Serum Levels of OPG, RANKL, and RANKL/OPG Ratio in Patients with Ankylosing Spondylitis: A Systematic Review and Meta-analysis

Mengya Chen, Xingxing Hu*, Meng Wu, Jiajia Yang, Renfang Han, Yubo Ma, Xu Zhang, Yaping Yuan, Rui Liu, Mengmeng Wang, Guangming Jiang, Jixiang Deng, Shengqian Xu, Jianhua Xu, Zongwen Shuai, and Faming Pan

Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China; The Key Laboratory of Major Autoimmune Diseases, Anhui Medical University, Hefei, Anhui, China; Department of Rheumatism and Immunity, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

ABSTRACT

Objectives: To investigate the role of osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL), and RANKL/OPG ratio in the pathogenesis of ankylosing spondylitis (AS).

Methods: Studies that compared serum levels of OPG, RANKL, and RANKL/OPG ratio between AS patients and healthy controls were gathered. Pooled standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated by the random-effects model.

Results: Twenty studies containing 1592 AS patients and 1064 healthy controls were included in this meta-analysis. Serum levels of OPG, RANKL, and RANKL/OPG ratio in AS patients were significantly higher than that in normal controls (OPG: SMD = 0.401, 95% CI = 0.026–0.777, p = 0.036; RANKL: SMD = 1.116, 95% CI = 0.510–1.723, p < 0.001; RANKL/OPG ratio: SMD = 0.691, 95% CI = 0.084–1.299, p = 0.026, respectively). Subgroup analysis suggested that Asian AS patients and patients with elevated ESR (ESR >20 mm/h) had higher serum OPG levels compared to normal controls. Asian patients, CRP >10 mg/L, ESR >20 mm/h, duration of disease ≤8 years, and BASDAI score >4 points subgroups showed increased RANKL levels compared to controls.

Conclusions: Serum levels of OPG, RANKL, and RANKL/OPG ratio may be used as potential susceptible biomarkers for AS, but they could be influenced by race, inflammatory factors, and disease activity of AS patients.

KEYWORDS
Ankylosing spondylitis; OPG; RANKL; bone metabolism; meta-analysis

Introduction

Ankylosing spondylitis (AS) is the most common form of spondyloarthritis that usually affects young adults, and the prevalence of AS is 0.1%–1.4% in general populations (Braun and Sieper, 2007; Tang et al., 2018). AS is an immune-mediated inflammatory autoimmune arthritis characterized by damaged axial skeleton, sacroiliac joints, and spine attachment points, which can lead to loss of joint function and disability (Mahmoudi et al., 2016).
Although the etiology of AS remains unclear, bone formation and bone loss have been proved to play a pivotal role in the pathogenesis of AS (Carter and Lories, 2011; Hinze and Louie, 2016; Schett, 2009). Accumulating evidence shows that biomarkers of bone metabolism, including sclerostin (Saad et al., 2012), Dickkopf-1 (DDK-1) (Wu et al., 2018), osteoprotegerin (OPG), and receptor activator of nuclear factor-kB ligand (RANKL) (Kim et al., 2006), are involved in bone formation and bone loss. 

OPG and RANKL (also called TNF-related activation-induced cytokine, TRANCE) are members of tumor necrosis factor-a superfamily (Simonet et al., 1997). RANKL is mainly secreted by osteoblasts and activated T cells. RANKL induces the maturation and activation of osteoclasts by binding to receptor activator of nuclear factor-kB (RANK) on osteoclast precursors and causes bone resorption (Gamal et al., 2018). Similarly, OPG is predominantly produced by osteoblasts, and it could inhibit the activation of osteoclast that competitively binds RANKL and blocks the interaction between RANKL and its receptors (Lacey et al., 1998). RANKL/RANK/OPG system is considered as the key signaling system in regulating osteoclast activity. Imbalance of RANKL/RANK/OPG system may be directly involved in bone remodeling and bone loss of many diseases, such as osteoporosis, osteopetrosis, rheumatoid arthritis, chronic arthritis (Liu et al., 2018; Mou et al., 2015), and the pathogenesis of osteoporosis in AS (Kim et al., 2006).

Current findings concerning serum levels of soluble OPG, RANKL (sRANKL), and RANKL/OPG ratio in AS patients versus healthy controls were inconsistent. Thus, the purpose of the present meta-analysis is to comprehensively evaluate an association between biomarkers of bone metabolism (serum OPG and RANKL levels and RANKL/OPG ratio) and AS susceptibility or clinical outcomes (inflammatory factors and disease activity).

Materials and methods

Literature search strategy

PubMed, Web of Science, and three Chinese databases—Wanfang, Chinese National Knowledge Infrastructure (CNKI), and VIP Database were searched and updated until February 2018 to identify relevant studies that investigated the relationships between RANKL/RANK/OPG system and AS. Searching keywords included “osteoprotegerin” or “OPG” or “receptor activator of nuclear factor-kB ligand” or “RANKL” or “TNFsf11” or “TRANCE” or “OPGL” or “ODF” and “Ankylosing spondylitis” or “Spondylitis, Ankylosing [MeSH Terms]” or “Ankylosing Spondylarthritis [Entry Terms]” or “Rheumatoid Spondylitis [Entry Terms]” or “AS.” To include all available studies, we contacted each corresponding author of articles that offered insufficient data for meta-analysis. All references of searched studies were also reviewed to identify additional studies. Finally, only published studies with full text were included.

Inclusion and exclusion criteria

Studies that met the following criteria were included: (1) PICOS criteria should be met in the studies. P: Ankylosing spondylitis; I: Enzyme-linked immunosorbent assay; C: Healthy control; O: Serum levels of OPG, RANKL; S: Case–control or Cross-sectional study or
Clinical cohort; (2) offered sufficient data to calculate the mean and standard deviation (SD) of serum levels of RANKL and OPG and/or RANKL/OPG ratio for each group; (3) AS diagnoses fulfilled the 1984 Modified New York criteria (mNYC) or 1987 American College of Rheumatology modified AS diagnostic criteria; (4) written in English or Chinese; (5) peer-reviewed studies with full text.

Studies that met the following criteria were excluded: (1) insufficient data; (2) comment, review, and abstracts; (3) animals or in vitro study; (4) quality score assessed by Newcastle–Ottawa Scale (NOS) score less than 5.

**Data extraction**

According to the selection criteria, two researchers (Mengya Chen and Xingxing Hu) independently extracted data. Any discrepancy in data extraction was discussed by a third researcher (Meng Wu). The following data were extracted if available: first author, publication year, sample size, geographic location, study design, measurement, and mean ± SD for serum OPG, RANKL concentrations and RANKL/OPG ratio in AS patients and healthy controls, and disease duration, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels and the Bath AS Disease Activity Index (BASDAI) in AS patients.

**Statistical analysis**

Serum levels of OPG and RANKL were presented as pg/mL. Standardized mean difference (SMD) and 95% confidence intervals (CIs) of serum OPG and RANKL levels were calculated for each study. In some studies, only median and range were obtained, so we transformed them into mean ± SD. Detailed description is provided in the previous article (Hozo et al., 2005). In short, \( m = \text{Median} \), \( a = \) the smallest value (minimum), \( b = \) the largest value (maximum), and \( n = \) the size of the sample. 

\[
\bar{x} = \frac{a+b}{2}, \quad s^2 = \frac{1}{12} \left[ \frac{(a-2m+b)^2}{4} + (b-a)^2 \right].
\]

Considering the possibility of heterogeneity among studies, the effect of heterogeneity was evaluated using Cochran’s Q-test and \( I^2 \) statistic (Lee and Bae, 2017). The fixed effect model was adopted if the heterogeneity was not statistically significant (\( I^2 < 50\% \) or \( p > 0.1 \)), otherwise the random effects model was used. Potential publication bias was investigated using Begg’s test or Egger’s linear regression test by visual examination of the funnel plot, and an asymmetric Funnel plot or \( p < 0.05 \) in Egger’s test suggests possible publication bias (Egger et al., 1997). Sensitivity analysis was performed to assess the stability of results after excluded an article. Newcastle–Ottawa Scale (NOS) was used to evaluate the quality of the included studies by two observers (Mengya Chen and Xingxing Hu) independently based on three main items: the selection of the study groups (0–4 points), the comparability of the two groups (0–2 points), and the determination of the exposure (0–3 points), with a perfect score of 9. A meta-regression was conducted to further explore the source of heterogeneity. Statistical analyses were performed using STATA 14.0 (StataCorp, College Station, TX, USA). A two-sided \( p \) value \( \leq 0.05 \) was considered statistically significant.
Results

Data source

The process of study selection is summarized in Figure 1. A total of 226 relevant papers were identified according to the searching strategy, and 108 duplicate records were initially excluded. After screening the titles and abstracts, 98 articles were excluded. Finally, 20 studies (Bai et al., 2017; Chen et al., 2010; Dhir et al., 2013; Franck et al., 2004; Grisar et al., 2002; Hou et al., 2018; Jadon et al., 2017; Klingberg et al., 2014; Kong et al., 2010; Korkosz et al., 2013; Kwon et al., 2012; Li et al., 2013; Mou et al., 2015; Serdaroğlu Beyazal et al., 2016; Sveaas et al., 2015; Taylan et al., 2012; Wen et al., 2016; Xu et al., 2014; Zhang et al., 2010, 2015) assessing serum OPG (n = 19), RANKL (n = 11) levels, and RANKL/OPG ratio (n = 7) in AS patients and healthy controls were included in the meta-analysis. All included studies were published between 2002 and 2018. Among the 20 studies, there were 1592 AS patients, mostly diagnosed by the modified New York classification criteria (mNYC) in 1984, and 1064 healthy controls. Serum levels of OPG and RANKL were measured by enzyme-linked immunosorbent assay (ELISA) in all studies. NOS scores of included studies mainly ranged from 5 to 8, and one article was excluded because of a NOS score <5. Detailed characteristics of the included studies are shown in Table 1.
### Table 1. Characteristic of including studies in this meta-analysis.

| Authors                  | Publication year | Region    | Study type          | Cases                      | Controls                   | Criteria for the classification of AS | Measurement type | NOS |
|--------------------------|------------------|-----------|---------------------|----------------------------|----------------------------|---------------------------------------|------------------|-----|
| Hou et al. [18]          | 2018             | China     | Case-control        | 40 | 111.3 ± 7.2          | 40 | 90.5 ± 7.0 | mNYC | ELISA | 8   |
| Bai et al. [19]          | 2017             | China     | Case-control        | 46 | 5.0 ± 1.6            | 38 | 1.87 ± 0.3 | 7.6 ± 1.2 | mNYC | ELISA | 7   |
| Jadon et al. [20]        | 2017             | UK        | Cross-sectional study | 157 | 162.0 ± 89.0 | 50 | 147 ± 43.8 | mNYC | ELISA | 7   |
| Serdaroglu et al. [21]   | 2016             | Turkey    | Cross-sectional study | 60 | 106.7 ± 50.9 | 50 | 58.1 ± 12.8 | mNYC | ELISA | 8   |
| Sveaas et al. [22]       | 2015             | Norway    | Cross-sectional study | 143 | 2300.0 ± 905.0 | 124 | 2000.0 ± 852.0 | mNYC | ELISA | 8   |
| Zhang et al. [23]        | 2015             | China     | Case-control        | 22 | 122.4 ± 10.9          | 22 | 114.0 ± 20.9 | 111.4 ± 21.7 | mNYC | ELISA | 7   |
| Mou et al. [13]          | 2015             | China     | Case-control        | 68 | 85.3 ± 44.6           | 32 | 59.5 ± 22.9 | 17105.0 ± 10045.6 | mNYC | ELISA | 7   |
| Wen et al. [24]          | 2014             | China     | Case-control        | 46 | 71.1 ± 28.8          | 20 | 106.2 ± 41.1 | mNYC | ELISA | 6   |
| Klingberg et al. [25]    | 2014             | Sweden    | Case-control        | 204 | 720 ± 19.9          | 80 | 68.9 ± 20.9 | 7478.8 ± 9756.5 | mNYC | ELISA | 7   |
| Li et al. [26]           | 2013             | China     | Case-control        | 44 | 57.0 ± 22.0          | 15 | 53.0 ± 15.0 | -       | ACR  | ELISA | 7   |
| Korkosz et al. [27]      | 2013             | Poland    | Case-control        | 50 | 95520.0 ± 47760.0    | 23 | 77610.0 ± 110644.0 | 133.0 ± 57.0 | mNYC | ELISA | 7   |
| Dhir et al. [28]         | 2013             | India     | Cross-sectional study | 85 | 649.7 ± 286.8          | 20 | 389.3 ± 244.8 | 554.7 ± 1850.1 | mNYC | ELISA | 6   |
| Xu et al. [29]           | 2012             | China     | Case-control        | 30 | 206.9 ± 37.8          | 30 | 232.8 ± 49.8 | mNYC | ELISA | 6   |
| Taylan et al. [30]       | 2012             | Turkey    | Case-control        | 55 | 339.0 ± 266.0          | 33 | 527.0 ± 253.5 | 3.6 ± 4.6 | mNYC | ELISA | 7   |
| Kwon et al. [31]         | 2012             | Korea     | Clinical cohort    | 55 | 69.7 ± 21.9           | 40 | 39.8 ± 19.9 | 103596.2 ± 5676.5 | mNYC | ELISA | 7   |
| Zhang et al. [32]        | 2010             | China     | Case-control        | 23 | 157.0 ± 49.0          | 17 | 105.0 ± 20.0 | 1.6 ± 0.8 | mNYC | ELISA | 6   |
| Kong et al. [33]         | 2010             | China     | Case-control        | 100 | 77.6 ± 24.7          | 100 | 66.3 ± 22.3 | 131.3 ± 40.4 | mNYC | ELISA | 7   |
| Study             | Year | Country | Study Type | Cases | Cases mean ± SD | Controls | Controls mean ± SD | Controls mean ± SD | Diagnostic Criteria | Method | Mixtures |
|-------------------|------|---------|------------|-------|-----------------|----------|--------------------|--------------------|-------------------|---------|----------|
| Chen et al. [34]  | 2010 | China   | Case-control | 42    | 103.1 ± 23.7    | -        | 26                 | 89.9 ± 16.9        | mNYC             | ELISA   | 7        |
| Franck et al. [35]| 2004 | Germany | Case-control | 264   | 36.6 ± 22.9     | -        | 240                | 70.4 ± 43.4        | mNYC             | ELISA   | 7        |
| Grisar et al. [36]| 2002 | Austria | Case-control | 30    | 44300.0 ± 19700.0 | -        | 41                 | 35200.0 ± 10000.0  | mNYC             | ELISA   | 7        |

OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kB ligand; ACR, American College of Rheumatology-modified AS diagnostic criteria; mNYC, modified New York diagnostic criteria; ELISA, enzyme-linked immunosorbent assay; NOS, Newcastle–Ottawa Scale; N, number of studies; NA, not available. Data are expressed as mean (SD).
Results of meta-analysis

Heterogeneity test results
Heterogeneity was significant in this meta-analysis (all \( p < 0.001 \), Table 2); therefore, the random-effect models were performed.

Overall effects
The pooled results are presented in Table 2. Serum levels of OPG, RANKL, and RANKL/OPG ratio in AS patients were significantly higher than that in normal controls (OPG: SMD = 0.401, 95%CI = 0.026–0.777, \( p = 0.036 \); RANKL: SMD = 1.116, 95% CI = 0.510–1.723, \( p < 0.001 \); RANKL/OPG ratio: SMD = 0.691, 95%CI = 0.084–1.299, \( p = 0.026 \), respectively) (Figure 2).

Subgroup analysis
Subgroup analyses stratified by region, duration of disease, CRP, ESR, and BASDAI were performed, and the results are detailed in Table 2.

Subgroup analysis showed that Asian AS patients and patients with elevated ESR (ESR >20 mm/h) had higher serum OPG levels compared to normal controls (Asian: SMD = 0.614, 95%CI = 0.049–1.179, \( p = 0.033 \); ESR: SMD = 0.952, 95%CI = 0.518–1.387, \( p < 0.001 \), respectively). The two subgroups of serum OPG levels in BASDAI and duration of disease were significantly higher than that in controls, respectively (All \( p < 0.05 \)). In AS patients with Asian origin, CRP >10 mg/L, ESR >20 mm/h, duration of disease \( \leq 8 \) years, or BASDAI score >4 points, the serum RANKL levels were significantly higher than in controls (Asian: SMD = 1.598, 95%CI = 0.911–2.285, \( p < 0.001 \); CRP: SMD = 2.053, 95%CI = 0.744–3.363, \( p = 0.002 \); ESR: SMD = 1.508, 95% CI = 0.521–2.494, \( p = 0.003 \); duration of disease: SMD = 2.474, 95%CI = 0.988–3.960, \( p = 0.001 \); BASDAI: SMD = 0.952, 95%CI = 0.518–1.387, \( p < 0.001 \), respectively). A significant difference in RANKL/OPG ratio was found in Asian origin and patients with high CRP (CRP >10 mg/L) (Asian: SMD = 1.111, 95%CI = 0.364–1.857, \( p = 0.004 \); CRP: SMD = 1.174, 95%CI = 0.101–2.247, \( p = 0.032 \), respectively).

Meta-regression analysis
Meta-regression for RANKL/OPG ratio was not conducted due to the limited number of studies. The source of heterogeneity was explored by adding country (China and others), publication year, NOS score, and sample size (subjects \( \leq 100 \) and \( >100 \)) in meta-regression. The results of meta-regression did not reveal the source of heterogeneity (Table 3). Meta-regression of race, disease, CRP, ESR, and BASDAI had performed to further explore the source of heterogeneity between different subgroups. However, no any heterogeneity was found between different subgroups (Table S1).

Publication bias and sensitivity analysis
A significant publication bias was found for serum RANKL levels (Egger’s test: \( p = 0.002 \), Begg’s test: \( p = 0.013 \)) but not for serum OPG levels and RANKL/OPG ratio using Egger’s and Begg’s tests. Sensitivity analysis showed that no significant changes were found by removing one single study in sequence, indicating that our results were statistically robust (Figure 3).
Table 2. Subgroup analysis of OPG, RANKL, and RANKL/OPG in AS.

| Subgroups | N | Studies | Case | Control | SMD(95%CI) | z | p | Test of heterogeneity |
|-----------|---|---------|------|---------|------------|---|---|-----------------------|
| **OPG**   |   |         |      |         |            |   |   |                       |
| Region    |   |         |      |         |            |   |   |                       |
| Asia      | 10| 476     | 340  |         | 0.614(0.049–1.179) | 2.13 | 0.033 | 92.5% <0.001          |
| Europe    | 10| 1076    | 684  |         | 0.196(–0.285–0.678) | 0.59 | 0.558 | 95.1% <0.001          |
| Combined  | 20| 1552    | 1024 |         | 0.401(0.026–0.777)  | 2.09 | 0.036 | 94.6% <0.001          |
| Duration of disease |   |         |      |         |            |   |   |                       |
| ≤8        | 6 | 311     | 273  |         | 1.049(0.401–1.698)  | 3.17 | 0.002 | 91.9% <0.001          |
| >8        | 8 | 766     | 397  |         | 0.281(0.003–0.559)  | 1.98 | 0.048 | 77.2% <0.001          |
| Combined  | 14| 1077    | 670  |         | 0.605(0.274–0.937)  | 3.58 | <0.001 | 89.8% <0.001          |
| CRP       |   |         |      |         |            |   |   |                       |
| ≤10       | 5 | 508     | 325  |         | 0.713(–0.071–1.498) | 1.78 | 0.075 | 96.0% <0.001          |
| >10       | 9 | 671     | 482  |         | 0.421(–0.215–1.058) | 1.30 | 0.194 | 95.3% <0.001          |
| Combined  | 14| 1179    | 807  |         | 0.527(0.042–1.012)  | 2.13 | 0.033 | 95.8% <0.001          |
| ESR       |   |         |      |         |            |   |   |                       |
| ≤20       | 4 | 666     | 477  |         | –0.298(–1.024–0.428) | 0.81 | 0.421 | 96.8% <0.001          |
| >20       | 10| 441     | 300  |         | 0.952(0.518–1.387)  | 4.30 | <0.001 | 85.8% <0.001          |
| Combined  | 14| 1107    | 777  |         | 0.582(0.073–1.091)  | 2.24 | 0.025 | 95.9% <0.001          |
| **RANKL** |   |         |      |         |            |   |   |                       |
| Region    |   |         |      |         |            |   |   |                       |
| Asia      | 9 | 315     | 220  |         | 1.598(0.911–2.285)  | 4.56 | <0.001 | 91.2% <0.001          |
| Europe    | 3 | 344     | 133  |         | –0.102(–0.304–0.100) | 0.99 | 0.324 | 0.0% 0.892          |
| Combined  | 12| 659     | 353  |         | 1.116(0.510–1.723)  | 3.61 | <0.001 | 94.0% <0.001          |
| Duration of disease |   |         |      |         |            |   |   |                       |
| ≤8        | 3 | 108     | 100  |         | 2.474(0.988–3.960)  | 3.26 | 0.001 | 93.5% <0.001          |
| >8        | 4 | 386     | 159  |         | 0.053(–0.269–0.375) | 0.32 | 0.746 | 59.9% 0.058          |
| Combined  | 7 | 494     | 259  |         | 1.065(0.228–1.901)  | 2.49 | 0.013 | 95.6% <0.001          |
| CRP       |   |         |      |         |            |   |   |                       |
| ≤10       | 3 | 305     | 151  |         | 0.317(–0.403–1.038) | 0.86 | 0.388 | 90.8% <0.001          |
| >10       | 4 | 127     | 105  |         | 2.053(0.744–3.363)  | 3.07 | 0.002 | 93.5% <0.001          |
| Combined  | 7 | 432     | 256  |         | 1.282(0.418–2.145)  | 2.91 | 0.004 | 95.5% <0.001          |
| ESR       |   |         |      |         |            |   |   |                       |
| ≤20       | 2 | 259     | 113  |         | –0.085(–0.307–0.137) | 0.75 | 0.454 | 0.0% 0.754          |
| >20       | 6 | 258     | 163  |         | 1.508(0.521–2.494)  | 3.00 | 0.003 | 94.2% <0.001          |
| Combined  | 8 | 517     | 276  |         | 1.090(0.324–1.857)  | 2.79 | 0.005 | 95.0% <0.001          |
| **RANKL/OPG** |   |         |      |         |            |   |   |                       |
| Region    |   |         |      |         |            |   |   |                       |
| Asia      | 4 | 131     | 80   |         | 1.111(0.364–1.857)  | 2.92 | 0.004 | 82.5% <0.001          |
| Europe    | 2 | 259     | 113  |         | –0.093(–0.315–0.129) | 0.82 | 0.410 | 0.0% 0.423          |
| Combined  | 6 | 390     | 193  |         | 0.691(0.084–1.299)  | 2.23 | 0.026 | 89.3% <0.001          |
| CRP       |   |         |      |         |            |   |   |                       |
| ≤10       | 2 | 259     | 113  |         | –0.093(–0.315–0.129) | 0.82 | 0.410 | 0.0% 0.423          |
| >10       | 3 | 87      | 65   |         | 1.174(0.101–2.247)  | 2.14 | 0.032 | 88.4% <0.001          |
| Combined  | 5 | 346     | 178  |         | 0.640(–0.037–1.317) | 1.85 | 0.064 | 90.3% <0.001          |
| ESR       |   |         |      |         |            |   |   |                       |
| ≤4        | 2 | 227     | 97   |         | 0.397(–0.751–1.546) | 0.68 | 0.497 | 90.3% 0.001          |
| >4        | 2 | 64      | 48   |         | 1.271(–0.603–3.144) | 1.33 | 0.184 | 94.1% <0.001          |
| Combined  | 4 | 291     | 145  |         | 0.817(–0.106–1.741) | 1.73 | 0.083 | 92.7% <0.001          |

AS, Ankylosing spondylitis; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand; N, number; SMD, standardized mean difference; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BASDAI, the Bath AS Disease Activity Index.

**Discussion**

Accumulating evidence shows that abnormal bone remodeling and osteoporosis are frequent even in the early stage of AS (Baek et al., 2005; Calin, 1991; Davey-Ranasinghe...
and Deodhar, 2013). There is some evidence that OPG and RANKL are the important bone-turnover biomarkers and the relative balance of RANKL/RANK and OPG plays a pivotal role in the regulation of bone remodeling and bone loss (Kim et al., 2006). However, previous studies showed inconsistent results when comparing serum levels of

![Figure 2](image)

**Figure 2.** Forest plots of serum levels of biomarkers of bone metabolism for AS patients versus healthy controls: (a) forest plots based on OPG serum levels; (b) forest plots based on RANKL serum levels; (c) forest plots based on RANKL/OPG ratio.

**Table 3.** Meta-regression analysis coefficients in the examined group of studies.

| Variables  | Coefficient (SE) | 95% Confidence Interval | p  |
|------------|------------------|--------------------------|----|
|            |                  |                          |    |
| OPG        |                  |                          |    |
| Country    | −0.129(0.464)    | [−1.118, 0.860]          | 0.785 |
| Publication year | 0.056(0.055)    | [−0.061, 0.173]          | 0.325 |
| NOS        | −0.040(0.374)    | [−0.386, 1.210]          | 0.289 |
| Total sample size | −0.306(0.511)  | [−1.397, 0.784]          | 0.558 |
|            |                  |                          |    |
| RANKL      |                  |                          |    |
| Country    | 1.049(0.778)     | [−0.854, 2.953]          | 0.226 |
| Publication year | 0.148(0.163)    | [−0.251, 0.548]          | 0.399 |
| NOS        | 0.297(0.686)     | [−1.382, 1.978]          | 0.680 |
| Total sample size | −0.085(0.812)  | [−2.882, 1.092]          | 0.393 |

OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kB ligand; SE, standard error; NOS, Newcastle–Ottawa Scale.

**Notes:** Weights are from random effects analysis.
OPG and RANKL between AS patients and healthy controls. In some studies, serum levels of OPG and RANKL were elevated in AS patients (Zhang et al., 2010); however, serum OPG and RANKL levels have been found to be reduced in AS patients in some other studies (Franck et al., 2004; Klingberg et al., 2014). In addition, some researchers suggested that there were no definite differences between AS patients and healthy controls in serum levels of OPG (Jadon et al., 2017) and RANKL (Taylan et al., 2012). Some reasons may explain these discrepancies among studies, such as small sample size, different detection methods, and different races. Therefore, we conducted the present meta-analysis to comprehensively evaluate the relationships of serum levels of OPG, RANKL, and RANKL/OPG ratio with the development of AS.

We found that serum OPG levels in AS patients were significantly higher than that in normal controls. High serum OPG concentrations might inadequately inhibit RANK-mediated bone resorption explaining the presence of excessive ossification in AS patients (such as paravertebral syndesmophytes). It has also been explained that high OPG levels in AS was a compensatory manifestation of the anti-bone resorption (Zhang et al., 2010). Serum RANKL levels were significantly higher in AS patients compared with normal controls, further validated that patients with AS was prone to osteoporosis (Klingberg et al., 2012). RANKL interacts with its receptor RANK as a trigger of downstream signaling pathways for osteoclastogenesis. These signaling pathways include three mitogen-activated protein kinases (p38 MAPK, ERK, and JNK), AKT, and auto amplified nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), which lead to stimulate the activation of critical genes for osteoclastogenesis (Wang et al., 2018). These are the potential specific molecular...
mechanisms of bone loss in the early stages of AS. \textit{RANKL/OPG} ratio shows a significant difference between AS patients and normal controls. An earlier study in RA demonstrated that the imbalance of \textit{RANKL/OPG} ratio plays an important role in bone metabolism (Haynes et al., 2003). Radiographic damage in early stage of AS is initially characterized by erosive changes followed by a distinct anabolic skeletal response, which results in excessive bone formation (Lories et al., 2009). Accordingly, early stage of AS was prone to osteoporosis \textit{while} AS with long disease duration was prone to paravertebral syndesmophytes.

When stratified by ethnicity, three biomarkers of bone metabolism showed no connections with AS risk in Caucasian, but existed in Asians. This may be related to ethnic differences in the predominant \textit{HLA-B27} alleles associated AS in which \textit{HLA-B27*05} and \textit{HLA-B27*02} are widely prevalent in European populations, and \textit{HLA-B27*04} is predominant in Asian populations (Bowness, 2015). The association between different \textit{HLA-B27} subtypes with AS patients is different. It is now widely believed that \textit{HLA-B27*02}, \textit{HLA-B27*04}, and \textit{HLA-B27*05} had positive associations with AS, but \textit{HLA-B27*06} and \textit{HLA-B27*09} had negative associations (Park et al., 2008; Lin and Gong, 2017). Patients with elevated ESR (ESR \textit{>20 mm/h}) had higher serum \textit{OPG} levels when compared to controls, suggesting that race and inflammatory factors may be associated with \textit{OPG}. Similarly, Asanuma et al. also found a positive correlation between serum \textit{OPG} levels and ESR (Asanuma et al., 2007). Serum \textit{RANKL} levels were significantly higher in patients with elevated ESR (ESR \textit{>20 mm/h}), high CRP (CRP \textit{>10 mg/L}), short duration of disease (duration of disease \textit{\leq 8 years}), and high BASDAI score (BASDAI score \textit{>4 points}) compared to healthy controls. Patients with higher bone erosion had short disease duration and higher inflammatory activity, because early stage of AS is acute stages of inflammation marked by mononuclear cell infiltrates and an increased number of osteoclasts (OCs) (Caparbo et al., 2018). Given these facts, we hypothesized that serum \textit{OPG} and \textit{RANKL} levels may be used as potential biomarkers to reflect inflammation and bone metabolism in AS.

Heterogeneity was significant in this meta-analysis, and a meta-regression was conducted to further explore the source of heterogeneity. However, the result of meta-regression does not reveal the source of heterogeneity.

Egger’s \((p = 0.002)\) and Begg’s test \((p = 0.013)\) found publication bias in \textit{RANKL}. Then, trim-and-fill method was used to correct the result, and two potential missing studies were added in the lower side of the funnel plot to make the plot symmetric (Figure 4). However, the correction did not significantly reduce the serum \textit{RANKL} levels in AS patients compared with controls \((\text{SMD} = 0.693, 95\% \text{CI} = 0.056–1.329, p = 0.033)\).

This is the first meta-analysis to comprehensively evaluate the role of serum \textit{OPG}, \textit{RANKL} levels and \textit{RANKL/OPG} ratio in the pathogenesis of AS. This study also had some limitations. First, we only included online published articles in this meta-analysis, some gray literature have not been taken into account and were missed. A significant publication bias has been found in this meta-analysis, but trim-and-fill method indicated that the publication bias did not significantly influence the pooled results, suggesting the results were robust. Second, we did not investigate the correlation of other factors, such as sex, smoking, body mass index (BMI), and drug use, with AS risk due to lack of availability of data. Meta-regression did not reveal any source of heterogeneity for \textit{OPG} and \textit{RANKL}, suggesting other uninvestigated factors may also contribute to heterogeneity. Third, the levels of \textit{OPG} and \textit{RANKL} affect the capacity to generate osteoclasts leading to the onset of
AS, but the capacity to generate osteoclasts has not been evaluated in our study. Therefore, we cannot draw such a conclusion that the changes in the levels of OPG and RANKL result in the occurrence of AS by affecting the capacity to generate osteoclasts.

**Conclusion**

In conclusion, serum levels of OPG, RANKL, and RANKL/OPG ratio may be used as potential susceptible biomarkers for AS, but they could be influenced by race, inflammatory factors, and disease activity of AS patients.

**Acknowledgments**

Thanks for the people who participated in this study. This work was supported by <the National Natural Science Foundation of China> under Grant <number 30972530, 81273169, 81573218 and 81773514>; <Funding for scientific research activities of academic and technical leaders in Anhui Province> under Grant <number 2017D140>.

**Disclosure of interest**

The authors report no conflict of interest.

**Funding**

This work was supported by the National Natural Science Foundation of China under Grant [30972530, 81273169, 81573218, 81773514]; the Funding for scientific research activities of academic and technical leaders in Anhui 265 Province under Grant [2017D140].

**ORCID**

Shengqian Xu [http://orcid.org/0000-0002-9559-1143](http://orcid.org/0000-0002-9559-1143)
References

Asanuma Y, Chung CP, Oeser A, et al. (2007). Serum osteoprotegerin is increased and independently associated with coronary-artery atherosclerosis in patients with rheumatoid arthritis. Atherosclerosis, 195(2), e135–41. doi:10.1016/j.atherosclerosis.2007.04.049

Baek HJ, Kang SW, Lee YJ, et al. (2005). Osteopenia in men with mild and severe ankylosing spondylitis. Rheumatol Int, 26(1), 30–34. doi:10.1007/s00296-004-0516-3

Bai J, Sun QH, Zhang Z, et al. (2017). Clinical significance of the levels of MIF, IL-23, RANKL, OPG, and DKK1 in patients with active ankylosing spondylitis. Hebei Med J, 39, 3071–3074. doi:10.3969/j.issn.1002-7386.2017.20.007

Bowness P. (2015). HLA-B27. Annu Rev Immunol, 33(1), 29–48. doi:10.1146/annurev-immunol-032414-112110

Braun J, Sieper J. (2007). Ankylosing spondylitis. Lancet, 369(9570), 1379–1390. doi:10.1016/s0140-6736(07)60494-9

Calin A. (1991). Osteoporosis and ankylosing spondylitis. Br J Rheumatol, 30(4), 318–319.

Caparbo VF, Saad CGS, Moraes JC, et al. (2018). Monocytes from male patients with ankylosing spondylitis display decreased osteoclastogenesis and decreased RANKL/OPG ratio. Osteoporosis Int, 29(11), 2565–2573. doi:10.1007/s00198-018-4629-z

Carter S, Lories RJ. (2011). Osteoporosis: a paradox in ankylosing spondylitis. Curr Osteoporos Rep, 9(3), 112–115. doi:10.1007/s11914-011-0058-z

Chen CH, Chen HA, Liao HT, et al. (2010). Soluble receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoprotegerin in ankylosing spondylitis: OPG is associated with poor physical mobility and reflects systemic inflammation. Clin Rheumatol, 29(10), 1155–1161. doi:10.1007/s10067-010-1543-y

Davey-Ranasinghe N, Deodhar A. (2013). Osteoporosis and vertebral fractures in ankylosing spondylitis. Curr Opin Rheumatol, 25(4), 509–516. doi:10.1097/BOR.0b013e3283620777

Dhir V, Srivastava R, Aggarwal A. (2013). Circulating levels of soluble receptor activator of NF-κB ligand and matrix metalloproteinase 3 (and their antagonists) in Asian Indian patients with ankylosing spondylitis. J Rheumatol, 2013, 1–4. doi:10.1155/2013/814350

Egger M, Davey Smith G, Schneider M, Minder C. (1997). Bias in meta-analysis detected by a simple, graphical test. BMJ (Clin Res Ed), 315(7109), 629–634.

Franck H, Meurer T, Hofbauer LC. (2004). Evaluation of bone mineral density, hormones, biochemical markers of bone metabolism, and osteoprotegerin serum levels in patients with ankylosing spondylitis. J Rheumatol, 31(11), 2236.

Gamal RM, Gamal WM, Ghandour AM, et al. (2018). Study of the osteoprotegerin/receptor activator of nuclear factor-κB ligand and matrix metalloproteinase 3 (and their antagonists) in systemic sclerosis. Immunol Invest, 47(3), 241–250. doi:10.1080/08820139.2017.1423499

Grisar J, Bernecker PM, Aringer M, et al. (2002). Ankylosing spondylitis, psoriatic arthritis, and reactive arthritis show increased bone resorption, but differ with regard to bone formation. J Rheumatol, 29(7), 1430.

Haynes DR, Barg E, Crotti TN, et al. (2003). Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology, 42(1), 123–134.

Hinze AM, Louie GH. (2016). Osteoprotegerin management in ankylosing spondylitis. Curr Treat Options Rheumatol, 2(4), 271–282. doi:10.1007/s40674-016-0055-6

Hou C, Luan L, Ren C. (2018). Oxidized low-density lipoprotein promotes osteoclast differentiation from CD68 positive mononuclear cells by regulating HMGB1 release. Biochem Biophys Res Commun, 495(1), 1356–1362. doi:10.1016/j.bbrc.2017.11.083

Hozo SP, Djulbegovic B, Hozo I. (2005). Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol, 5, 13. doi:10.1186/1471-2288-5-13

Jadon DR, Sengupta R, Nightingale A, et al. (2017). Serum bone-turnover biomarkers are associated with the occurrence of peripheral and axial arthritis in psoriatic disease: a prospective cross-sectional comparative study. Arthritis Res Ther, 19(1), 210. doi:10.1186/s13075-017-1417-7

M. CHEN ET AL.
Park KS, Kang SY, Lee WI. (2008). HLA-B27 subtypes in Korean patients with ankylosing spondylitis. Korean J Lab Med, 28(1), 46–52. doi:10.3343/kjlm.2008.28.1.46
Kim HR, Lee SH, Kim HY. (2006). Elevated serum levels of soluble receptor activator of nuclear factors-kappaB ligand (sRANKL) and reduced bone mineral density in patients with ankylosing spondylitis (AS). Rheumatology, 45(10), 1197–1200. doi:10.1093/rheumatology/kei072
Klingberg E, Geijer M, Gothlin J, et al. (2012). Vertebral fractures in ankylosing spondylitis are associated with lower bone mineral density in both central and peripheral skeleton. J Rheumatol, 39(10), 1987–1995. doi:10.3899/jrheum.120316
Klingberg E, Nurkkala M, Carlsten H, Forsblad-d’Elia H. (2014). Biomarkers of bone metabolism in ankylosing spondylitis in relation to osteoproliferation and osteoporosis. J Rheumatol, 41(7), 1349–1356. doi:10.3899/jrheum.131199
Kim HR, Lee SH, Kim HY. (2006). Elevated serum levels of soluble receptor activator of nuclear factors-kappaB ligand (sRANKL) and reduced bone mineral density in patients with ankylosing spondylitis (AS). Rheumatology, 45(10), 1197–1200. doi:10.1093/rheumatology/kei072
Korkosz M, Gąsowski J, Leszczyński P, et al. (2013). High disease activity in ankylosing spondylitis is associated with increased serum sclerostin level and decreased wingless protein-3a signaling but is not linked with greater structural damage. BMC Musculoskelet Disord, 14(1), 99. doi:10.1186/1471-2474-14-99
Kwon SR, Lim MJ, Suh CH, et al. (2012). Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. Rheumatol Int, 32(8), 2523–2527. doi:10.1007/s00296-011-1981-0
Lee YH, Bae SC. (2017). Association between functional CYP2D6 polymorphisms and susceptibility to autoimmune diseases: a meta-analysis. Immunol Invest, 46(2), 109–122. doi:10.1080/08820139.2016.1226898
Li XJ, Huang SQ, Wang AN, et al. (2013). The serum levels of interleukin-17 and receptor activator of nuclear factors -κB ligand in ankylosing spondylitis. Chin J Osteoporosis, 16(1), 27–30,38. doi:10.3969/j.issn.1006-7108.2010.01.007
Lin H, Gong YZ. (2017). Association of HLA-B27 with ankylosing spondylitis and clinical features of the HLA-B27-associated ankylosing spondylitis: a meta-analysis. Rheumatol Int, 37(8), 1267–1280. doi:10.1007/s00296-017-3741-2
Li XJ, Huang SQ, Wang AN, et al. (2013). The serum levels of interleukin-17 and receptor activator of nuclear factors -κB ligand in ankylosing spondylitis. Chin J Osteoporosis, 17(11), 769–771. doi:10.3760/ema.j.issn.1007-7480.2013.11.011
Lin H, Gong YZ. (2017). Association of HLA-B27 with ankylosing spondylitis and clinical features of the HLA-B27-associated ankylosing spondylitis: a meta-analysis. Rheumatol Int, 37(8), 1267–1280. doi:10.1007/s00296-017-3741-2
Liu LN, Mao YM, Zhao CN, et al. (2018). Circulating levels of osteoprotegerin, osteocalcin and osteopontin in patients with rheumatoid arthritis: a systematic review and meta-analysis. Immunol Invest, 6, 1–14. doi:10.1080/08820139.2018.1510957
Lories RJ, Luyten FP, de Vlam K. (2009). Progress in spondyloarthritis. Mechanisms of new bone formation in spondyloarthritis. Arthritis Res Ther, 11(2), 221. doi:10.1186/ar2642
Mahmoudi M, Jamshidi AR, Karami J, et al. (2016). Analysis of killer cell immunoglobulin-like receptor genes and their HLA ligands in Iranian patients with ankylosing spondylitis. Iran J Allergy Asthma Immunol, 15(1), 27–38.
Mou YK, Zhang PP, Li QX, et al. (2015). Changes of serum levels of MMP-3, sRANKL, and OPG in juvenile-onset ankylosing spondylitis patients carrying different HLA-B27 subtypes. Clin Rheumatol, 34(6), 1085–1089. doi:10.1007/s10067-015-2940-z
Saad CGS, Ribeiro ACM, Moraes JCB, et al. (2012). Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? Arthritis Res Ther, 14(5), R216–R. doi:10.1186/ar4055
Schett G. (2009). Bone formation versus bone resorption in ankylosing spondylitis. Adv Exp Med Biol, 649, 114–121.
Serdaroğlu Beyazal M, Erdoğan T, Türkyılmaz AK, et al. (2016). Relationship of serum osteoprotegerin with arterial stiffness, preclinical atherosclerosis, and disease activity in patients with ankylosing spondylitis. Clin Rheumatol, 35(9), 2235–2241. doi:10.1007/s10067-016-3198-9
Simonet WS, Lacey DL, Dunstan CR, et al. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell, 89(2), 309–319. doi:10.1016/S0092-8674(00)80209-3
Sveaas SH, Berg IJ, Provan SA, et al. (2015). Circulating levels of inflammatory cytokines and cytokine receptors in patients with ankylosing spondylitis: a cross-sectional comparative study. Scand J Rheumatol, 44(2), 118–124. doi:10.3109/03009742.2014.956142

Tang Y, Yang P, Wang F, et al. (2018). Association of polymorphisms in ERAP1 and risk of ankylosing spondylitis in a Chinese population. Gene, 646, 8–11. doi:10.1016/j.gene.2017.12.050

Taylan A, Sari I, Akinci B, et al. (2012). Biomarkers and cytokines of bone turnover: extensive evaluation in a cohort of patients with ankylosing spondylitis. BMC Musculoskelet Disord, 13, 191. doi:10.1186/1471-2474-13-191

Wang B, Hao D, Zhang Z, et al. (2018). Inhibition effects of a natural inhibitor on RANKL downstream cellular signalling cascades cross-talking. J Cell Mol Med, 22(9), 4236–4242. doi:10.1111/jcmm.13703

Wen L, Wang GL, Jiang W, Zhou MQ. (2016). Research on changes of bone metabolism in male patients with ankylosing spondylitis and the effect of small dose of hormone. China Med Her, 13(22), 44–47.

Wu M, Chen M, Ma Y, et al. (2018). Dickkopf-1 in ankylosing spondylitis: review and meta-analysis. Clin Chim Acta, 481, 177–183. doi:10.1016/j.cca.2018.03.010

Xu Y, Yan XP, Zhang WJ. (2014). The function of bushen qiangdu recipe containing serum in OPG/RANKL pathway of ankylosing spondylitis patients. Chin J Integr Traditional West Med (Chin), 32(4), 521–524.

Zhang PY, Zhou SF, Wang MY, et al. (2015). Expression of chemokine CXCL16 and its receptor CXCR6 can be suppressed by recombinant human TNF receptor α-Ig fusion protein in ankylosing spondylitis. J Shandong Univ (Health Sci), 53(12), 51–56. doi:10.6040/j.issn.1671-7554.0.2015.253

Zhang WH, Huang XH, Chen JM. (2010). The activity of osteoclast precursor cells in peripheral blood of ankylosing spondylitis. Chin J Rheumatol, 14(6), 273–276. doi:10.3760/cma.j.issn.1007-7480.2010.06.004