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Impact of homologous recombination deficiency biomarkers on outcomes in patients with primary breast cancer: a systematic review protocol

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ABSTRACT

Introduction Breast cancer patients with homologous recombination deficiency (HRD) such as germline BRCA1/2 mutations would respond to DNA-damaging drugs. Several clinical studies have revealed that HRD biomarkers were associated with the outcomes of patients with primary breast cancer (PBC). However, no systematic review has determined the prognostic role of HRD biomarkers in PBC patients. Therefore, this study will systematically combine and analyze the results of previous studies, to facilitate the clinical use of HRD detection in PBC.

Methods and analysis We will search five databases including PubMed, Cochrane Library, EMBASE, OVID, and Web of Science through December 2021, with language restriction of English. Two reviewers will independently screen all records based on pre-established inclusion and exclusion criteria. The main outcomes include pathological Complete Response, Disease-free Survival, and Overall Survival. In addition, all studies included must contain the detection of HRD biomarkers. Data extraction will be carried out by two reviewers independently according to a self-designed template. The Newcastle-Ottawa Quality Assessment Scale and Jadad scale will be used for quality assessment for cohort studies and randomized clinical trials, respectively. Review Manager version 5.3.5 will be utilized to perform meta-analysis. Both the Q test and I^2 statistic will be used to assess heterogeneity. Subgroup and sensitivity analysis will be conducted if significant heterogeneity appears and cannot be reduced by using a random-effect model.

Ethics and dissemination Ethical approval is not required for a systematic review. The results will be disseminated through international and national conferences or peer-reviewed publications.

PROSPERO registration number CRD42021286522.

Strengths and limitations of this study

- This systematic review will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.
- Stringent inclusion and exclusion criteria will be used to select clinical studies assessing the impact of homologous recombination deficiency biomarkers on outcomes of patients with primary breast cancer.
- Internationally recognized scales will be used for the quality assessment, to exclude low-quality studies and enhance the credibility of pooled results.
- Only studies published in English will be included in this systematic review.
- Differences in patient cohort, sample size, treatment regimen, and measure of homologous recombination deficiency biomarkers may yield significant heterogeneity.

INTRODUCTION

Cancer refers to a disease in which cells divide uncontrollably and invade normal tissues[1]. Cells must undergo two genetic changes to become cancerous: activation of proto-oncogenes and inactivation of tumor suppressor genes[2]. Endogenous (replication stress, oxygen radicals, and cell metabolism) and exogenous (radiation, viral infection, and chemotherapy) damaging factors continually act on the genome of cells and caused different degrees of DNA lesions[3]. And what protects organisms from cancer is that cells have inherent repair mechanisms to eliminate these damaging events. DNA damages that occur on a single strand are to be dealt with by a number of simple repair pathways including base-excision repair (BER), nucleotide-excision repair (NER), direct repair (DR), and mismatch repair (MMR). On the contrary, DNA double-strand break (DSB), which is the most severe DNA lesion and the main driver of cancer, requires sophisticated repair pathways such as NHEJ (non-homologous end joining) and HR (homologous recombination)[4]. The HR system utilizes a homologous sister chromatid (available in the S and G2 phases of cell cycle) as a template to copy and replace damaged DNA in a relatively error-free manner compared with NHEJ[5]. A number of key genes including BRCA1/2, RAD51, and PALB2 will encode functional proteins and get involved in the process of repair[6]. If these genes

are mutated, the HR system will fail to perform the repair function, which is so-called HR deficiency (HRD), leading to the accumulation of somatic mutations, chromosomal aberrations, and genomic scars (heritable genomic changes resulted from DNA repair defeat), as well as the development of cancer, especially breast cancer (BC)[7, 8].

BC is a highly heterogeneous disease and treated mainly based on the receptor expression status. In recent years, with the development of sequencing technology and our further understanding of genetic variation of cancer, numerous genes are being used to screen for available therapeutic targets[9]. For example, DNA-damaging drugs such as PARP inhibitors and platinums have been shown to significantly improve Progression-free Survival in patients with advanced triple-negative BC (TNBC) with germline BRCA1/2 mutations[10, 11]. Therefore, as key genes in the process of HR, BRCA1/2 are generally detected to determine the HRD status of BC patients. However, only 4% and 22% HRD can be attributed to germline BRCA1/2 mutations in BC and TNBC, respectively[12-14]. On this condition, biomarkers with wider coverage are needed to identify more BC patients with HRD. The HRD score is an algorithmic assessment of three measures of loss of heterozygosity (LOH), large-scale transition (LST), and telomeric allelic imbalance (TAI)[15]. This kind of assessment along with BRCA mutation detection is now widely used to define the HRD status.

Several studies have investigated the prognostic role of HRD score in primary BC (PBC)[16-19]. Telli et al assessed the HRD score in three neoadjuvant TNBC trials and found that a HRD score ≥42 or the presence of BRCA1/2 mutations were correlated with the objective response rate to platinum-based therapy[16]. SWOG S9313 is a phase III randomized study, comparing the efficacy of simultaneous anthracycline (A) and cyclophosphamide (C) and sequential A→C in more than 3000 stage I/II BC patients. Sharma et al investigated the prognostic role of HRD status in a subset of patients from SWOG S9313. The results indicated that HRD positive status was associated with better Disease-free Survival (DFS) (hazard ratio 0.72; 95% confidence interval 0.51–1.00; P=0.049)[17]. Significant associations between HRD positive status and higher pathological Complete Response (pCR) rates of PBC patients were also revealed in two studies by Loibl et al and Telli et al, respectively[18, 19]. Despite

all the above efforts, the detection of HRD biomarkers has not been incorporated into the clinical practice of BC. In addition, no systematic review has explored the relationship between HRD biomarkers and the prognosis of PBC patients. Therefore, we will firstly systematically combine and analyze the results of previous studies in this study, to facilitate the clinical use of HRD detection in PBC.

METHODS AND ANALYSIS

This systematic review will be conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement[20]. This review's protocol has been registered in the International Prospective Register of Systematic Reviews.

Search strategy

Five databases including PubMed, Cochrane Library, EMBASE, OVID, and Web of Science will be searched through December 2021, with language restriction of English. Medical Subject Headings and free text will be combined to search for concepts such as 'Breast Neoplasms' and 'Primary' and 'Recombinational DNA Repair'. The detailed example of the search strategy applied in PubMed is available in online **Supplemental File 1**. In addition, we will search the reference lists of recognized studies to identify additional papers.

Study selection

All records identified through database searching will be imported into EndNote version 9.1 software. Firstly, duplicates will be removed using the built-in recognition function of the software by the lead author. Then, all records will be screened by two reviewers independently according to the title and abstracts. After that, the potentially relevant full-text articles will be reviewed by the same two reviewers independently based on pre-established inclusion and exclusion criteria. Finally, the remaining full-text articles will be assessed for eligibility by the team. Disagreements between the two reviewers will be settled by discussion. The particular reason for exclusion of each reviewed article will be recorded and presented in the final manuscript. In addition, the

reference lists of recognized studies will be searched to make sure that no potentially eligible article is missed.

Inclusion criteria

Types of studies

Clinical studies investigating the impact of HRD biomarkers (HRD score, BRCA1/2 mutational status, and HRD status) on outcomes in patients with PBC will be included. This review will only include articles published in English, with no restriction of date. The treatment regimens in all studies included should be reasonable. For studies involving grouping, the treatment regimen received by patients in each group should be comparable.

Types of participants

Patients with histologically confirmed PBC.

Interventions/exposures

High HRD score, positive BRCA1/2 mutational status, and positive HRD status. Specifically, the assessment of HRD score should include three measures of tumor genomic instability (LOH, LST, and TAI), with a cutoff of 42[16].

Comparators/control

Low HRD score, negative BRCA1/2 mutational status, and negative HRD status.

Main outcomes

Each study should contain at least one of the following outcomes:

1. pCR: after neoadjuvant therapy, the lesions disappeared completely, or all symptoms and signs of unmeasurable lesions disappeared completely;

2. DFS: the time from randomization to disease recurrence or death due to disease progression;

3. Overall Survival (OS): the time from randomization to death from any cause.

Exclusion criteria

Articles that meet the following criteria will be excluded:

1. Non-clinical studies including reviews, conference abstracts, case reports and series, and comments;

2. Patients with metastatic or advanced BC;

3. No detection of HRD, wrong evaluation methods of HRD score, or other cutoff values;

4. Non-human experiments.

Data extraction

Two reviewers will independently extract data from the included studies into a self-designed data extraction template. If some important data are not available in the articles, we will make contact with the first or corresponding authors for potential support. Differences in opinion between the two reviewers will be settled by discussion. The study selection process is shown in **Figure 1**. The following study characteristics will be collected:

Study details

First author, year of publication, country/region, study design, and setting (neoadjuvant and adjuvant);

Patients characteristics

Patient subtype (hormone receptor-positive, human epidermal growth factor receptor 2-positve, and TNBC), number of patients, and treatment regimen;

Evaluation indicators

Main outcomes (pCR, DFS, and OS), HRD biomarkers (HRD score, BRCA1/2 mutational status, and HRD status), and score of quality assessment.

Quality assessment

Two reviewers will independently conduct quality assessment using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies and Jadad scale for randomized clinical trials, respectively[21, 22]. The NOS consists of three key items: 1. Selection; 2. Comparability; and 3. Outcome. One point will be added when there is enough support information for an item. One study that obtains at least 6 points will be considered as high quality, with a full score of 9 points[21]. The Jadad scale includes four key items: 1. Randomization; 2. Double blinding; 3. Concealment of allocation; and 4. Withdrawals and dropouts. If the description of one item is described and

appropriate, two points will be added to this item. On the contrary, if the description is not described or inappropriate, the score for this item will be zero. If the rating falls between the two situations, one point will be added. Specially, for the item of withdrawals and dropouts, only 1 and 0 point can be chosen. The full score of Jadad scale is 7 points, and a score of more than 3 points means high quality[22].

Statistical analysis

Data synthesis

All data will be synthesized narratively and quantitatively. If there are sufficient studies, meta-analysis will be further conducted. Otherwise, we will only carry out systematic review with descriptive analysis. Review Manager version 5.3.5 (Cochrane Collaboration, Oxford, UK) will be used to pool the results. Odds ratios and hazard ratios along with 95% confidence intervals will be calculated using the Mantel–Haenszel method and inverse variance method, respectively. Forest plots will be used to present the pooled results. For all statistical tests, a two-tailed *P*-value of <0.05 will be considered statistically significant.

Heterogeneity assessment

Before pooling the results, both the Q test and I^2 statistic will be used to assess heterogeneity. A *P*-value of <0.1 and an I^2 value of >50% indicate significant heterogeneity across studies. A fixed-effect model will be used unless considerable heterogeneity arises. Alternatively, a random-effect model will be used.

Subgroup and sensitivity analysis

If significant heterogeneity appears and cannot be reduced by using a random-effect model, subgroup analysis or sensitivity analysis will be conducted to find possible source of heterogeneity. The grouping methods of subgroup analysis will be based on the study characteristics, patient subtypes, chemotherapy regimens, or HRD detection methods, while the sensitivity analysis will be conducted by omitting the data of individual studies. The potential source of heterogeneity can be identified if the heterogeneity decreases significantly when carrying out subgroup analysis based on one factor or discarding data from one study.

Publication bias

Stata version 12.0 (Stata Corporation, College Station, TX, USA) will be used to evaluate potential publication bias using Egger's and Begg's test. A *P*-value of <0.05 will be considered a significant publication bias.

Patient and public involvement

Patients and/or the public are not involved in the design, conduct, reporting or dissemination plans of this research.

ETHICS AND DISSEMINATION

Ethical approval is not required in this study because no data are related to an individual patient. The results will be disseminated through international and national conferences or peer-reviewed publications.

Author contributions HaL, WP, and JZ were responsible for the conception of the study plan, and preparation of the manuscript. HuL reviewed the study plan and manuscript and offered comments and edits.

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Competing interests None declared.

Patient consent for publication Not required.

Data sharing No additional data available.

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Figure legend

Figure 1 Flow diagram of study selection process.

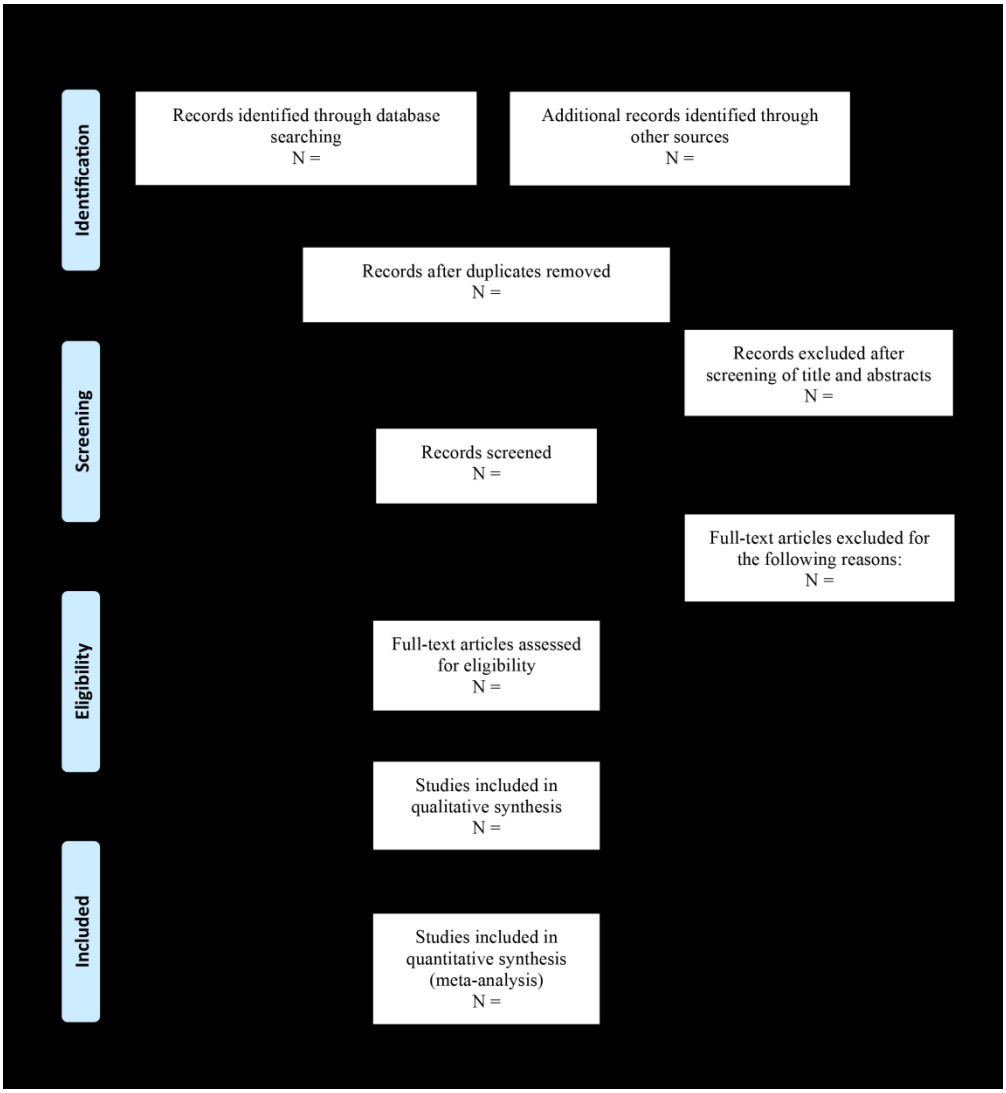

Figure 1 Flow diagram of study selection process.

Main search algorithm:

((("Breast Neoplasms"[Mesh]) OR ((Breast Neoplasm) OR (Neoplasm, Breast)) OR (Breast Tumors)) OR (Breast Tumor)) OR (Tumor, Breast)) OR (Tumors, Breast)) OR (Neoplasms, Breast)) OR (Breast Cancer)) OR (Cancer, Breast)) OR (Mammary Cancer)) OR (Cancer, Mammary)) OR (Cancers, Mammary)) OR (Mammary Cancers)) OR (Malignant Neoplasm of Breast)) OR (Breast Malignant Neoplasm)) OR (Breast Malignant Neoplasms)) OR (Malignant Tumor of Breast)) OR (Breast Malignant Tumor)) OR (Breast Malignant Tumors)) OR (Cancer of Breast)) OR (Cancer of the Breast)) OR (Mammary Carcinoma, Human)) OR (Carcinoma, Human Mammary)) OR (Carcinomas, Human Mammary)) OR (Human Mammary Carcinomas)) OR (Mammary Carcinomas, Human)) OR (Human Mammary Carcinoma)) OR (Mammary Neoplasms, Human)) OR (Human Mammary Neoplasm)) OR (Human Mammary Neoplasms)) OR (Neoplasm, Human Mammary)) OR (Neoplasms, Human Mammary)) OR (Mammary Neoplasm, Human)) OR (Breast Carcinoma)) OR (Breast Carcinomas)) OR (Carcinoma, Breast)) OR (Carcinomas, Breast))) AND (((((((Primary) OR (Early)) OR (Operable)) OR (Resectable)) OR (Curable)) OR (Non-metastatic)) OR (Non-advanced))) AND (("Recombinational DNA Repair"[Mesh]) OR ((((((((((((((((((Recombinational DNA Repair) OR (DNA Repair, Recombinational)) OR (Repair, Recombinational DNA)) OR (Recombinational Repair of DNA)) OR (DNA Recombinational Repair)) OR (Homologous Recombinational Repair)) OR (Homologous Recombinational Repairs)) OR (Recombinational Repair, Homologous)) OR (Repair, Homologous Recombinational)) OR (Homologous Recombination Repair)) OR (Recombination Repair, Homologous)) OR (Homologous Recombination Repair of DNA)) OR (Homologous Recombination DNA Repair)) OR (Recombination Repair)) OR (Repair, Recombination)) OR (Homologous Recombination Double-Stranded Break DNA Repair)) OR (Homologous Recombination Double Stranded Break DNA Repair)) OR (Homology-Directed dsDNA Break Repair)) OR (Homology Directed dsDNA Break Repair)))

Sources:

Total N =
PubMed N =
Cochrane Library N =
EMBASE N =
OVID N =
Web of Science N =
Other sources N =
N records after duplications removed
N records after screening of titles and abstracts
N records after further evaluation (N records were excluded for the following reasons: Non-clinical studies; Patients with metastatic or advanced BC; No detection of HRD, wrong evaluation methods of HRD score, or other cutoff values; and Non-human studies)
N records included in qualitative synthesis
N records included in quantitative synthesis (meta-analysis)

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Line 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Line 7
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Line 102
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Line 114
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Line 144
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Line 124
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Line 124
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Line 132
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Line 175
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Line 182
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Line 182
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Line 191
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Line 206
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Line 206
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Line 206
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 206
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Line 206
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Line 215
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Line 220
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	NA
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	NA
Study characteristics	17	Cite each included study and present its characteristics.	NA
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	NA
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	NA
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	NA
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	NA
	23b	Discuss any limitations of the evidence included in the review.	NA
	23c	Discuss any limitations of the review processes used.	NA
	23d	Discuss implications of the results for practice, policy, and future research.	NA
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Line 50
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Line 243
Competing interests	26	Declare any competing interests of review authors.	Line 244
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NA

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi:

PRISMA 2020 Checklist

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For more information, visit: http://www.prisma-statement.org/

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Genetics and genomics
Keywords:	Breast tumours < ONCOLOGY, Adult oncology < ONCOLOGY, Cancer genetics < GENETICS

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ABSTRACT

Introduction Breast cancer patients with homologous recombination deficiency (HRD) such as germline BRCA1/2 mutations would respond to DNA-damaging drugs. Several clinical studies have revealed that HRD biomarkers were associated with the outcomes of patients with early breast cancer (EBC). However, no systematic review has determined the prognostic role of HRD biomarkers in EBC patients. Therefore, this study will systematically combine and analyze the results of previous studies, to facilitate the clinical use of HRD detection in EBC.

Methods and analysis We will search five databases including PubMed, Cochrane Library, EMBASE, OVID, and Web of Science through December 2021, with no language restriction. Two reviewers will independently screen all records based on pre-established inclusion and exclusion criteria. The main outcomes include pathological Complete Response, Disease-free Survival, and Overall Survival. In addition, all studies included must contain the detection of HRD score, HRD status, or HRD-related gene mutational status and protein expression. Data extraction will be carried out by two reviewers independently according to a self-designed template. The Newcastle-Ottawa Quality Assessment Scale and Jadad scale will be used for quality assessment for cohort studies and randomized clinical trials, respectively. Review Manager version 5.3.5 will be utilized to perform meta-analysis. Both the Q test and I^2 statistic will be used to assess heterogeneity. Subgroup and sensitivity analysis will be conducted if significant heterogeneity appears and cannot be reduced by using a random-effect model.

Ethics and dissemination Ethical approval is not required for a systematic review. The results will be disseminated through international and national conferences or peer-reviewed publications.

PROSPERO registration number CRD42021286522.

Strengths and limitations of this study

- This systematic review will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.
- Stringent inclusion and exclusion criteria will be used to select clinical studies assessing the impact of homologous recombination deficiency (HRD) biomarkers (HRD score, HRD status, and HRD-related gene mutational status and protein expression) on outcomes of patients with early breast cancer.
- Internationally recognized scales will be used for the quality assessment, to exclude low-quality studies and enhance the credibility of pooled results.
- Differences in patient cohort, sample size, treatment regimen, and measure of homologous recombination deficiency biomarkers may yield significant heterogeneity.

INTRODUCTION

Cancer refers to a disease in which cells divide uncontrollably and invade normal tissues[1]. In the pathogenesis of most cancers, normal cells need to undergo certain genetic changes to become cancerous such as activation of proto-oncogenes and inactivation of tumor suppressor genes[2]. For example, the occurrence of retinoblastoma is often companied by the mutation of the tumor suppressor gene RB1[3]. Endogenous (replication stress, oxygen radicals, and cell metabolism) and exogenous (radiation, viral infection, and chemotherapy) damaging factors continually act on the genome of cells and caused different degrees of DNA lesions[4]. And what protects organisms from cancer is that cells have inherent repair mechanisms to eliminate these damaging events. DNA damages that occur on a single strand are to be dealt with by a number of simple repair pathways including base-excision repair (BER), nucleotide-excision repair (NER), direct repair (DR), and mismatch repair (MMR). On the contrary, DNA double-strand break (DSB), which is the most severe DNA lesion and the main driver of cancer, requires sophisticated repair pathways such as NHEJ (non-homologous end joining) and HR (homologous recombination)[5]. The HR system utilizes a homologous sister chromatid (available in the S and G2 phases of cell cycle) as a template to copy and replace damaged DNA in a relatively error-free

manner compared with NHEJ[6]. A number of key genes including BRCA1/2, RAD51, and PALB2 will encode functional proteins and get involved in the process of repair[7]. If these genes are mutated, the HR system will fail to perform the repair function, which is so-called HR deficiency (HRD), leading to the accumulation of somatic mutations, chromosomal aberrations, and genomic scars (heritable genomic changes resulted from DNA repair defeat), as well as the development of cancer, especially breast cancer (BC)[8, 9].

BC is a highly heterogeneous disease and treated mainly based on the receptor expression status. In recent years, with the development of sequencing technology and our further understanding of genetic variation of cancer, numerous genes are being used to screen for available therapeutic targets[10]. For example, DNA-damaging drugs such as PARP inhibitors and platinums have been shown to significantly improve Progression-free Survival in patients with advanced triple-negative BC (TNBC) with germline BRCA1/2 mutations[11, 12]. Moreover, based on the latest data from phase III OlympiaA trial, adjuvant olaparib was shown to significantly improve the primary endpoint of invasive Disease-free Survival (DFS) vs. placebo in patients with germline BRCA1/2-mutated high-risk EBC (3-year invasive DFS rate: 85.9% vs. 77.1%; hazard ratio 0.58, 95% confidence interval 0.41-0.82, $P<0.001$)[13]. Therefore, as key genes in the process of HR, BRCA1/2 are generally detected to determine the HRD status of BC patients. However, only 4% and 22% HRD can be attributed to germline BRCA1/2 mutations in BC and TNBC, respectively[14-16]. On this condition, biomarkers with wider coverage are needed to identify more BC patients with HRD. The HRD score is an algorithmic assessment of three measures of loss of heterozygosity (LOH), large-scale transition (LST), and telomeric allelic imbalance (TAI)[17]. This kind of assessment along with BRCA mutation detection is now widely used to define the HRD status.

Several studies have investigated the prognostic role of HRD score in early BC (EBC)[18-21]. Telli et al assessed the HRD score in three neoadjuvant TNBC trials and found that a HRD score ≥42 or the presence of BRCA1/2 mutations were correlated

with the objective response rate to platinum-based therapy[18]. SWOG S9313 is a phase III randomized study, comparing the efficacy of simultaneous anthracycline (A) and cyclophosphamide (C) and sequential A→C in more than 3000 stage I/II BC patients. Sharma et al investigated the prognostic role of HRD status in a subset of patients from SWOG S9313. The results indicated that HRD positive status was associated with better DFS (hazard ratio 0.72, 95% confidence interval 0.51–1.00, *P*=0.049)[19]. Significant associations between HRD positive status and higher pathological Complete Response (pCR) rates of EBC patients were also revealed in two studies by Loibl et al and Telli et al, respectively[20, 21]. Despite all the above efforts, the detection of HRD biomarkers has not been incorporated into the clinical practice of BC. In addition, no systematic review has explored the relationship between HRD biomarkers and the prognosis of EBC patients. Therefore, we will firstly systematically combine and analyze the results of previous studies in this study, to facilitate the clinical use of HRD detection in EBC.

METHODS AND ANALYSIS

This systematic review is expected to begin on December 1, 2021 and end on June 30, 2022 and will be conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement[22]. This review's protocol has been registered in the International Prospective Register of Systematic Reviews.

Search strategy

Five databases including PubMed, Cochrane Library, EMBASE, OVID, and Web of Science will be searched from December 1, 2021 to March 31, 2022, with no language restriction. Medical Subject Headings and free text will be combined to search for concepts such as 'Breast Neoplasms' and 'Early' and 'Recombinational DNA Repair' and 'Biomarkers'. The detailed example of the search strategy applied in PubMed is available in online **Supplemental File 1**. In addition, we will search the reference lists of recognized studies to identify additional papers.

Study selection

All records identified through database searching will be imported into EndNote version 9.1 software. Firstly, duplicates will be removed using the built-in recognition function of the software by the lead author. Then, all records will be screened by two reviewers independently according to the title and abstracts. After that, the potentially relevant full-text articles will be reviewed by the same two reviewers independently based on pre-established inclusion and exclusion criteria. Finally, the remaining full-text articles will be assessed for eligibility by the team. Disagreements between the two reviewers will be settled by discussion. The particular reason for exclusion of each reviewed article will be recorded and presented in the final manuscript. In addition, the reference lists of recognized studies will be searched to make sure that no potentially eligible article is missed.

Inclusion criteria

Types of studies

Clinical studies investigating the impact of HRD biomarkers (HRD score, HRD status, and HRD-related gene mutational status and protein expression) on outcomes in patients with EBC will be included. Concretely, the HRD-related genes/proteins mainly include ATM, ATR, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C, RAD51D, RBBP8, SLX4, and XRCC2[23]. This review will include articles published in any language, with no restriction of date. Non-English articles potentially eligible for inclusion will be translated to obtain enough data. The rationality of treatment regimens in all included studies will be confirmed by the lead author based on the recommendations of NCCN clinical practice guidelines[24]. For studies involving grouping, the treatment regimen received by patients in each group should be comparable.

Types of participants

Patients with histologically confirmed EBC.

Interventions/exposures

High HRD score, positive HRD status, positive gene mutational status, and positive

protein expression. Specifically, the assessment of HRD score should include three measures of tumor genomic instability (LOH, LST, and TAI), with a cutoff of 42[18].

Comparators/control

Low HRD score, negative HRD status, negative gene mutational status, and loss of protein expression.

Main outcomes

Each study should contain at least one of the following outcomes:

1. pCR: no invasive carcinoma in primary site and negative regional lymph node (ypT0/ypTis ypN0) after neoadjuvant therapy[25];

2. DFS: the time from randomization to disease recurrence or death due to disease progression;

3. Overall Survival (OS): the time from randomization to death from any cause.

Exclusion criteria

Articles that meet the following criteria will be excluded:

1. Non-clinical studies including reviews, conference abstracts, case reports and series, and comments;

2. Patients with metastatic or advanced BC;

3. No detection of HRD, wrong evaluation methods of HRD score, or other cutoff values;

4. Non-human experiments.

Data extraction

Two reviewers will independently extract data from the included studies into a self-designed data extraction template. If some important data are not available in the articles, we will make contact with the first or corresponding authors for potential support. Differences in opinion between the two reviewers will be settled by discussion. The study selection process is shown in **Figure 1**. The following study characteristics will be collected:

Study details

First author, year of publication, country/region, study design, and setting (neoadjuvant

and adjuvant);

Patients characteristics

Patient subtype (hormone receptor-positive, human epidermal growth factor receptor 2-positve, and TNBC), number of patients, and treatment regimen;

Evaluation indicators

Main outcomes (pCR, DFS, and OS), HRD biomarkers (HRD score, HRD status, and HRD-related gene mutational status and protein expression), and score of quality assessment.

Quality assessment

Two reviewers will independently conduct quality assessment using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies and Jadad scale for randomized clinical trials, respectively[26, 27]. The NOS consists of three key items: 1. Selection; 2. Comparability; and 3. Outcome. One point will be added when there is enough support information for an item. One study that obtains at least 6 points will be considered as high quality, with a full score of 9 points[26]. The Jadad scale includes four key items: 1. Randomization; 2. Double blinding; 3. Concealment of allocation; and 4. Withdrawals and dropouts. If the description of one item is described and appropriate, two points will be added to this item. On the contrary, if the description is not described or inappropriate, the score for this item will be zero. If the rating falls between the two situations, one point will be added. Specially, for the item of withdrawals and dropouts, only 1 and 0 point can be chosen. The full score of Jadad scale is 7 points, and a score of more than 3 points means high quality[27].

Statistical analysis

Data synthesis

All data will be synthesized narratively and quantitatively. If there are more than two studies for one outcome, meta-analysis will be further conducted[28]. Otherwise, we will only carry out systematic review with descriptive analysis. Review Manager version 5.3.5 (Cochrane Collaboration, Oxford, UK) will be used to pool the results. Odds ratios and hazard ratios along with 95% confidence intervals will be calculated

using the Mantel–Haenszel method and inverse variance method, respectively. Forest plots will be used to present the pooled results. For all statistical tests, a two-tailed *P*-value of <0.05 will be considered statistically significant.

Heterogeneity assessment

Before pooling the results, both the *Q* test and I^2 statistic will be used to assess heterogeneity. A *P*-value of <0.1 and an I^2 value of >50% indicate significant heterogeneity across studies. A fixed-effect model will be used unless considerable heterogeneity arises. Alternatively, a random-effect model will be used.

Subgroup and sensitivity analysis

If significant heterogeneity appears and cannot be reduced by using a random-effect model, subgroup analysis or sensitivity analysis will be conducted to find possible source of heterogeneity. The grouping methods of subgroup analysis will be based on the study characteristics, patient subtypes, chemotherapy regimens, or HRD detection methods, while the sensitivity analysis will be conducted by omitting the data of individual studies. The potential source of heterogeneity can be identified if the heterogeneity decreases significantly when carrying out subgroup analysis based on one factor or discarding data from one study.

Publication bias

Stata version 12.0 (Stata Corporation, College Station, TX, USA) will be used to evaluate potential publication bias using Egger's and Begg's test. A *P*-value of <0.05 will be considered a significant publication bias.

Patient and public involvement

Patients and/or the public are not involved in the design, conduct, reporting or dissemination plans of this research.

ETHICS AND DISSEMINATION

Ethical approval is not required in this study because no data are related to an individual patient. The results will be disseminated through international and national conferences or peer-reviewed publications.

Author contributions HaL, WP, and JZ were responsible for the conception of the

study plan, and preparation of the manuscript. HuL reviewed the study plan and manuscript and offered comments and edits.

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Competing interests None declared.

Patient consent for publication Not required.

Data sharing No additional data available.

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Figure legend

Figure 1 Flow diagram of study selection process.

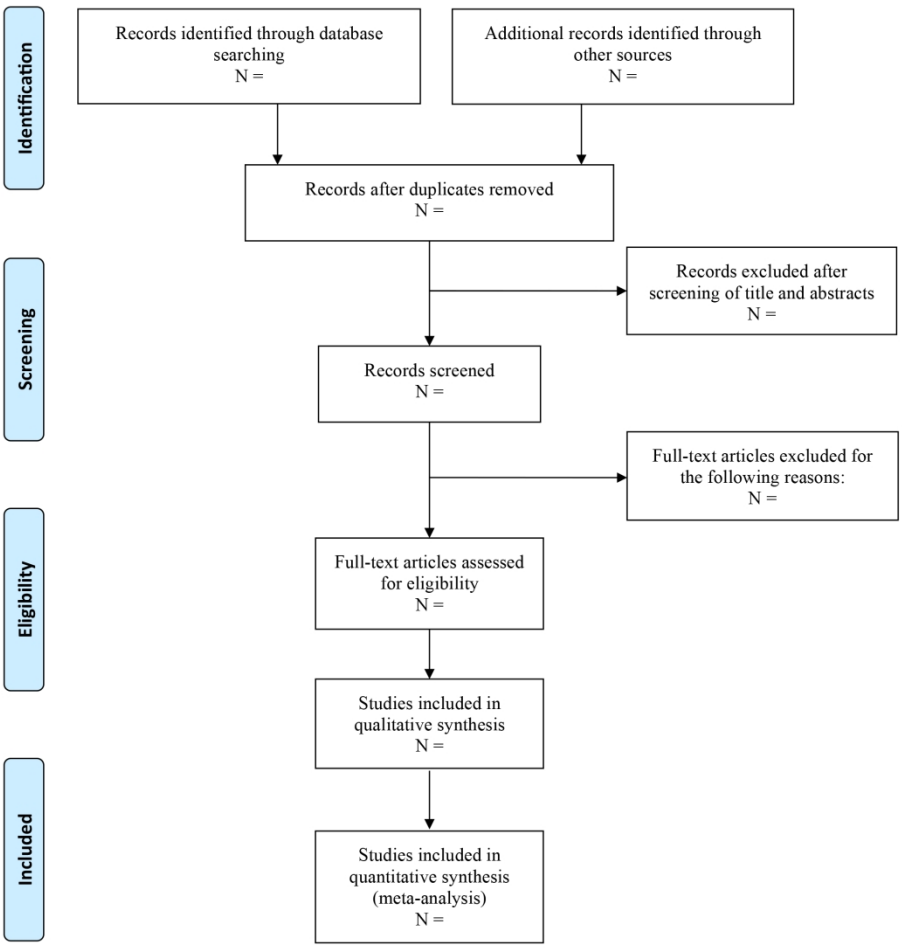

Figure 1 Flow diagram of study selection process.

Main search algorithm:

((((("Breast Neoplasms"[Mesh]) OR ((Breast Neoplasm) OR (Neoplasm, Breast)) OR (Breast Tumors)) OR (Breast Tumor)) OR (Tumor, Breast)) OR (Tumors, Breast)) OR (Neoplasms, Breast)) OR (Breast Cancer)) OR (Cancer, Breast)) OR (Mammary Cancer)) OR (Cancer, Mammary)) OR (Cancers, Mammary)) OR (Mammary Cancers)) OR (Malignant Neoplasm of Breast)) OR (Breast Malignant Neoplasm)) OR (Breast Malignant Neoplasms)) OR (Malignant Tumor of Breast)) OR (Breast Malignant Tumor)) OR (Breast Malignant Tumors)) OR (Cancer of Breast)) OR (Cancer of the Breast)) OR (Mammary Carcinoma, Human)) OR (Carcinoma, Human Mammary)) OR (Carcinomas, Human Mammary)) OR (Human Mammary Carcinomas)) OR (Mammary Carcinomas, Human)) OR (Human Mammary Carcinoma)) OR (Mammary Neoplasms, Human)) OR (Human Mammary Neoplasm)) OR (Human Mammary Neoplasms)) OR (Neoplasm, Human Mammary)) OR (Neoplasms, Human Mammary)) OR (Mammary Neoplasm, Human)) OR (Breast Carcinoma)) OR (Breast Carcinomas)) OR (Carcinoma, Breast)) OR (Carcinomas, Breast))) AND (((((((Primary) OR (Early)) OR (Operable)) OR (Resectable)) OR (Curable)) OR (Non-metastatic)) OR (Non-advanced))) AND (("Recombinational DNA Repair"[Mesh]) OR ((((((((((((((((((Recombinational DNA Repair) OR (DNA Repair, Recombinational)) OR (Repair, Recombinational DNA)) OR (Recombinational Repair of DNA)) OR (DNA Recombinational Repair)) OR (Homologous Recombinational Repair)) OR (Homologous Recombinational Repairs)) OR (Recombinational Repair, Homologous)) OR (Repair, Homologous Recombinational)) OR (Homologous Recombination Repair)) OR (Recombination Repair, Homologous)) OR (Homologous Recombination Repair of DNA)) OR (Homologous Recombination DNA Repair)) OR (Recombination Repair)) OR (Repair, Recombination)) OR (Homologous Recombination Double-Stranded Break DNA Repair)) OR (Homologous Recombination Double Stranded Break DNA Repair)) OR (Homology-Directed dsDNA Break Repair)) OR (Homology Directed dsDNA Break Repair)))) AND ((((biomarkers) OR (biomarker)) OR (gene)) OR (protein))

Sources:

Total N =
PubMed N =
Cochrane Library N =
EMBASE N =
OVID N =
Web of Science N =
Other sources N =
N records after duplications removed
N records after screening of titles and abstracts
N records after further evaluation (N records were excluded for the following reasons: Non-clinical studies; Patients with metastatic or advanced BC; No detection of HRD, wrong evaluation methods of HRD score, or other cutoff values; and Non-human studies)
N records included in qualitative synthesis
N records included in quantitative synthesis (meta-analysis)

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Location where item is reported
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Line 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	Not applicable
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Line 51
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Line 5-19
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Line 256-258
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	Not applicable
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Line 259
Sponsor	5b	Provide name for the review funder and/or sponsor	Not applicable
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Not applicable
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Line 67-120
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Line 121-126, line 166-181
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Line 154-181
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Line 134-135
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Line 136-140

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Line 142-143
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Line 143-152
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Line 191-195
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Line 198-206
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Line 176-181
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Line 208-220
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Line 223-225
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Line 225-230
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Line 237-244
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Line 224-225
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Line 246-248
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Not applicable

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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