Prognostic value of miR-21 for prostate cancer: a systematic review and meta-analysis

MY Cynthia Stafford¹, Colin E Willoughby¹, Colum P Walsh¹,², Declan J McKenna¹*

¹Genomic Medicine Research Group, Ulster University, Cromore Road, Coleraine, UK, BT52 1SA

²Centre for Research and Development, Region Gävleborg/Uppsala University, Gävle, Sweden

*Correspondence:

Declan McKenna
Biomedical Science Research Institute
University of Ulster
Cromore Road
Coleraine
Northern Ireland
BT52 1SA
Tel. +44 (0)2870 124356
E. dj.mckenna@ulster.ac.uk

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ABSTRACT:

Elevated levels of miR-21 expression are associated with many cancers, suggesting it may be a promising clinical biomarker. In prostate cancer (PCa), however, there is still no consensus about the usefulness of miR-21 as an indicator of disease progression. This systematic review and meta-analysis was conducted to investigate the value of miR-21 expression as a prognostic measurement in PCa patients. Medline (Ovid), EMBASE, Web of Science, Scopus and Cochrane Library databases were systematically searched for relevant publications between 2010 to 2021. Studies exploring the relationship between miR-21 expression, PCa prognosis and clinicopathological factors were selected for review. Those reporting hazard ratio (HR) and 95% confidence intervals (CIs) were subject to meta-analyses. Fixed-effect models were employed to calculated pooled HRs and 95% CIs. Risk of bias in each study was assessed using QUIPS tool. Certainty of evidence in each meta-analysis was assessed using GRADE guidelines. A total of 64 studies were included in the systematic review. Of these, 11 were eligible for inclusion in meta-analysis. Meta-analyses revealed that high miR-21 expression was associated with poor prognosis: HR=1.58 (95% CI=1.19-2.09) for biochemical recurrence, MODERATE certainty; HR=1.46 (95% CI=1.06-2.01) for death, VERY LOW certainty; and HR=1.26 (95% CI=0.70-2.27) for disease progression, VERY LOW certainty. Qualitative summary revealed elevated miR-21 expression was significantly positively associated with PCa stage, Gleason score and risk groups. This systematic review and meta-analysis suggests that elevated levels of miR-21 are associated with poor prognosis in PCa patients. miR-21 expression may therefore be a useful prognostic biomarker in this disease.

Key Words: Prostate cancer, microRNA, miR-21, systematic, meta-analysis, prognostic
INTRODUCTION:

Prostate cancer (PCa) is the most commonly diagnosed cancer for males in 105 countries including North and South America, Western Europe and Australia. The majority of PCa cases are localised disease with very high survival rate after initial treatment (~100% 5-year survival), but recurrence may occur in about 40% as biochemical recurrence (BCR) or distant metastasis that has a significantly poorer prognosis (~30% 5-year survival). Additionally, some may progress as castration-resistant prostate cancer (CRPC) or develop chemoresistance.

Currently, prognosis is predicted by considering cancer stage, Gleason score, prostate-specific antigen (PSA) level, patient’s health condition, treatment choice and treatment response. However, these clinicopathological factors still have certain limitations. For example, Gleason score is a histological method which is subject to inter-observer variability, and clinicians can find the grading system confusing. Staging may vary between clinical and pathological estimation, forcing clinicians to alter treatment regime, and prognosis for lower stage cancer is less than predictable. PSA lacks specificity and BCR, defined by rise in PSA level, does not necessarily predict clinical recurrence or metastasis with sufficient accuracy. Therefore, there is still a clear clinical need for novel molecular markers that may overcome some of these limitations.

MicroRNAs (miRNAs) are a class of non-coding molecules which have emerged as strong candidates for useful clinical biomarkers. Over the past decade, they have been actively researched in a wide range of diseases, including prostate cancer. miRNAs are estimated to regulate 60% of gene expression in human and some specifically target oncogenes or tumour suppressor genes. The aberrant expression of miRNAs can therefore contribute to cancer development and several dysregulated miRNAs have been associated with PCa progression. Importantly, miRNAs can be detected in blood and urine, as well as tissue. Indeed, they are known to be more stable in biofluids than other nucleic acids which gives them potential as diagnostic or prognostic markers.
However, more research is needed to understand which miRNAs are most relevant in prostate cancer.

miR-21 is one of the most studied miRNAs and there is a large body of evidence to suggest that it has a predominantly oncogenic function since it is over-expressed in many cancers\textsuperscript{14}. As one of the first miRNAs to be categorized as an ‘oncomiR’, it has been subsequently evaluated for its potential use as a clinical biomarker in various cancers\textsuperscript{15, 16, 17}. Several recent systematic reviews have found evidence that circulating miR-21 levels can predict poor prognosis in oesophageal, pancreatic, colorectal and breast cancers\textsuperscript{18, 19}. In urological cancers, including PCa, Chen et al. found some evidence that miR-21 over-expression was significantly associated with unfavourable prognosis in their integrated analysis\textsuperscript{20}. However, despite evidence that it can contribute to PCa development, no systematic review or meta-analysis to date has been carried out specifically for miR-21 in this setting. Therefore, the purpose of this paper is to systematically evaluate studies related to prognostic value of miR-21 in PCa, appraising study qualities and synthesising evidence by meta-analyses, data association and qualitative summary.
MATERIALS & METHODS:

Protocol and registration

This review was conducted following a protocol which was registered with the International Prospective Register of Systematic Reviews (PROSPERO; https://www.crd.york.ac.uk/prospero/) under the registration ID: CRD42020183408 on 23rd June 2020. The protocol was developed following guidance on PRISMA-P \(^\text{21}\), systematic review and meta-analysis of prognostic factor studies \(^\text{22}\) and the checklist of items recommended in the PRISMA statement \(^\text{23}\).

Search Strategy

Electronic databases from which records were retrieved include Medline (Ovid), EMBASE, Web of Science, Scopus and Cochrane Library, covering publications from 2010 to 2021 and they were last searched on 8th November 2021. Additionally, reference lists of included studies and relevant review papers were searched manually. Prognostic factor studies were prone to selective reporting in that miRNAs with insignificant findings might not be reported \(^\text{24}\), therefore a high-sensitivity approach was used in the search strategy as shown in Supplementary Table ST 1. Key words related to miRNAs, in addition to miR-21, were included to broaden the search to cover relevant studies that measured miR-21 but did not report the result. Retrieved records from databases were exported to systematic review manager Rayyan where duplicates were removed \(^\text{25}\). Titles and abstracts of remaining records were screened for relevance independently by two reviewers. Full text of studies selected for inclusion were subsequently imported into another systematic review manager Covidence (www.covidence.org) where studies were assessed for eligibility in duplicate. Any disagreements were resolved through discussion.

Eligibility criteria

For inclusion in the systematic review, original peer-reviewed human studies published in English from year 2010 to 2021 with full-text available online or from Ulster University Library were
included. *In vitro, in silico and in vivo* studies that did not include human participants were excluded. Studies without original human data which analysed publicly available human data (e.g., from The Cancer Genome Atlas repository) were not included to avoid multiple counting of sample size. Review-type studies and duplicate reports were excluded for the same reason. If the same study was published in multiple journals, only the most informative or the most recent one was included. Studies published before 2010 were excluded due to advances in miRNA technology.

For meta-analyses, studies with characteristics specified by PICOT (*Table 1*) were eligible for inclusion in meta-analysis. Length of follow-up was not restricted to broaden the number of inclusions and increase the number of eligible studies.

### Data collection process

A data extraction form adapted from CHARMS-PF checklist was created within *Covidence* to capture information about each study, source of data, PICOT details, sample size, missing data, statistical analysis methods, survival outcome results and/or association analysis results (*Supplementary Table ST 2*). Data was extracted independently in duplicate into separate forms. Completed forms were compared, and conflicts were resolved through discussion. Authors of 12 studies were contacted for missing data or clarifications (*Supplementary Table ST 3*). Only data relevant to prognosis were considered, therefore data related to diagnosis and healthy or benign prostatic hyperplasia (BPH) controls were disregarded.

### Risk of bias in individual studies

Judgment was made independently in duplicate using the Quality in Prognostic Factor Studies (QUIPS) tool which assesses risk of bias as HIGH, MODERATE, LOW or UNCLEAR in six domains (*Supplementary Table ST 4*) (*Table 2*). For domain 3 “Prognostic factor measurement”, methods accepted as reliable for miR-21 measurement were qPCR, sequencing and array technology. For domain 5
“Adjustment for covariates”, the core set of desired adjustment covariates was predefined as Gleason score/grade and pathological/clinical stage.

**Statistical Analysis**

The principal summary measure for meta-analysis was hazard ratio (HR), presented with 95% confidence interval (CI) and p-value. Kaplan Meier plot presented with log-rank p-value was also accepted. Eligible studies of similar design in terms of outcome and handling of miR-21 data were grouped into separate meta-analyses. For each meta-analysis effect estimates were pooled as HR (95% CI) based on fixed-effect inverse variance method in the review manager RevMan5.4 27. Statistical heterogeneity was assessed by visual inspection of the forest plot, chi-square (Chisq) test and I² test (Chisq p≤0.1 indicates significant heterogeneity; I² <30% denotes low/unimportant heterogeneity, 30-60% moderate heterogeneity, 50-90% substantial heterogeneity, and 75-100% considerable heterogeneity). Impact on the robustness of analyses by the presence of an outlier and the inclusion of a study that introduced clinical heterogeneity was assessed by sensitivity analyses.

For qualitative summary, association measure included but was not limited to correlation, fold change (FC) or mean difference.

**Certainty of evidence**

For each analysis the certainty of evidence was rated according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines 28. This review estimated the prognostic value of miR-21 in PCa as an exploratory study without direct association with clinical decision making, therefore, certainty was rated based on the non-contextualised setting as HIGH, MODERATE, LOW or VERY LOW certainty. Starting from HIGH certainty, evidence could be rated down in five domains: risk of bias, inconsistency, indirectness, imprecision and publication bias; or rated up in three domains: large effect, dose response and plausible confounding. Assessment of publication bias was not possible due to low number of studies eligible for each analysis, which meant any test of bias would be underpowered.
RESULTS:

Study selection

Study selection was as shown in the flow diagram (Figure 1). Up until 23rd July 2020, 4859 records were retrieved from database searching and a further 90 were identified from manual searching of reference lists of included studies and relevant reviews. After duplicates were removed (n=2800), record screening identified 76 eligible studies for full-text assessment. 13 full-text articles were ineligible due to lack of prognostic data (n=8), lack of miR-21 data (n=4) and lack of original human prognostic data (n=1) (Supplementary Table ST 5). The remaining 63 studies 29-77, 78-92 were included in the systematic review, with 10 eligible for meta-analysis. On 8th November 2021, an update screening for meta-analysis identified one more eligible study 78, bringing the total number of included studies to 64, with 11 eligible for meta-analysis.

Study characteristics

Characteristics of all 64 studies included in this systematic review are summarised in Supplementary Table ST 6. Each included study was assigned a Study ID composed of first author’s name and publication year. The PICOT eligibility criteria (Table 1) identified studies on PCa patient cohorts which could be stratified against measurable parameters and outcomes for inclusion in the meta-analysis. A total of 11 studies, with study sizes ranging from 31 to 478 participants, encompassing 1485 PCa patients total, were eligible for meta-analysis (Tables 2 & 3). Amankwah2013 31 indicated that the recurrent group was oversampled, no rationale was provided. Sharova2021 78 was clearly indicated as prospective; Zedan2017 85 and Zhao2019a 89 were clearly indicated as retrospective studies. Cohort types were projected for the rest judging by the details contained. Thus, six studies appeared to be prospective (Guan2016 42; Leite2015 60; Lin2014 64; Lin2017 65; Sharova2021 78; Yang2016 84) and four were retrospective (Amankwah2013 31; Melbø-Jørgensen2014 68; Zedan2017 85; Zhao2019a 89); it was unclear for Li2012 61.
For population “P”, two studies from the same research group (Lin2014 & Lin2017) included male patients diagnosed with CRPC that underwent docetaxel chemotherapy (a different set of patients was used for each study, therefore no double counting). Participants of Guan2016 and Sharova2021 received androgen deprivation therapy (ADT) and androgen receptor-targeted agents (ARTA) respectively; However, Sharova2021 only included metastatic castration-resistant prostate cancer (mCRPC) patients. The rest of the studies (n=7) included male PCa patients that underwent resection surgeries such as radical prostatectomy (RP) and/or regional lymph node dissection. Not all studies reported the age range of participants, but it is apparent from available information that they were all around middle to old age groups at baseline (≥ 40 years).

For index prognostic factor “I”, Lin2014, Lin2017, Sharova2021 and Yang2016 measured circulating miR-21 in plasma, serum or peripheral blood mononuclear cell (PBMC) samples while the rest (n=7) measured tissue miR-21 in formalin-fixed paraffin-embedded (FFPE) tumour samples; Li2012 and Zedan2017 measured miR-21 level by in situ hybridisation (ISH) methods that are semi-quantitative, while the rest (n=9) used real-time quantitative polymerase chain reaction (RT-qPCR) techniques that are highly sensitive and specific.

For comparator prognostic factors “C”, the most frequently included ones were Gleason score/grade (GS/GG; n=10 except Lin2017), PSA (n=10 except Amankwah2013) and pathological/clinical stage (pT/cT; n=8 except Lin2014, Lin2017 and Sharova2021). These were followed by age (n=6), haemoglobin (n=3), surgical margin (n=3), lymph node metastasis (pN; n=2) and alkaline phosphatase (n=2). Body mass index (BMI), capsular invasion, visceral metastasis, perineural infiltration, tumour size, vascular infiltration, digital rectal examination (DRE), prostate volume, neutrophil/lymphocyte ratio and time to CRPC were each included once between 6 studies (Amankwah2013; Li2012; Lin2014; Melbø-Jørgensen2014; Sharova2021; Zhao2019a).

For outcomes of interest “O”, Lin2014, Lin2017, Sharova2021 and Yang2016 observed for overall survival (OS) defined as time from the date of treatment to the date of death; Guan2016.
and Sharova2021 78 observed for progression-free survival (PFS), defined as time to development of CRPC from initiation of ADT by Guan2016 42, and as time to radiological/clinical progression from initiation of ARTA by Sharova2021 78. The rest (n=6) observed for recurrence-free survival (RFS), generally defined as time from the date of treatment to the date of biochemical recurrence (BCR) with slight variations as indicated in Table 2 footnotes d, f and g. Latest follow-up times across studies ranged from 45 months (Lin2017 65) to 254 months (Amankwah2013 31), averaging up to 125 months (~10 years). Not enough information was provided in Zedan2017 85 to estimate the follow-up period.

Risk of bias within studies

Risk of bias within each eligible study was assessed using the QUIPS tool 26; two independent judgments were made before reaching consensus. Final ratings of risk of bias within the 11 studies eligible for meta-analyses are summarised in Table 4.

Overall, no eligible study achieved LOW risk of bias in all domains. Most concerns in risk of bias were around domain 5 and 6 mainly due to inadequate adjustment for predefined important prognostic factors and selective reporting. The lack of rationale for sample size appears to be a common problem across the majority of eligible studies.

Meta-analyses & Sensitivity Analyses

For all outcomes, results of each study eligible for meta-analyses are summarised in Table 5 (n=11). Six studies observed RFS, four observed OS, and two observed PFS. Effect estimates were pooled as HR (95% CI) based on fixed-effect inverse variance method. Statistical heterogeneity was determined by visual inspection of the forest plot, Chi² test and I² test (Chi² p≤0.1 indicates significant heterogeneity; I² <30% denotes low/unimportant heterogeneity, 30-60% moderate heterogeneity, 50-90% substantial heterogeneity, and 75-100% considerable heterogeneity).

Analysis 1: Recurrence-free survival; dichotomous miR-21 data (n=4)
This analysis includes Amankwah2013 31, Leite2015 60, Leite2012 61 and Melbø-Jørgensen2014 68 as they have observed RFS as outcome and dichotomised tissue miR-21 expression data into high and low groups (median as cut-off for Amankwah2013 31, Leite2015 60 and Li2012 61; 4th quartile for Melbø-Jørgensen2014 68). Unadjusted and adjusted effect estimates of all four studies were combined in Analysis 1.1 (Figure 2a) and Analysis 1.2 (Figure 3a) respectively for comparison to examine the effect of heterogeneity caused by differences in covariate adjustment. Overall number of participants is 838 (364 with BCR; 474 without BCR).

The overall effect of unadjusted estimates, as shown in the forest plot of Analysis 1.1, favours low miR-21, suggesting high miR-21 expression is associated with higher risk of BCR (HR=1.54, 95% CI=1.23-1.92). Statistical heterogeneity tests indicate significantly considerable heterogeneity (Chi^2 p<0.00001; I^2=90%), most likely caused by the presence of an outlier (Amankwah2013 31) which showed an opposite direction of effect estimate to the other studies. To probe this further, the impact of the outlier on this meta-analysis was assessed by sensitivity analysis. Results of sensitivity analysis (Figure 2b) confirmed the data from Amankwah2013 31 as the source of statistical heterogeneity (I^2=0% without outlier). However, the inclusion of the outlier did not change the effect estimate significantly, therefore the results of Analysis 1.1 are still valid.

The overall effect of adjusted estimates (Analysis 1.2) is very close to that of unadjusted estimates (Analysis 1.1) supporting the same conclusion, i.e., it favours low miR-21, suggesting high miR-21 expression is associated with higher risk of BCR (HR=1.58, 95% CI=1.19-2.09; Figure 3a). However, different from Analysis 1.1, Melbø-Jørgensen2014 68 now occupied over half of the overall weight (52.8%) with Li2012 61 weighing only 18.8%. Amankwah2013 31 still appears to be outlying, and statistical heterogeneity tests also indicate significantly substantial heterogeneity (Chi^2 p=0.05; I^2=62%). Again, sensitivity analysis repeating Analysis 1.2 without Amankwah2013 31 reduced statistical heterogeneity to insignificant and low/unimportant (I^2=30%; Figure 3b), verifying the outlying estimate as the source of statistical heterogeneity. The slight difference in overall effect
reveals that the inclusion of the outlier has limited impact, and that the results of Analysis 1.2 are robust.

Comparing the two analyses, covariate adjustment in Analysis 1.2 had brought Amankwah2013 closer to the other studies with the upper CI arm crossing the line of no effect and overlapping with others', that might explain the lower statistical heterogeneity indicated by $I^2$ values compared to Analysis 1.1 (62% vs 90%). However, eliminating the effect of outlier, higher $I^2$ value of adjusted estimates compared to unadjusted (30% vs 0%) implies that differences in covariate adjustment might have introduced some heterogeneity, though low and insignificant.

**Analysis 2: Recurrence-free survival; continuous miR-21 data (n=2)**

This analysis includes Zedan2017 and Zhao2019a as both have observed RFS as outcome against continuous miR-21 expression in tissue samples. Only unadjusted effect estimates were combined in Analysis 2 (Figure 4) because of lack of multivariate analysis data for Zedan2017. Overall number of participants is 255 (117 with BCR; 138 without BCR).

The overall effect estimate (HR=1.12, 95% CI=1.01-1.26) favours lower miR-21, indicating that higher miR-21 expression is associated with higher risk of BCR. The overall effect in the forest plot showed high precision from the tight CI and statistical heterogeneity is very low (Chi$^2$ p=0.75; $I^2$=0%). However, the data points are very close to the line of no effect with the lower CI of Zedan2017 across. The overall weight is dominated by Zhao2019a (96.2%) between only two studies.

**Analysis 3: Overall survival; dichotomous miR-21 data (n=4)**

This analysis included Lin2014, Lin2017, Sharova2021 and Yang2016 as they are similar in outcome observed (OS), handling of miR-21 data (dichotomised) and source of miR-21 (circulating samples). Only unadjusted effect estimates were combined in Analysis 3 (Figure 5a) because of lack of multivariate analysis data for Lin2014 and differences in covariate adjustment and handling of
miR-21 data in multivariate analysis for Lin2017. Overall number of participants is 307 (163 dead; 144 alive).

The overall effect in *Analysis 3* favours low miR-21, suggesting high miR-21 expression is associated with higher risk of death (HR=1.46, 95% CI=1.06-2.01; *Figure 5a*). Sharova2021 was outlying in the opposite direction to the rest and mostly likely have caused the considerable heterogeneity (Chi² p=0.0008; I²=82%); Therefore the impact of including Sharova2021 in *Analysis 3* was examined in sensitivity analysis (*Figure 5b*). Sensitivity analysis repeating *Analysis 3* without Sharova2021 significantly reduced heterogeneity to low/unimportant level (Chi² p=0.25; I²=27%; *Figure 5b*), confirming an outlier as the main source of heterogeneity, and that had brought the overall effect estimate closer to the line of no effect.

*Analysis 4: Progress-free survival; dichotomous miR-21 data (n=2)*

*Analysis 4* included Guan2016 and Sharova2021 because both studies observed PFS as outcome. Overall number of participants is 116 (73 with progression; 43 without progression). *Figures 6a* and 6b showed meta-analysis results along with forest plots of combined unadjusted and adjusted effect estimates respectively (*Analyses 4.1* and 4.2). Neither analysis reached a significant overall effect (CIs crossing line of no effect), most likely since only two studies with opposite effect estimates were available, which also contributed to considerable heterogeneities (Chi²<0.1; I²>80%). Therefore, no meaningful conclusion could be drawn from *Analysis 4*.

**Qualitative Summary & Associations**

Most of the 64 studies included in this review compared the association of miR-21 with commonly used clinicopathological prognostic factors (*Table 6*). These included Gleason score/grade (n=28); pathological/clinical stage (n=18); serum PSA level (n=18); risk stratification (n=12); and age at diagnosis (n=9). Association of miR-21 expression with recurrence (n=19) and metastasis (n=14) were also examined in many included studies. A few studies have compared miR-21 levels in/with...
prostate volume (n=4), chem-response (n=3), digital rectal examination (DRE) result (n=3), ethnicity (n=2) and surgical margin (n=2). Other comparisons made include genitourinary radiotoxicity (Kopcalic2019 53), neuroendocrine-like vs Adeno PCa (Ostano2020 71), follow-up time, family history (Shen2012 79) and reclassification (Zhao2019b 90).

Results were grouped according to statistical significance (p<0.05/p>0.05), association direction (positive/negative) and sample source (tissue/circulating). Association measures varied between studies, these include fold change (FC), mean difference and correlation, meaning it was impractical to summarise findings according to comparison methods. Therefore, findings were summarised according to association directions. When higher miR-21 expression was associated with higher degree/presence of the comparators it was indicated as positive; when it was associated with lower degree/absence of the comparators it was negative.

Additional figures demonstrating association results can be found in Supplementary Figure SF 1a-g.

Twelve out of 28 studies (43%) that compared miR-21 levels in different Gleason scores/grades found significant positive association of miR-21 levels from tissue and circulating samples. Twelve out of 18 studies (67%) that compared miR-21 levels in different pathological/clinical stages found significant positive association of miR-21 mostly from circulating samples as well as tissue. In contrast, only three studies reported significant positive association in circulating miR-21 and serum PSA. Seven out of 19 studies (37%) found significant positive association between tissue/circulating miR-21 and biochemical recurrence, defined generally as biochemical recurrence determined by rise in serum PSA≥0.2-0.4 ng/ml after treatment. Ten out of 14 studies (71%) that compared miR-21 levels in samples of metastatic vs localised PCa patients found significant positive association between metastatic PCa and miR-21 mostly in circulating samples (n=8; tissue n=2). 11 out of 12 studies (92%) that examined risk stratification reported positive association of higher risk with elevated miR-21 expression, although only 4 (33%) of these were found to be statistically significant.

Certainty of evidence – GRADE
Publication bias was not assessed due to low number of studies eligible for each analysis. No analysis was rated up for large effect, dose response or plausible confounding. Table 7 presented judgments of rate-downs and overall certainties of each analysis. Overall certainty is MODERATE for Analysis 1.2; LOW for Analyses 1.1 and 2; VERY LOW for Analyses 3, 4.1 and 4.2. See Supplementary Table ST 7 for full rationales for rating down certainty of evidence.
DISCUSSION:

In this report, we have performed the first systematic review and meta-analysis of miR-21 as a prognostic factor in PCa. miR-21 is one of the most studied miRNAs in cancer and has been shown to play a role in many different cellular mechanisms which can contribute to cancer progression, including PCa. Although miR-21 targets many genes and thus regulates many genetic pathways, it appears to act in a primarily oncogenic fashion with many studies reporting elevated levels in samples taken from cancer patients. Despite this body of evidence, there is still doubt about whether it may be a useful biomarker for cancer prognosis, so robust analyses of existing studies are needed to determine its value for clinical application and to inform the optimal design of future studies.

The pooled results of all meta-analyses reported here supported an association between high miR-21 expression and poor prognosis in PCa. Regarding RFS, Analysis 1.2 estimated a 58% increased risk of BCR for high baseline expression of tissue miR-21 (HR=1.58, 95% CI=1.19-2.09) with MODERATE certainty of evidence. For OS, Analysis 3 estimated a 75% increased risk of death for high baseline expression of circulating miR-21 with VERY LOW certainty of evidence (HR=1.75, 95% CI=1.26-2.45). No meaningful conclusion could be drawn for PFS in Analysis 4 due to considerable heterogeneity between only two eligible studies. The heterogeneity could be attributed to differences in population, miR-21 source and PFS definition. Guan2016 recruited pathologically confirmed PCa patients while Sharova2021 only included mCRPC patients; Guan2016 detected miR-21 from FFPE tissue samples while Sharova2021 examined it in plasma samples; Guan2016 defined PFS as time to development of CRPC while Sharova2021 defined it as time to radiological/clinical progression. Analysis 4 demonstrated the importance of only combining results of similar studies as a basic principle of meta-analysis. The limited certainty in OS result and lack of similar studies in PFS for a meaningful meta-analysis indicated that more high-quality prognostic studies are needed for OS and PFS. Nevertheless, our systematic approach and meta-analyses found consistent evidence...
that miR-21 may have prognostic value in PCa. This data suggests miR-21 can be put forward as a strong candidate for the prognosis of the disease, although further work is clearly needed to prove its value more conclusively as a biomarker.

Our results agreed with systematic reviews in other cancers such as non-small cell lung, pancreatic and colorectal cancers. These suggested high tissue miR-21 as an unfavourable prognostic biomarker. Circulating miR-21 overexpression was also associated with poor prognosis in digestive system and breast cancers. This is not unexpected, given that it is generally agreed to act as an oncogene, but this understanding of its functional role in the cell can only be translated into medical application when the literature available is subject to methodical evaluation in studies such as these.

However, it is worth noting that the authors of the papers subject to meta-analysis here all indicated limitations with their studies. We recorded this as part of our data gathering process and further probed it through our quality assessment of individual studies. Pooled evidence by QUIPS and GRADE methodologies revealed sources of risk of bias and down-rate of certainty of evidence. In several studies, selective reporting and failure to adjust for the core set of covariates increased risk of bias and imprecision, thus decreased certainty of evidence. Furthermore, publication bias could not be properly assessed due to inadequate number of studies included in individual analysis. This was mainly due to high heterogeneity across studies, such as differences in outcome, handling of miR-21 data and sample source. The limited similarities meant that eligible studies had to be split into separate small analyses, therefore reducing the impact of meta-analyses. It was unfortunate that so few of the published studies met the required criteria for inclusion in meta-analysis, which limits the strength of the analyses and our subsequent ability to draw firm conclusions. Although the very nature of a properly conducted meta-analysis is to be robust and consistent in the application of the methodology, limitations in selected studies are inevitably reflected in the limitations of the subsequent meta-analyses, since the patient numbers and/or measured parameters are less than
ideal. Perhaps that is to be expected since miRNAs as biomarkers is a relatively recent field of research, but it is clear that a lack of standardised approach to these type of biomarker studies makes it difficult to evaluate the clinical usefulness of miRNAs as prognostic biomarkers. Therefore, for any researchers carrying out future cancer prognostic studies of this type, it is highly recommended that they adhere to the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) guidelines for proper study design, conduct, analysis and reporting. This will reduce risk of bias and heterogeneity across studies to generate higher quality evidence and more opportunity for comparison in meta-analyses like the ones presented here. Evidently, Zhao2019a was the only included study that followed the guidelines and achieved LOW risk of bias in most QUIPS domains.

Although several of the full-text studies reviewed were not eligible for meta-analysis, they nevertheless contained useful data about the association of miR-21 with PCa, which is important to discuss since it can inform future study design. Overall, several studies in this review supported the hypothesis that there is a significant positive association between miR-21 expression and various clinical measurements of PCa progression, such as stage, Gleason score, risk groups, metastasis and recurrence. Notably, very few studies found a significant association between miR-21 expression and serum PSA level or age at diagnosis.

However, for clinical application of miR-21 analysis, several barriers must be overcome. A standardised method for measuring miR-21 must be decided upon. RT-qPCR, as used in many of the studies reported here, would seem the most appropriate technique at present in terms of sensitivity and applicability. Nevertheless, agreement is needed on common normalisation approaches and comparable internal controls, such as reference genes. Even with these measures in place, a consensus would then be needed on an appropriate cut-off value for prognostic outcome, which was very variable in the studies evaluated here. Another important consideration is that the correct miR-21 strand is being measured, since there is no guarantee that expression of miR-21-3p and miR-21-
5p will be similar. The majority of the studies in this review did not specify miR-21 strand, which is also another reason to be cautious about the interpretation of the results presented here.

Even if standardised approaches meant RT-qPCR was accepted as suitably sensitive and accurate method, the sample type in which to measure the miR-21 target is a further complication. Among 63 studies included in this review, 31 measured miR-21 levels in circulating samples, including plasma, serum, PBMC, urine, exosome and whole blood; 30 measured miR-21 levels in tissue samples; Zedan2018 86 measured from both sample types; and Samaan2014 74 did not clearly state the sample source. Zedan2018 86 found significant correlation of miR-21 levels between matched tissue and plasma samples from 25 healthy patients (r=0.58, p<0.01) but not in 21 PCa patients (p=0.42). It is not certain that tissue and biofluid levels of miR-21 will be directly comparable, and it is also possible that different outcomes might be better predicted by miR-21 expression in one particular sample type. Thus, further inter- and intra-individual analyses would be needed to determine the relative value of these different sample types. It is therefore clear that for miR-21, or any other miRNA, to gain clinical acceptance as disease biomarker, it requires well-designed, prospective clinical studies to validate the findings reported here. Ideally, these studies should utilise the same PICOT criteria, ensuring common outcomes and measurements can then be compared between studies and across different research centres.

Nevertheless, even though there are not yet enough well-designed studies to conclusively prove biomarker potential of miRNAs, it does appear increasingly likely that they will be used in future as non-invasive, liquid biomarkers for cancer and other diseases.101, 102 With this in mind, miR-21 is a very attractive candidate to profile, since it is abundantly expressed in both tissue and biofluids, making it easy to measure.14, 103 In relation to PCa specifically, its involvement in promoting cancer growth, and related roles in important pathological changes, such as epithelial-to-mesenchymal transition (EMT), is now well established.14, 104, so there is a strong biological rationale for measuring its expression as a marker of disease progression. It is worth remembering however that miRNAs
often work synergistically as a regulatory network for gene expression, so the involvement of miR-21 with other miRNAs should be considered. For instance, while this paper was being prepared, another systematic review and meta-analysis was published which reported the prognostic significance of 15 microRNAs related to metastasis and EMT process in PCa patients. Surprisingly, miR-21 was not included among them, but the authors did acknowledge the link between their selected miRNAs and miR-21 in their discussion, and they concluded that a miRNA panel of biomarkers would be optimal to determine progression risk. Similarly, another recent paper used meta-analysis methods to identify miR-21 as one of several miRNAs which could predict response to ADT. Profiling different miRNAs in parallel makes sense, since many miRNAs are known to be involved in PCa development. It is also unlikely that miR-21 (or any other miRNA) as a single biomarker would be sufficient to accurately predict any given patient outcome. Therefore, the ability to measure expression levels of other miRNAs, or other genetic parameters, in combination with miR-21 should be built into the design of future studies investigating its prognostic value in cancer. A multivariate profiling approach to PCa prognosis, which includes measurement of miR-21, would be a sensible approach to take.

CONCLUSIONS:

Meta-analyses of 11 studies in this report showed that high miR-21 expression was associated with poor prognosis in PCa. Qualitative summary of all 64 studies also found positive association of miR-21 expression with various prognostic factors for PCa. These findings corroborate data from other systematic reviews which have shown similar findings for miR-21 in various cancers. However, further research is needed, including more high-quality investigations that follow standardised guidelines for study design. With continued effort, miR-21 could prove to be a clinically useful prognostic biomarker in prostate cancer.

DATA AVAILABILITY:

The datasets analysed in the present study are available from the published papers that have been cited in this manuscript.
AUTHOR CONTRIBUTIONS:

MYCS and DJM were responsible for study design, literature search, data extraction, data analysis and drafting the manuscript. CEW and CPW reviewed the manuscript and contributed intellectual input to the study. All authors approved the final version of the manuscript.

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Figure 1. Study flow diagram (Adapted from Moher et al.23).
Figure 2. **Analysis 1.1: Meta-analysis of dichotomous miR-21 expression with recurrence-free survival (unadjusted).**

(a) Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Amankwah2013).
### (a)

| Study or Subgroup          | log(Hazard Ratio) | SE  | With BCR Total | Without BCR Total | Weight | Hazard Ratio IV, Fixed, 95% CI |
|---------------------------|-------------------|-----|---------------|-------------------|--------|-----------------------------|
| Amankwah 2013             | -0.6882           | 0.5315 | 28            | 37                | 7.3%   | 0.50 [0.18, 1.42]           |
| Leite 2015                | 0.9203            | 0.3127 | 50            | 57                | 21.1%  | 2.51 [1.36, 4.63]           |
| Li 2012                   | 0.7227            | 0.331  | 116           | 109               | 18.8%  | 2.06 [1.08, 3.94]           |
| Melbø-Jørgensen 2014      | 0.3365            | 0.1978 | 170           | 205               | 52.8%  | 1.40 [0.95, 2.06]           |
| **Total (95% CI)**        | **364**           | **474** | **100.0%**    |                   |        | 1.58 [1.19, 2.09]           |

Heterogeneity: $\chi^2 = 7.85$, df = 3 ($P = 0.05$); $I^2 = 62$
Test for overall effect: $Z = 3.19$ ($P = 0.001$)

### (b)

| Analysis 1.2 | Chi² p-value | $I^2$ | HR  | LCI | UCI  |
|--------------|--------------|-------|-----|-----|------|
| All studies (with outlier) | 0.05         | 62%   | 1.58| 1.19| 2.09 |
| Without outlier         | 0.24         | 30%   | 1.73| 1.29| 2.32 |

**Figure 3. Analysis 1.2: Meta-analysis of dichotomous miR-21 expression with recurrence-free survival (adjusted).**

(a) Adjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Amankwah 2013).

**BCR:** Biochemical recurrence; CI: Confidence interval; HR: Hazard ratio; IV: Inverse variance; LCI: Lower confidence interval; SE: Standard error; UCI: Upper confidence interval
| Study or Subgroup | log[Hazard Ratio] | SE | Total | Total | Weight | Hazard Ratio IV, Fixed, 95% CI |
|-------------------|------------------|----|-------|-------|--------|-------------------------------|
| Zedan 2017        | 0.2078           | 0.2903 | 19    | 30    | 3.8%  | 1.23 [0.70, 2.17]             |
| Zhao 2019a        | 0.1133           | 0.0576 | 98    | 108   | 96.2% | 1.12 [1.00, 1.25]             |
| Total (95% CI)    |                  |     | 117   | 138   | 100.0%| 1.12 [1.01, 1.26]             |

Heterogeneity: Chi² = 0.10, df = 1 (P = 0.75); I² = 0%
Test for overall effect: Z = 2.07 (P = 0.04)

Figure 4. Analysis 2: Meta-analysis of continuous miR-21 expression with recurrence-free survival.

Unadjusted results and forest plot, RevMan5.4 snapshot.

**BCR**: Biochemical recurrence; **CI**: Confidence interval; **HR**: Hazard ratio; **IV**: Inverse variance; **SE**: Standard error
Figure 5. Analysis 3: Meta-analysis of miR-21 expression with Overall Survival.

(a) Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Sharova2021).  

CI: Confidence interval; HR: Hazard ratio; IV: Inverse variance; LCI: Lower confidence interval; SE: Standard error; UCI: Upper confidence interval.
Figure 5. Meta-analyses of miR-21 expression with progression-free survival.

(a) Analysis 4.1: Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Analysis 4.2: Adjusted results and forest plot, RevMan5.4 snapshot.

CI: Confidence interval; HR: Hazard ratio; IV: Inverse variance; SE: Standard error
FIGURE LEGENDS:

Figure 1. Study flow diagram (Adapted from Moher et al.23).

Figure 2. Analysis 1.1: Meta-analysis of dichotomous miR-21 expression with recurrence-free survival (unadjusted).
(a) Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Amankwah201331).

**BCR:** Biochemical recurrence; **CI:** Confidence interval; **HR:** Hazard ratio; **IV:** Inverse variance; **LCI:** Lower confidence interval; **SE:** Standard error; **UCI:** Upper confidence interval

Figure 3. Analysis 1.2: Meta-analysis of dichotomous miR-21 expression with recurrence-free survival (adjusted).
(a) Adjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Amankwah201331).

**BCR:** Biochemical recurrence; **CI:** Confidence interval; **HR:** Hazard ratio; **IV:** Inverse variance; **LCI:** Lower confidence interval; **SE:** Standard error; **UCI:** Upper confidence interval

Figure 4. Analysis 2: Meta-analysis of continuous miR-21 expression with recurrence-free survival.
Unadjusted results and forest plot, RevMan5.4 snapshot.

**BCR:** Biochemical recurrence; **CI:** Confidence interval; **HR:** Hazard ratio; **IV:** Inverse variance; **SE:** Standard error
Figure 5. Analysis 3: Meta-analysis of miR-21 expression with Overall Survival.

(a) Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Sensitivity analysis of impact of outlier (Sharova2021 [78]).

Ci: Confidence interval; HR: Hazard ratio; IV: Inverse variance; LCI: Lower confidence interval; SE: Standard error; UCI: Upper confidence interval

Figure 6. Meta-analyses of miR-21 expression with progression-free survival.

(a) Analysis 4.1: Unadjusted results and forest plot, RevMan5.4 snapshot. (b) Analysis 4.2: Adjusted results and forest plot, RevMan5.4 snapshot.

Ci: Confidence interval; HR: Hazard ratio; IV: Inverse variance; SE: Standard error
Table 1: PICOT eligibility criteria

| P | Population | Male patients of any age worldwide diagnosed with PCa. |
| I | Index prognostic factor | Measurement of miR-21 levels in tissue or circulating/fluid samples such as tumour tissue, blood, plasma, serum, urine and seminal fluid. |
| C | Comparator prognostic factors | Clinicopathological factors such as stage, grade, Gleason score, PSA level and health condition (e.g., recurrence, metastasis). |
| O | Outcomes of interest | Survival outcomes of any type (e.g., OS, RFS) estimated in HR, 95% CI, p-value and/or survival curves with log-rank p-value. |
| T | Timing | Samples taken as baseline at the start of follow-up of any length. |

Studies with characteristics specified by PICOT were eligible for inclusion in meta-analysis.

CI: Confidence interval; HR: Hazard ratio; OS: Overall survival; PCa: Prostate cancer; PSA: Prostate-specific antigen; RFS: Recurrence-free survival
Table 2: Characteristics of 11 studies eligible for meta-analyses

| Study ID, Type | Study size | P | I | C | O | Follow-up period |
|---------------|------------|---|---|---|---|-----------------|
| Amankwah 2013 * | 65 | Retrospective | PCa histologically confirmed; Underwent RP | High/low miR-21, -221 & -222 in FFPE tissue (TaqMan RT-qPCR) | Age; BMI; cT; GS | RFS | 3-254 months |
| Guan2016 * | 85 | Prospective | PCa pathologically confirmed; Underwent ADT | High/low levels of 7 miRNAs (including miR-21) in FFPE tissue (TaqMan RT-qPCR) | Age; cT; GS; PSA | PFS | 14-95 months |
| Leite2015 * | 127 | Prospective | Localised PCa; Underwent RP | High/low miR-21 in FFPE tissue (TaqMan RT-qPCR) | GG; PSA; pT | RFS | 2-120 months |
| Li2012 * | 168 | (unclear) | PCa pathologically confirmed; Underwent RP & regional lymph node dissection | High/low miR-21 in FFPE tissue (LNA-ISH) | Age; Capsular invasion; GS; pN; PSA; pT; Surgical margin | RFS | 2-80 months |
| Lin2014 * | 97 | Prospective | CRPC patients; Underwent docetaxel chemotherapy | High/low levels of 46 miRNAs (including miR-21) in plasma/serum (TaqMan RT-qPCR) | Age; Alkaline phosphatase; GS; Haemoglobin; PSA; Visceral metastasis | OS | 3-62 months |
| Lin2017 * | 87 | Prospective | CRPC patients; Underwent docetaxel chemotherapy | High/low levels of 14 miRNAs (including miR-21) in plasma (TaqMan RT-qPCR) | Alkaline phosphatase; Haemoglobin; PSA | OS | 0.7-45 months |
| Melbs-Jørgensen 2014 * | 478 | Retrospective | PCa patients; Underwent RP | High/low levels of 7 miRNAs (including miR-21-5p) in FFPE tissue (RT-qPCR) | GG; Perineural infiltration; PSA; pT; Surgical margins; Tumour size; Vascular infiltration | RFS | 6-188 months |
| Sharova 2021 * | 31 | Prospective | mCRPC patients; Treated with ARTA | High/low levels of miR-21, -141 & -223 in plasma (TaqMan RT-qPCR) | GS; Haemoglobin; Neutrophil/lymphocyte ratio; PSA; Time to CRPC | OS | Median = 36.6 months |
| Yang2016 * | 92 | Prospective | PCa pathologically confirmed; Underwent resection | High/low miR-21 in PBMC (TaqMan RT-qPCR) | Age; cT; GS; PSA | OS | 21-69 months |
| Zedan2017 * | 49 | Retrospective | Localised PCa; Underwent RP & regional lymph node dissection | Continuous levels of 6 miRNAs (including miR-21) in FFPE tissue (ISH analysed by computer software) | GS; PSA; pT | RFS | (Not stated) |
| Zhao2019a * | 206 | Retrospective | PCa patients; Underwent RP | Continuous levels of 20 miRNAs (including miR-21-5p) in FFPE tissue (TaqMan RT-qPCR) | Age; DRE; PSA; ISUP grade; pN; Prostate volume; pT; Surgical margin | RFS | 17-180 months |

ADT: Androgen deprivation therapy; ARTA: Androgen receptor-targeted agents; BMI: Body mass index; C: Comparator prognostic factor; CRPC: Castration-resistant prostate cancer; cT: Clinical tumour stage; DRE: Digital rectal examination; FFPE: Formalin-fixed paraffin-embedded; GG: Gleason grade; GS: Gleason score; I: Index prognostic factor; ISH: In situ hybridisation; ISUP: International Society of Urological Pathology; LNA-ISH: Locked nucleic acid in situ hybridisation; mCRPC: Metastatic castration-resistant prostate cancer; O: Outcomes of interest; OS: Overall survival; P: Population; PBMC: Peripheral blood mononuclear cell; PCa: Prostate cancer; PFS: Progression-free survival; pN: Lymph node metastasis; PSA: Prostate-specific antigen; pT:
Pathological tumour stage; **RFS**: Recurrence-free survival; **RP**: Radical prostatectomy; **RT-qPCR**: Real-time quantitative polymerase chain reaction

aNADT included surgical castration or luteinising hormone-releasing hormone agonist combined with an antiandrogen according to **Guan2016**42.
bnARTA included abiraterone (n=10) and enzalutamide (n=21) according to **Sharova2021**78.
cISUP grading system was based on Gleason score according to **Zhao2019a**89.
dEndpoint included biochemical recurrence defined as serum PSA≥0.2ng/ml after treatment, clinical metastasis or PCa-specific death.
ePFS defined as time to development of CRPC from initiation of ADT where progression to CRPC was defined as three consecutive monthly increases in serum PSA level against ADT according to **Guan2016**42.
fBiochemical recurrence defined as serum PSA≥0.2ng/ml after treatment.
gBiochemical recurrence defined as serum PSA≥0.4ng/ml after treatment.
hPFS defined as time to radiological/clinical progression from initiation of ARTA according to **Sharova2021**78.
Table 3: Allocation of eleven studies into 4 meta-analyses

| Outcome | Handling of miR-21 data | No. of studies | Total no. of participants | Study IDs | Analysis |
|---------|-------------------------|----------------|---------------------------|-----------|----------|
| RFS     | Dichotomous             | 4              | 838                       | Amankwah2013<sup>31</sup>; Leite2015<sup>60</sup>; Li2012<sup>61</sup>; Melbø-Jørgensen2014<sup>68</sup> | 1         |
|         | Continuous              | 2              | 255                       | Zedan2017<sup>85</sup>; Zhao2019<sup>a89</sup> | 2         |
| OS      | Dichotomous             | 4              | 307                       | Lin2014<sup>64</sup>; Lin2017<sup>65</sup>; Sharova2021<sup>78</sup>; Yang2016<sup>84</sup> | 3         |
| PFS     | Dichotomous             | 2              | 116                       | Guan2016<sup>82</sup>; Sharova2021<sup>78</sup> | 4         |

Eleven eligible studies were allocated into four separate meta-analyses according to outcomes and handlings of miR-21 data. Note: Sharova2021<sup>78</sup> with two outcomes was allocated into Analyses 3 and 4.

**OS**: Overall survival; **PFS**: Progression-free survival; **RFS**: Recurrence-free survival
Table 4: Risk of bias within studies assessed using QUIPS tool

| Study ID                  | QUIPS domains       | 1 Study participation | 2 Study attrition | 3 Prognostic factor measurement | 4 Outcome measurement | 5 Adjustment for covariates | 6 Statistical analysis & reporting |
|---------------------------|---------------------|-----------------------|-------------------|---------------------------------|-----------------------|-------------------------------|-----------------------------------|
| Amankwah2013              | HIGH                | LOW                   | LOW               | LOW                             | LOW                   | LOW                           | LOW                               |
| Guan2016                  | UNCLEAR             | LOW                   | LOW               | LOW                             | LOW                   | HIGH                          | LOW                               |
| Leite2015                 | UNCLEAR             | LOW                   | LOW               | LOW                             | HIGH                  | HIGH                          | MODERATE                         |
| Li2012                    | UNCLEAR             | LOW                   | MODERATE          | LOW                             | LOW                   | HIGH                          | MODERATE                         |
| Lin2014                   | UNCLEAR             | LOW                   | LOW               | LOW                             | HIGH                  | MODERATE                      | LOW                               |
| Lin2017                   | UNCLEAR             | LOW                   | LOW               | LOW                             | HIGH                  | MODERATE                      | LOW                               |
| Melbø-Jørgensen2014       | LOW                 | LOW                   | LOW               | LOW                             | MODERATE             | LOW                           | MODERATE                         |
| Sharova2021               | MODERATE            | LOW                   | LOW               | LOW                             | HIGH                  | LOW                           | LOW                               |
| Yang2016                  | UNCLEAR             | MODERATE             | LOW               | LOW                             | LOW                   | UNCLEAR                       | UNCLEAR                           |
| Zedan2017                 | MODERATE            | LOW                   | MODERATE          | LOW                             | UNCLEAR               | UNCLEAR                       | UNCLEAR                           |
| Zhao2019a                 | LOW                 | LOW                   | LOW               | LOW                             | HIGH                  | LOW                           | LOW                               |
| Outcome (Analysis) | Study ID | Event/Total | Univariate analysis: Unadjusted HR (95% CI) | Multivariate analysis: Adjusted HR (95% CI) | Covariates adjusted for * |
|-------------------|----------|-------------|---------------------------------------------|---------------------------------------------|--------------------------|
| **RFS (1)**       | Amankwah 2013 31 | 28/65 (43%) | (Cut-off=median; log-rank p<0.0001) | 1.99 (0.70-5.64), p=0.20 | Age GS ct |
|                   |          |             | KM plot favouring high miR-21             |                                             |                          |
|                   |          |             | Estimated HR (95% CI) a: =4.83 (2.26-10.35), p=0.00005 |                                             |                          |
|                   |          |             | Inverse b: =0.21 (0.10-0.44), p=0.00005 |                                             |                          |
|                   | Leite2015 60 | 50/127 (39%) | (Cut-off=median; log-rank p=0.003) | 2.505 (1.356-4.629), p=0.003 | GG PSA pT |
|                   |          |             | KM plot favouring low miR-21             |                                             |                          |
|                   |          |             | Estimated HR (95% CI) a: =2.32 (1.33-4.03), p=0.003 |                                             |                          |
|                   | Li2012 61 | 116/168 (69%) | (Cut-off=median; log-rank p<0.001) | 2.059 (1.075-3.944), p=0.029 | Age Capsular invasion GS pN PSA pT Surgical margin |
|                   |          |             | KM plot favouring low miR-21             |                                             |                          |
|                   |          |             | Estimated HR (95% CI) a: =1.91 (1.33-2.75), p=0.0005 |                                             |                          |
|                   | Melbø-Jørgensen 2014 68 | 170/478 (36%) | (Cut-off=4th quartile; log-rank p=0.006) | 1.4 (1.0-1.9), p=0.089 | Apical PSM GG Non-apical PSM Perineural infiltration PSA pT Vascular infiltration |
| **RFS (2)**       | Zedan2017 85 | 19/49 (39%) | (Continuous miR-21) 1.231 (0.697-2.177), p=0.474 | (No multivariate analysis data) | (N/A) |
|                   | Zhao2019a 89 | 98/206 (48%) | (Continuous miR-21) 1.12 (1.01-1.24), p=0.049 | (Continuous miR-21) 1.35 (0.86-2.12), p=0.188 | 15 other miRNAs of interest |
| **OS (3)**        | Lin2014 64 | 55/97 (57%) | (High vs low miR-21, cut-off=median) 2.3 (1.3-3.9), log-rank p=0.004 | (No multivariate analysis data) | (N/A) |
|                   | Lin2017 65 | 53/87 (61%) | (High vs low miR-21, cut-off=median) 1.2204 (0.7028-2.1192), p=0.477 | (Continuous miR-21) 1.1488 (0.8849-1.4916), p=0.303 | Alkaline phosphatase Haemoglobin PSA |
|                   | Sharova2021 78 | 13/31 (42%) | (Cut-off=2.69; log-rank p=0.0067) | 5.8 (1.0-33.1), p=0.049 | Haemoglobin Time to CRPC |
| Study          | Cases/Total (Percentage) | PFS (Years) | Estimated HR (95% CI) | Inverse^b: |
|---------------|-------------------------|-------------|-----------------------|------------|
| Yang2016^a     | 42/92 (46%)             | 3.567 (1.287-9.882), p=0.014 | =0.192 (0.064-0.588), p=0.0191 |
| Guan2016^b     | 47/85 (55%)             | 1.985 (1.032-3.817), p=0.040 | Age BCR Bone metastasis |
| Sharova2021^c  | 26/31 (84%)             | 4.8 (1.3-17.8), p=0.019 | Inverse^b: =0.208 (0.056-0.769), p=0.019 |

**Notes:**
- KM plot favouring high miR-21  
  5.2 (1.7-15.7), p=0.0191  
  Inverse^b:  
  =0.172 (0.03-1.0), p=0.049
- Estimated HR (95% CI)^a:  
  =2.02 (1.09-3.73), p=0.025
- Estimated HR (95% CI)^a:  
  =2.381 (1.25-4.537), p=0.008
- Estimated HR (95% CI)^a:  
  =7.4 (2.6-21.2), p=0.0021
- Estimated HR (95% CI)^a:  
  =4.8 (1.3-17.8), p=0.019
- Estimated HR (95% CI)^a:  
  =0.135 (0.047-0.385), p=0.0021

*GS/GG and pT/cT were predefined as important prognostic factors that should be adjusted for in multivariate analysis.*

**Abbreviations:**  
BCR: Biochemical recurrence; CI: Confidence interval; CRPC: Castration-resistant prostate cancer; cT: Clinical tumour stage; GG: Gleason grade; GS: Gleason score; HR: Hazard ratio; KM: Kaplan Meier; N/A: Not applicable; OS: Overall survival; PFS: Progression-free survival; pN: Lymph node metastasis; PSA: Prostate-specific antigen; PSM: Positive surgical margins; pT: Pathological tumour stage; RFS: Recurrence-free survival

^a Unadjusted HR (95% CI) was not reported; hence it was estimated using an Excel calculator.  
^b The direction of effect estimates in Amankwah2013^31 and Sharova2021^78 were opposite to the rest of eligible studies; hence they were inverted (i.e. divided by 1) to obtain the complimentary value.
Most of the 64 studies included in this review compared the association of miR-21 with commonly used clinicopathological prognostic factors (Gleason score/grade; pathological/clinical stage; serum PSA level; risk stratification; age at diagnosis), as well as recurrence and metastasis.

Study IDs in **bold** were eligible for meta-analysis (n=11). Possible part overlap of participants between Ibrahim2019a 48 and Ibrahim2019b 49.

* Zedan2018 86 was counted twice as both tissue and plasma miR-21 expressions were measured.
** 3p strand of miR-21 was measured.

C: Circulating miR-21; corr: Correlation; diff: Difference; Neg: Negative association; Pos: Positive association; PSA: Prostate-specific antigen; T: Tissue miR-21; U: Unknown miR-21 source

### Table 6: Summary of association results of included studies

| Association result | Gleason (n=28) * | Stage (n=18) | PSA (n=18) * |
|--------------------|-----------------|-------------|-------------|
| p<0.05 Pos T       | Arisan2020; Guan2016; Li2012; Melbø-Jørgensen2014; Zhao2019a | Li2012; Melbø-Jørgensen2014; Reis2012; Zhao2019a |                        |
| C                  | Al-Qatari2017; Gurbuz2020; Ibrahim2019a; Ibrahim2019b; Ju2019; Yang2016 | Al-Qatari2017; Gurbuz2020; Ibrahim2019a; Ibrahim2019b; Ju2019; Stuopelyte2016; Yang2016 | Al-Qatari2017; Gurbuz2020; Ibrahim2019b |
| U                  | Samaan2014      |                          |                        |
| p>0.05 Neg T       | Katz2014; Kurui2019; Lichner2015; Reis2012; Zedan2017; Zedan2018 | Zedan2017 | Li2012; Reis2012; Zedan2018; Zhao2019a |
| C                  | Shen2012       | Shen2012; Zedan2019     | Ju2019; Shen2012; Zedan2018; Zedan2019 |
| Neg T              | Kristensen2016 ** | Katz2014                | Zedan2017              |
| C                  | Kotb2014; Zedan2018; Zedan2019 |                       |                          |
| No diff T          | Amankwah2013   | Guan2016; Katz2014      |                          |
| C                  | Farran2018; Foj2017; Stuopelyte2016 |                       |                          |
| No pos T           | Hart2014       |                          | Agaoglu2011             |
| p-value            | No corr T       |                          |                          |

### Association result | Recurrence (n=19) | Metastasis (n=14) | Risk (n=12) | Age (n=9) |
|----------------------|-------------------|-------------------|-------------|-----------|
| p<0.05 Pos T         | Leite2015; Li2012; Melbø-Jørgensen2014; Reis2012 | Guan2016; Li2012 | Zhu2019    |           |
| C                    | Huang2015b; Ju2019; Yang2016 | Agaoglu2011; Brase2011; Huang2015b; Ibrahim2019a; Ibrahim2019b; Watahiki2013; Yang2016; Ju2019 | Foj2017; Ju2019; Shen2012; Zedan2019 | Zedan2019 |
| Neg T                | Suer2019 **; Amankwah2013 | Ren2014           | Ren2014     |           |
| C                    |                             |                   |                          |           |
| p>0.05 Pos T         | Kurui2019; Leite2011; Ren2014 | Katz2014; Leite2013; Zedan2017 |                                      |           |
| C                    | Stuopelyte2016 | Al-Qatari2017; Hoey2019; Sapre2014; Zedan2019 |                                   | Huang2015b; Yang2016 |
| Neg T                | Katz2014          | Leite2011          | Lichner2013   | Zhao2019a; Li2012 |
| C                    | Selth2013; Shen2012 |                                            |                                   |                                   |
| No diff/corr T       | Kristensen2016 **; Zheng2014 | | | Guan2016 |
| C                    | Singh2014         |                   |               |           |
| No p-value Pos T     | Bonci2016         |                   |               |           |

Downloaded from http://portlandpress.com/bioscirep/article-pdf/doi/10.1042/BSR20211972/926844/bsr-2021-1972.pdf by guest on 21 December 2021
| Analysis | Outcome | Pooled result (HR 95% CI) | No. of participants | Certainty rate-downs | Overall certainty* |
|----------|---------|---------------------------|---------------------|----------------------|-------------------|
| 1.1      | RFS a, c| 1.54 (1.23-1.92)          | 838 (4 studies)     | - RoB: High RoB in 3 studies - Imprecision: Estimated HR in all studies | LOW               |
| 1.2      | RFS b, c| 1.58 (1.19-2.09)          | 838 (4 studies)     | - RoB: High RoB in 3 studies | MODERATE          |
| 2        | RFS a, d| 1.12 (1.01-1.26)          | 255 (2 studies)     | - RoB: Unadjusted HR & high RoB in 1 study - Imprecision: CI close to HR 1 | LOW               |
| 3        | OS a, c | 1.46 (1.06-2.01)          | 307 (4 studies)     | - RoB: Unadjusted HR & high RoB in 3 studies - Indirectness: Lin 2014 & Lin 2017 recruited CRPC patients to address chemo-response - Imprecision: Estimated HR in 1 study; CI close to HR 1 | VERY LOW          |
| 4.1      | PFS a, c| 1.09 (0.63-1.88)          | 116 (2 studies)     | - RoB: High RoB in both studies - Inconsistency: Opposite direction results - Imprecision: Wide CI crossing HR 1 | VERY LOW          |
| 4.2      | PFS b, c| 1.26 (0.70-2.27)          | 116 (2 studies)     | - RoB: High RoB in both studies - Inconsistency: Opposite direction results - Imprecision: Wide CI crossing HR 1 | VERY LOW          |

CI: Confidence interval; CRPC: castration-resistant prostate cancer; HR: Hazard ratio; OS: Overall survival; RFS: Recurrence-free survival; RoB: Risk of bias

a Unadjusted effect estimates
b Adjusted effect estimates
c Dichotomised miR-21 levels
d Continuous miR-21 levels
e HIGH: We are very confident that the variation in risk associated with miR-21 expression lies close to that of the estimate
MODERATE: We are moderately confident that the variation in risk associated with miR-21 expression is likely to be close to the estimate, but substantial difference is possible
LOW: We have limited certainty in the estimate, the variation in risk associated with miR-21 expression may be substantially different from the estimate
VERY LOW: We have very little certainty in the estimate, the variation in risk associated with miR-21 expression is likely to be substantially different from the estimate (GRADE 28).
### ST 1: Search strategies in electronic databases

#### Medline (Ovid)
1. exp MicroRNAs/
2. (microRNA or miRNA or microRNA-21 or microRNA21 or miRNA-21 or miR-21 or miR21)
3. exp Prostatic Neoplasms/
4. (prostat* cancer* or prostat* carcinoma* or prostat* tumo?r* or prostat* neoplasm* or prostat* adenocarcinoma* or PRAD)
5. exp Biomarkers/
6. exp Prognosis/
7. exp Survival Analysis/
8. (biomarker* or marker* or prognos* or survival)
9. 1 or 2
10. 3 or 4
11. 5 or 6 or 7 or 8
12. 9 and 10 and 11
13. limit 12 to yr="2010-Current"
14. limit 13 to english language
15. limit 14 to (case reports or editorial or english abstract or letter or meta analysis or "review" or "systematic review")
16. 14 not 15

#### EMBASE
1. exp microRNA 21/
2. exp microRNA/
3. (microRNA or miRNA or microRNA-21 or microRNA21 or miRNA-21 or miR-21 or miR21)
4. 1 or 3
5. exp prostate cancer/
6. (prostat* cancer* or prostat* carcinoma* or prostat* tumo?r* or prostat* neoplasm* or prostat* adenocarcinoma* or PRAD)
7. 5 or 6
8. exp prognosis/
9. exp biological marker/
10. exp survival/ or exp survival analysis/
11. (biomarker* or marker* or prognos* or survival)
12. 8 or 9 or 10 or 11
13. 4 and 7
14. 12 and 13
15. limit 14 to yr="2010-Current"
16. limit 15 to english language
17. limit 16 to (meta analysis or "systematic review")
18. limit 16 to (books or chapter or conference abstract or editorial or letter or "review" or short survey)
19. 17 or 18
20. 16 not 19

#### Web of Science (Core Collection)
1. TOPIC: ("microRNA-21" OR "microRNA21" OR "miRNA-21" OR "miRNA21" OR "miR-21" OR "miR21" OR microRNA OR miRNA)
2. TOPIC: ("prostat* cancer*" OR "prostat* carcinoma*" OR "prostat* tumo?r*" OR "prostat* neoplasm*" OR "prostat* adenocarcinoma*" OR PRAD)
3. TOPIC: (biomarker* OR marker* OR prognostic OR survival)
4. #3 AND #2 AND #1 Refined by: [excluding] PUBLICATION YEARS: ( 2008 OR 2007 OR 2006 OR 2009 ) AND LANGUAGES: ( ENGLISH ) AND [excluding] DOCUMENT TYPES: ( EDITORIAL MATERIAL OR LETTER OR REVIEW OR PROCEEDINGS PAPER OR RETRACTED PUBLICATION OR RETRACTION OR MEETING ABSTRACT OR BOOK CHAPTER )

#### Scopus
(TITLE-ABS-KEY ( biomarker* OR marker* OR prognostic OR survival ) ) AND ( ( TITLE-ABS-KEY ("microRNA-21" OR "microRNA21" OR "miRNA-21" OR "miRNA21" OR "miR-21" OR "miR21" OR "circulating microRNA" ) ) AND ( TITLE-ABS-KEY ("prostat* cancer*" OR "prostat* carcinoma*" OR "prostat* tumo?r*" OR "prostat* neoplasm*" OR "prostat* adenocarcinoma*" OR PRAD ) ) ) AND ( EXCLUDE ( PUBYEAR , 2009 ) OR EXCLUDE ( PUBYEAR , 2008 ) ) AND ( LIMIT-TO ( LANGUAGE , "English" ) ) AND ( EXCLUDE ( DOCTYPE , "ch" ) OR EXCLUDE ( DOCTYPE , "ed" ) OR EXCLUDE ( DOCTYPE , "sh" ) OR EXCLUDE ( DOCTYPE , "no" ) ) AND ( LIMIT-TO ( DOCTYPE , "ar" ) )

#### Cochrane Library
microRNA-21 or microRNA21 or miRNA-21 or miRNA21 or miR-21 or miR21 or microRNA or miRNA or miR in All Text AND prostate or prostatic in Title Abstract Keyword AND cancer or carcinoma or tumour or tumor or neoplasm or adenocarcinoma or PRAD in Title Abstract Keyword AND biomarker or marker or prognostic or prognosis or survival in Title Abstract Keyword
ST 2: Data items included in Covidence data extraction form (Adapted from CHARMS-PF checklist\textsuperscript{22})

| General information |
|---------------------|
| Study ID            |
| Title               |
| Lead author and contact details |
| Country in which the study conducted |
| Study funding sources |
| Possible conflicts of interest for study authors |
| Notes               |

| Source of data |
|----------------|
| Source of data (e.g., cohort, case control, randomised trial or registry data) |

| Participants |
|--------------|
| Participant eligibility and recruitment method |
| Participant description |
| Details of treatments received (if relevant) |
| Study dates |

| Outcomes to be predicted |
|--------------------------|
| Definition and method for measurement of outcomes |
| Was the same outcome definition (and method for measurement) used in all participants? |
| Types of outcomes |
| Were the outcomes assessed without knowledge of the candidate prognostic factors (i.e., blinded)? |
| Were candidate prognostic factors part of the outcome? |
| Time of outcome occurrence or summary of duration of follow-up |

| Prognostic factors (index and comparator) |
|------------------------------------------|
| Number and type of prognostic factors |
| Definition and method for measurement of prognostic factors |
| Timing of prognostic factor measurement |
| Were prognostic factors assessed blinded for outcome, and for each other (if relevant)? |
| Handling of prognostic factors in the analysis |

| Sample size |
|-------------|
| Was a sample size calculation conducted and, if so, how? |
| Number of participants and number of outcomes or events |
| Number of outcomes or events in relation to the number of candidate prognostic factors (events per variable) |

| Missing data |
|--------------|
| Number of participants with any missing value |
| Number of participants with missing data for miR-21 expression |
| Details of attrition (loss to follow-up) and, for time-to-event outcomes, number of censored observations |
| Handling of missing data |

| Analysis (N/A for studies excluded from meta-analysis) |
|-----------------------------------------------------|
| Modelling method |
| How modelling assumptions were checked; the method for assessing non-proportional hazards |
| Method for selection of prognostic factors for inclusion in multivariable modelling |
| Method for selection or exclusion of prognostic factors during multivariable modelling, and criteria used for any selection or exclusion |
| Method of handling each continuous prognostic factor, including values of any cut points used and their justification |

| Results of studies included in meta-analysis |
|---------------------------------------------|
| Unadjusted and adjusted prognostic effect estimates for miR-21 expression, the corresponding 95\% confidence interval with p-value. |
| For the extracted adjusted prognostic effect estimate of interest, the set of adjustment factors used |

| Results of studies excluded from meta-analysis |
|-----------------------------------------------|
| Prognostic factors or stratification used for association analysis |
| Type of association analysis and estimates with p-value |

| Interpretation and discussion |
|-------------------------------|
| Interpretation of presented results |
| Comparison with other studies, discussion of generalisability, strengths and limitations |
### ST 3: Records of authors contacted (12 studies)

| Study ID     | Author contacted                                                                 | Response | Additional data                                      |
|--------------|----------------------------------------------------------------------------------|----------|------------------------------------------------------|
| Bryant2012   | Freddie Hamdy<br><freddie.hamdy@nds.ox.ac.uk><richard.bryant@nds.ox.ac.uk>      | Yes      | miR-21 raw data excel file including 78 PCa patients  |
| Fendler2011  | Klaus Jung<br><klaus.jung@charite.de>                                             | Yes      | No (Communication stopped without useful data)       |
| Huang2015a   | Liang Wang<br>liwang@mcw.edu                                                     | No       |                                                      |
| Kelly2015    | Brian Kelly<br>dbriankelly@hotmail.com                                            | Yes      | No (Communication stopped without useful data)       |
| Leite2013    | Katia Ramos Moreira Leite<br>katiaramos@usp.br                                  | Yes      | Clarification on results reported                    |
| Leite2015    | Updated: <katiaramos@usp.br>                                                     | Yes      | Details of multivariate analysis                     |
| Lin2014      | Hui-Ming Lin<br>b.lin@garvan.org.au                                             | Yes      | Clarification on analysis method                     |
| Lin2017      | Updated: <b.lin@garvan.org.au>                                                   | Yes      | Results of univariate & multivariate analyses        |
| McDonald2019 | Alicia McDonald<br>amcdonald3@phs.psu.edu                                        | Yes      | No (miR-21 measured but not analysed because it did not meet criteria) |
| Mortensen2014| Lars Dyrskjet Andersen<br>lars@clin.au.dk                                        | Yes      | Raw unanalysed data                                  |
| Schubert2013 | Maria Schubert<br>schubert_m@klinik.uni-wuerzburg.de<br>Burkhard Kneitz<br>kneitz_b@klinik.uni-wuerzburg.de | No       |                                                      |
| Stuopelyte2016| Sonata Jarmalaite<br>sonata.jarmalaite@gf.vu.lt<br>sonata.jarmalaite@nvi.lt      | No       |                                                      |
| ST 4: QUIPS (Quality in Prognostic Factor Studies) risk of bias classification tool |
|---|---|---|
| **Signalling items** | **1. Study participation** | 
| | (a) Adequate participation in the study by eligible persons | HIGH |
| | (b) Description of the target population or population of interest | MODERATE |
| | (c) Description of the baseline study sample | LOW |
| | (d) Adequate description of the sampling frame and recruitment | 
| | (e) Adequate description of the period and place of recruitment | 
| | (f) Adequate description of inclusion and exclusion criteria | 
| **Risk of bias ratings** | The relationship between the PF and outcome is very likely to be different for participants and eligible non-participants | 
| | The relationship between the PF and outcome may be different for participants and eligible non-participants | 
| | The relationship between the PF and outcome is unlikely to be different for participants and eligible non-participants | **
| **Signalling items** | **2. Study attrition** | 
| | (a) Adequate response rate for study participants | HIGH |
| | (b) Description of attempts to collect information on participants who dropped out | MODERATE |
| | (c) Reasons for loss to follow-up are provided | LOW |
| | (d) Adequate description of participants lost to follow-up | 
| | (e) There are no important differences between participants who completed the study and those who did not | 
| **Risk of bias ratings** | The relationship between the PF and outcome is very likely to be different for completing and non-completing participants | 
| | The relationship between the PF and outcome may be different for completing and non-completing participants | 
| | The relationship between the PF and outcome is unlikely to be different for completing and non-completing participants | **
| **Signalling items** | **3. Prognostic factor measurement** | 
| | (a) A clear definition or description of the PF is provided | HIGH |
| | (b) Method of PF measurement is adequately valid and reliable | MODERATE |
| | (c) Continuous variables are reported or appropriate cut-points are used | LOW |
| | (d) The method and setting of measurement of PF is the same for all study participants | 
| | (e) Adequate proportion of the study sample has complete data for the PF | 
| | (f) Appropriate methods of imputation are used for missing PF data | 
| **Risk of bias ratings** | The measurement of the PF is very likely to be different for different levels of the outcome of interest | 
| | The measurement of the PF may be different for different levels of the outcome of interest | 
| | The measurement of the PF is unlikely to be different for different levels of the outcome of interest | **
| **Signalling items** | **4. Outcome measurement** | 
| | (a) A clear definition of the outcome is provided | HIGH |
| | (b) Method of outcome measurement used is adequately valid and reliable | MODERATE |
| | (c) The method and setting of outcome measurement is the same for all study participants | LOW |
| **Risk of bias ratings** | The measurement of the outcome is very likely to be different related to the baseline level of the PF | 
| | The measurement of the outcome may be different related to the baseline level of the PF | 
| | The measurement of the outcome is unlikely to be different related to the baseline level of the PF | **
| **Signalling items** | **5. Adjustment for covariates** | 
| | (a) All other important covariates are measured | HIGH |
| | (b) Clear definitions of the important covariates measured are provided | MODERATE |
| | (c) Measurement of all important covariates is adequately valid and reliable | LOW |
| | (d) The method and setting of covariate measurement are the same for all study participants | 
| | (e) Important covariates are accounted for in the analysis | 
| | (f) Important covariates are accounted for in the analysis | 
| **Risk of bias ratings** | The observed effect of the covariate on the outcome is very likely to be distorted by another factor related to PF and outcome | 
| | The observed effect of the covariate on outcome may be distorted by another factor related to PF and outcome | 
| | The observed effect of the covariate on outcome is unlikely to be distorted by another factor related to PF and outcome | **
| **Signalling items** | **6. Statistical analysis and reporting** | 
| | (a) Sufficient presentation of data to assess the adequacy of the analytic strategy | HIGH |
| | (b) Strategy for model building is appropriate and is based on a conceptual framework or model | MODERATE |
| | (c) The selected statistical model is adequate for the design of the study | LOW |
| | (d) There is no selective reporting of results | 
| **Risk of bias ratings** | The reported results are very likely to be spurious or biased related to analysis or reporting | 
| | The reported results may be spurious or biased related to analysis or reporting | 
| | The reported results are unlikely to be spurious or biased related to analysis or reporting | **

* Risk of bias is rated as **Unclear** when there is insufficient information to inform judgment.

**PF:** Prognostic factor
**ST 5: Reasons for exclusion of 13 full-text articles**

| Reason for exclusion                  | Full-text articles                                                                 |
|--------------------------------------|------------------------------------------------------------------------------------|
| No prognostic data (n=4)              | Benoist2020; Egidi2013; Li2015; Liu2018; Martens-Uzunova2012; Osipov2016; Valera2020; Yang2015 |
| miR-21 not studied (n=4)              | Haldrup2014; Knyazev2016; Moltzahn2011; Nam2015                                     |
| Non-original human prognostic data (n=1) | Kumar2018                                                                         |

**Benoist2020**
Benoist, G.E., van Oort, I.M., Boerigter, E., Verhaegh, G.W., van Hooij, O., Groen, L., Smit, F., de Mol, P., Hamberg, P., Dezentjé, V.O. and Mehra, N., 2020. Prognostic Value of Novel Liquid Biomarkers in Patients with Metastatic Castration-Resistant Prostate Cancer Treated with Enzalutamide: A Prospective Observational Study. *Clinical Chemistry*, 66(6), pp.842-851.

**Egidi2013**
Egidi, M.G., Cochetti, G., Serva, M.R., Guelfi, G., Zampini, D., Mechelli, L. and Mearini, E., 2013. Circulating microRNAs and kallikreins before and after radical prostatectomy: are they really prostate cancer markers? *BioMed research international*, 2013.

**Haldrup2014**
Haldrup, C., Kosaka, N., Ochiya, T., Borre, M., Høyer, S., Orntoft, T.F. and Sorensen, K.D., 2014. Profiling of circulating microRNAs for prostate cancer biomarker discovery. *Drug delivery and translational research*, 4(1), pp.19-30.

**Knyazev2016**
Knyazev, E., Samatov, T., Fomicheva, K., Nyushko, K., Alekseev, B. and Shkurnikov, M., 2016. MicroRNA hsa-miR-4674 in hemolysis-free blood plasma is associated with distant metastases of prostatic cancer. *Bulletin of Experimental Biology & Medicine*, 161(1).

**Kumar2018**
Kumar, B., Rosenberg, A.Z., Choi, S.M., Fox-Talbot, K., De Marzo, A.M., Nnon, L., Brennen, W.N., Marchionni, L., Halushka, M.K. and Lupold, S.E., 2018. Cell-type specific expression of oncogenic and tumor suppressive microRNAs in the human prostate and prostate cancer. *Scientific reports*, 8(1), pp.1-13.

**Li2015**
Li, M., Rai, A.J., DeCastro, G.J., Zeringer, E., Barta, T., Magdaleno, S., Setterquist, R. and Vlassov, A.V., 2015. An optimized procedure for exosome isolation and analysis using serum samples: application to cancer biomarker discovery. *Methods*, 87, pp.26-30.

**Liu2018**
Liu, R.S., Olikhov-Mitsel, E., Jeyapala, R., Zhao, F., Commissos, K., Klotz, L., Loblaw, A., Liu, S.K., Vesprini, D., Fleshner, N.E. and Bapati, B., 2018. Assessment of serum microRNA biomarkers to predict reclassification of prostate cancer in patients on active surveillance. *The Journal of urology*, 199(6), pp.1475-1481.

**Martens-Uzunova2012**
Martens-Uzunova, E.S., Jalava, S.E., Dits, N.F., Van Leenders, G.J.L.H., Møller, S., Trapman, J., Bangma, C.H., Litman, T., Visakorpi, T. and Jenster, G., 2012. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. *Oncogene*, 31(8), pp.978-991.

**Moltzahn2011**
Moltzahn, F., Olshen, A.B., Baehner, L., Peek, A., Fong, L., Stöppler, H., Simko, J., Hilton, J.F., Carroll, P. and Blelho, R., 2011. Microfluidic-based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in the sera of prostate cancer patients. *Cancer research*, 71(2), pp.550-560.

**Nam2015**
Nam, R.K., Amemiya, Y., Benatar, T., Wallis, C.J., Stoijic-Bendavid, J., Bapopulos, S., Sherman, C., Sugar, L., Naeim, M., Yang, W. and Zhang, A., 2015. Identification and validation of a five microRNA signature predictive of prostate cancer recurrence and metastasis: a cohort study. *Journal of Cancer*, 6(11), p.1160.

**Osipov2016**
Osipov, I.D., Zaporozhchenko, I.A., Bondar, A.A., Zaripov, M.M., Voyitsitskii, V.E., Vlassov, V.V., Laktionov, P.P. and Morozkin, E.S., 2016. Cell-free miRNA-141 and miRNA-205 as prostate cancer biomarkers. In *Circulating Nucleic Acids in Serum and Plasma–CNAPS IX* (pp. 9-12). Springer, Cham.

**Valera2020**
Valera, V.A., Parra-Medina, R., Walter, B.A., Pinto, P. and Merino, M.J., 2020. microRNA expression profiling in young prostate cancer patients. *Journal of Cancer*, 11(14), p.4106.

**Yang2015**
Yang, C.H., Pfeffer, S.R., Sims, M., Yue, J., Wang, Y., Linga, V.G., Paulus, E., Davidoff, A.M. and Pfeffer, L.M., 2015. The oncogenic microRNA-21 inhibits the tumor suppressive activity of FBXO11 to promote tumorigenesis. *Journal of Biological Chemistry*, 290(10), pp.6037-6046.
| Ref no. | Study ID | Study size | miR-21 source | miR-21-5p/-3p | Comparator | Association |
|---------|----------|------------|---------------|---------------|------------|-------------|
| 29      | Agaoglu2011 | 51         | plasma        | Not specified | PSA, metastasis | Correlation, median diff |
| 30      | Al-Qatati2017 | 79         | plasma        | miR-21-5p     | GS, pT, PSA, risk groups | FC |
| 31      | Amankwah2013 | 65         | tissue        | Not specified | Aggressiveness (determined by GS or stage), recurrence (BCR/clinical metastasis/PCa death) | % diff |
| 32      | Arisan2020  | 40         | tissue        | Not specified | GS         | % diff |
| 33      | Bell2015*   | 43         | tissue        | Not specified | (Raw data of m-R-21 in GEO not analysed. No other miR-21 data available.) |         |
| 34      | Bonci2016   | 15         | tissue        | Not specified | Metastasis | % diff |
| 35      | Brase2011   | 21         | serum         | Not specified | Metastasis | FC |
| 36      | Bryant2012* | 78         | plasma        | Not specified | (Author provided miR-21 raw data excel file.) |         |
| 37      | Danarto2020 | 60         | urine         | miR-21-5p     | Metastasis | Mean diff |
| 38      | Endzelinski2017* | 50     | plasma or exosome | miR-21-5p | (Comparison and ROC curve of miR-21 expression between GS≥8 & ≤6 were done but not shown due to insignificant result.) |         |
| 39      | Farran2018  | 114        | plasma        | Not specified | Aggressiveness (determined by GS) | OR |
| 40      | Fendler2011* | 52        | tissue        | Not specified | (Communication with authors failed to obtain full list of differentially expressed miRNAs.) |         |
| 41      | Foj2017     | 60         | urine, urine exosome | miR-21-5p | GS, D'Amico risk groups | Mean diff |
| 42      | Guan2016    | 85         | tissue        | Not specified | GS, PSA, metastasis, age | Correlation |
| 43      | Gurbuz2020  | 65         | whole blood   | Not specified | GS, TNM, PSA | FC diff |
| 44      | Hart2014    | 20         | tissue        | Not specified | pT         | FC diff |
| 45      | Hoey2019    | 75         | serum         | miR-21-5p     | Risk groups | FC |
| 46      | Huang2015a* | Screening =23 Follow-up =100 | plasma exosome | miR-21-5p | (miR-21 raw data in supplemental materials; overall survival might have been analysed but contact author failed.) |         |
| 47      | Huang2015b  | 75         | PBMC          | Not specified | pT, ct, pN, metastasis, recurrence, age | Mean diff |
| 48      | Ibrahim2019a| 100        | plasma        | Not specified | GS, pT, metastasis, DRE, prostate volume | Correlation, mean diff |
| 49      | Ibrahim2019b| 80         | plasma        | Not specified | GS, pT, PSA, metastasis, DRE, prostate volume | Median diff |
| 50      | Ju2019      | 88         | serum         | Not specified | GS, pT, PSA, metastasis, BCR, risk groups | Mean diff |
| 51      | Katz2014    | 51         | tissue        | Not specified | GS, pT, PSA, BCR, risk groups | Mean diff |
| 52      | Kelly2015*  | 75         | whole blood   | Not specified | (miR-21 was among the 12 selected for expression profiling, but data wasn't presented. Author stopped communication.) |         |
| 53      | Kopcalic2019| 15         | PBMC          | Not specified | Acute genitourinary radiotoxicity | Mean diff |
| 54      | Kotb2014    | 10         | serum         | Not specified | GS | Correlation |
| 55      | Kristensen2016 | Training =134 | tissue        | miR-21-3p     | GS, BCR | FC, correlation |
| Validation | Leite2015 | Discovery 53 | tissue | miR-21-5p, miR-21-3p | BCR | FC, mean diff |
|---|---|---|---|---|---|---|
| Leite2011a | 22 | tissue | Not specified | Metastasis | Mean diff |
| Leite2011b | 49 | tissue | Not specified | BCR | Mean diff |
| Leite2013 ** | 48 | tissue | Not specified | Risk groups (favourable vs non-favourable) | Mean diff |
| Leite2015 | Discovery 53 | tissue | miR-21-5p, miR-21-3p | BCR | FC, mean diff |
| Li2012 | 168 | tissue | Not specified | GS, pT, PSA, pN, BCR, age, surgical margin, capsular invasion, organ confined disease | % diff |
| Lichner2013 | Discovery 41 | tissue | miR-21-5p, miR-21-3p | Risk groups | FC |
| Lichner2015 | Discovery 45 | tissue | miR-21-5p, miR-21-3p | GG | FC |
| Lin2014 * | 97 | plasma or serum | Not specified | (Pre-docetaxel median diff and post-docetaxel median FC in responder vs non-responder compared. Results for miR-21 not shown due to insignificant p-values.) | (No association analysis with comparator.) |
| Lin2017 * | 87 | plasma | Not specified | (miR-21 expression relating to BCR prediction raw data in supplemental materials.) |
| Long2011 * | Training 70 | tissue | Not specified | (miR-21 expression measured but not analysed because it did not meet study criteria.) |
| McDonald 2019 * | 66 | plasma | Not specified | (miR-21 expression measured but not analysed.) |
| Melbø-Jørgensen 2014 | 535 | tissue | miR-21-5p | GS, pT, BCR, perineural infiltration, vascular infiltration | Correlation, FC |
| Mortensen2014 * | 36 | tissue | Not specified | (miR-21 expression measured but not analysed.) |
| Nam2018 * | 38 | tissue | miR-21-5p, miR-21-3p | (miR-21 normalised read count available in GEO, not analysed.) |
| Ostano2020 | 48 | tissue | miR-21-3p | Neuroendocrine-like vs Adeno PCa | FC |
| Reis2012 | 53 | tissue | Not specified | GS, pT, PSA, BCR | Mean diff |
| Ren2014 | 204 | tissue | Not specified | GS, pT, metastasis, BCR, age, ethnicity, survival, tissue type, hormone therapy | FC, mean diff |
| Samaan2014 | 95 | Not stated | Not specified | GG | FC |
| Sapre2014 | 36 | urine | Not specified | Risk groups | Ct FC |
| Schubert 2013 * | 13 | tissue | Not specified | (miR-21 tested in microarray; raw data deposited in GEO (GSE18671); not included in further tests because of insignificant differential expression in high-risk PCa compared to BPH.) |
|   | Study Year | Study Type | Sample Type | Sample Source | Biomarkers | Endpoints |
|---|------------|------------|-------------|---------------|-------------|-----------|
| 77 | Selth2013 | Screening | serum | Not specified | BCR | FC |
| 78 | Sharova2021 | Validation | plasma | miR-21-5p | Haemoglobin; Neutrophil/lymphocyte ratio; PSA; Time to CRPC | Correlation |
| 79 | Shen2012 | Validation | plasma | Not specified | GS, pT, PSA, BCR, risk groups (CAPRA, D’Amico), age, prostate volume, ethnicity, follow-up time, family history of PCa | Mean diff (copy number) |
| 80 | Singh2014 | Validation | serum | Not specified | Biochemical progression | Mean diff (delta Ct) |
| 81 | Stuopelyte 2016 | Validation | urine | Not specified | GS, pT, BCR | FC |
| 82 | Suer2019 | Validation | tissue | miR-21-3p | BCR | FC |
| 83 | Watahiki2013 | Validation | plasma | Not specified | mCRPC | Mean diff |
| 84 | Yang2016 | Validation | PBMC | Not specified | GS, cT, PSA, metastasis (bone), BCR, age | Mean diff |
| 85 | Zedan2017 | Validation | tissue | Not specified | GS, pT, PSA, risk groups (D’Amico, NCCN) | Correlation |
| 86 | Zedan2018 | Validation | tissue or plasma | Not specified | GS, PSA | Mean diff |
| 87 | Zedan2019 | Validation | plasma | Not specified | GS, cT, PSA, risk groups (EAU), age, prostate volume | Correlation |
| 88 | Zhang2011 | Validation | serum | Not specified | Chemo-resistance | |
| 89 | Zhao2019a | Validation | tissue | miR-21-5p | ISUP (based on GS), pT, PSA, age, DRE, margin | Correlation |
| 90 | Zhao2019b | Validation | urine | Not specified | PSA, age, %core, reclassification | Correlation |
| 91 | Zheng2014 | Validation | tissue | Not specified | Recurrence (CBR/local recurrence/systemic metastases/PCa death) | Mean diff, OR |
| 92 | Zhu2019 | Validation | tissue | Not specified | Risk groups (identified by GAS5 SNPs) | FC |

Studies in bold are eligible for meta-analyses (n=11). Possible part overlap of participants between Ibrahim2019a and Ibrahim2019b.

* miR-21 expression measured but no useful data for narrative summary (n=13).

** (Leite2013) A corrigendum would be published in Urologic Oncology.

ARTA: Androgen receptor-targeted agents; BCR: Biochemical recurrence; BPH: Benign prostate enlargement; CAPRA: Cancer of the Prostate Risk Assessment; CRPC: Castration-resistant prostate cancer; cT: Clinical tumour stage; Ct: Threshold cycle; diff: Difference; DRE: Digital rectal examination; EAU: European Association of Urology; FC: Fold change; GAS5: Growth Arrest Specific 5; GEO: Gene Expression Omnibus; GG: Gleason grade; GS: Gleason score; ISUP: International Society of Urological Pathology; mCRPC: Metastatic castration resistant prostate cancer; miRNAs: microRNAs; NCCN: National Comprehensive Cancer Network; OR: Odds ratio; PBMC: Peripheral blood mononuclear cell; PCA: Prostate cancer; pN: Lymph node metastasis; PSA: Prostate-specific antigen; pT: Pathological tumour stage; ROC: Receiver operating characteristic; SNPs: Single-nucleotide polymorphisms; TNM: Tumour, Node, Metastasis staging.
Agaoglu2011
Agaoglu, F.Y., Kovancilar, M., Dizdar, Y., Darendeliler, A., Holdenrieder, S., Dalay, N. and Gezer, U., 2011. Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumor Biology, 32(3), pp.583-588.

Al-Qatati2017
Al-Qatati, A., Akrong, C., Stevic, I., Pantel, K., Awe, J., Saranchuk, J., Drachenberg, D., Mai, S. and Schwarzenbach, H., 2017. Plasma microRNA signature is associated with risk stratification in prostate cancer patients. International journal of cancer, 141(6), pp.1231-1239.

Amankwah2013 (Analysis 1)
Amankwah, E.K., Anegbe, E., Park, H., Pow-Sang, J., Hakam, A. and Park, J.Y., 2013. miR-21, miR-221 and miR-222 expression and prostate cancer recurrence among obese and non-obese cases. Asian journal of andrology, 15(2), p.226.

Arisan2020
Arisan, E.D., Rencuzogullari, O., Freitas, I.L., Radzali, S., Keskin, B., Kothari, A., Warford, A. and Uysal-Onganer, P., 2020. Upregulated Wnt-11 and miR-21 expression trigger epithelial mesenchymal transition in aggressive prostate cancer cells. Biology, 9(3), p.52.

Bell2015
Bell, E.H., Kirste, S., Fleming, J.L., Stegmaier, P., Drendel, V., Mo, X., Ling, S., Fabian, D., Manring, I., Jill, C.A. and Schultze-Seemann, W., 2015. A novel miRNA-based predictive model for biochemical failure following post-prostatectomy salvage radiation therapy. Plos one, 10(3), p.e0118745.

Bonci2016
Bonci, D., Coppola, V., Patrizii, M., Addario, A., Cannistraci, A., Francescangeli, F., Pecchi, R., Muto, G., Collura, D., Bedini, R. and Zeuner, A., 2016. A microRNA code for prostate cancer metastasis. Oncogene, 35(9), pp.1180-1192.

Brase2011
Brase, J.C., Johannes, M., Schlom, T., Fälth, M., Haese, A., Steuber, T., Beissbarth, T., Kuner, R. and Sültemann, H., 2011. Circulating miRNAs are correlated with tumor progression in prostate cancer. International journal of cancer, 128(3), pp.608-616.

Bryant2012
Bryant, R., Pawlowski, T., Catto, J.W.F., Marsden, G., Vessella, R.L., Rhee, B., Kuslich, C., Visakorp, T. and Hamdy, F.C., 2012. Changes in circulating microRNA levels associated with prostate cancer. British journal of cancer, 106(4), pp.768-774.

Danarto2020
Danarto, R., Astuti, I., Umbas, R. and Haryana, S.M., 2020. Urine miR-21-5p and miR-200c-3p as potential non-invasive biomarkers in patients with prostate cancer. Turkish journal of urology, 46(1), p.26.

Endzelis2017
Endzelis, E., Berger, A., Melne, V., Bajo-Santos, C., Sobolovska, K., Ābols, A., Rodríguez, M., Šantare, D., Rudnickiha, A., Lietuvietis, V. and Llorente, A., 2017. Detection of circulating miRNAs: comparative analysis of extracellular vesicle-incorporated miRNAs and cell-free miRNAs in whole plasma of prostate cancer patients. BMC cancer, 17(1), pp.1-13.

Farran2018
Farran, B., Dyson, G., Craig, D., Dombkowski, A., Beebe-Dimmer, J.L., Powell, I.J., Podgorski, I., Heilbrun, L., Bolton, S. and Bock, C.H., 2018. A study of circulating microRNAs identifies a new potential biomarker panel to distinguish aggressive prostate cancer. Carcinogenesis, 39(4), pp.556-561.

Fendler2011
Fendler, A., Jung, M., Stephan, C., Honey, R.J., Stewart, R.J., Pace, K.T., Erbersdobler, A., Samaan, S., Jung, K. and Yousef, G.M., 2011. miRNAs can predict prostate cancer biochemical relapse and are involved in tumor progression. International journal of oncology, 39(5), pp.1183-1192.

Foj2017
Foj, L., Ferrer, F., Serra, M., Arévalo, A., Gavagnach, M., Giménez, N. and Filella, X., 2017. Exosomal and non-exosomal urinary miRNAs in prostate cancer detection and prognosis. The Prostate, 77(6), pp.573-583.

Guan2016 (Eligible for meta-analysis but no similar studies)
Guan, Y., Wu, Y., Liu, Y., Ni, J. and Nong, S., 2016. Association of microRNA-21 expression with clinicopathological characteristics and the risk of progression in advanced prostate cancer patients receiving androgen deprivation therapy. The Prostate, 76(11), pp.986-993.

Gurbuz2020
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### ST 7: Rationales for rating down certainty of evidence - GRADE

| Domains | Analysis 1.1 | Analysis 1.2 | Analysis 2 | Analysis 3 | Analysis 4.1 | Analysis 4.2 |
|---------|--------------|--------------|------------|------------|--------------|--------------|
| **RoB** | Estimate was unadjusted but sensitivity analysis showed limited difference in HR, rate-down not necessary. | Visual inspection of the point estimates and CI showed limited difference caused by difference in covariate adjustments, rate-down not necessary. | Unadjusted estimate and high RoB in 1 study (Zhao2019a – Domain 5), rate down 1 point. | Unadjusted estimate and high RoB in 3 studies (Lin2014, Lin2017 & Sharova2021 – Domain 5), rate down 1 point. | High RoB in both studies (Domain 5), rate down 1 point. | High RoB in both studies (Domain 5), rate down 1 point. |
| **Inconsistency** | Amankwah2013 outlying but low weight (8.5%), rate-down not necessary. | Amankwah2013 outlying but low weight (8.5%), rate-down not necessary. | Both studies showed positive association and CI overlapped, no rate-down. | Sharova2021 outlying but low weight (8.2%), rate-down not necessary. | The two studies showed opposite direction results, rate down 1 point. | The two studies showed opposite direction results, rate down 1 point. |
| **Indirectness** | Amankwah2013 RFS endpoint included clinical metastasis and PCa death but low weight, rate-down not necessary. | Amankwah2013 RFS endpoint included clinical metastasis and PCa death but low weight, rate-down not necessary. | No rate-down. | Lin2014 & Lin2017 included CRPC patients, not representing entire PCa population; main aim was to address chemo-response, rate down 1 point. | No rate-down. | No rate-down. |
| **Imprecision** | Pooled CI well excluded HR of 1 but individual HRs were not reported and hence estimated from available data, rate down 1 point. | Pooled CI well excluded HR of 1, no rate-down. | Pooled CI close to HR of 1 (CI: 1.01-1.26), rate down 1 point. | HR was not reported and hence estimated from available data in Yang 2016. Pooled CI close to HR of 1 (CI: 1.06-2.01), rate down 1 point. | Wide pooled CI crossing HR of 1 (CI: 0.63-1.88), rate down 1 point. | Wide pooled CI crossing HR of 1 (CI: 0.70-2.27), rate down 1 point. |

**Publication bias**

- Publication bias was not assessed because there was inadequate number of studies for proper assessment by funnel plot and statistical tests.

**Overall certainty**

| LOW | MODERATE | LOW | VERY LOW | VERY LOW | VERY LOW |

**Note:**
- CI: Confidence interval; CRPC: Castration-resistant prostate cancer; HR: Hazard ratio; mCRPC: metastatic castration-resistant prostate cancer; PCa: Prostate cancer; RFS: Recurrence-free survival; RoB: Risk of bias
SUPPLEMENTARY FIGURES

SF 1: Associations of miR-21 expression with clinicopathological measurements. (a) Gleason score/grade, (b) Stage, (c) PSA, (d) Recurrence, (e) Metastasis, (f) Risk stratification and (g) Age at diagnosis.
# PRISMA 2009 Checklist

| Section/topic          | # | Checklist item                                                                                                                                                                                                 | Reported on page # |
|------------------------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| **TITLE**              |   |                                                                                                                                                                                                                 |                   |
| Title                  | 1 | Identify the report as a systematic review, meta-analysis, or both.                                                                                                                                              | 1                 |
| **ABSTRACT**           |   |                                                                                                                                                                                                                 |                   |
| Structured summary     | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2                 |
| **INTRODUCTION**       |   |                                                                                                                                                                                                                 |                   |
| Rationale              | 3 | Describe the rationale for the review in the context of what is already known.                                                                                                                                     | 3                 |
| Objectives             | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).                                                       | 4                 |
| **METHODS**            |   |                                                                                                                                                                                                                 |                   |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.                                          | 5                 |
| Eligibility criteria   | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.                       | 5-6               |
| Information sources    | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.                                              | 5                 |
| Search                 | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.                                                                                     | 5, ST1            |
| Study selection        | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).                                                         | 5                 |
| Data collection process | 10| Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.                                                | 6, ST3            |
| Data items             | 11| List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.                                                                             | 6, ST2            |
| Risk of bias in individual studies | 12| Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 6-7, ST4          |
| Summary measures       | 13| State the principal summary measures (e.g., risk ratio, difference in means).                                                                                                                                       | 7                 |
| Synthesis of results   | 14| Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I² for each meta-analysis).                                                                  | 7                 |
| Section/topic                     | #  | Checklist item                                                                                                                                                                                                 | Reported on page # |
|----------------------------------|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| Risk of bias across studies      | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).                                                                 | 7                |
| Additional analyses              | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.                                                                  | 7                |

**RESULTS**

| Study selection                   | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.                                               | 8                |
| Study characteristics             | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.                                                                                 | 8-9, Table 2     |
| Risk of bias within studies       | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).                                                                                                                 | 10, Table 4      |
| Results of individual studies     | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.   | 10, Table 5      |
| Synthesis of results              | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency.                                                                                                             | 10-13            |
| Risk of bias across studies       | 22 | Present results of any assessment of risk of bias across studies (see Item 15).                                                                                                                               | 15, Table 7      |
| Additional analysis               | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).                                                                                             | 11-13            |

**DISCUSSION**

| Summary of evidence               | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 16               |
| Limitations                       | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).                                                      | 16-20            |
| Conclusions                       | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research.                                                                                          | 20               |

**FUNDING**

| Funding                           | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.                                                                   | 1, 21            |

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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