial decortication and received an injection of sclerosing agent into the intramedullary cavity. The next process was proceeded in two steps to observe the detectability of osteomyelitis-induced bone destruction (step 1) and treatment response (step 2) using the variables examined in our study. In step 1, the study group received 1 mL solution containing *Staphylococcus aureus* (*S. aureus*) bacteria (2×108 per mL) into the intramedullary cavity. Five mice from each group were sacrificed every seven days for three weeks and tibia and blood samples were obtained. In step 2, the remaining 30 infected mice were further divided into two groups to investigate the possible value of RANKL plasma level as a marker of treatment response. Fifteen of these mice received teicoplanin 20 mg/kg for four weeks, while the rest did not receive antibiotics. Eight mice from each group were sacrificed at the end of the second week and the remaining 14 mice were sacrificed at the end of four weeks. Complete blood count, procalcitonin level, C-reactive protein (CRP), and RANKL concentrations were measured from blood samples of each sacrificed mouse.

Results: Median RANKL concentration of the control subjects was significantly higher than recipients of intervention at the first and third weeks in step 1 where bone destruction of osteomyelitis was examined. No significant changes occurred in groups receiving and not receiving antimicrobial treatment in terms of RANKL, CRP, and procalcitonin levels throughout four weeks in step 2. The RANKL concentration was significantly correlated with colony growth in subjects allocated to the *S. aureus* inoculation group (r=-0.547, p=0.035).

Conclusion: The RANKL levels in mice with *S. aureus* osteomyelitis are not correlated with colony growth or other markers of inflammation and not useful for monitoring the response to antimicrobial treatment during osteomyelitis.

Keywords: Bone destruction, c-reactive protein, osteomyelitis, procalcitonin, *RANKL*.

the severity of the infectious process itself and not the severity of bone destruction due to osteomyelitis. Although the presence of osteomyelitis in clinical practice is demonstrated by various biomarkers and imaging methods, these parameters have various disadvantages such as non-specificity, radiation exposure, long duration of procedures, and limited accessibility.[4,5] Therefore, it would be valuable in clinical practice to demonstrate that a bone-specific parameter can be used in osteomyelitis cases. One of the bone specific markers is the receptor activator of nuclear factor kappa-B ligand (RANKL), which is an indicator of bone resorption. The RANKL is a type II membrane protein that is encoded by the TNFSF11 (Tumor necrosis factor ligand superfamily member 11) gene. It is an important member of the tumor necrosis factor (TNF) superfamily of cytokines, and therefore, has significant influence on immune regulation; however, RANKL is also known as the osteoclast differentiation factor (ODF), underlining its significant role in osteoclast function and regulation of bone tissue.[6]

Given its critical role in osteoclast differentiation and activation, we hypothesized that circulating levels of RANKL may be associated with the degree of bone destruction in osteomyelitis. Based on this hypothesis, the aim of this study was to investigate the relationship between RANKL plasma levels, bone destruction and classical markers of inflammation in mice with osteomyelitis. For this purpose, we investigated the usability of RANKL as a bone-specific marker in addition to the general infection parameters in bone infections and, thus, the detectability of the extent of the damage caused by the infection to the bone. In addition, we aimed to discuss the use of this marker, which we believe is specific for bone damage, in the monitoring of treatment, and in the regulation of antibiotic treatment regimens.

MATERIALS AND METHODS

This experimental study was performed at Mersin University, Faculty of Medicine, Center of
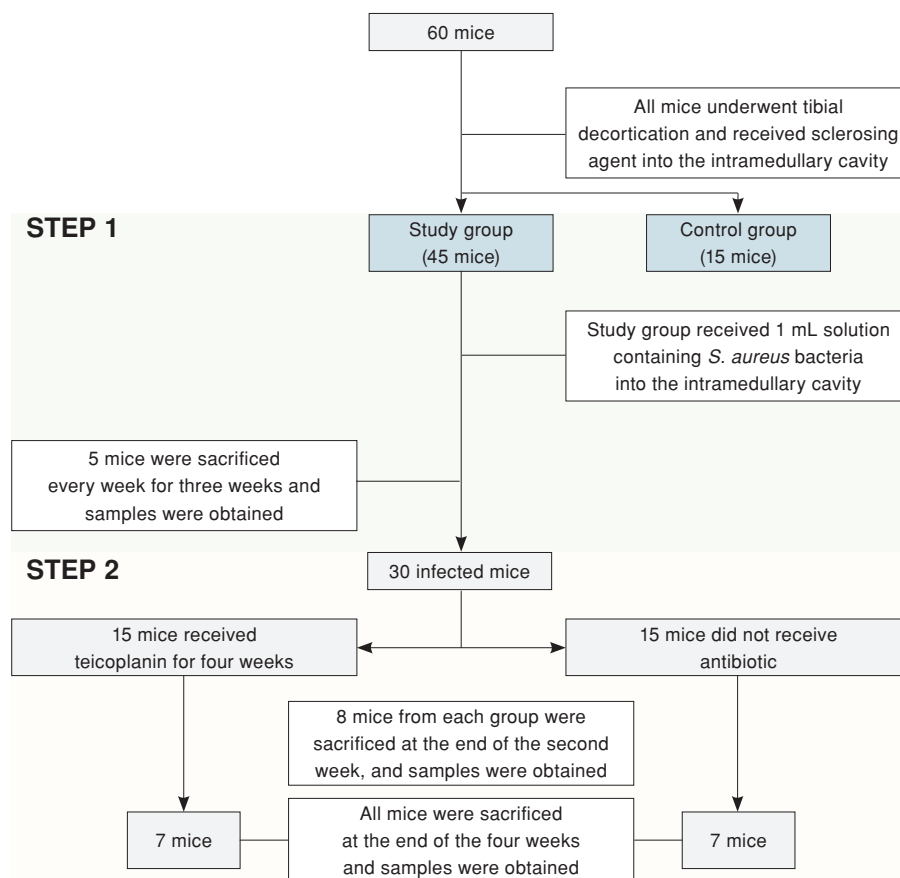

FIGURE 1. Study flow chart.

Experimental Medicine. The study protocol was approved by the Mersin University Animal Ethics Committee (Date: 26.02.2015, No: 2015/07). Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) were followed. Sixty Balb/c female mice that weighed 30 to 40 g and were between 3.5 to 4 months of age were included in the study. Animals were housed in 12-h/12-h light/dark (06.00 to 18.00) cycle in rooms that were automatically kept at a temperature of 22±1°C. Animals were maintained on *ad libitum* rodent chow and tap water.

Experimental osteomyelitis

The gold-standard method for diagnosis of osteomyelitis is to demonstrate microbial growth in culture. Growth in culture was accepted as osteomyelitis, and *RANKL* values were measured after growth was observed. The correlation relationship of the next process with other infection markers was examined. In addition, damage to the bone was confirmed macroscopically both in sacrifice and sampling of the animal. The diagnosis of tibial osteomyelitis was considered as acute osteomyelitis. In our study, the osteomyelitis model used in the study of Kandemir et al.[7] was used.

Study groups

A total of 60 mice were initially divided into two groups: controls (n=15) and study group (n=45). Following adequate anesthesia with intraperitoneal ketamine (90 mg/kg), all mice underwent tibial decortication and received an injection of 50 μL sclerosing agent (Lauromacrogol 200, Aethoxysklerol®, Assos Pharma, Turkey) into the intramedullary cavity. The next process was proceeded in two steps to observe the detectability of osteomyelitis-induced bone destruction (step 1) and treatment response (step 2) using the variables examined in our study. The flow chart of the study is shown in Figure 1.

Step 1: All mice received an injection of 50 μL sclerosing agent (Lauromacrogol 200, Aethoxysklerol®, Assos Pharma, Turkey) into the intramedullary cavity. After 5 min, 45 mice allocated to the study group received 1 mL solution containing *Staphylococcus aureus (S. aureus)* bacteria (2×108 per mL) into the intramedullary cavity. When macroscopic findings and microbial growth were observed, it was accepted as an infection. After completion of these procedures, five of these mice

TABLE I									
Summary of measurements with regard to groups and weeks									
	Groups								
	Control				Infected				Between groups
	n	Mean±SD	Median	Min-Max	n	Mean±SD	Median	Min-Max	*p*
RANKL									
Total	15	8.8±1.7	8.25	6.9-13.5	15	7.1±0.6	7.21	5.99-8.39	<0.001
1st week	5	9.3±2.5	8.01	7.74-13.5	5	7.2±0.4	7.27	6.75-7.69	0.009
2nd week	5	8.8±1.5	8.44	6.9-10.71	5	7.4±0.9	7.57	5.99-8.39	0.117
3rd week	5	8.5±1.1	8.25	7.49-10.35	5	6.8±0.3	6.68	6.5-7.3	0.009
p (between weeks)	0.914				0.210				
C-reactive protein (pg/mL)									
Total	15	8.2±1.6	8.2	5.6-11.8	15	7.9±1.8	7.2	5.6-12	0.560
1st week	5	8.8±2.0	8.8	6.2-11.8	5	9.1±2.1	9.2	6.4-12	0.599
2nd week	5	8.3±1.4	8	6.8-10.6	5	7.8±1.6	7.2	7-10.6	0.399
3rd week	5	7.4±1.4	7	5.6-8.8	5	6.6±0.7	6.8	5.6-7.4	0.461
p (between weeks)	0.420				0.087				
Procalcitonin (pg/mL)									
Total	15	48.8±36.0	37.4	17.95-155.95	15	115.4±122.3	47.65	30.4-445	0.044
1st week	5	36.4±15.6	35.95	23.35-61.75	5	45.4±14.7	42.5	30.85-69.15	0.251
2nd week	5	31.2±11.2	30.8	17.95-45.65	5	177.3±155.0	124.3	45.5-445	0.016
3rd week	5	78.8±49.5	69.6	20.2-155.95	5	123.6±130.9	43.85	30.4-331.45	0.917
p (between weeks)	0.134				0.151				
SD: Standard deviation; RANKL: Receptor activator of nuclear factor kappa-B ligand.									

(n=45) were sacrificed every seven days for three weeks and tibia and blood samples were obtained (n=15).

Step 2: After three weeks, the remaining 30 infected mice were further divided into two groups to investigate the possible value of *RANKL* plasma level as a marker of treatment response: Fifteen of these mice received teicoplanin 20 mg/kg for four weeks, the remaining 15 mice did not receive antibiotic treatment. After this second step, eight mice from each grou p were sacrificed at the end of the second week and the remaining 14 mice (n=7 each group) were sacrificed at the end of the four weeks. Tibia and blood samples of these mice were also obtained.

Tibia samples of the sacrificed mice were excised and cultivated in blood agar. Following incubation at 37°C, the number of reproduced colonies was recorded (CFU/mL). All blood samples obtained from sacrificed mice were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes and centrifuged at 4,000 rpm for 10 min to obtain plasma; these samples were, then, aliquoted into the Eppendorf® tubes and stored at -20°C until analysis. After sacrification of all mice, plasma samples were thawed at room temperature. Complete blood count (Sysmex XT-2000i, Roche Diagnostics, USA), procalcitonin level (ELISA kit, Uscn Life Science Inc., Wuhan, Hubei, China), CRP (ELISA kit, Dynex Tecnologies, Virginia, USA), and *RANKL* concentration (DSX™ Four-PlateAutomated ELISA Processing System, Uscn Life Science Inc., Wuhan, Hubei, China) were measured using plasma samples.
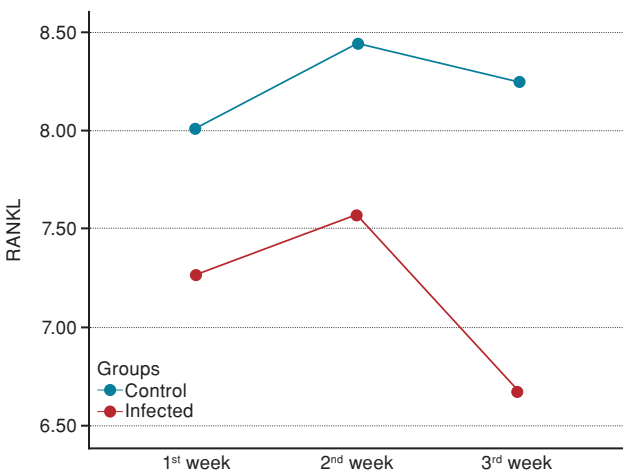
Primary outcome

The primary outcome measure of this study was the relationship between *RANKL* concentration and bone destruction occurring during osteomyelitis. The relationships between *RANKL* concentration and other markers of osteomyelitis were the secondary outcome measure.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). For the normality check, the Shapiro-Wilk test was used. Data were expressed
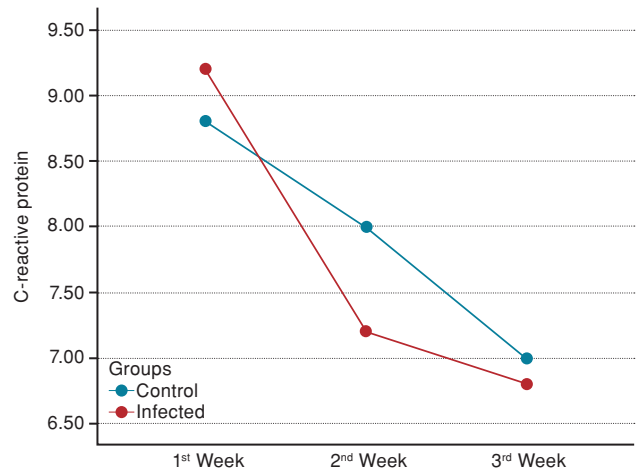
FIGURE 3. The change in the C-reactive protein level form baseline to the third week in controls and in mice that were infected with *S. aureus*.

FIGURE 2. The change in the *RANKL* level form baseline to the third week in controls and in mice that were infected with *S. aureus*.
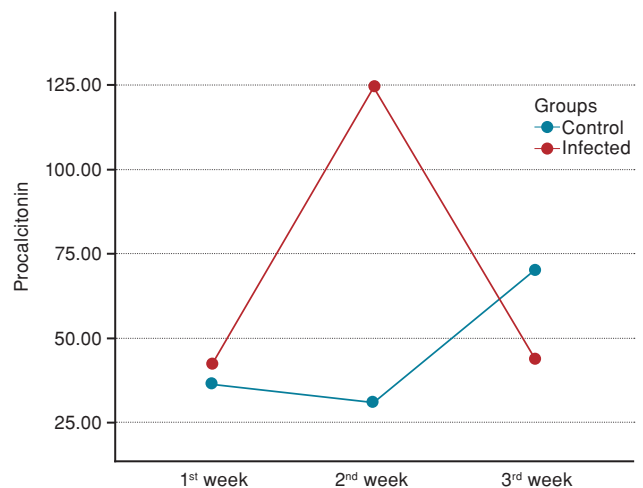*RANKL*: Receptor activator of nuclear factor kappa-B ligand.

FIGURE 4. The change in the procalcitonin level form baseline to the third week in controls and in mice that were infected with *S. aureus*.

TABLE II								
Summary of measurements with regard to presence of treatment and weeks								
	Treatment							Between groups
	Absent				Present			
	n	Mean±SD	Median	Min-Max	n	Mean±SD	Median	Min-Max	*p*
RANKL								
Total	15	6.3±0.5	6.11	5.7-7.38	15	6.7±1.5	6.31	5.71-10.5	0.206
2nd week	8	6.3±0.5	6.21	5.7-7.38	8	6.6±1.4	6.28	5.71-9.91	0.916
4th week	7	6.2±0.6	5.84	5.73-7.18	7	7.2±1.7	6.34	6.13-10.5	0.064
p (between weeks)	0.487				0.298			
C-reactive protein (pg/mL)								
Total	15	5.4±0.6	5.4	4.6-6.8	15	5.5±0.9	5.2	4.6-7.8	0.737
2nd week	8	5.2±0.4	5.2	4.6-5.6	8	5.4±1.0	5.2	4.6-7.8	1.000
4th week	7	5.7±0.7	5.4	5-6.8	7	5.6±0.7	5.2	4.8-6.6	0.519
p (between weeks)	0.098				0.558			
Procalcitonin (pg/mL)								
Total	15	47.0±37.3	29.95	15.05-131.95	15	59.9±50.0	47.55	20.85-223.1	0.152
2nd week	8	54.8±43.1	36.55	22.1-131.95	8	63.0±65.4	42.3	20.85-223.1	0.753
4th week	7	38.1±30.1	28.1	15.05-103.4	7	56.2±28.5	55.75	24.05-108.25	0.110
p (between weeks)	0.417				0.355			

SD: Standard deviation; RANKL: Receptor activator of nuclear factor kappa-B ligand.

in mean ± standard deviation (SD) and median (min-max). Non-normally distributed variables were analyzed using the Mann-Whitney U test or with the Kruskal-Wallis test depending on the number of groups compared. The Spearman correlation coefficients were calculated for the assessment of relationships between variables. A two-tailed *p* value of <0.05 was considered statistically significant.

RESULTS

Results of step 1 (effect of osteomyelitis on bone without treatment)

The *RANKL*, CRP, and procalcitonin levels of the intervention group and control group measured at the end of the first, second, and third weeks after *S. aureus* inoculation are presented in Table I. No significant changes were found in either group in terms of changes in *RANKL*, CRP, and procalcitonin levels during the first three weeks (Figures 2-4). However, median *RANKL* concentration of the controls was significantly higher than the intervention subjects at the first and third weeks. Procalcitonin level of the study group was also significantly higher than that of the control group at the end of the second week [124.3 (45.5-445) *vs.* 30.8 (17.95-45.65), p=0.009].

Results of step 2 (effect of treatment on bone)

The *RANKL*, CRP, and procalcitonin levels of groups with respect to antimicrobial treatments (step 2) are presented in Table II. No significant changes occurred in groups with or without antimicrobial treatment in terms of *RANKL*, CRP, and procalcitonin levels throughout four weeks (Figures 5-7).
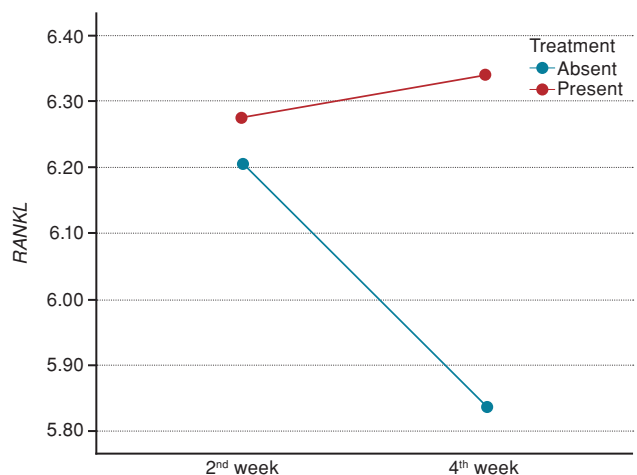

FIGURE 5. The change in *RANKL* level in mice receiving and not receiving antimicrobial treatment.
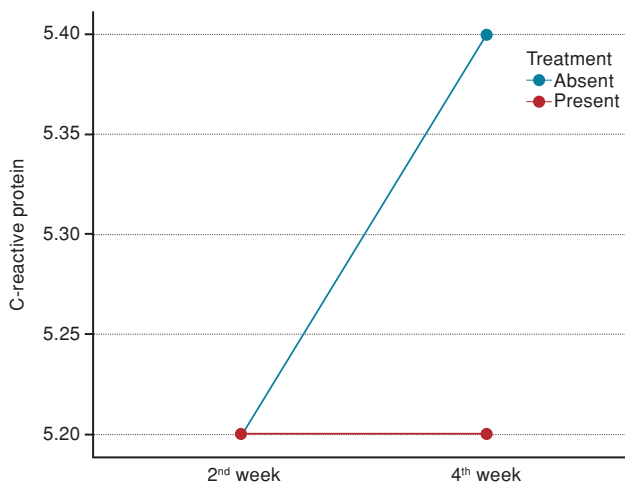*RANKL*: Receptor activator of nuclear factor kappa-B ligand.

FIGURE 6. The change in C-reactive protein level in mice receiving and not receiving antimicrobial treatment.
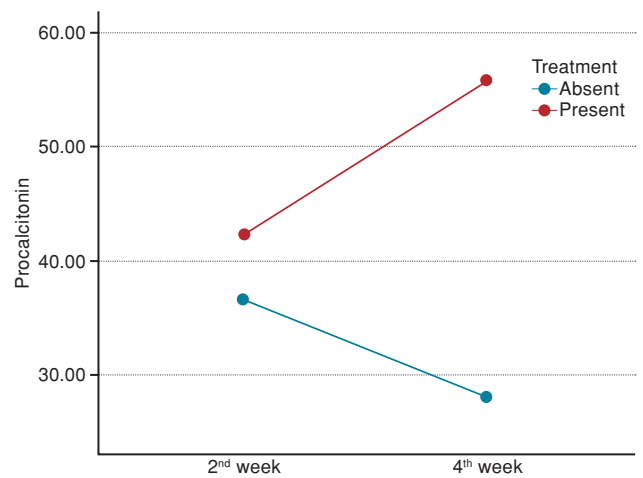

FIGURE 7. The change in procalcitonin level in mice receiving and not receiving antimicrobial treatment.

Table III demonstrates the results of correlation analysis. There were no significant correlations between *RANKL* concentrations and levels of CRP, procalcitonin and colony growth in control subjects. However, *RANKL* concentration was significantly correlated with colony growth in mice allocated to the *S. aureus* inoculation group (r=-0.547, p=0.035). In subjects not receiving antimicrobial treatment, *RANKL* concentration was significantly correlated with procalcitonin level (r=0.691, p=0.004). On the other hand, CRP was correlated with colony growth in subjects receiving antimicrobial treatment after inoculation of *S. aureus* (r=0.517, p=0.048).

DISCUSSION

We found that plasma *RANKL* level is not a reliable marker to monitor the bone destruction. Plasma *RANKL* level was not correlated with colony growth or any markers of inflammation in osteomyelitis. In addition, *RANKL* was not useful as a marker of treatment response in osteomyelitis.

Active inflammation leads to an increase in plasma interleukin-6 (IL-6) concentration which induces the expression of *RANKL*-the primary factor influencing osteoclast differentiation and proliferation (osteoclastogenesis).[8] In addition to its well-characterized role in the control of bone homeostasis, mostly through the RANK receptor, *RANKL* can also play important roles in the regulation of the immune system.[9] The relationships between the activation of immune cells, their *RANKL* expression and, in turn, the activity of osteoclasts and osteoblasts have long been shown in various studies.[10-12] Furthermore, an interesting *in vitro* study conducted by Widaa et

al.[13] demonstrated that *S. aureus* protein A (SpA) induced *RANKL* secretion in osteoblasts, leading to the activation of osteoclasts.[13] They concluded that this effect could inhibit osteoblast activity for a three-week period. However, the circulating concentrations of *RANKL* and its relationship with inflammatory markers and treatment outcomes have not been researched in osteomyelitis.

In this study, we sought possible associations between *RANKL* and other markers of the infection, including leukocyte count, CRP, and procalcitonin. Leukocytes are the primary immune cells initiating inflammation during the acute phase of osteomyelitis.[14] Despite this relationship, we failed to demonstrate a significant correlation between leukocyte count and the *RANKL* in this study. Although the crosstalk between osteoclasts and *RANKL*-expressing T/B cells are well known, it is interesting that many clinical studies have failed to determine significant differences between leukocyte counts and *RANKL* concentrations. This may be related to the fact that leukocyte counts may be within reference ranges during the subacute phase of the disease, a characteristic of osteomyelitis that has been shown in both rodents and humans.[15,16]

C-reactive protein is an acute phase protein synthesized in the liver by the induction of IL-6.[17] Since the biological half-life of CRP is 19 h, which is relatively shorter than other acute phase reactants, elimination of the inflammatory source leads to a significant decline in CRP level within hours.[18] The CRP level can, therefore, be used to monitor the activity of various kinds of infection. Due to its characteristic response to inflammatory activity,

		C-reactive protein	Procalcitonin	WBC	Colony growth
TABLE III					
Correlations between measurements with regard to groups and presence of treatment					
Groups					
Control					
RANKL	r	0.126	0.100	0.446	-
	p	0.655	0.723	0.095	-
C-reactive protein	r		-0.144	0.016	-
	p		0.610	0.954	-
Procalcitonin	r			0.046	-
	p			0.869	-
Infected					
RANKL	r	-0.101	-0.032	-0.238	-0.547
	p	0.721	0.909	0.394	0.035
C-reactive protein	r		0.163	-0.448	0.166
	p		0.561	0.094	0.553
Procalcitonin	r			-0.038	-0.134
	p			0.894	0.633
WBC	r				0.154
	p				0.584
Presence of treatment					
Absent					
RANKL	r	-0.142	0.691	-0.100	0.312
	p	0.612	0.004	0.722	0.277
C-reactive protein	r		-0.099	0.399	0.245
	p		0.726	0.141	0.398
Procalcitonin	r			0.063	0.118
	p			0.824	0.688
WBC	r				0.089
	p				0.762
Present					
RANKL	r	0.172	-0.232	0.136	-0.211
	p	0.541	0.405	0.630	0.451
C-reactive protein	r		0.215	-0.067	0.517
	p		0.442	0.813	0.048
Procalcitonin	r			-0.211	0.220
	p			0.451	0.431
WBC	r				-0.179
	p				0.524

r: Spearman correlation coefficient; RANKL: Receptor activator of nuclear factor kappa-B ligand.

CRP level is utilized as a sensitive, but relatively non-specific marker for the identification of subjects with osteomyelitis.[5] Our findings have shown that there was no significant correlation between CRP and *RANKL* level in any of our groups, strongly suggesting that *RANKL* is insufficient for the identification of bone destruction occurring in osteomyelitis.

Procalcitonin is another fast-responding acute phase protein secreted from the liver, lung, and the neuroendocrine cells of the intestine.[19,20] There is also some evidence that leukocytes and monocytes can also secrete procalcitonin.[21] Procalcitonin levels increase as a response to severe bacterial infections and also in burns and sepsis; however, viral infections rarely induce a prominent procalcitonin response.[22] In this study, we observed a significant correlation between procalcitonin and *RANKL* levels in infected mice that did not receive antimicrobial treatment. However, there was no correlation between *RANKL* and procalcitonin levels in mice receiving antimicrobial treatment. Although these two results seem to suggest that the relationship between procalcitonin and *RANKL* levels prior to treatment may be important in

identifying osteomyelitis and, possibly, the degree of bone destruction; it is critical to note that the literature on the topic of procalcitonin and osteomyelitis is controversial. While some studies fail to find any important relationship between procalcitonin levels and osteomyelitis (either for diagnosis or severity),[23] others report varying values of procalcitonin cut-off values with rather high sensitivity and specificity for the diagnosis of osteomyelitis.[24-26] In a very informative review that assessed the roles of various acute phase reactants in infectious diseases, it was concluded that ESR remained an ideal follow-up marker in osteomyelitis; furthermore, the article also noted that procalcitonin should be seen more as a biomarker that could be utilized for the rule-in of osteomyelitis, particularly while employing a lower-than-usual cut-off value in the range of 0.2-0.3 ng/mL.[27] Although our results do suggest a relationship between *RANKL* and procalcitonin in mice without treatment, we would be mistaken to conclude that this relationship could be used to determine the degree of bone destruction, as the relationship is not apparent in the overall group or in the presence of treatment.

White blood cell (WBC) count, which is one of the acute phase markers, increases in cases of acute osteomyelitis, but does not increase in subacute cases, and this may lead to incorrect and inadequate treatments in the follow-up of the infection.[8] In our study, no statistically significant correlation was observed between WBC and *RANKL*. In addition, the ESR starts to increase two days after the onset of inflammation in the case of acute osteomyelitis. The ESR is also initially high in chronic post-traumatic osteomyelitis, but with appropriate treatment it returns to the normal range.[28] The ESR measurement is not a sensitive test, as it affects many conditions in the diagnosis and follow-up of acute or chronic osteomyelitis. It also increases in collagen vascular diseases, inflammatory arthritis, previous surgical procedures, and malignancies.[29] In our study, since the ESR was below the measurable value, it was excluded from the study.

Current evidence regarding *RANKL* levels during osteomyelitis is controversial. Okahashi et al.[30] showed that infection of mice osteoblasts by *S. pyogenes* stimulated *RANKL* production and triggered bone destruction in infected bone tissue. Findings of the study conducted by Widaa et al.[13] also support these results. In their study, the authors showed that *S. aureus* induced *RANKL* secretion and activates osteoclasts. Additionally, evidence from the study by Claro et al.[31] indicates that binding of SpA to osteoblasts increases

the expression of *RANKL*, activating bone resorption. The major difference in study design between our study and the aforementioned studies is that blood sampling for *RANKL* in those studies were performed within 24 h of the induction of osteomyelitis. However, in our study, we performed intermittent blood sampling throughout the study until the seventh week. Our study is, therefore, has a unique property that helps to demonstrate the long-term change in *RANKL* levels following induction of osteomyelitis with *S. aureus*. However, given the short half-life of *RANKL*, weekly blood sampling and relatively long periods between blood sampling may have led to loss of data, particularly in terms of earlier changes due to bone destruction. However, it is quite impossible to determine the exact time at which any animal would demonstrate maximal amount of bone destruction, and we believe the sampling method of our study has significant advantages compared to other studies that used one or two measurements. Finally, it is also possible that small amounts of circulating *RANKL* induced the production of antibodies against *RANKL*, thereby leading to a decline in levels of the already-low levels of circulating *RANKL*.

Computed tomography (CT) is not recommended routinely for the diagnosis of osteomyelitis, but it is preferred as the second option in cases where magnetic resonance imaging (MRI) cannot be used.[4] During CT, the patient receives radiation and this is a carcinogenic condition for the patient. Furthermore, performing of MRI could be difficult in children and required analgesia, and also is not cost-effective. In the study of Llewellyn et al.,[32] it was reported that, despite the high sensitivity of MRI, it had lower sensitivity compared to positron emission tomography (PET)/CT. If the *RANKL* value was significant in our study, we would be able to understand the severity of osteomyelitis-induced bone destruction and the severity of the disease. In other words, in our study, the advantages of *RANKL* over other blood parameters were investigated rather than the superiority of *RANKL* over imaging. However, theoretically, the advantages of *RANKL* over imaging are that it does not contain radiation and it is an easy procedure that can be performed by taking blood samples at the bedside without the need for patient mobilization. In addition, X-ray graphies are not used in the diagnosis of acute hematogenous osteomyelitis, as the changes of osteomyelitis are not reflected in the radiographs in the acute stage.[33] Therefore, we did not use direct radiography, as we evaluated acute osteomyelitis cases in our study. Magnetic resonance imaging has disadvantages such as being expensive, showing only

one area, and long procedure time. In summary, the study was inspired by question "Can *RANKL* be used in place of imaging modalities and other biochemical markers in the clinical determination of osteomyelitis-induced bone resorption?". We attempted to answer the question of whether *RANKL* can be used as a more specific marker for bone damage, rather than biomarkers that increase in other infections such as CRP, ESR, and WBC. We believe that, if the results of our study were significant, both the bone destruction and the severity of osteomyelitis could be determined by looking only *RANKL* level instead of general infection markers in the blood samples. However, our results were insignificant.

The lack of radiological examination is one of the main limitations of our study. Coordinated sampling with radiological examinations can give more effective and reliable results in terms of bone destruction. The presence of other conditions that increase inflammation markers in mice was not studied. These situations may have affected the levels of *RANKL* and other biomarkers examined in our study. This study is one of the preclinical studies in the field. Their results show that the diagnosis of osteomyelitis would be supported by laboratory parameters. However, it is not possible to make a definitive judgment without clinical studies.

In conclusion, *RANKL* levels in mice with *S. aureus* osteomyelitis are not correlated with colony growth or other markers of inflammation and not useful for monitoring the bone destruction and response to antimicrobial treatment during osteomyelitis. Further studies are required to determine whether there are relationships between the *RANKL* pathway of bone regulation and the bone loss seen in osteomyelitis.

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