Non-genetic biomarkers for ankylosing spondylitis: An umbrella review

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81973663; Talent project of Zhejiang Association for Science and Technology, Grant/Award Number: 2018YCGC003

Abstract
Objective: The objective of the study was to provide an overview of the existing evidence on non-genetic biomarkers for ankylosing spondylitis (AS).

Methods: In this umbrella review, we searched PubMed and Web of Science from database inception to October 31, 2020. Systematic reviews and meta-analyses of observational studies investigating the associations between any non-genetic biomarkers and AS were included. We estimated summary standardized mean difference (SMD) along with 95% confidence interval (CI), I² statistic, 95% prediction interval (PI), and assessed small-study effects and excess significance bias. The study was registered in PROSPERO with registration number of CRD42020218240.

Results: A total of 1276 publications were identified, of which 21 articles covering 43 non-genetic biomarkers were eligible for inclusion. Evidence of 22 (51%) non-genetic biomarkers exhibited a nominally significant effect (p < 0.05) on AS, and 7 associations (14%) showed small-study effects. The associations of platelet count (SMD: 0.53, 95% CI: 0.36 to 0.71) and serum interleukin (IL)-23 levels (SMD = 2.03, 95% CI: 1.27 to 2.79) with AS presented highly suggestive evidence, while circulating IL-17 levels (SMD = 2.36, 95% CI: 1.71, 3.01) and Treg/PBMC ratio (SMD = −0.75, 95% CI: −1.06 to −0.44) presented suggestive evidence. However, these associations showed large or very large between-study heterogeneity, suggesting an indefinite direction for the effect when 95% PIs were considered.

Conclusion: No convincing evidence supported the existence of a non-genetic biomarker for AS. Some highly suggestive associations might be affected by bias, therefore, promising non-genetic biomarkers for AS remain limited at least based on the current evidence from observational studies.

Key words
ankylosing spondylitis, biomarker, meta-analysis, systematic review, umbrella review

Changming Lv and Ding Ye contributed equally to this study.

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1 | INTRODUCTION

Ankylosing spondylitis (AS) is an inflammatory rheumatic disease which affects the axial spine and sacroiliac joints and leads to new bone formation and ankylosis. The prevalence of AS varies considerably across countries from 6.5/100,000 in Japan to 540/100,000 in Turkey. The disorder has a profound impact on the quality of life, affecting patients' physical function and mental health and can lead to significant loss of work productivity. Meanwhile, it poses a heavy burden for the society and the health system. Despite this, the etiology and pathogenesis of AS remains largely unknown. Growing evidence has suggested that both genetic predisposition and environmental factors contribute substantially to its development, and molecular biomarkers might reflect the effect of these factors. For example, a wide variety of molecules and immune cells have been implicated to be involved in AS development, including serum macrophage migration inhibitory factor and T lymphocytes. Recent studies have also attempted to find metabolites-based panels so as to aid in the diagnosis and treatment of AS.

In the past few decades, there are numerous systematic reviews and meta-analyses evaluating the associations between various biomarkers and AS that have been published. For example, circulating interleukin (IL)-17 levels were found to be elevated in patients with AS, and they also positively associated with disease activity. Besides, a meta-analysis of twenty studies involving 1592 patients with AS and 1064 healthy controls showed that levels of osteoprotegerin (OPG), receptor activator of nuclear factor-kB ligand (RANKL), and RANKL/OPG ratio were higher in patients relative to controls, and they could be used as potential AS susceptibility biomarkers. However, these studies are usually restricted to one or several specific biomarker(s), and the results are often vulnerable to diverse biases, such as publication bias and reporting bias. Moreover, these studies do not generate a hierarchy of existing evidence to examine the magnitude, direction, and significance of the associations.

Therefore, we performed an umbrella review of the evidence across existing systematic reviews and meta-analyses of observational studies for AS-related biomarkers. Considering that genetic risk factors have been recently explored in a number of meta-analyses of genome-wide association studies, genetic biomarkers were not considered in this work. We aimed to summarize the potential non-genetic biomarkers for AS, assess the quality of methodology, and determine the associations supported by robust epidemiological evidence.

2 | METHODS

We conducted an umbrella review, which is a systematic collection and assessment of multiple systematic reviews and meta-analyses on a wide range of non-genetic biomarkers associated with AS. We applied the standardized methods and followed the same principles as previous umbrella reviews for other medical conditions. Our study protocol was registered with PROSPERO (registration number: CRD42020218240).

2.1 | Search strategy

We systematically searched PubMed and Web of Science from database inception to October 31, 2020, to identify systematic reviews and meta-analyses of observational studies assessing the associations between non-genetic biomarkers and AS. Relevant keywords for the search strategy were (ankylosing spondylitis OR spondyloarthritis OR spondyloarthritides OR spondyloarthropathies OR spondylarthritides OR spondylarthropathies OR AS OR SpA) AND (systematic review OR meta-analysis). We also screened the references of the retrieved articles manually to identify eligible articles for additional inclusion.

2.2 | Eligibility criteria

Two investigators (Changming Lv and Yi Zhu) retrieved and scrutinized independently the full texts of potentially eligible articles. We included systematic reviews and meta-analyses of observational studies examining non-genetic biomarkers with relation to AS, of any ethnicity or sex in all countries and settings. We excluded meta-analyses that investigated the association of genetic markers with AS because these factors are not modifiable. We did not consider meta-analyses with the outcomes related to organ involvement, severity of symptoms, or progression of AS. We also excluded meta-analyses that regarded AS as a risk factor for other medical conditions and examined the association between AS and other diseases. We further excluded meta-analyses including less than three component studies. When the same association was investigated by two or more meta-analyses, we synthesized the largest evidence by including all relevant component articles of these meta-analyses.

2.3 | Data extraction

Two researchers (Changming Lv and Yi Zhu) performed data extraction independently, with discrepancies solved by a third researcher (Ding Ye). For each eligible article, we extracted the first author’s name, journal and year of publication, study design, involved biomarker(s), and the number of studies included. We also extracted the study-specific risk estimates (i.e., standardized mean difference [SMD]) along with their corresponding confidence interval (CI), p-value, and the number of cases and controls in each meta-analysis for the biomarker.
2.4 | Data analysis

We used a series of statistical analyses to evaluate the robustness of each association. First, we re-analyzed the summary effect size and its 95% CI by pooling SMD from individual studies under the random-effects model. The level of statistical significance was claimed at $p<0.05$, and we further evaluated $p$-values below two thresholds: 0.001 and $1 \times 10^{-6}$. We also evaluated whether the summary effect size of each association and the effect size of its largest study (smallest standard error [SE]) was consistent in terms of statistical significance.

The $I^2$ metric was used to evaluate between-study heterogeneity, which ranged between 0% and 100%. It quantifies the variability of effect estimates as a result of heterogeneity rather than sampling error. Values exceeding 50% or 75% are usually regarded as large and very large heterogeneity, respectively. We then estimated the 95% prediction interval (PI), which is the range in which we expect the effect size will lie for 95% of future studies on the same topic. The value further accounts for the between-study heterogeneity.

Furthermore, we assessed small-study effects (i.e., smaller studies exaggerated a reported effect compared with larger studies), using the regression asymmetry test proposed by Egger and colleagues. A $p<0.10$ combined with a more conservative result in the largest component study than that in random-effects meta-analysis suggested the presence of small-study effects.

Finally, we further applied the excess statistical significance test to assess the reporting bias. We performed a chi-square test to evaluate whether the observed (O) number of studies with nominally significant results ($p<0.05$) is larger than the expected (E) number. We calculated the power of each study in each meta-analysis using an algorithm from a noncentral t distribution. Excess statistical significance was claimed at two-sided $p<0.10$ with $O>E$.

2.5 | Quality of articles

The methodological quality of each meta-analysis was assessed with AMSTAR 2 (i.e., A Measurement Tool to Assess Systematic Reviews 2), which was a critical and well-founded appraisal instrument to evaluate the strength of systematic reviews and meta-analyses. It was composed of 16 items with seven critical domains which contain protocol registration, comprehensive literature search strategy, list of excluded studies and reason for the exclusion, satisfactory technique for assessing the risk of bias, appropriate method for statistical combination of results, discussion for the risk of bias in primary studies, adequate investigation, and discussion of publication bias. The methodological quality was classified into four grades: high, moderate, low, and critically low, instead of creating an overall score.

2.6 | Strength of epidemiological evidence

We followed the well-established criteria as previous umbrella reviews and classified the eligible studies into following levels: convincing (class I), highly suggestive (class II), suggestive (class III), weak (class IV), and non-significant associations. Convincing evidence should fulfill all the following criteria: statistical significance by a random-effects model at $p<10^{-6}$, based on more than 1000 cases; without large between-study heterogeneity ($I^2<50%$); 95% PI excluding the null value (0 in the case of SMDs); and no presence of small-study effects and excess significance bias. Highly suggestive evidence required statistical significance with $p<10^{-6}$, more than 1000 cases, and largest study with a statistically significant effect. The associations supported by more than 1000 cases and $p<10^{-3}$ were graded as suggestive. The remaining nominally significant peripheral biomarkers ($p<0.05$) were considered as having weak evidence. Non-significant associations were those with $p>0.05$.

The statistical analysis was performed by STATA version 15.1, and the power calculation was conducted by PASS version 15.

3 | RESULTS

3.1 | Search results

Overall, the search returned 1276 articles and 21 articles were deemed eligible for inclusion (Figure 1). Of the 29 articles screened in full text, five were excluded because they had no quantitative synthesis, three were excluded because all its included studies, and datasets were overlapped with the larger meta-analyses.

A total of 21 articles including 428 primary observational studies corresponded to 43 unique meta-analyses of the association between non-genetic biomarkers and AS from 2015 to 2020 were included. The number of meta-analyses per non-genetic biomarker ranged from 1 to 3. The median number of original studies per meta-analysis was 7 (interquartile range, 4–12) and that of AS cases was 355 (interquartile range, 197–765) per meta-analysis. In particular, the association of serum IL-23 levels, regulatory T cells (Treg)/PBMC ratio, mean platelet volume and serum dickkopf-1 levels with AS were reported in two meta-analyses, and for the same association, the meta-analysis with smaller datasets had some component studies that the larger meta-analysis did not cover, so we included all eligible individual studies to get a more precise estimate. All included meta-analyses used summary-level data from published articles, and none of them had access to individual participant data.

3.2 | Summary effects, heterogeneity, and bias tests

Across all the meta-analyses, the number of AS cases was more than 1000 in 8 meta-analyses (Table 1). For the 43 non-genetic
biomarkers, 22 (51%) biomarkers presented a nominally statistically significant effect ($p < 0.05$) on AS, and 12 (28%) biomarkers reached statistical significance at $p < 0.001$. However, only five (12%) associations reached $p < 1 \times 10^{-6}$. These potential non-genetic biomarkers pertained to platelet count (SMD = 0.53, 95% CI: 0.36, 0.71), serum IL-23 levels (SMD = 2.03, 95% CI: 1.27, 2.79), circulating IL-17 levels (SMD = 2.36, 95% CI: 1.71, 3.01), matrix metalloproteinase-3 levels (SMD = 1.29, 95% CI: 0.82, 1.75), and serum malondialdehyde (MDA) levels (SMD = 1.78, 95% CI: 1.10, 2.47), which were positively associated with AS.

Among the meta-analyses that were included, 6 of these associations showed publication bias according to the $p$-value of Egger's test (Table 1). Of the 22 significant associations, 14 (64%) non-genetic biomarkers showed a significant effect in the largest component study. All the 43 associations had PIs including the null value; four (9%) associations had large between-study heterogeneity ($50% < I^2 \leq 75%$), and 37 (86%) associations presented very large heterogeneity ($I^2 > 75%$). Moreover, seven (14%) associations showed evidence for small-study effects, and none of the associations presented evidence for excess statistical significance.

3.3 | Quality assessment

We assessed the quality of included articles according to the AMSTAR 2 criteria. The ratings of included articles were classified into two levels: high quality and low quality. Of the 21 articles, three (14%) articles were scored as high quality, and the remaining 18 (86%) articles were rated as low quality. The most critical flaws were the absence of a registered protocol (18 studies, 86%), resulting in downgrading to low quality. None of the articles reported the sources of funding for the studies included in the meta-analysis. The detailed results of each item of AMSTAR 2 for the included articles are presented in Table 2.

3.4 | Strength of epidemiological evidence

By applying the predefined methodological criteria, none of the potential non-genetic biomarkers were graded as convincing evidence. The association of platelet count and serum IL-23 levels with AS were supported by highly suggestive evidence (more than 1000 cases, $p < 10^{-6}$ and $p < 0.05$ in the largest study). Furthermore, circulating IL-17 levels and Treg/PBMC ratio presented suggestive evidence. A total of 18 (42%) associations presented weak evidence with $p < 0.05$. However, 21 (49%) associations did not present a nominally statistically significant effect ($p > 0.05$).

4 | DISCUSSION

In this umbrella review, we synthesized data from 43 different non-genetic biomarkers and summarized the evidence of their associations with AS. About half of the examined associations presented a nominally significant effect, while no associations met criteria for convincing evidence. Moreover, most of these associations were rated as weak evidence due to either a small number of cases or beyond the reach of significance threshold. Nevertheless, the associations of platelet count and serum IL-23 levels with AS were
| Reference      | Biomarker                                      | Study design                  | Number of cases/controls | Number of datasets | Effect size metric | Largest study effect size | Random effects summary effect size (95% CI) | P random   | 95% PI   | I²        | Egger p value | Small-study effects/excess statistical significance | Level of evidence |
|----------------|-----------------------------------------------|-------------------------------|--------------------------|--------------------|---------------------|--------------------------|--------------------------------------------|------------|----------|-----------|--------------|------------------------------------------------|-------------------|
| Han, 2017,38   | Serum IL-23 levels                            | Case–control/cross-sectional | 2392/2152                | 23                 | SMD                 | 4.48 (4.32 to 4.65)      | 2.03 (1.27 to 2.79)                       | 1.89 × 10⁻⁷ | 1.87 to 5.93 | 98.8      | 0.39         | No/No                                           | Highly suggestive   |
| Lee, 2020      |                                               |                               |                          |                    |                     |                          |                                           |            |           |           |              |                                                 |                   |
| Zhou, 2020     | Platelet count                                | Case–control/cross-sectional | 1197/845                 | 12                 | SMD                 | 0.39 (0.21 to 0.56)      | 0.53 (0.36 to 0.71)                       | 1.54 × 10⁻⁹ | -0.03 to 1.10 | 67.1      | 0.03         | Yes/No                                          | Highly suggestive   |
| Lai, 2019,52   | Treg/PBMC ratio                               | Case–control                 | 2676/2003                | 65                 | SMD                 | -0.74 (-0.99 to -0.49)   | -0.75 (-1.06 to -0.44)                    | 2.14 × 10⁻⁶ | -3.21 to 1.71 | 95.2      | 0.47         | No/No                                           | Suggestive          |
| Li, 2020       |                                               |                               |                          |                    |                     |                          |                                           |            |           |           |              |                                                 |                   |
| Zhang, 2019    | Circulating IL-17 levels                      | Case–control/cross-sectional | 1335/1002                | 26                 | SMD                 | -0.03 (-0.27 to 0.22)    | 2.36 (1.71 to 3.01)                       | 1.34 × 10⁻¹² | -1.11 to 5.82 | 97.4      | <0.01       | Yes/No                                          | Suggestive          |
| Cai, 2015      | Serum total vitamin D levels                  | Case–control                 | 725/646                  | 12                 | SMD                 | -0.52 (-0.81 to -0.23)   | -0.70 (-1.11 to -0.29)                    | 8.08 × 10⁻⁴ | -2.28 to 0.88 | 91.8      | 0.12         | No/No                                           | Weak                |
| Cai, 2015      | Serum 25-OHD levels                           | Case–control                 | 533/478                  | 8                  | SMD                 | -0.52 (-0.81 to -0.23)   | -0.67 (-1.10 to -0.24)                    | 0.002      | -2.17 to 0.83 | 89.8      | 0.15         | No/No                                           | Weak                |
| Chen, 2019     | Serum osteoprotegerin levels                  | Case–control/cross-sectional | 1524/1001                | 19                 | SMD                 | -0.99 (-1.17 to -0.80)   | 0.42 (0.03 to 0.81)                       | 0.04       | -1.39 to 2.22 | 94.9      | 0.02         | Yes/No                                          | Weak                |
| Chen, 2019     | Serum receptor activator of nuclear factor-κB ligand levels | Case–control/cross-sectional | 748/445                  | 11                 | SMD                 | -0.11 (-0.36 to 0.15)    | 1.12 (0.55 to 1.68)                       | 1.09 × 10⁻⁴ | -1.06 to 3.29 | 94.4      | 0.01         | Yes/No                                          | Weak                |
| Chen, 2019     | RANKL/OPG ratio                               | Case–control                 | 390/193                  | 6                  | SMD                 | -0.15 (-0.41 to 0.11)    | 0.69 (0.08 to 1.30)                       | 0.026      | -1.45 to 2.83 | 89.3      | 0.009       | Yes/No                                          | Weak                |
| Deng, 2019     | Red cell distribution width                   | Case–control/cross-sectional | 775/972                  | 9                  | SMD                 | -0.02 (-0.25 to 0.22)    | 0.68 (0.30 to 1.05)                       | 4.65 × 10⁻⁴ | -0.71 to 2.06 | 92.6      | 0.87         | No/No                                           | Weak                |
| Gao, 2015      | Serum matrix metalloproteinase-3 levels       | Case–control                 | 707/412                  | 13                 | SMD                 | 1.27 (0.99 to 1.54)      | 1.29 (0.82 to 1.75)                       | 5.33 × 10⁻⁸ | -0.55 to 3.13 | 90.5      | 0.36         | No/No                                           | Weak                |
| Huang, 2016    | Serum/plasma pentraxin 3 levels               | Case–control/cross-sectional | 99/79                    | 3                  | SMD                 | 0.30 (-0.18 to 0.78)     | 0.93 (0.17 to 1.68)                       | 0.02       | -7.83 to 9.68 | 77.0      | 0.29         | No/No                                           | Weak                |
| Reference | Biomarker | Study design | Number of cases/controls | Number of datasets | Effect size metric | Largest study effect size | Random effects summary effect size (95% CI) | P random | 95% PI | $I^2$ | Egger p value | Small-study effects/excess statistical significance | Level of evidence |
|-----------|-----------|--------------|--------------------------|--------------------|--------------------|--------------------------|--------------------------------------------|----------|-------|------|----------------|-----------------------------------------------|------------------|
| Liu, 2015<sup>58</sup> | Serum IL-6 levels | Case–control | 320/226 | 8 | SMD | 0.50 (0.11 to 0.89) | 2.51 (1.32 to 3.69) | $3.47 \times 10^{-5}$ | -1.74 to 6.75 | 96.5 | 0.02 | Yes/No | Weak |
| Li, 2020<sup>59</sup> | Serum TOS levels | Case–control/cross-sectional | 323/281 | 7 | SMD | 0.31 (-0.03 to 0.65) | 1.02 (0.48 to 1.57) | $2.43 \times 10^{-4}$ | -0.98 to 2.94 | 89.3 | 0.17 | No/No | Weak |
| Li, 2020<sup>59</sup> | Serum MDA levels | Case–control/cross-sectional | 429/359 | 10 | SMD | 1.18 (0.89 to 1.48) | 1.78 (1.10 to 2.47) | $3.40 \times 10^{-7}$ | -0.72 to 4.29 | 93.5 | 0.11 | No/No | Weak |
| Li, 2020<sup>59</sup> | Serum NO levels | Case–control/cross-sectional | 205/171 | 6 | SMD | 0.50 (0.19 to 0.81) | 0.44 (0.08 to 0.80) | 0.02 | -0.63 to 1.51 | 57.4 | 0.80 | No/No | Weak |
| Yang, 2017<sup>62</sup> | Serum resistin levels | Case–control | 172/126 | 4 | SMD | 0.53 (0.08 to 0.98) | 1.41 (0.29 to 2.53) | 0.01 | -3.93 to 6.75 | 93.7 | 0.19 | No/No | Weak |
| Yang, 2019<sup>63</sup> | Serum bone morphogenetic protein-2 levels | Case–control | 300/155 | 5 | SMD | 0.30 (-0.06 to 0.66) | 1.18 (0.21 to 2.16) | 0.02 | -2.59 to 4.96 | 94.7 | 0.16 | No/No | Weak |
| Zhu, 2018<sup>64</sup> | Serum/plasma high mobility group box 1 levels | Case–control/cross-sectional | 248/130 | 3 | SMD | 2.85 (2.45 to 3.26) | 1.56 (0.22 to 2.90) | 0.02 | -15.58 to 18.69 | 96.3 | 0.43 | No/No | Weak |
| Cai, 2015<sup>54</sup> | Serum 1,25OHD levels | Case–control | 192/168 | 4 | SMD | -0.11 (-0.48 to 0.27) | -0.73 (-1.81 to 0.35) | 0.19 | -5.91 to 4.46 | 95.4 | 0.56 | No/No | NS |
| Cai, 2015<sup>54</sup> | Serum PTH levels | Case–control | 507/465 | 7 | SMD | 0.22 (-0.06 to 0.51) | -0.10 (-0.57 to 0.37) | 0.67 | -1.77 to 1.57 | 91.8 | 0.45 | No/No | NS |
| Cai, 2015<sup>54</sup> | Serum calcium levels | Case–control | 311/297 | 4 | SMD | 0.07 (-0.07 to 0.31) | 0.12 (-0.07 to 0.31) | 0.22 | -0.48 to 0.72 | 25.2 | 0.72 | No/No | NS |
| Cai, 2015<sup>54</sup> | Serum ALP levels | Case–control | 307/197 | 4 | SMD | 0.17 (-0.16 to 0.49) | 0.21 (-0.01 to 0.43) | 0.06 | -0.49 to 0.91 | 28.6 | 0.46 | No/No | NS |
| Deng, 2019<sup>65</sup>; Zhou, 2020<sup>51</sup> | Mean platelet volume | Case–control/cross-sectional | 1105/847 | 12 | SMD | 0.01 (-0.23 to 0.24) | 0.08 (-0.27 to 0.43) | 0.65 | -1.27 to 1.43 | 92.3 | 0.58 | No/No | NS |
| Reference | Biomarker                     | Study design          | Number of cases/controls | Number of datasets | Effect size metric | Largest study effect size | Random effects summary effect size (95% CI) | P random | 95% PI | I² | Egger p value | Small-study effects/excess statistical significance | Level of evidence |
|-----------|-------------------------------|-----------------------|--------------------------|--------------------|-------------------|-------------------------|---------------------------------------------|----------|-------|----|----------------|-----------------------------------------------|------------------|
| Li, 2020  | Treg/CD4+ T cell ratio       | Case–control         | 626/394                  | 16                 | SMD               | -0.28 (-0.70 to -0.14) | -0.18 (-0.72 to -0.36)                  | 0.51     | -2.48 to 2.11 | 93.2 | 0.87          | No/No                             | NS                |
| Li, 2020  | Serum MPO levels             | Case–control         | 95/106                   | 3                  | SMD               | 0.57 (0.14 to 0.99)    | 0.92 (-0.71 to 2.56)                   | 0.27     | -19.88 to 21.73 | 95.9 | 0.85          | No/No                             | NS                |
| Li, 2020  | Erythrocytes MDA levels      | Case–control         | 77/77                    | 3                  | SMD               | 0.67 (0.26 to 1.08)    | 0.71 (-0.38 to 1.79)                   | 0.20     | -12.56 to 13.97 | 87.4 | 0.86          | No/No                             | NS                |
| Li, 2020  | Serum TAS levels             | Case–control/cross-sectional | 355/229                  | 7                  | SMD               | -0.13 (-0.47 to 0.21)  | -1.14 (-2.32 to 0.04)                  | 0.06     | -5.43 to 3.15    | 97.1 | 0.65          | No/No                             | NS                |
| Li, 2020  | Serum ARE levels             | Case–control         | 112/90                   | 3                  | SMD               | 0.05 (-0.47 to 0.58)   | -1.34 (-2.77 to 0.09)                  | 0.07     | -19.51 to 16.84 | 94.9 | 0.42          | No/No                             | NS                |
| Li, 2020  | Serum CAT levels             | Case–control         | 150/92                   | 3                  | SMD               | -0.60 (-0.92 to -0.29) | 0.10 (-0.82 to 1.03)                   | 0.83     | -11.25 to 11.45 | 87.0 | 0.22          | No/No                             | NS                |
| Li, 2020  | Serum SOD levels             | Case–control         | 270/226                  | 7                  | SMD               | -0.83 (-1.16 to -0.51) | -0.34 (-1.21 to 0.53)                  | 0.44     | -3.47 to 2.78   | 94.5 | 0.94          | No/No                             | NS                |
| Li, 2020  | Erythrocytes CAT levels      | Case–control/cross-sectional | 182/201                  | 4                  | SMD               | -0.99 (-1.28 to -0.70) | 0.14 (-0.92 to 1.20)                  | 0.80     | -4.94 to 5.22   | 95.2 | 0.33          | No/No                             | NS                |
| Li, 2020  | Erythrocytes GPx levels      | Case–control/cross-sectional | 195/214                  | 5                  | SMD               | -0.95 (-1.24 to -0.66) | -0.64 (-1.50 to 0.22)                  | 0.14     | -3.90 to 2.62   | 92.7 | 0.88          | No/No                             | NS                |
| Li, 2020  | Erythrocytes SOD levels      | Case–control/cross-sectional | 166/166                  | 3                  | SMD               | 0.49 (0.09 to 0.9)     | -2.22 (-6.10 to 1.65)                  | 0.26     | -52.36 to 47.91 | 99.2 | 0.59          | No/No                             | NS                |
| Ma, 2020  | Fecal calprotectin levels    | Case–control         | 164/240                  | 3                  | SMD               | 0.26 (-0.06 to 0.59)   | 2.11 (-0.34 to 4.56)                   | 0.09     | -29.43 to 33.66 | 98.7 | 0.24          | No/No                             | NS                |
| Song, 2020| Platelet-to-lymphocyte ratio| Case–control/cross-sectional | 460/292                  | 5                  | SMD               | 0.02 (-0.21 to 0.25)   | 0.20 (-0.04 to 0.44)                   | 0.11     | -0.56 to 0.96   | 57.0 | 0.80          | No/No                             | NS                |
| Wu, 2018; Zhang, 2017 | Serum dickkopf-1 levels | Case–control/cross-sectional | 1433/982                  | 25                 | SMD               | -1.48 (-1.82 to -1.14) | 0.45 (-0.16 to 1.05)                  | 0.15     | -2.75 to 3.64   | 97.5 | 0.06          | Yes/No                            | NS                |
| Yang, 2017| Serum leptin levels          | Case–control/cross-sectional | 619/481                  | 15                 | SMD               | -0.310 (-0.619 to -0.001) | 0.83 (-0.12 to 1.77)                  | 0.09     | -3.27 to 4.93   | 97.6 | 0.29          | No/No                             | NS                |
Platelet count, which is a common index of platelet activation, was found to be higher in AS patients with highly suggestive evidence. For the largest component study, there was also statistically significant differences in platelet count between AS patients and controls. In our umbrella review, we observed that the association between platelet count and AS had large between-study heterogeneity, source of which might be erythrocyte sedimentation rate (ESR) and the disease activity score. Besides, the impact of medication compliance and preanalytical factors might also account for the heterogeneity. In addition, the SMD was 0.53 (95% CI: 0.36 to 0.71) in a meta-analysis of 12 studies with evidence of small-study effects. As for the underlying mechanism of platelets in the development and progression of AS, there were several possible explanations. First, platelet count is a marker of inflammation, which plays a pivotal role in the development of AS. Previous evidence has shown that the platelet count in AS patients was decreased through anti-inflammatory therapy. Second, platelet count might regulate the progress of AS through gut microbiome. Recently, Yoon et al. found that the increased platelet counts could have an adverse effect on gut microbiome diversity and composition. It is well recognized that gut microbiome was vital for protection against invading bacteria, regulation of host physiological functions, and homeostasis of immunity. To be noted, gut microbiome was different between AS patients and healthy controls including 23,709 genes and 12 metagenomic species, and the alterations of the gut microbiome were correlated with the development of AS. Third, endothelial dysfunction (ED) has been found in the process of AS, then platelet count had a compensatory increase with the repair in the endothelial cells, which reflected ED in AS patients. Specifically, ED was the first step in the development of atherosclerosis and closely associated with disease activity and disease duration of AS. Therefore, platelet count could be an indicator of endothelial repair activity and might participate in the development of AS. However, further studies are needed to elucidate the mechanism how the platelet participates in the development and progression of AS.

IL-23, a pro-inflammatory cytokine mainly produced by macrophages and dendritic cells, plays a vital role in the differentiation and activation of T helper 17 (Th17) cells. Two related meta-analyses showed statistically significant associations, and the overall summary effect in our umbrella review suggested that serum IL-23 was higher in patients with AS than in healthy controls, which was supported by highly suggestive evidence. Of the largest study in the umbrella review including 1001 AS patients and 1011 controls presented consistent results with the summary effect. It was considered that sample size, ethnicity, covariates adjusted, and publication year might be the sources of the very large between-study heterogeneity. Moreover, the combination of the two meta-analyses might also result in the presence of heterogeneity.
TABLE 2 AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews 2) scoring of the 21 articles retained in the umbrella review.

| Author, Year | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12 | Q13 | Q14 | Q15 | Q16 | Total |
|--------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------|
| Lai, 2019    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | High quality |
| Li, 2020     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | High quality |
| Li, 2020     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | High quality |
| Cai, 2015    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Chen, 2019   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Deng, 2019   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Gao, 2015    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Huang, 2016  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Han, 2017    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Liu, 2015    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Lee, 2020    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Ma, 2020     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Song, 2020   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Wu, 2018     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Xu, 2020     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Yang, 2017   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Yang, 2019   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Zhang, 2017  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Zhu, 2018    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Zhang, 2019  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |
| Zhou, 2020   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | Low quality |

Note: Green, yellow and red were regarded as “Yes,” “Partial Yes,” and “No,” respectively.
the pathophysiology of IL-23 in the development of AS remained incompletely understood, there were some established concepts. First, IL-23 might play the regulatory role of AS mediated by the binding to IL-23 receptor (IL-23R) complex. A recent meta-analysis showed that IL-23R polymorphisms were associated with the risk of AS. Second, IL-23 contributed to the differentiation from CD4+ T cells into Th17 cells and the latter can cause tissue damage in multiple organs containing joints through producing IL-17. Third, it has been reported that the damaged function of endoplasmic reticulum aminopeptidase 1 (ERAP1) led to the accumulation of human leukocyte antigen (HLA)-B27 in endoplasmic reticulum (ER), subsequently resulting in the excessive production of unfolded protein response (UPR) in cells and elevated level of IL-23 due to the activated UPR.

IL-17 is also a pro-inflammatory cytokine secreted by Th17 cells. In the umbrella review, IL-17 was higher in AS patients, which was supported by suggestive evidence with very large between-study heterogeneity and small-study effects. Elevated IL-17 might contribute to the development of AS in the following ways. First, it has been established that IL-17 could recruit other leukocytes to participate in acute and chronic inflammation, which were signal features of the pathogenesis of AS. Second, IL-17 could suppress matrix production in chondrocytes and osteoblasts, leading to the damage of joint and the defect of tissue repair. Third, recently, Hu et al. found that serum tumor necrosis factor-alpha stimulated gene-6 (TSG-6) can effectively evaluate the severity of rheumatoid arthritis (RA), and can serve as a potential biomarker for the diagnosis of RA. Xu et al. also found that etanercept, the TNF-alpha inhibitor, can effectively improve the clinical indicators of AS patients. All these indicate that TNF may play an important role in the occurrence and development of rheumatologic disease. The combination of IL-17 and tumor necrosis factor (TNF) could cause irreversible cartilage damage, and in this process, IL-17 greatly contributed to the capacity of TNF, including inflammation and destruction in joints.

Tregs are important subsets of CD4+ T cells and play a pivotal role in the suppression of immune response. In the umbrella review, Treg/PBMC ratio was negatively correlated with AS. The association presented suggestive evidence with very large between-study heterogeneity, due to the identification of various Treg phenotypes with different biomarkers and inconsistencies in the degree of disease activity. Here were some possible mechanisms. First, functional defects of Tregs might play important roles in AS. These defective Tregs cells from AS patients are less able to control the proliferation of effector CD4+ T cells (Teff), due to defect of using IL-2, decreased STAT5 phosphorylation and higher CpG methylation levels in CNS2 region of the foxp3 gene. Second, Tregs could suppress other immune cells and mediate self-tolerance via releasing the immunosuppressive cytokines including TGF-β, IL-10, and IL-35, thus decreased Tregs might enhance the pathological process of AS.

Briefly, this is the first umbrella review applying established quantitative criteria on observational evidence summarizing the potential non-genetic biomarkers associated with AS. We have presented the most extensive critical appraisal of published associations to date. A wide variety of statistical tests were used to evaluate the strength and validity of the evidence, in attempt to find the most robust evidence. In addition, we endeavored to discuss the underlying mechanisms for each non-genetic biomarker with highly suggestive and suggestive evidence, which may be used as biomarker for the diagnosis and the treatment of AS.

Our work has several limitations. First, the study designs of articles included in this umbrella review are mainly case-control and cross-sectional, which are susceptible to various biases. Second, the number of AS cases is small, which is more than 1000 only in eight meta-analyses. Therefore, large-scale prospective cohort studies are warranted in the future. Third, we included only associations that had been examined in meta-analyses, thus some potential associations with adequate evidence that had not been meta-analyzed were missed. We may also miss the most recent individual studies as the identification of individual studies is beyond the scope of the umbrella review. Fourth, we use AMSTAR 2 to assess the methodological quality and we found that only three out of the 21 eligible articles being of high quality and others being of low quality mainly due to a lack of registered protocol, indicating more high-quality studies are needed in the future. Finally, due to the nature of observational studies, though we have identified several associations with highly suggestive or suggestive evidence, the findings we identified do not necessarily imply causation.

5 | CONCLUSION

In conclusion, our umbrella review provided a comprehensive summary of the published systematic reviews and meta-analyses examining multiple non-genetic biomarkers of AS. Platelet count and serum IL-23 levels were supported by highly suggestive evidence, while circulating IL-17 levels and Treg/PBMC ratio presented suggestive evidence. Prospective cohort studies and randomized controlled trials are needed to confirm the findings of observational studies, and further studies are warranted to clarify the underlying mechanisms of these non-genetic biomarkers in the development and progression of AS.

FUNDING STATEMENT
The work was jointly supported by grants from the National Natural Science Foundation of China (81973663) and the Talent project of Zhejiang Association for Science and Technology (2018YGC003).

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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How to cite this article: Lv C, Ye D, Zhu Y, et al. Non-genetic biomarkers for ankylosing spondylitis: An umbrella review. *J Clin Lab Anal*. 2022;36:e24759. doi: [10.1002/jcla.24759](https://doi.org/10.1002/jcla.24759)