Clinicopathological characteristics and prognostic value of cancer stem cell marker CD133 in breast cancer: a meta-analysis

Zhan Li1, * Songcheng Yin2, * Lei Zhang3 Weiguang Liu1 Bo Chen1 Hua Xing2

1Department of Breast Surgery, 2Department of Surgical Oncology, The First Hospital of China Medical University, Shenyang, Liaoning, 3Department of Breast Surgery, China-Japan Union Hospital of Jilin University, Changchun, Jilin, People’s Republic of China

*These authors contributed equally to this work

Background: The association of CD133 overexpression with clinicopathological significance and prognosis in patients with breast cancer remains controversial. We thus performed a meta-analysis to evaluate the role of CD133 expression in the development and prognosis of breast cancer.

Methods: The databases PubMed, Embase, and Cochrane Library (updated to August 1, 2016) were searched. Pooled odds ratios (ORs) or hazard ratios (HRs) with 95% confidence intervals (95% CI) were used to evaluate the impact of CD133 expression on clinicopathological features, overall survival, and disease-free survival.

Results: A total of 1,734 patients from 13 studies were subject to final analysis. The results showed a significant association between overexpression of CD133 and estrogen receptor status (OR 0.35, 95% CI 0.18–0.70), progesterone receptor status (OR 0.56, 95% CI 0.43–0.74), human epidermal growth factor-2 status (OR 1.81, 95% CI 1.33–2.45), lymph node metastasis (OR 1.98, 95% CI 1.34–2.92), and tumor histological grade (OR 1.79, 95% CI 1.26–2.54) in breast cancer. However, no significant correlation was found between upregulation of CD133 expression and onset age (OR 1.03, 95% CI 0.70–1.53) or tumor size (OR 1.29, 95% CI 0.80–2.09). Moreover, CD133-positive breast cancer patients had a higher risk of mortality (HR 1.91, 95% CI 1.21–3.03) and disease progression (HR 2.70, 95% CI 1.26–2.54).

Conclusion: This meta-analysis suggested that CD133 might be a predictor of clinical outcomes as well as prognosis and could be a potentially new gene therapy target for breast cancer patients.

Keywords: CD133, CSCs, breast cancer, prognosis, biomarker, meta-analysis

Introduction

Breast cancer is the most commonly occurring malignant tumor in women, with ~1.67 million new cases (25% of all cancers) diagnosed worldwide in 2012. It is the most frequent cause of cancer death (522,000 deaths, 14.7% of total) in females.1 From the time that distinct molecular subtypes were proposed by Perou et al in 2000,2 the combination of traditional pathological morphological classification and molecular subtyping has been applied to determine the optimal therapy for breast cancer patients. However, the prognosis of breast cancer patients remains unsatisfactory. Consequently, it is critical to predict prognosis through novel biomarkers that can serve as potential therapeutic targets in breast cancer patients.

There is a growing realization that a small subpopulation of cells with stem cell-like features resides in the tumor tissue and is known as cancer stem cells (CSCs).3
Quality assessment
The selected cohort studies were analyzed from three perspectives, selection, comparability, and outcomes, by two investigators independently, according to the Newcastle–Ottawa scale (NOS). The details of NOS table are shown in Table S1.

Data extraction
The following details were extracted using a predefined form: first author’s name, publication year, country, mean age, tumor stage, total number of included patients, median follow-up time, cutoff value, survival outcome, outcome method, and estimated HR.

Statistical analysis
This meta-analysis was performed using Stata Version 12.0 (Stata Corporation, College Station, TX, USA). For the pooled analysis of clinicopathological features, OR was evaluated. HR was applied as a measure of the prognostic value. Study heterogeneity was evaluated using the chi-square-based Q test and I² statistic. Studies with an I²>50% or a P<0.05 was considered to have significant heterogeneity, and a random-effects model test was conducted. Otherwise, the fixed-effects model test was selected. Sensitivity analysis was performed to evaluate the stability of the pooled results. Publication bias was assessed using Begg’s funnel plots and Egger’s test. All P-values were two-sided and P<0.05 was considered statistically significant.

Results
Search results
A total of 424 citations were potentially identified for inclusion using the described search strategies. Through reviewing the title and abstracts, 381 papers were excluded. We then systematically read the full text of the remaining 43 articles and filtered out an additional 30 papers. Among the excluded papers, 13 studies were experimental studies, six studies were not correlated with target protein, three studies had overlapped data with other published trials, six studies had no sufficient survival data to analyze, and two studies are reviews. Ultimately, 13 studies11–23 were included (Figure 1).

Characteristics of included studies
The details of 13 included studies selected from the literature search are summarized in Table 1. In total, 13 eligible articles with 1,734 patients were analyzed for clinicopathological features, and five qualified studies with 879 patients were analyzed for survival outcomes. These cohort studies

The activity is achieved by self-renewal, unlimited proliferation, and high tumorigenicity. Recently, it has been found that CSCs have similar specific cell surface molecular markers to stem cells such as CD44, CD24, ALDH1, and CD133. CD133, which is known as prominin-1, a pentaspan transmembrane cell surface glycoprotein with a molecular weight of 120 kDa, is located in the plasma membrane protrusions. It was initially considered to be a marker of hematopoietic stem cells by Yin et al.5 Biological functions of CD133 include tumor initiation, cellular migration, vasculogenic mimicry, and drug resistance.6 Although CD133 has been studied intensely in various types of solid tumors, including lung cancer,7 renal cancer,8 esophageal carcinoma,9 and gastric cancer,10 the role of CD133 in breast cancer has not been verified.

In this meta-analysis, we aimed to evaluate the relationship between CD133 expression in breast cancer and clinicopathological features, including tumor size, lymph node metastasis, histological grade, onset age, receptor status (estrogen receptor [ER], progesterone receptor [PR], and human epidermal growth factor-2 [HER2]) as well as prognostic significance.

Methods
Literature search
The literature in the following electronic databases – PubMed, Embase, and the Cochrane Library (updated to August 1, 2016) – was systematically searched. We performed our search using the medical subject heading (MeSH) term “CD133” and its synonyms: “fudene”, “prominin”, “PROML1”, and “AC141 antigen”. These keywords were then combined with “breast”, “mammary”, “cancer”, “neoplasm”, “carcinoma”, “prognosis”, and “survival” using the Boolean “OR” term or the Boolean “AND” term.

Study selection criteria
Study selection inclusion criteria were as follows: 1) patients diagnosed with breast cancer using pathological and histological examinations, 2) full text and published in English, 3) clinicopathological and survival (overall survival [OS] and disease-free survival [DFS]) outcomes were recorded, 4) CD133 expression was detected in primary breast tumors, and 5) outcomes were recorded using odds ratios (ORs) or hazard ratios (HRs) with 95% confidence intervals (CIs).

Exclusion criteria were as follows: 1) meeting abstracts, comments, case reports, reviews, and meta-analyses; 2) experiments on cell lines and animals; 3) metastatic or recurrent cancer; and 4) duplicate studies.
were conducted in eight regions (Italy, Taiwan, China, New Zealand, Japan, Turkey, Egypt, and Korea) and were published between 2009 and 2016 with a mean patient age ranging from 45.6 years to 61.8 years. Univariate analysis was applied for the survival data.

### Meta-analysis of clinicopathological parameters

**CD133 expression and ER, PR, and HER2 status**

The pooled ORs indicated that overexpression of CD133 was significantly associated with ER status (positive vs negative: OR 0.35, 95% CI 0.18–0.70; Figure 2A), PR status (positive vs negative: OR 0.56, 95% CI 0.43–0.74; Figure 2B), and HER2 status (≥2+ vs 1+, OR 1.81, 95% CI 1.33–2.45; Figure 2C). In the subgroup analysis of ER status, there were no significant heterogeneity in the group of non-Asian ($I^2=11.1\%$, $P=0.289$) and group of mean age was ≥50 years ($I^2=0.0\%$, $P=0.689$). The details are shown in Table 2.

**CD133 expression and age, tumor, node, and grade**

Our results showed that there was no significant association between CD133 high expression and onset age (≥50 vs <50 OR 1.03, 95% CI 0.70–1.53; Figure 3A) and tumor size (≥2 cm vs <2 cm, OR 1.29, 95% CI 0.80–2.09; Figure 3B). However, breast cancer with CD133 expression was associated with lymph node metastasis (positive vs negative: OR 1.98, 95% CI 1.34–2.92; Figure 3C) and tumor histological grade (III vs I–II: OR 1.79, 95% CI 1.26–2.54; Figure 3D). We further performed subgroup analysis from Table 1 characteristics of studies included in our meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>No of patients</th>
<th>Mean age (years)</th>
<th>Follow-up, months (range)</th>
<th>Stage</th>
<th>No of patients</th>
<th>Mean age (years)</th>
<th>Follow-up, months (range)</th>
</tr>
</thead>
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<tr>
<td>Lin et al.</td>
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<td>49</td>
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<td>L II</td>
<td>49</td>
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<td>Taiwan</td>
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<td>52</td>
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<td>I–III</td>
<td>49</td>
<td>52</td>
<td>41.6 (3–49.3)</td>
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<tr>
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<td>New Zealand</td>
<td>204</td>
<td>64.2</td>
<td>48.0 (9–83)</td>
<td>L II</td>
<td>204</td>
<td>64.2</td>
<td>48.0 (9–83)</td>
</tr>
<tr>
<td>Kapucuoglu et al.</td>
<td>2015</td>
<td>Turkey</td>
<td>204</td>
<td>56</td>
<td>46.8 (2–89)</td>
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<td>204</td>
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<td>46.8 (2–89)</td>
</tr>
<tr>
<td>Zhao et al.</td>
<td>2011</td>
<td>China</td>
<td>67</td>
<td>47</td>
<td>36 (1–64)</td>
<td>I–III</td>
<td>67</td>
<td>47</td>
<td>36 (1–64)</td>
</tr>
<tr>
<td>Mansour &amp; Atwa</td>
<td>2015</td>
<td>Egypt</td>
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<td>49</td>
<td>46.5 (1–108)</td>
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<tr>
<td>Kim et al.</td>
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<td>Korea</td>
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<td>Mansour &amp; Awad</td>
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<td>Lv et al.</td>
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<td>45.6</td>
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<td>Han et al.</td>
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<td>China</td>
<td>54</td>
<td>45.6</td>
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<td>L II</td>
<td>54</td>
<td>45.6</td>
<td>46.5 (1–108)</td>
</tr>
</tbody>
</table>

**Abbreviations:** NR, not reported; AB, antibody; ID, intensity distribution; CFC, clinicopathological features; OS, overall survival; DFS, disease-free survival; NOS, Newcastle-Ottawa scale.
Figure 2 Forest plots of ORs for the correlation between cD133 overexpression and ER, PR, and HER2.

Notes: (A) OR for the relation between cD133 overexpression and ER; (B) OR for the relation between cD133 overexpression and PR; and (C) OR for the relation between cD133 overexpression and HER2. Weights are from random-effects analysis.

Abbreviations: OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor-2.
Table 2 Stratified analysis of pooled hazard ratios or odds ratios of breast cancer patients with CD133 expression on survival data and clinicopathological features

<table>
<thead>
<tr>
<th>Categories</th>
<th>Region</th>
<th>Sample sizes</th>
<th>Mean age</th>
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</thead>
<tbody>
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<td>non-Asian</td>
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<td>HR/OR</td>
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<td></td>
<td></td>
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<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DFS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ER</td>
<td></td>
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<td></td>
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<tr>
<td>PR</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HER2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>1.50(0.86, 2.61)</td>
<td></td>
<td>1.24(0.48, 2.27)</td>
</tr>
<tr>
<td>N</td>
<td>2.42(1.67, 3.51)</td>
<td></td>
<td>0.72(1.43, 3.44)</td>
</tr>
<tr>
<td>G</td>
<td>1.85(1.40, 2.46)</td>
<td></td>
<td>0.58(2.30, 6.32)</td>
</tr>
</tbody>
</table>

**Abbreviations:** OS, overall survival; DFS, disease-free survival; HR, hazard ratio; OR, odds ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor-2; T, tumor size; N, lymph node; G, tumor histological grade; H, heterogeneity.
and are the source that enables the tumor to keep growing and spreading. This recognition of the importance of CSCs in tumors has not only led to new directions and perspectives that resulted in a reexamination of the causes of tumor initiation, development, and therapeutic resistance but also provided new ideas for early diagnosis and treatment.

CSCs can be distinguished from tumor cells through identification of specific molecular surface markers such as CD24, CD44, ALDH1, and ESA (epithelial specific antigen). Several meta-analysis studies have been performed to evaluate the association between biomarkers of CSCs and prognosis in various malignancies. A meta-analysis performed by Wang et al revealed that CD24 overexpression was significantly correlated with shortened OS in breast cancer patients. Wei et al conducted a pooled analysis of ALDH1 expression in lung cancer; the results showed that higher ALDH1 levels were associated with decreased DFS and OS.

CD133, a transmembrane cell surface glycoprotein, was initially found in hematopoietic stem cells and is considered to be a specific molecular biomarker of hematopoietic stem

![Figure 3 (Continued)](image-url)
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C  Study ID                      OR (95% CI)  % weight
Lin et al\(^{15}\) (2015)         2.10 (0.61, 7.20) 5.75
Liu et al\(^{12}\) (2009)         3.42 (1.31, 8.91) 7.48
Currie et al\(^{14}\) (2013)     0.76 (0.29, 2.00) 7.41
Di Bonito et al\(^{15}\) (2012)  0.65 (0.30, 1.39) 8.99
Aomatsu et al\(^{10}\) (2012)    2.39 (1.02, 5.60) 8.28
Kapucuoglu et al\(^{11}\) (2015) 0.83 (0.29, 2.33) 6.94
Zhao et al\(^{18}\) (2011)       6.04 (2.05, 17.85) 6.64
Mansour and Atwa\(^{19}\) (2015) 2.63 (1.20, 5.76) 8.81
Kim et al\(^{20}\) (2015)         1.90 (1.11, 3.28) 10.81
Liu et al\(^{21}\) (2013)         4.71 (2.16, 10.27) 8.82
Lv et al\(^{22}\) (2016)          1.09 (0.48, 2.47) 8.52
Han et al\(^{23}\) (2015)        3.11 (1.98, 4.89) 11.54
Overall (\(\chi^2=64.7\%, P=0.001\)) 1.98 (1.34, 2.92) 100

D  Study ID                      OR (95% CI)  % weight
Leni et al\(^{11}\) (2011)        1.87 (0.55, 6.29) 5.96
Lin et al\(^{12}\) (2015)         3.76 (0.72, 19.52) 3.72
Currie et al\(^{14}\) (2013)     10.94 (2.37, 50.54) 4.19
Di Bonito et al\(^{15}\) (2012)  0.77 (0.32, 1.86) 8.94
Aomatsu et al\(^{10}\) (2012)    3.15 (0.58, 17.08) 3.57
Kapucuoglu et al\(^{11}\) (2015) 1.01 (0.38, 2.68) 7.95
Zhao et al\(^{18}\) (2011)       2.27 (0.81, 6.36) 7.46
Mansour and Atwa\(^{19}\) (2015) 1.25 (0.60, 2.60) 10.90
Kim et al\(^{20}\) (2015)         1.55 (0.90, 2.64) 13.97
Liu et al\(^{21}\) (2013)         4.31 (2.08, 8.94) 10.94
Lv et al\(^{22}\) (2016)          1.56 (0.57, 4.32) 7.56
Han et al\(^{23}\) (2015)        1.30 (0.80, 2.11) 14.83
Overall (\(\chi^2=44.8\%, P=0.046\)) 1.79 (1.26, 2.54) 100

Figure 3  Forest plots of ORs for the correlation between CD133 overexpression and age, tumor size, lymph node metastasis, and tumor histological grade.

Notes: (A) OR for the relation between CD133 overexpression and age; (B) OR for the relation between CD133 overexpression and tumor size; (C) OR for the relation between CD133 overexpression and lymph node metastasis; and (D) OR for the relation between CD133 overexpression and tumor histological grade. Weights are from random-effects analysis.

Abbreviations: OR, odds ratio; CI, confidence interval.

cells.\(^{28}\) In recent years, CD133 as a stem cell marker was demonstrated to be expressed in many types of solid tumors, such as liver, colorectal, and ovarian cancers.\(^{29-31}\) However, the prognostic role of CD133 expression in breast cancer is still controversial. Kim et al\(^{20}\) suggested that CD133 high-expression patients had shorter OS and DFS than CD133 low-expression cases. Conversely, Currie et al\(^{14}\) found no significant difference between CD133 high expression and CD133 low expression in breast cancer patients regarding survival time. In view of the inconsistent conclusions on
the impact of CD133 expression in breast cancer patients, it was necessary to conduct a meta-analysis to evaluate the prognostic value of CD133 in breast cancer.

Based on our comprehensive analysis of published studies, we found that overexpression of CD133 was significantly associated with ER-positive status, PR-positive status, HER2-positive status, lymph node metastasis, and high histological grade. However, there was no significant association between CD133 high expression and large tumor size or late onset age. Furthermore, the overall analysis of prognosis revealed that CD133-positive breast cancer patients had a higher risk of mortality and a poorer DFS. In subgroup analysis by region, there was a difference between the Asian and non-Asian groups. In the Asian group, there was a significant association between CD133-positive breast cancer and poorer OS and DFS.
It was gradually discovered that several signaling pathways such as Hedgehog, Wnt, Notch, and NF-κB were involved in the CSC development, progression, differentiation, and metastasis. Based on the blockade of these signaling pathways, targeted therapy provides a new method to attack surface molecules of CSCs. Before 2010, gemtuzumab/ozogamicin, an antibody–drug conjugate of a recombinant humanized anti-CD33 monoclonal antibody, had been used in targeted therapy for clinical applications in acute myeloid leukemia patients, but has been pulled out of market due to high toxicity. Moreover, a recently published report in *Nature Communications* revealed that self-renewal of CD133 cells by IL6/Notch3 signaling regulates therapeutic resistance in metastatic breast cancer. Similarly, the results of our meta-analysis demonstrated that patients with high CD133-expressing tumors tended to have poorer survival. Consequently, targeted drugs that act on CD133 have the potential to be applied in the clinic, and breast cancer patients with high CD133 expression levels may benefit from them.

Previously, there was a similar meta-analysis published in 2010, which evaluated the association of CSCs with clinical outcome. They presented statistics on two indicators, CD44+/CD24−/low and ALDH1. However, the key point of our study focused on CD133 expression and its association with clinicopathological features and prognosis. Furthermore, statistical analysis of earlier studies on survival data was calculated using risk ratios (RRs). It is believed that the statistics of HRs take into consideration differences in end events, and also take into account the time to reach the end point and censored data. Consequently, the survival data statistic of HRs is calculated in our meta-analysis.

There were limitations in our meta-analysis. First, eligible studies were incorporated with diverse TNM stage and histological grade that may have potentially influenced the results. Second, although we collected all eligible studies for evaluating the association between CD133 expression and survival data, the sample size was not large enough, which in turn weakened the statistical power of the results. Finally, in this present analysis, the influence of bias could not be completely excluded.

### Conclusion

The present results provide some evidence on the clinical outcome and prognostic value of CD133 in breast cancer patients. High CD133 expression predicted a worse OS and DFS. CD133 markers may potentially serve as prognostic markers and novel potential therapeutic targets in breast cancer. Large-scale and standard cohort studies are required for further confirmation.

### Acknowledgment

This study was supported by the grants from the National Natural Science Foundation of China (no 81372811) and Science and Technology Agency of Liaoning Province (no 2013225049).

### Disclosure

The authors report no conflicts of interest in this work.

### References


## Table S1 Newcastle–Ottawa quality assessment scale (cohort studies)

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection Representativeness of the exposed cohort</th>
<th>Selection of the nonexposed cohort</th>
<th>Ascertainment of exposure</th>
<th>Demonstration that outcome of interest was not present at start of study</th>
<th>Comparability Comparability of cohorts on the basis of the design or analysis</th>
<th>Outcome Assessment of outcome</th>
<th>Was follow-up long enough for outcomes to occur?</th>
<th>Adequacy of follow-up of cohorts</th>
<th>Score</th>
</tr>
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<tbody>
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<td>Leni et al11</td>
<td>b★</td>
<td>★</td>
<td>★</td>
<td>★</td>
<td>a★</td>
<td>a★</td>
<td>★</td>
<td>b★</td>
<td>8</td>
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<td>Lin et al12</td>
<td>b★</td>
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<td>★</td>
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<td>a★</td>
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<td>★</td>
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<tr>
<td>Liu et al13</td>
<td>b★</td>
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<td>b★</td>
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<td>★</td>
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<td>Aomatsu et al16</td>
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<td>Zhao et al18</td>
<td>b★</td>
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<td>Mansour and Atwa19</td>
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**Notes:** A study can be awarded a maximum of one star for each numbered item within the “selection” and “outcome” categories. A maximum of two stars can be given for “comparability.”