MESOTHELIN EXPRESSION AND TRIPLE-NEGATIVE BREAST CANCER

ASSOCIATION BETWEEN MESOTHELIN EXPRESSION AND SURVIVAL OUTCOMES IN PATIENTS WITH TRIPLE–NEGATIVE BREAST CANCER: A SYSTEMATIC REVIEW

BY MEI WANG, B.Sc. (Honours), MMed

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science

McMaster University MASTER OF SCIENCE (2016) Hamilton, Ontario

(Health Research Methodology – Clinical Epidemiology Specialization)

TITLE: Association between mesothelin expression and survival outcomes in patients with triple-negative breast cancer: a systematic review

AUTHOR: Mei Wang, Bachelor of Science (Shandong University). MMed. (Qingdao University)

SUPERVISOR: Professor Lehana Thabane

NUMBER OF PAGES: xi, 66

LAY ABSTRACT

It is unclear whether mesothelin expression in triple-negative breast cancer (TNBC) is an independent prognostic marker for survival. To the best of our knowledge, no systematic review or meta-analysis has ever been done on this topic. The present systematic review aims to evaluate the role of mesothelin as a prognostic marker for TNBC. The primary objective of this review is to synthesize available evidence on the association between the expression of mesothelin and overall survival (OS) of patients with TNBC. The secondary objectives include determining the relationship between the expression of mesothelin and disease-free survival (DFS), distant metastases, and mortality. Despite some limitations, this study shows a significant association between mesothelin expressions and long-term OS rate as well as DFS rate and mortality rate in patients with TNBC. Mesothelin has a prognostic significance for patients with mesothelin based on our findings. Patients with mesothelin-positive TNBC could benefit from mesothelin-targeted immunotherapies in development.

ABSTRACT

Background and Objectives:

Mesothelin, identified as a tumor-associated biomarker, is more often overexpressed in triple receptor-negative breast cancer (TNBC) than in common luminal breast tumor subtype or normal tissues. The objective of this systematic review is to determine the association between the expressions of mesothelin with survival outcomes in patients with TNBC.

Methods

We searched the following electronic databases: MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, PubMed, and Web of Science with no time or language restriction till May 19, 2016. Any prospective or retrospective longitudinal studies that investigate the prognosis of TNBC with mesothelin baseline measurement were selected. Two reviewers independently assessed every article for inclusion, extracted data, and assessed the methodological quality of every eligible trial. Pooled measures of associations were summarized with meta-analyses.

Results and conclusions

Among the 592 patients with TNBC included in the four eligible studies, 269 patients (45.4%) demonstrated mesothelin expression. For the primary outcome OS, we found the trend toward decreased survival for patients with mesothelin-positive TNBC than those without mesothelin expression. We also found that for long-term OS, the association was statistically significant (OR = 0.46; 95% CI= 0.30 to 0.73; P< 0.001). For the secondary outcomes, we found that mesothelin expression in patients with TNBC was associated with lower DFS and higher overall mortality than those without mesothelin expression. Despite the limitations of sample size, this present study shows a significant association between mesothelin expressions and survival outcomes in patients with TNBC. Patients with mesothelin-positive TNBC could benefit from mesothelin-targeted immunotherapies recently in the development.

ACKNOWLEDGEMENTS

I would first like to express my deepest gratitude to my supervisor, Dr. Lehana Thabane. He has been providing outstanding and patient mentorship, inspiration, encouragement and guidance throughout my graduate study at McMaster University.

I am also grateful to my thesis committee, including Dr. Lehana Thabane, Dr. Lawrence Mbuagbaw, and Dr. Peter J. Lovrics, for their invaluable and timely guidance to the research presented in this thesis.

My husband, Jason and my son, Kevin, gave me a continuing support and encouragement to keep me moving forward.

Finally, I would like to thank my partners, Aihua Li and Guangwen Sun; The thesis would not have been possible without their cooperation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

DFS: Disease-free survival; **ER**: estrogen receptor; **PRISMA**: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **HER2**: human epidermal growth factor receptor 2; **OS**: overall survival; **PR**: progesterone receptor; **NOS**: The Newcastle-Ottawa Assessment Scale; **TNBC**: triple-negative breast cancer; **MOOSE**: the Meta-Analysis of Observational Studies in Epidemiology; **PROSPERO**: the international prospective register of systematic reviews.

MW: Mei Wang; **AL**: Aihua Li; **GS**: Guangwen Sun; **SR**: Susan Reid; **PL**: Peter Lovrics; **LM**: Lawrence Mbuagbaw; **LT**: Lehana Thabane.

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis was prepared for publication in peer-reviewed journals. The protocol of this project was confirmed for publication in Systematic Reviews. The contributions of Mei Wang in the project are carrying out the initial background research, developing the research question; writing the protocol; doing the literature screening, data extraction, and management; conducting the statistical and qualitative analyses; writing all the manuscripts; submitting the papers and responding to reviewers' comments. Dr. Lehana Thabane is the guarantor and involved in all aspects of the project. My partner Aihua Li contributed in extracting and analyzing data; My co-authors participated in drafting the manuscript or revising it critically for important methodological content. The work in this thesis was conducted between January 2016 and July 2016.

CHAPTER 1

INTRODUCTION[1]

BACKGROUND

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast neoplasms. It is characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). TNBC has a relatively higher rate of relapse and worse overall survival rate than other forms of breast cancer[2].

Targeted therapies on specifically overexpressed antigens have been successful in the treatment of some malignant diseases. Targeted therapies offer the potential of better selecting patients that will benefit from treatment, and with potentially less toxic therapy. Mesothelin, a membrane-bound glycoprotein, is identified as a biomarker for TNBC. Overexpression in TNBC and limited expression in the typical luminal breast tumor subtype and healthy tissues, mesothelin becomes a potential candidate for targeted therapies for TNBC.[4] Although TNBC is sensitive to chemotherapy, cytotoxic agents often result in numerous adverse side effects in patients. Therefore, a better understanding of the prognostic value of mesothelin in TNBC may lead to improving health outcomes of patients with TNBC. Li et al. and Tozbikian et al. showed that the level of mesothelin expression in TNBC was an independent prognostic marker

associated with low survival rates [5, 6]. However, two other studies failed to demonstrate such an association between the presence of mesothelin and poor clinical outcome[7, 8].

We performed the first systematic review and meta-analysis of the literature to evaluate the frequency of mesothelin expression and the value of mesothelin as a prognostic marker for TNBC.

OVERALL OBJECTIVES

The overall objective of this systematic review is to determine the association between the expressions of mesothelin with survival outcomes in patients with TNBC.The primary objective of this project is to synthesize available evidence on the association between the expression of mesothelin and overall survival of patients with TNBC. The secondary objectives include determining the relationship between the expression of mesothelin and disease-free survival (DFS), relapse- free survival (RFS), distant metastases, and mortality in patients with TNBC.

SCOPE

The scope of this thesis is as follows: Chapter 2: Protocol, which gives out the plan and guidelines of the project. Chapter 3: Results, which includes the details of final data we got from our systematic review. Chapter 4: Conclusions. Which explains the key findings, implications, comparison with similar studies, and limitations.

REFERENCES

1. Wang M, Li A, Sun G, Mbuagbaw L, Reid S, Lovrics PJ, Thabane L. Association between mesothelin expression and survival outcomes in patients with triple-negative breast cancer: a protocol for a systematic review. Syst Rev. 2016, Aug 11; 5(1):133.

2. Tan AR, Swain SM (2008) Therapeutic strategies for triple-negative breast cancer. Cancer J 14:343–351.

3. Wu JM, Fackler MJ, Halushka MK et al. (2008) Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res 14:1938–1946.

4. Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. Breast Cancer Res Treat. 2012; 133:799–804.

5. Yun R. Li, Rena R. Xian et al. Mesothelin expression is associated with poor outcomes in breast cancer. Breast Cancer Res Treat (2014) 147:675–684

6. Tozbikian G, Brogi E et al. Mesothelin Expression in Triple Negative Breast Carcinomas Correlates Significantly with Basal-Like Phenotype, Distant Metastases and Decreased Survival. PLoS ONE 9(12): e114900.

CHAPTER 2

PROTOCOL

Association between mesothelin expression and survival outcomes in patients with triple-negative breast cancer: a protocol for a systematic review

Mei Wang[1], Aihua Li[1], Guangwen Sun[8], Lawrence Mbuagbaw[1, 2, 3], Susan Reid[9], Peter J. Lovrics[10], and Lehana Thabane*[1, 4, 5, 6, 7]

Email: Mei Wang. wangm59@mcmaster.ca - Aihua Li. liaih@mcmaster.ca - Guangwen Sun. gsun5903@hwdsb.on.ca – Lawrence Mbuagbaw. mbuagblc@mcmaster.ca -Susan Reid. reid@mcmaster.ca -Peter Lovrics. lovricsp@mcmaster.ca

*Correspondence: thabanl@mcmaster.ca Department of Clinical Epidemiology and Biostatistics. Biostatistics Unit, Father Sean O'Sullivan Research Centre. St. Joseph's Healthcare Hamilton, 3rd floor Martha Wing. 50 Charlton Avenue East. Hamilton ON Canada L8N 4A6

A full list of author information is available at the end of the article.

Cited as: Mei Wang, Aihua Li, Guangwen Sun, Lawrence Mbuagbaw, Susan Reid, Peter J. Lovric, and Lehana Thabane. Association between mesothelin expression and survival outcomes in patients with triple-negative breast cancer: a protocol for a systematic review. Syst Rev. 2016 Aug 11; 5(1):133

Abstract

Background: Mesothelin is a membrane-bound glycoprotein. Although the biologic function of mesothelin is not clear, researchers have found that it plays a role in the survival, proliferation, and migration of tumor cells. In some studies, Mesothelin has been identified as a tumor-associated biomarker, and is more often overexpressed in triple receptor-negative breast cancer (TNBC) than in common luminal breast tumor subtype or normal tissues. The objective of this review is to determine the association between the expression of mesothelin and overall survival in patients with TNBC.

Methods/design: We will search the following electronic databases: MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, PubMed, and Web of Science with no time or language restriction. Prospective or retrospective longitudinal studies that investigate mesothelin expression in TNBC or the prognosis of TNBC with mesothelin baseline measurement will be selected. Two reviewers will independently assess every abstract or full text for inclusion. Data on clinical outcomes, as well as on study design, research setting, study population, demographic characteristics of the participants, and methodological quality, will be extracted using a structured codebook developed by the authors. A pooled measure of associations will be assessed through meta-analyses if appropriate. Heterogeneity across the included studies will be

evaluated using the I^2 statistics. Findings will be reported according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. The quality of evidence and risk of bias of the studies will be evaluated.

Discussion: The aim of this systematic review is to synthesize the evidence regarding the association between the expression of mesothelin and the survival outcomes of patients with TNBC. A better understanding of the prognostic value of mesothelin in TNBC will be essential to identifying a novel therapeutic target.

Systematic review registration: PROSPERO CRD42016036212

Keywords: Triple negative breast cancer, mesothelin, meta-analysis, Survival, systematic review

BACKGROUND

Description of the condition

TNBC, which is characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), is an aggressive subtype of breast neoplasms. About 15% of breast cancers are TNBC[1], and they tend to be more

common in younger women[2]. TNBC has a relatively higher rate of relapse and worse overall survival rate than other forms of breast cancer[3].

Description of the prognostic factor

Mesothelin is a membrane-bound glycoprotein. Its expression is limited to mesothelial cells and some malignant neoplasms[4]. The biological function of mesothelin is not very clear. However, researchers have found that it has a role in the survival, proliferation, and migration of tumor cells [5].

How the prognostic factor may be related to health outcomes

Mesothelin is identified as a biomarker for TNBC because it is overexpressed in TNBC while its expression is limited in the typical luminal breast tumor subtype and normal tissues [6]. As such, it is hypothesized that the expression of mesothelin in TNBC is of relevance for patients' outcome.

Although TNBC is more sensitive to chemotherapy than other types of breast cancer, cytotoxic agents often result in numerous adverse side effects, and disease progression and recurrence can still occur despite treatment with cytotoxic agents. Targeted therapies on specifically overexpressed antigens have been successful in the treatment of some malignant diseases[7]. These therapies allow offer greater specificity (treating only patients that will benefit) and generally have fewer toxicities.

TNBC lacks effective targeted therapies that can improve its chemotherapy. Mesothelin is a potential candidate for targeted therapies for TNBC because of its overexpression in TNBC cells and limited expression in normal tissues. Therefore, a better understanding of the expression levels of mesothelin and its prognostic value in TNBC may provide evidence necessitating the role of mesothelin as a therapeutic target, thus leading the path of improving health outcomes of patients with TNBC.

Why it is important to do this review

Li et al. and Tozbikian et al. showed that the levels of mesothelin expression in TNBC was an independent prognostic marker that associated with low survival rates [8, 9]. However, two other studies failed to demonstrate such an association between the presence of mesothelin and poor clinical outcome [10, 11]. These controversial results may result from insufficient samples and different study designs. To the best of our knowledge, no systematic review or meta-analysis has ever been done on this topic. The present systematic review aims to evaluate the frequency of mesothelin expression and the value of mesothelin as a prognostic marker for TNBC.

OBJECTIVES

The primary objective of this review is to synthesize available evidence on the association between the expression of mesothelin and overall survival of patients with TNBC. The secondary objectives include determining the association between the expression of mesothelin and disease-free survival (DFS), relapse- free survival (RFS), distant metastases, and mortality. Where feasible, to combine the results of original studies using meta-analysis, critically evaluate the literature and identify future research needs.

METHODS

Study registration

This project has been registered with the international prospective register of systematic reviews (PROSPERO) as number CRD42016036212. This systematic review protocol has been designed and reported using Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 statement[12]and the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines[14].

Eligibility criteria

Types of study designs

We will include both prospective and retrospective longitudinal studies in this systematic review. The studies will be eligible if they

evaluated mesothelin expression in TNBC or the prognosis of TNBC with mesothelin at baseline measurement. We will consider both exploratory and confirmatory prognostic factor investigation studies. When overlapping data of the same patient population are included in more than one publication, only the most recent or complete study will be used in the meta-analysis.

Types of participants and setting

Studies would be included in this systematic review if the participants had TNBC. No age, intervention or setting restrictions will be placed.

Exposure variable

For the prognostic studies, our exposure will be mesothelin expression measured by immunohistochemical analysis.

Types of outcome measures

Our primary outcome will be overall survival (OS). If the OS is not reported in the study, we will collect its relevant surrogate outcomes, which include Disease-free survival (DFS) or relapse-free survival (RFS), distant metastases, and mortality.

Timeframe

The prognostic studies should be at least 1-year duration for follow-up. We will group outcome data into three time periods for analysis purposes: short-term (≤3 years), medium-term (3-5 years), and long-term follow-up (≥5 years).

Hypothesis

The research hypothesis of this systematic review is that the expression of mesothelin in patients with TNBC is associated with adverse health outcomes.

Primary outcome

The primary outcome of interest is the OS in patients with mesothelin-positive TNBC, which were estimated from the date of surgery to the date of the final follow-up visit, or the date of the end of the study, or the date of death.

Secondary outcomes

DFS and RFS—time-to-event data which were measured from the date of surgery to the date of first documented local or distant recurrence. Patients who died or lost to follow-up before experiencing the relevant events were considered censored at their dates of the last follow-up.

Distant metastases, estimated by subsequent distant metastases, interval to metastases.

Mortality, measured by mortality rate.

Search methods for identification of studies

Electronic searches

A comprehensive literature search will be done for original articles analyzing the expression and prognostic value of mesothelin in TNBC. The electronic database sources we will search are MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, and Web of Science with no restrictions on time and languages. As there may be limited published research on mesothelin in TNBC, we will increase our search sensitivity by finding any articles related to TNBC and mesothelin. The search terms will be “triple negative breast cancer”, “triple negative breast carcinoma”, “triple negative breast neoplasm”, “TNBC” and “mesothelin”.

A three-step search strategy will be utilized in this review. First, we will explore database subject headings and text words. An initial pilot search in MEDLINE will be undertaken followed by analysis of the text words contained in the title and abstract and of the subject headings used to describe the article. Secondly, we will search using all identified keywords and index terms across all included databases. And finally, the reference list of all identified reports and articles will be searched for additional studies. Studies published in all languages will be considered for inclusion in this review. (Please refer to **Appendix 1** for the full search

strategy and **Appendix 2** for the primary literature screening form we will use during this stage.)

Searching other resources

We will search for additional references by cross-checking bibliographies of retrieved studies or relevant reviews. We will also contact researchers in the field to identify additional trials that may have been eligible for inclusion. Mediconf (www.mediconf.com/) will be used to search conference abstracts.

Data collection and analysis

Selection of studies

Our selection of studies will follow the four-phase flow diagram (**Figure 1**) referring to the PRISMA Statement. The literature search results will be uploaded to EndNote X7 Software, which will facilitate the collaboration among reviewers during the study selection process.

The screening will be conducted using a pretested screening form. Titles and abstracts will first be screened for relevance and presence of the eligible criteria listed above (the second phase). Full-text articles for potentially eligible titles or abstracts will be downloaded for further assessment. Articles will be classified as 1) included, 2) excluded or 3) uncertain. For each phase of screening, the Kappa statistic will be

performed to calculate inter-rater agreement. Two authors (MW and AL) will independently carry out the filtering of the selected pieces of literature. Disagreements will be resolved by consensus or by contacting a third author when consensus cannot be reached (LM or LT).

Data extraction and management

Data will be collected using a predesigned data collection form which will be pilot tested beforehand (**Appendix 3**). The following details will be extracted: study characteristics (such as the first author, the country, the year of publication, the study design, and the sample size); participants' characteristics (the age, the stage of breast cancer, and the interventions); study exposure (the technique used to quantify mesothelin, the cut-off to determine mesothelin positivity, and the number of mesothelin positivity and controls); and outcome variables (median OS, median disease-free survival (DFS) or relapse-free survival (RFS), number of distant metastases, number of deaths, and 95% confidence intervals, as well as events rates and numbers at risk during short-term (≤3 years), medium-term (3-5 years), and long-term follow-up (>5 years more)interval. If a study has insufficient details to extract the number of events, Hazard Ratio (95% CI) or Odds Ratio (95% CI), but provides the survival curves, we will use the survival curve approach to extract required data to obtain estimates of association.[15]

If necessary, modifications will be done to the data collection form after testing the reliability of data abstraction on a random sample. The review authors (WM and AL) will independently extract the data, resolve discrepancies by discussion or refer to the third reviewer (LM or LT).

Breast carcinoma was defined as TNBC when nuclear staining is < 1% for both ER and PR, and membranous staining is < 10% of HER2 on immunohistochemistry (IHC) staining or negative gene amplification was found by fluorescence in situ hybridization (FISH) [16, 17]. An H-score is used to quantify the mesothelin staining. H-score (final score ranging between 0 and 300) is the combination of the percentage of tumor cells with positive staining (0-100%) and the intensity of staining (1+, 2+, and 3+)[18]. We will choose H- score ≤ 10 as mesothelin-negative and H-score> 10 as mesothelin-positive [18]. The survival data will be extracted from the tables or texts of eligible articles or estimated from Kaplan - Meier curves where it is applicable[19]. If we encounter any missing or unclear data, study authors will be contacted for additional details.

Quality assessment of included studies

The Newcastle-Ottawa Assessment Scale (NOS) [18] will be employed by two independent reviewers (MW and AL) to assess the methodological quality of all eligible trials. The adapted version of the NOS will be used to meet the unique needs of this systematic review[20]. Included

studies will be assessed on three perspectives: the selection of the study arms; the comparability of the arms; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies respectively[20]. Reviewers will classify the risk of biases in the studies on a scale from 0 to 3, where 0 indicates a high risk of bias and 3 indicates low risk. The adapted version of the NOS includes seven questions through four domains of evaluation; selection bias- methods for selecting study participants, performance bias - methods to control for confounding, detection bias - statistical methods, and information bias - methods for measuring exposure and outcome variables.These scales will be used to gauge the risk of bias in every study (see **Appendix 4**)[21]. Reviewers will resolve disagreements by discussion, and one arbitrator will adjudicate unresolved disputes (LM or LT).

Data synthesis and statistical analysis

The statistical reporting will be performed according to the guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group[22]. Descriptive statistics will be used to describe the characteristics of all eligible studies. Data will be presented as mean (standard deviation) or median (first quartile, third quartile) for both continuous variables and frequencies (percent) for categorical variables. If there are a sufficient number of studies (at least three) suitable for pooling, we will perform meta-analyses of the studies to obtain a pooled estimate

of the association between the expression levels of mesothelin and the survival of TNBC expressed as odds ratio (at similar follow-up points) or hazard ratio with 95% confidence interval (CIs). This analysis will be conducted using STATA. When heterogeneity is present, a random effect model (Der Simonian and Laird method) will be applied, while the fixed effect model will be used in the absence of between-study heterogeneity ($P>0.10$ or I^2 <50%). We will consider the clinical heterogeneity of included studies based on outcome measurements, follow-up length, and methodological heterogeneity due to study design (prospective versus retrospective).

We will synthesize data within these clinically-relevant subgroups. Between-study heterogeneity will be measured using the Cochrane's Q test and Higgins I-squared statistic (P value of <0.10 or I^2 > 50% will be considered as statistically significant heterogeneity). If there is a large number study, we will use meta- regression or subgroup analyses to explain the heterogeneity by lymph node metastasis, pathological stage, and adjuvant chemotherapy.

Dealing with missing participant data, sensitivity analyses, and publication bias

For missing data, we will contact study authors for clarification and attempt to retrieve any missing information. We will conduct a complete

case analysis (excluding those with missing data) as the primary analysis[24], and a sensitivity analysis will be performed to determine the articles with more than 25% missing data influence the overall result. If feasible, we will also perform a sensitivity analysis by excluding trials with a high risk of bias to test the robustness of our results.

If the number of eligible studies is too small (<10), publication bias will not be assessed. If there are 10 or more eligible studies, publication bias will be examined for each meta-analysis by visually examining asymmetry of funnel plots and testing for asymmetry at the 10% level, using Egger's test for hazard ratios, and Peters' test for odds ratios [25].

DISCUSSION

The lack of expression of conventional prognostic markers (estrogen, progesterone, and Her2 receptors) in TNBC denies those patients the benefit of targeted therapy against these receptors. The search for a molecular therapeutic target for TNBC is ongoing. Mesothelin is identified as a biomarker for TNBC because some authors have identified its overexpression in TNBC and limited expression in the common luminal breast tumor subtype and normal tissues [6]. A better understanding of the expression frequency and prognostic value of mesothelin in TNBC will be essential to identify a novel therapeutic target with the goal of improving health outcomes of patients with TNBC.

This proposed review will serve several purposes. First, we hope to identify the expression level of mesothelin in TNBC. Secondly, we will document survival rates linked to the levels of mesothelin in TNBC. Our results will inform disease prognosis and potentially highlight better therapeutic and diagnostic timing strategies. There are ongoing investigations of agents that target mesothelin-expressing tumors. Even if we find mesothelin has little prognostic value in patients with TNBC, it may still be a promising target for novel drug therapies in other cancers. Finally, we hope the results of this review will encourage future research in identifying mesothelin as a novel target for improving the outcomes for patients with TNBC.

Our systematic review will be conducted according to recommended standards including explicit eligibility criteria, duplicate independent assessment of eligibility and a comprehensive search. We will use the NOS approach to assessing methodological quality including duplicate independent assessment of the validity of the individual studies. Our results are only likely to be restricted by limitations in the primary studies. To our knowledge, this review will be the first systematic review about the association between the expression levels of mesothelin and clinical outcomes in patients with TNBC.

References

1. Elias AD. Triple-negative breast cancer: a short review. Am J Clin Oncol. 2010 Dec; 33(6):637-45.

2. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, et al. (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13: 4429–4434.

3. Tan AR, Swain SM (2008) Therapeutic strategies for triple-negative breast cancer. Cancer J 14:343–351

4. Chang K, Pastan I, Willingham MC. Isolation and char¬acterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int.J.Cancer. 1992; 50:373–381.

5. Li M, Bharadwaj U, Zhang R, Zhang S, Mu H, Fisher WE, et al. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther 2008; 7:286–96.

6. Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. Breast Cancer Res Treat. 2012; 133:799–804.

7. Wu JM, Fackler MJ, Halushka MK et al. (2008) Heterogeneity of breast cancer metastases: comparison of therapeutic target expression

and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res 14:1938–1946.

8. Yun R. Li, Rena R. Xian et al. Mesothelin expression is associated with poor outcomes in breast cancer. Breast Cancer Res Treat (2014) 147:675–684

9. Tozbikian G, Brogi E. et al. Mesothelin Expression in Triple Negative Breast Carcinomas Correlates Significantly with Basal-Like Phenotype, Distant Metastases and Decreased Survival. PLoS ONE 9(12): e114900.

10. Napa Parinyanitikul, George R. Blumenschein et al. Mesothelin Expression and Survival Outcomes in Triple Receptor-Negative Breast Cancer. Clinical Breast Cancer, Vol. 13, No. 5, 378-84.

11. Ibrahim Vedat Bayoglu, Betul Bolat Kucukzeybek et al. Prognostic value of mesothelin expression in patients with triple negative and HER2-positive breast cancers. Biomedicine & Pharmacotherapy 70 (2015) 190–195

12. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, and Stewart LA: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015, 349:g7647

13. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283:2008–12.

14. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses:* The PRISMA Statement. BMJ 2009; 339:b2535.

15. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Statistics in Medicine 1998; 17:2815–34

16. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, et al. (2010) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemically testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med 134: 907–922.

17. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, et al. (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 131: 18–43.

18. Wang L, Niu Z, Zhang L, Liu X, Wang X, Li F, et al. Clinicopathological significance of mesothelin expression in invasive breast cancer. J Int Med Res 2012; 40:909–16.

19. Michiels S, Piedbois P, Burdett S, et al. Meta-analysis when only the median survival times are known: a comparison with individual patient data results. *Int J Technol Assess Health Care*. 2005; 21(1):119–125.

20. Wells GA, Shea B, O'connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000.

21. Bawor M, Dennis BB, Anglin R, Steiner M, Thabane L, Samaan Z. Sex differences in outcomes of methadone maintenance treatment for opioid addiction: a systematic review protocol. Syst Rev. 2014; 3:45.

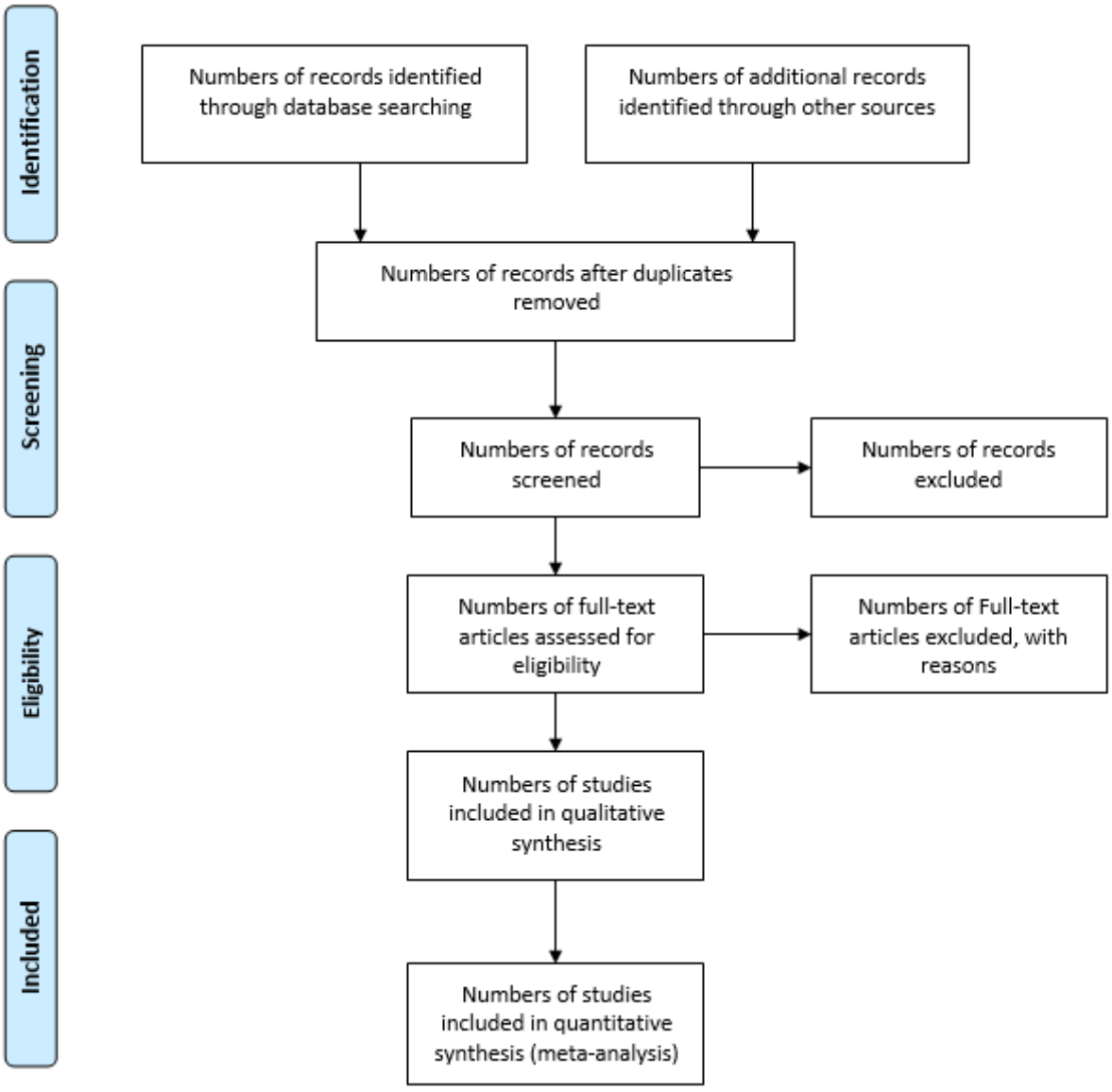
22. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, et al. (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283: 2008–2012.

23. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007; 8:16.

24. Akl EA, Johnston BC, Alonso-Coello P, Neumann I, Ebrahim S, Briel M, Cook DJ, Guyatt GH. Addressing dichotomous data for participant's exluded from trial analysis: a guide for systematic reviewers. PLoS One 2013; 8(2):e57132.

25. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 2011; 343:d4002.

Figure 1. PRISMA flow diagram[14].



CHAPTER 3

RESULTS

Association between mesothelin expression and survival outcomes in patients with triple-negative breast cancer: a systematic review

Mei Wang[1], Aihua Li[1], Lawrence Mbuagbaw[1, 2, 3], Susan Reid[8], Peter J. Lovrics[9], and Lehana Thabane*[1, 4, 5, 6, 7]

Email: Mei Wang. wangm59@mcmaster.ca - Aihua Li. liaih@mcmaster.ca - Guangwen Sun. gsun5903@hwdsb.on.ca – Lawrence Mbuagbaw. mbuagblc@mcmaster.ca -Susan Reid. reid@mcmaster.ca -Peter Lovrics. lovricsp@mcmaster.ca

*Correspondence: Lehana Thabane

Email: thabanl@mcmaster.ca

Department of Clinical Epidemiology and Biostatistics. Biostatistics Unit, Father Sean O'Sullivan Research Centre. St. Joseph's Healthcare Hamilton, 3rd floor Martha Wing. 50 Charlton Avenue East. Hamilton ON Canada L8N 4A6A

A full list of author information is available at the end of the article.

ABSTRACT

Background: Mesothelin is a tumor-associated biomarker that is more often overexpressed in triple receptor-negative breast cancer (TNBC) than in common luminal breast tumor subtype or normal tissues. The objective of this systematic review is to determine the association between the expressions of mesothelin with survival outcomes in patients with TNBC.

Methods: We searched the following electronic databases: MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, and Web of Science with no time or language restriction till May 19, 2016. Any prospective or retrospective longitudinal studies that investigated the prognosis of TNBC with mesothelin baseline measurement were selected. Two reviewers independently assessed every article for inclusion, extracted data, and assessed the methodological quality of every eligible trial. A pooled measure of association was assessed through meta-analyses.

Results: Among the 592 patients with TNBC included in the four eligible retrospective cohort studies, 269 patients (45.4%) demonstrated mesothelin expression. For the primary outcome OS, we found a trend toward decreased survival for patients with mesothelin-positive TNBC than those without mesothelin expression. And we found for long-term OS, the association is statistically significant (OR 0.46; 95% CI, 0.30 to 0.73; P <0.001).

Conclusion: Despite the limitations of small sample size, this present study shows a significant correlation between mesothelin expressions and long-term OS rate as well as DFS rate in patients with TNBC. Patients with mesothelin-positive TNBC could benefit from MSLM-targeted immunotherapies recently in the development.

Funding: We do not have any funding for this project.

Systematic review registration: PROSPERO CRD42016036212

Keywords: Triple negative breast cancer, mesothelin, meta-analysis, Survival, systematic review

INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast neoplasms. It is characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2).[1] TNBC has a relatively higher rate of relapse and worse overall survival rate than other forms of breast cancer[2].

Targeted therapies on uniquely overexpressed antigens have been successful in the treatment of some malignant diseases[3]. Mesothelin, a membrane-bound glycoprotein, is identified as a biomarker for TNBC. Being Overexpressed in TNBC and limited in the common luminal breast

tumor subtype and normal tissues, mesothelin becomes a potential candidate for targeted therapies for TNBC.[4] Although TNBC is very sensitive to chemotherapy, cytotoxic agents often result in numerous adverse side effects in patients. Therefore, a better understanding of the mesothelin expressions and its prognostic value in TNBC may lead the path of improving health outcomes of patients with TNBC.

Mesothelin may involve in the prognosis of several human tumors. Servais et al. reported that mesothelin plays a role in promoting tumor invasion and matrix metalloproteinase-9 expression in mesothelioma.[5] Rump et al. indicated that mesothelin is a novel binding protein to CA125, which might contribute to the metastasis of ovarian cancer.[6] Bharadwaj et al. indicated that overexpression of mesothelin in pancreatic cancer induced expression of cyclin E and cyclin binding complex, which resulted in promoting tumor cell proliferation and tumor cell cycle progression.[7]

There is controversy about wether mesothelin can play a prognostic marker for TNBC. Li et al. and Tozbikian et al. showed that the levels of mesothelin expression in TNBC was an independent prognostic marker that correlates with low survival rates [8, 9]. However, two other studies failed to demonstrate such an association between the presence of mesothelin and poor clinical outcome [10, 11].

We performed the first systematic review and meta-analysis of the literature to evaluate the frequency of mesothelin expression and the value of mesothelin as a prognostic marker for TNBC.

OBJECTIVES

The primary objective of this review is to synthesize available evidence on the association between the expression of mesothelin and overall survival of patients with TNBC. The secondary objectives include determining the association between the expression of mesothelin and disease-free survival (DFS), relapse- free survival (RFS), distant metastases, and mortality in patients with TNBC.

METHODS

Data source

This systematic review followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[12] Two reviewers conducted a search of MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, and Web of Science with no restrictions on time and languages. Bi-weekly email alerts were used until May 19, 2016, to identify any newly published trials. Search terms included “triple negative breast cancer/ triple negative breast carcinoma/ triple negative breast neoplasm/ TNBC” and “mesothelin”. We searched for additional references by cross-checking bibliographies of retrieved studies or relevant reviews. We also contacted

researchers in the field to identify additional trials that may have been eligible for inclusion.

Study selection

We included both prospective and retrospective longitudinal studies in this systematic review. The studies are eligible if they evaluated the prognosis of TNBC with mesothelin at baseline measurement, in addition to the eligibility criteria below:

1) Participants: Studies were included in this systematic review if the participants had TNBC.

2) Exposure variable: The exposure is mesothelin expression measured by immunohistochemical analysis.

3) Outcomes: Our primary outcome is overall survival (OS). We also collected its relevant surrogate outcomes, which include Disease-free survival (DFS) or relapse-free survival (RFS), distant metastases, and mortality as our secondary outcomes.

4) Timeframe: The prognostic studies should be at least 3-year duration for follow-up. We grouped outcome data into three time periods for analysis purposes: short-term (3 years), medium-term (5 years), and long-term follow-up (≥5 years).

No age, intervention, setting or language restrictions are placed.

Titles and abstracts were first screened for relevance by two independent reviewers (WM and AL) and full-text articles with potential eligibility were downloaded for further assessment. Disagreements were resolved by consulting a third author when consensus cannot be reached (LM or LT).The Kappa statistic was performed to calculate inter-rater agreement. [13]

Data extraction

The review authors (WM and AL) independently extracted the data, resolved discrepancies by discussion or referral to a third reviewer (LM or LT). The following data items were recorded for each study: 1) study characteristics (such as the first author, the country, the year of publication, the study design, and the sample size). 2) Participants' characteristics (the age, the stage of breast cancer, and the interventions). 3) Study exposure (the technique used to quantify mesothelin expression, the cut-off to determine mesothelin positivity, and the number of mesothelin positivity). 4) Survival data (e.g., OS, DFS, mortality and distant metastasis as long as they are available). Two independent reviewers (MW and AL) employed the Newcastle-Ottawa Assessment Scale (NOS) to assess the methodological quality of all eligible trials.[14]

Statistical analysis

We performed meta-analyses of the studies to obtain a pooled estimate of the association between the expression levels of mesothelin,

and the OS (as well as DFS and mortality) of TNBC expressed as odds ratio (at similar follow-up points) with 95% confidence interval (CIs). Between-study heterogeneity was measured using the Cochrane's Q test and Higgins I-squared statistic (P value of <0.10 or I^2 > 50% was considered as statistically significant heterogeneity).[15] When heterogeneity was present, a random effect model (Der Simonian and Laird method) was applied, while the fixed effect model was used in the absence of between-study heterogeneity (P>0.10 or I^2 <50%). We performed a sensitivity analysis to confirm the robustness of our results using random effects model when fixed effects model was performed. Because the number of eligible studies is too small (<10), publication bias was not assessed. We did not do the subgroup analysis and meta-regression for the same reason.

RESULTS

Literature search results

The literature search identified the following number of studies from the various databases: PubMed (n=8), Medline (n=201), Embase (n=1134), the Cochrane Library (n=0), and Web of Science (n=14), giving a total of 1357 potentially relevant records. An additional five records were identified from other sources. After de-duplication, 1214 studies were left (Figure 1). By reading titles and abstracts we excluded 1199 articles that both review authors considering 15 references to needing closer scrutiny,

and the full text of the papers or conference abstracts of these papers were obtained. By reading full-text versions, we eliminated four articles from the 15 based on our inclusion/exclusion criteria. Ultimately, 4 articles were included in the final analysis (weighted kappa of 0.83. 95% confidence interval (CI), 0.52 to 1). The primary reasons for exclusion of studies were ineligible populations, ineligible exposure variable, as well as duplicate conference abstracts of full publications. (See Table 1. for characteristics of sample of excluded studies).

Description of study characteristics

There are four cohort design studies included in the review. The study of Ibrahim et al.[11] was conducted in Turkey, but the other three were conducted in U.S.A. The total sample size is 592. Among the four trials included, two compared the prognosis of mesothelin-positive expression with that of mesothelin negative expression in patients with TNBC[8, 10], and the other two compared that in patients with breast cancer (including TNBC [9, 11]. We only extracted data related to patients with TNBC, and we obtained validated data of one of the trials by contacting the authors[11]. The most commonly assessed clinical outcomes were OS and DFS. All studies have more than three years' follow-up. (See Table 2 for characteristics of included studies).

An H-score was used to quantify the mesothelin staining. H-score (final score ranging between 0 and 300) is the combination of the

percentage of tumor cells with positive staining (0-100%) and the intensity of staining (1+, 2+, and 3+)[16]. We choose H- score ≤ 10 as mesothelin-negative and H-score> 10 as mesothelin-positive [16]. Among the 592 patients with TNBC included in eligible studies, 269 patients (45.4%) had mesothelin expression (mesothelin-positive).

Risk of bias assessment

We evaluated bias for all four included studies.[16] The risk of bias was low (see details in Table 4). All the studies were judged to be at unclear risk of bias as no calculation of sample size were performed and there was no mention of dealing with missing data. In the outcome measurement domain, in the outcome measurement domain, there was a potential bias as pathologists were not blinded for determining the prognostic factors. In the Tozbikian et al. study[2] here is a limitation as confounders were not clearly identified.

Primary outcome

We extracted data by analyzing the original context or Kaplan-Meier curve in the articles or contacted the authors.[17]

Three-year OS

The heterogeneity of OS at 3-years between studies was statistically significant [Cochran's Q, Chi^2=8.29, DF=3(P = 0.04), I^2 =64%].Then a random effect model (Der Simonian and Laird method) was applied. In the patients with TNBC, compared with tumors without

mesothelin expression, those with mesothelin expression had a tendency toward reduction in OS (OR, 0.46; 95% CI, 0.17 to 1.23; P =0.12), but the association is not statistically significant (See Figure 2).

Five-year OS

The heterogeneity of OS at 5-years between studies is statistically significant [Cochran's Q, Chi^2=14.32, DF=2(P = 0.0008), I^2 =86%]. Then a random effect model was applied. In the patients with TNBC, compared with tumors without mesothelin expression, those with mesothelin expression still had a tendency toward reduction in OS (OR, 0.69; 95% CI, 0.15 to 3.20; P =0.63), but the association is not statistically significant (See figure 3).

More than five-year OS

The heterogeneity of OS at > 5-years between studies is not statistically significant [Cochran's Q test, Chi^2=3.79, DF=2(P = 0.15), I^2 =47 %]. Therefore, a fixed effect model was applied. In the patients with TNBC, compared with tumors without mesothelin expression, those with mesothelin expression associated with the reduction in OS (OR=0.46; 95% CI=0.30 to 0.73; P <0.001). The association is statistically significant (See figure 4).

Secondary outcomes

For DFS or RFS, we can only extract data from 2 studies, which included 335 patients with TNBC. The heterogeneity of DFS between those two studies was decreased as time went by［I^2 = 85% (three years), I^2 = 78% (five years) and I^2 = 15% (long-term)］. In the patients with TNBC, compared with tumors without mesothelin expression, those with mesothelin expression were associated with reduction in >5 years DFS (OR = 0.37; 95% CI = 0.23 to 0.60; P < 0.0001). The association is statistically significant.

Only one study[9] reported on distant metastasis data in 198 patients with TNBC. In those patients, compared with mesothelin-negative patients, those with mesothelin expression associated with more distant metastasis events (OR=2.86; 95% CI=1.27 to 6.47; P <0.05), the association is statistically significant.

For the overall mortality of all 592 patients with TNBC, compared with mesothelin-negative patients, those with mesothelin expression were associated with higher mortality (OR=1.88; 95% CI=1.27 to 2.78; P=0.002, using fixed model for heterogeneity is low). The association is statistically significant (see figure 5).

Additional analysis

There were not enough studies for us to do the meta- regression analysis. For sensitivity analysis, we compared the pooled estimates

between the fixed effects model (where ever available) and the random effects model. The direction of effect did not change for any estimate (See Figure 6 and Figure 7).

DISCUSSION

Main results

In this study, we evaluated the expression of mesothelin and its prognostic value for patients with TNBC. Among the 592 patients with TNBC included in eligible studies, 269 patients (45.4%) had mesothelin expression (mesothelin-positive). For the primary outcome OS, we found a trend toward decreased survival for patients with mesothelin-positive TNBC than those without mesothelin expression. And we found for long-term OS, the association is statistically significant (OR = 0.46; 95% CI = 0.30 to 0.73; P <0.001). For the secondary outcomes, we also found the mesothelin expression in patients with TNBC associated with lower DFS and high overall mortality than those without mesothelin expression. To our knowledge, this meta-analysis is the first study which systematically estimates the association between mesothelin expression and clinical outcomes in patients with TNBC.

Strengths and limitations

We extracted the data mainly by analyzing the Kaplan-Meier Curve, which may result in inaccuracy of the results.

There are limitations on our review. We found that the proportion of patients with mesothelin-positive TNBC group is 45.4%, and the overall relative hazard (196/592) is 1.18. Given that α (two-tailed) is 0.05, β (type II error rate) is 0.20, the entire events we needed to check the difference of the two-group survival analysis is 1,156.[22]Our results should be confirmed in larger studies. Ideally, levels of mesothelin expression should be determined retrospectively from large, prospective clinical trials in breast cancer where patient, treatment and outcome information would be available. This could lead to the development of valuable targeted therapies, with all of their potential benefits. Other limitations include that we did not have enough data to do confounfders analysis (e.g. clinical stage of the participants and the treatments of the participants) and this is not a causility result.

We are confident for not missing any trials as the screening strategy was guided by a professional librarian, and we searched any gray literature as much as we can. However, despite an extensive literature search, we were able to include only four studies with 592 eligible participants and 196 events. In the same time, for there are not enough studies, we cannot use meta- regression or subgroup analyses to explain the heterogeneity by any confounder. The relatively small number of studies and their small sample sizes may have influenced the reliability of our results. However, our studies strictly followed the standard for

systematic review methods. The methods of our review included explicit eligibility criteria, duplicate independent assessment of eligibility and a comprehensive search. We used the Newcastle-Ottawa Assessment Scale (NOS) approach to duplicate assessing the methodological quality of the individual studies, which show that all four included studies are at low risk of bias.

Over recent years, overwhelming data have revealed that some cell gene expression(e.g. CD8+T cell gene expression, CD4+ T cell gene expression) have been associated with prognosis in breast cancer. [20, 21] There are not specific prognostic predictor for patients with TNBC. Despite some inevitable limitations, we are confident that the results of our study can make a valuable contribution to the literature on TNBC.

CONCLUSIONS

There is growing interest in target therapies for mesothelin-expressing tumors in practice. [21] Some of the therapies have shown promising results without apparent toxicities in preclinical or early clinical trials, which should be tested in patients with TNBC. Despite the limitations listed above, the present study shows a significant correlation between mesothelin expressions and long-term OS rate as well as DFS rate in patients with TNBC. Mesothelin may have prognostic significance for patients with mesothelin based on currently obtained data. Patients

with mesothelin-positive TNBC could benefit from mesothelin-targeted immunotherapies recently in development. [23]

The evidence reviewed suggests that compared with tumors without mesothelin expression, those with mesothelin expression are associated with worse clinical outcomes in patients with TNBC. Based on the limitations listed above, there is a need for more practical research to confirm the association, especial the possibility of mesothelin as an independent prognostic marker for patients with TNBC.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LT is the guarantor. MW carried out the initial background research. MW, LT, and LM conceived of the study. MW also draft the manuscript. AL and MW acted as the independent reviewers in screening literature, extracting data, assessing the quality of each study.LT, LM, AL, SR, and PL helped in developing the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published.

Authors' information

1Department of Clinical Epidemiology and Biostatistics, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada. 2Biostatistics Unit, Father Sean O'Sullivan Research Centre, St. Joseph's Healthcare, Hamilton, ON, Canada. 3Centre for Development of Best Practices in Health, Yaoundé Central Hospital, Yaoundé, Cameroon. 4Department of Pediatrics and Anesthesia, McMaster University, Hamilton, Canada. 5Centre for Evaluation of Medicine, St Joseph's Healthcare Hamilton, Hamilton, Canada. 6Biostatistics Unit, Father Sean O'Sullivan Research Centre, St Joseph's Healthcare, 3rd Floor, Martha Wing, 50 Charlton Avenue East, Hamilton, Ontario L8N 4A6, Canada. 7Population Health Research Institute, Hamilton Health Sciences, Hamilton, Canada. 8General Surgery, Juravinski Hospital, 711 Concession St. E. Hamilton Ontario L8V 1C3. 9 Department of Surgery, Surgical Outcomes Research Centre, McMaster University and St. Joseph's Healthcare, 50 Charlton Avenue East, Hamilton, Ontario, L8N 4A6, Canada

REFERENCES

1. Elias AD. Triple-negative breast cancer: a short review. Am J Clin Oncol. 2010 Dec; 33(6):637-45.

2. Tan AR, Swain SM. Therapeutic strategies for triple-negative breast cancer. Cancer J. 2008; 14:343–351.

3. Wu JM, Fackler MJ, Halushka MK et al. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res. 2008; 14:1938–1946.

4. Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. Breast Cancer Res Treat. 2012; 133:799–804.

5. Servais EL, Colovos C, Rodriguez L, Bograd AJ, Nitadori J, et al. Mesothelin overexpression promotes mesothelioma cell invasion and MMP-9 secretion in an orthotopic mouse model and in epithelioid pleural mesothelioma patients. Clin Cancer Res. 2012; 18: 2478–89.

6. Rump A, Morikawa Y, Tanaka M, Minami S, Umesaki N, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem. 2004; 279: 9190–8.

7. Bharadwaj U, Li M, Chen C, Yao Q. Mesothelin-induced pancreatic cancer cell proliferation involves alteration of cyclin E via activation of signal transducer and activator of transcription protein 3.

Mol Cancer Res. 2008; 6: 1755–65.

8. Yun R. Li, Rena R. Xian et al. Mesothelin expression is associated with poor outcomes in breast cancer. Breast Cancer Res Treat. 2014; 147:675–684

9. Tozbikian G, Brogi E. et al. Mesothelin Expression in Triple Negative Breast Carcinomas Correlates Significantly with Basal-Like Phenotype, Distant Metastases and Decreased Survival. PLoS ONE. 2014; 9(12): e114900.

10. Parinyanitikul N, George R. Blumenschein et al. Mesothelin Expression and Survival Outcomes in Triple Receptor Negative Breast Cancer. Clinical Breast Cancer. 2013; Vol. 13, No. 5, 378-84.

11. Ibrahim Vedat Bayoglu, Betul Bolat Kucukzeybek et al. Prognostic value of mesothelin expression in patients with triple-negative and HER2-positive breast cancers. Biomedicine & Pharmacotherapy. 2015; 70,190–195

12. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, and Stewart LA: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015, 349:g7647

13. A.J. Viera, J.M. Garrett. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005, 37: 360–363

14. Wells GA, Shea B, O'connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000.

15. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7: 177–188.

16. Wang L, Niu Z, Zhang L, Liu X, Wang X, Li F, et al. Clinicopathological significance of mesothelin expression in invasive breast cancer. J Int Med Res 2012; 40:909–16.

17. Zhihui Liu, Benjamin Rich and James A Hanley. Recovering the raw data behind a non-parametric survival curve. Systematic Reviews 2015, 3:151.

18. Bawor M, Dennis BB, Anglin R, Steiner M, Thabane L, Samaan Z. Sex differences in outcomes of methadone maintenance treatment for opioid addiction: a systematic review protocol. Syst Rev. 2014; 3:45.

19. Schmoor C, Sauerbrei W, Schumacher M. Sample size considerations for the evaluation of prognostic factors in survival analysis. Statistics in Medicine. 2000; 19(4):441-52.

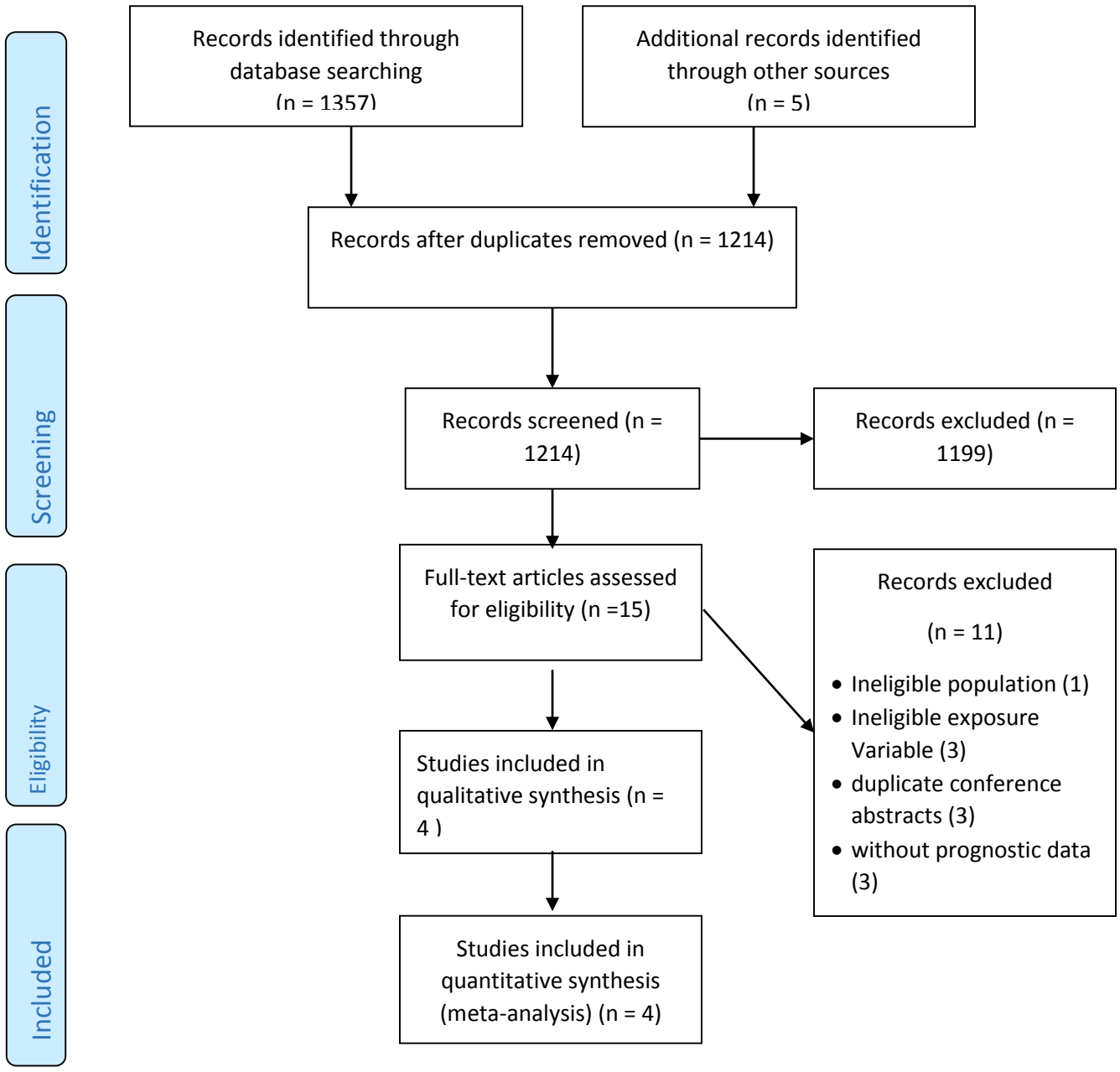
20. Mahmoud, S., Paish, E., Powe, D., Macmillan, R., Grainge, M., Lee, A. et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011; 29: 1949–1955.

21. DeNardo, D., Brennan, D., Rexhepaj, E., Ruffell, B., Shiao, S., Madden, S. et al. (2011) Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discovery 1: 54–67.

22. Hassan R, Ho M. Mesothelin targeted cancer immunotherapy. Eur J Cancer 2008; 44:46-53.

23. Raffit Hassana, Mitchell Hob. Mesothelin targeted cancer immunotherapy. European journal of cancer. 2008; 4 4:4 6 –5 3

Figure 1. PRISMA Study flow diagram (From Moher D, et al..[5])



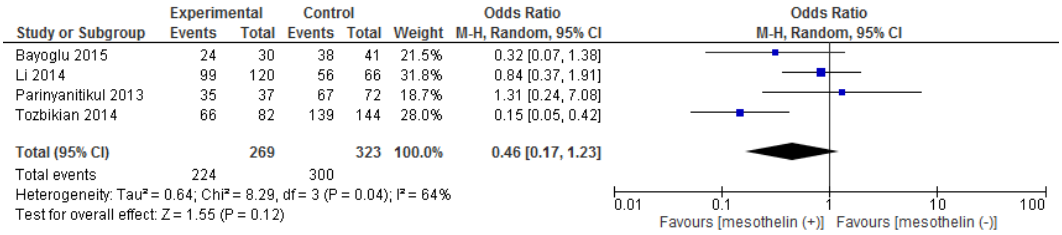


Figure 2. Forest plots of odds ratios about mesothelin expression compared with no mesothelin expression for three-year overall OS.

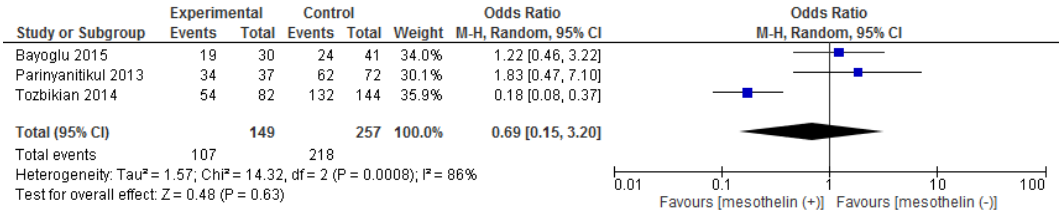


Figure 3. Forest plots of odds ratios about mesothelin expression compared with no mesothelin expression for five-year overall OS.

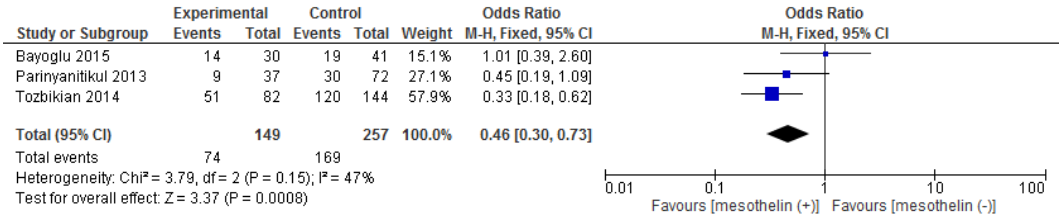


Figure 4. Forest plots of odds ratios about mesothelin expression compared with no mesothelin expression for long-term overall OS.

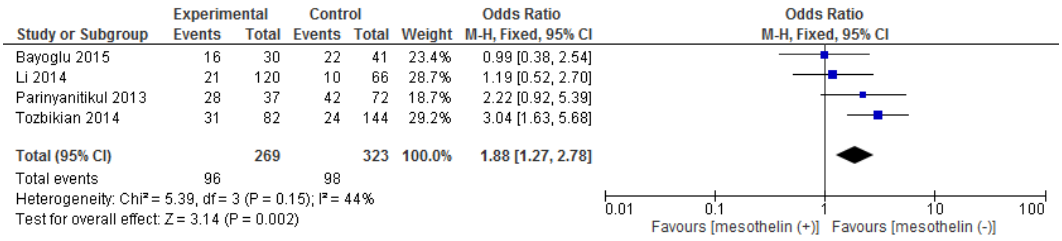


Figure 5. Forest plots of odds ratios about mesothelin expression compared with no mesothelin expression for mortality

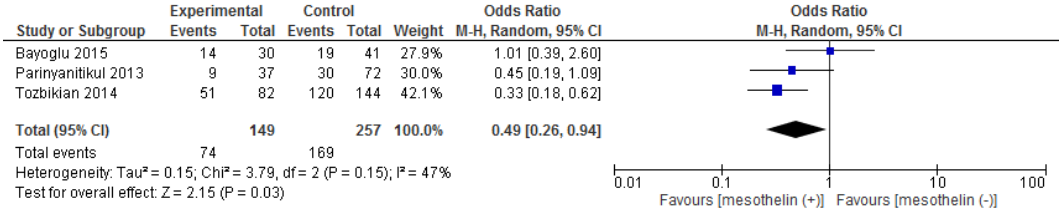


Figure 6. The Forest Plot of sensitivity analysis for long-term OS.

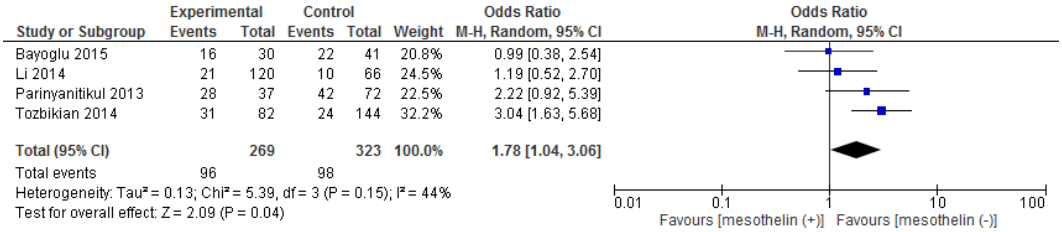


Figure 7. The Forest Plot of sensitivity analysis for mortality.

Table 1: Characteristics of excluded studies.

Description of reasons for exclusion of studies excluded due to ineligible populations, interventions, or comparators.

Reference	Reason for Exclusion
Atik, 2013	Without the interested prognostic factor-mesothelin.
Li 2014	Duplicate conference abstract of a full publication.
Rakha 2007	Without the interested prognostic factor-mesothelin.
Xian 2012	Duplicate conference abstract of a full publication
Tozbikian 2013	Duplicate conference abstract of a full publication
Minckwitz & Martin. 2012	Without the interested prognostic factor-mesothelin.
Ordonez 2014	Without the interested prognostic factor-mesothelin.
WANG 2012	Not population of interest
Lamberts, 2015	Without prognosis outcomes

Table 2. Characteristics of included studies.

Author (Year) (country)	Study design	Study Population	Prognostic factor	The technique used to determine mesothelin-positive	Follow-up period	Outcomes
Tozbikian 2014 (U.S.A)	Cohort	226 TNBC cases from consecutive patients	Mesothelin expression	H score ≥10	Median follow-up time: 5.3 years	OS DFS
Bayoglu 2015 (Turkey)	Retrospective Cohort	71 TNBC cases were included in analysis	Mesothelin expression	H score ≥ 10	Follow-up time ≥ 5 years 9 from 2006)	OS DFS
Parinyanitikul 2013 (U.S.A)	Retrospective cohort	109 TNBC cases	Mesothelin expression	H score ≥ 10	Median follow-up of 75.8 months	OS RFS
Li 2014 (U.S.A)	Cohort	186 TNBC cases from consecutive patients	Mesothelin expression	H score ≥ 10	Follow-up time 3 years	OS

Note: OS for overall survival. RFS for relapse free survival.

Table 3. Risk of bias assessment for each included trial (Bawor M. et al.[15])

	Selection bias	Performance bias		Detection bias		Information bias		
Author (Year)	Is the source Population representative?	Is the sample size adequate and is there sufficient power?	Did the study adjust for confounder?	Did the study use appropriate statistics for the outcome of interest?	Is there little missing data and was it handled appropriately?	Are the methods or outcome measurements explicitly stated and is it appropriate?	Is there an objective assessment of outcomes?	Total (out of 21)
Gary 2014	2	2	0	3	3	3	3	16
Ibrahim 2015	1	1	3	2	3	3	2	15
Napa 2013	2	1	3	3	3	3	2	17
Yun 2014	3	2	3	3	3	3	2	19

CHAPTER 4

CONCLUSIONS

Summary of main results

In this study, we evaluated the literature regarding the expression of mesothelin and its prognostic value for patients with TNBC. Among the 592 patients with TNBC included in eligible studies, 269 patients (45.4%) demonstrated mesothelin expression (mesothelin-positive). For the primary outcome OS, we found a trend toward decreased survival for patients with mesothelin-positive TNBC than those without mesothelin expression. For long-term OS, the association was statistically significant (OR = 0.46; 95% CI = 0.30 to 0.72; P =0.0007<0.05). For the secondary outcomes, we also found that the mesothelin expression in patients with TNBC was associated with lower DFS and higher overall mortality than those without mesothelin expression. To our knowledge, this meta-analysis is the first study which systematically estimates the association between mesothelin expression and clinical outcomes in patients with TNBC.

Comparison with similar studies

To the best of our knowledge, we performed the first systematic review and meta-analysis of the literature to evaluate the frequency of mesothelin expression and the value of mesothelin as a prognostic marker for TNBC. Although there are studies reporting the correlation between

mesothelin expression and final clinical outcomes in patients with gastric cancer, ovarian cancer, and pancreatic cancer.[1, 2, and 3], there is no systematic analysis of those results.

Implications

Target therapies for mesothelin-expressing tumors are being investigated in the clinical practice.[4] Some of the therapies have shown promising results with decreased toxicities in preclinical or early clinical trials, which should be tested in patients with TNBC. Despite the limitations listed above, this present study shows a significant correlation between mesothelin expression and long-term OS rate as well as DFS rate in patients with TNBC. Mesothelin expression may have prognostic significance for patients with TNBC based on currently obtained data. Patients with mesothelin-positive TNBC could benefit from mesothelin-targeted immunotherapies recently in development. [5]

The evidence reviewed suggests that compared with tumors without mesothelin expression, those with mesothelin are association with worse clinical outcomes in patients with TNBC. Based on the limitations listed above, there is a need for more research to confirm and further study the association, especial the possibility of mesothelin as an independent prognostic marker for patients with TNBC.

Strengths and weaknesses of the review

There are limitations to our review. We found that the proportion of patients with mesothelin-positive TNBC group is 45.4%, and the overall relative hazard (196/592) is 1.18. Given that α (two-tailed) is 0.05, β (type II error rate) is 0.20, the entire events we needed to check the difference of the two-group survival analysis is 1,156.[6] However, despite an extensive literature search, we were able to include only four studies with 592 eligible participants and 196 events. As there are not enough studies, we cannot use meta- regression or subgroup analyses to explain the heterogeneity by any confounder. The relatively small number of studies and their small sample sizes may also have influenced the reliability of our results. however, our studies strictly followed the standard for systematic review methods. The methods of our review included explicit eligibility criteria, duplicate independent assessment of eligibility and a comprehensive search. We used the Newcastle-Ottawa Assessment Scale (NOS) approach to duplicate assessing the methodological quality of the individual studies, which show that all four included studies are at low risk of bias. The potential bias is that we extracted the data mainly by analyzing the Kaplan-Meier Curve, which may result in inaccuracy of the results

Nevertheless, despite some inevitable limitations, we are confident that the results of our study can still make a valuable contribution to the literature on TNBC.

REFERENCES

1. Einama T, Kamachi H, Nishihara H, Homma S, Kanno H, Takahashi K, Sasaki A, Tahara M, Okada K, Muraoka S, et al. Co-expression of mesothelin and CA125 correlates with unfavorable patient outcome in pancreatic ductal adenocarcinoma. Pancreas. 2011; 40:1276–1282.

2. Einama T, Homma S, Kamachi H, Kawamata F, Takahashi K, Takahashi N, Taniguchi M, Kamiyama T, Furukawa H, Matsuno Y, et al. Luminal membrane expression of mesothelin is a prominent poor prognostic factor for gastric cancer. Br J Cancer. 2012; 107:137–142.

3. Cheng WF, Huang CY, Chang MC, et al. High mesothelin correlates with chemoresistance and poor survival in epithelial ovarian carcinoma. Br J Cancer 2009; 100:1144-53.

4. Hassan R, Ho M. Mesothelin targeted cancer immunotherapy. Eur J Cancer 2008; 44:46-53.

5. Hassana R, Hob M. Mesothelin targeted cancer immunotherapy. European journal of cancer. 2008; 4 4:4 6 –5 3

6. Schmoor C, Sauerbrei W, Schumacher M. Sample size considerations for the evaluation of prognostic factors in survival analysis. Statistics in Medicine. 2000; 19(4):441-52.

Apendix 1. Search strategies in MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials, and web of science databases

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:
---
1 ((Triple$ or triple negative) adj6 (breast cancer$ or breast neoplasm$ or breast tumo$ or carcinoma$)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (3999)
2 exp Membrane Glycoproteins/ or exp GPI-Linked Proteins/ or mesothelin.mp. (553957)
3 1 and 2 (181)

Database: Embase <1974 to present>
Search Strategy:
---
1 ((Triple$ or triple negative) adj6 (breast cancer$ or breast neoplasm$ or breast tumo$ or carcinoma$)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (9404)
2 mesothelin.mp. or exp mesothelin/ (1282)
3 1 and 2 (17)

Cochrane search results: 0 article

Database: web of science
# 1 799 TOPIC: (mesothelin)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI
Timespan=All years
# 2 14 TOPIC: (mesothelin)
Refined by: TOPIC: (triple negative breast)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI
Timespan=All years

Database: PubMed
#1 “mesothelin"[Supplementary Concept] OR "mesothelin"[All Fields] (702)

#2 "triple negative breast neoplasms"[MeSH Terms] OR ("triple"[All Fields] AND "negative"[All Fields] AND "breast"[All Fields] AND "neoplasms"[All Fields]) OR "triple negative breast neoplasms"[All Fields] OR ("triple"[All Fields] AND "negative"[All Fields] AND "breast"[All Fields] AND "cancer"[All Fields]) OR "triple negative breast cancer"[All Fields] (5238) #1 and #3 (8)

Appendix 2: Primary literature screening form

First author, Year:_____ abstractor initials:			
Eligible criteria			
1. Types of study designs: Measures the relationship between mesothelin expression and an outcome (i.e., not a descriptive study), or measures the mesothelin expression level in TNBC.	Yes	No	Not sure
2. Reports original research. NOTE: If the publication appears relevant to the topic, consider whether it should be retained for "review for references".	Yes	No	Not sure
3. Population is patients with triple negative breast cancer.	Yes	No	Not sure
4. Exposure variable: For the prognostic studies, our main exposure is mesothelin expression measured by immunohistochemically analysis.			

5. Types of outcome measures: Primary outcome: OS. Relevant surrogate outcomes, which include Disease-free survival (DFS) or relapse-free survival (RFS), distant metastases, and mortality.			
6. Time Frame: follow-up time ≥3 years	Yes	No	Not sure
Retain for: □ Background/Discussion □ Review of references □ Harms data □ Other_____ COMMENTS:			

Appendix 3. Data extraction form

Study ID: _____ Reviewer Initials: _____

STUDY INFORMATION

First Author: _____ Year of Publication_____

Title of Article:

_____

_____

Journal Name: _____ Country:

_____

METHODS

Study Setting: _____ Study Design:

_____

Sample Size: Total_____. The number of mesothelin (+)

_____and the # of mesothelin (-) _____.

Mean Age (SD): _____, the stage of breast

cancer_____, and the

interventions_____

.

The technique used to quantify mesothelin_____ ____. The cut-off to determine mesothelin positivity_____

RESULTS (survival data)

	Positive mesothelin expression				Negative mesothelin expression				Total
	total	≤3 years	3-5 years	>5 years	total	≤3 years	3-5 years	>5 years	
Sample size									
Median overall survival (OS) (95%CI)									
Median disease-free survival (DFS) (95%CI)									

number of distant metastases									
number of deaths									

COMMENTS

Appendix 4. Modified Newcastle-Ottawa Scale (NOS) (Bawor M. et al.)[15]

Legend

0 = definitely no (high risk of bias) 1 = Mostly no

2 = Mostly yes 3 = definitely yes (low risk of bias)

Domain of evaluation: Methods for selecting study participants (i.e. Selection bias)

Is the source population (cases, controls, cohorts) appropriate and representative of the population of interest? (0 1 2 3)

Is the sample size adequate and is there sufficient power to detect a meaningful difference in the outcome of interest? (0 1 2 3)

Domain of evaluation: Methods to control confounding (i.e. Performance bias)

Did the study identify and adjust for any variables or confounders that may influence the outcome?

(0 1 2 3)

Did the study use appropriate statistical analysis methods relative to the outcome of interest?

(0 1 2 3)

Is there little missing data and did the study handle it accordingly?

(0 1 2 3)

Domain of evaluation: Statistical methods (i.e. Detection bias)

Is the methodology of the outcome measurement explicitly stated and is it appropriate?

(0 1 2 3)

Is there an objective assessment of the outcome of interest?

(0 1 2 3)