REVIEW

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Calreticulin Expression in Human Carcinomas: A Systematic Review and Meta-Analysis

Mariana Paravani Palacon[1], Tulio Morandin Ferrisse[2], Camila de Oliveira Barbeiro[1], Elaine Maria Sgavioli Massucato[1], Andreia Bufalino[1]*

Abstract

Objective: The present study performed a systematic review and meta-analysis of observational studies on whether calreticulin levels could represent a prognostic factor in carcinoma patients. Calreticulin (CRT) is a multifunctional protein in the endoplasmic reticulum that can play distinct roles in different cancers. **Methods:** The search was performed in PubMed, Scopus, the Cochrane Library, Web of Science, Lilacs, Science Direct, Embase, Bireme, and SciELO databases. After a full-text evaluation, only 14 articles remained. The RoBANS tool assessed the risk of bias. The meta-analysis was performed with R software, and the odds ratio (OR) was the effect measure. The random effects model was chosen, and the quality of evidence was evaluated according to GRADE. **Result:** The most frequent carcinomas were in the breasts and the colon. CRT expression varied according to carcinoma origin and type, but these diseases had a prevalence of high CRT levels, indicating tumor progression. The high CRT levels were associated with lymph node metastasis (OR = 3.06 [1.71; 5.48]/p = 0.0002/I2 = 0%). All included articles had a blinding bias. **Conclusion:** High CRT levels may represent a prognostic factor for metastatic lymph nodes in carcinoma patients.

Keywords: Calreticulin- carcinoma- neoplasms- prognosis- systematic review

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Introduction

Calreticulin (CRT) is a multifunctional protein first described in 1974. It has a molecular weight of 46 kDa, mainly found in the lumen of the endoplasmic reticulum (ER), and it can move to the plasma membrane, cytosol, and nucleus (Lu et al., 2015; Chiang et al., 2013; Fucikova et al., 2018). Thus, the CRT function can be divided according to location. It plays a specific role in calcium storage and homeostasis when located in the ER and participates in cell adhesion, complement system activation, apoptotic cell phagocytosis, and RNA stability when located outside the ER (Chiang et al., 2013; Venkateswaran et al., 2018; Yang et al., 2013).

The CRT structure consists of three domains (Lu et al., 2015): the N-domain has a globular shape, interacting with α-integrins and binding with DNA receptors; the P-domain has low capacity and a high-affinity calcium-binding region; and the C-domain is where calcium molecules interact with other chaperone proteins in the ER (Venkateswaran et al., 2018).

CRT expression as a phagocytic signal stands out among its functions in cancer, acting at the beginning of the adaptive immune response, specifically in immunogenic cell death (ICD), cell proliferation, integrin activity regulation, and cell adhesion and migration (Venkateswaran et al., 2018; Fucikova et al., 2021). ICD is the capacity of specific stimuli to kill cells, mediated by damage-associated molecular patterns (DAMPs), i.e., molecules inaccessible by the immune system under normal conditions. CRT exposure is a characteristic of ICD, working on prophagocytic signal emission or through antigen presentation, particularly by immature dendritic cells (DC) and later CD8+ T cells (Fucikova et al., 2021).

The oncogenic properties related to mutations occurring in the gene that synthesizes CRT also stand out. More than 50 mutations involve CRT, and all are found in exon 9. There are two types of mutation: deletion (type 1), which removes 52 base pairs (bp), and insertion (type 2), which adds five bp. These alterations represent 84% (50% type 1 and 34% type 2) of those involving CRT (Accetta et al., 2020; Holmstrom et al., 2020). They produce modified proteins at the C-terminal, causing the lack of a retention signal in the ER (KDEL signal), and their location may change, resulting in secretion in the Golgi complex (Liu et al., 2020). This modification in protein structure complicates binding with calcium and triggers increased cell growth. That is because the mutant CRT will bind to DC, inhibiting tumor cell phagocytosis and showing inefficiency in antigen presentation by MHC

[1]*Department of Diagnosis and Surgery, São Paulo State University (UNESP), School of Dentistry, Araraquara, São Paulo, Brazil.*
[2]*Department of Dental Prothesis, São Paulo State University (UNESP), School of Dentistry, Araraquara, São Paulo, Brazil.*
**For Correspondence: andreia.bufalino@unesp.br*

class I molecules, favoring tumor immune evasion (Lu et al., 2015; Liu et al., 2020). Considering this background, the World Health Organization (WHO) established CRT mutations as diagnostic biomarkers for myeloproliferative disorders (Prins et al., 2020). However, the precise action mechanism related to CRT in other neoplasms and the possibility of this protein representing a clinical biomarker remains unclear.

The main challenge to establishing CRT levels as clinical biomarkers may occur due to overlapping functions from pro- and anti-oncogenic CRT activities over the population (Lu et al., 2015; Chiang et al., 2013; Fucikova et al., 2018). Moreover, CRT may have a high or low expression in tumor tissues compared to normal ones and be positively or negatively associated with the prognosis of these tumors (Lu et al., 2015; Chiang et al., 2013; Fucikova et al., 2018). Therefore, our systematic review and meta-analysis evaluated CRT levels in different human carcinomas and compared them to normal tissues, analyzing whether high CRT levels could be associated with clinicopathological and prognostic factors in carcinoma patients.

Materials and Methods

Protocol and registration

The present systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews (PRISMA) statement (Shamseer et al., 2015). This study was registered in the Open Science Framework (OSF) (registration DOI: 10.17605/OSF.IO/9DQWC).

Data extraction and research question

The research question was based on the PECO strategy for exploratory systematic reviews, where P= human carcinoma patients; E= CRT expression in patients with and without human carcinoma; C= patients without human carcinoma and different CRT expressions in clinicopathological and prognostic parameters of carcinoma patients; O= CRT expression in human carcinoma patients. Secondary outcomes regarded CRT expression levels in cancer patients associated with clinicopathological and prognostic parameters. The present study aimed to answer the following focused question: Are there differences in CRT expression levels in human carcinomas, and could they be related to clinicopathological and prognostic distinction parameters?

Eligibility criteria

The inclusion criteria for the present systematic review were human observational studies, carcinoma patients, and studies evaluating CRT expression in human carcinoma. There were no restrictions on cancer location (e.g., breasts, colon, esophagus) and publication language. The exclusion criteria were studies in vitro and with animals, studies evaluating CRT expression in other cancers (e.g., adenocarcinoma, lymphoma, leukemia), patients diagnosed with human carcinoma and affected by immunodeficiency conditions, case reports, and book chapters.

Search strategy

The keywords to search the databases were (Calreticulin) OR (CALR)) AND (Squamous cell carcinoma). The searched databases were PUBMED, SCOPUS, COCHRANE LIBRARY, WEB OF SCIENCE, LILACS, SCIENCE DIRECT, BIREME, and SciELO. Additionally, the search term "(calreticulin AND squamous AND cell AND carcinoma)" helped to access potential articles on Embase. Researchers (M.P.P and T.M.F) performed the first article selection by reading their titles and abstracts. The same researchers independently reviewed the studies selected for a full reading and summarized the extracted data in a table. Rayyan for Systematic Reviews Software™ deleted duplicates (Mourad et al., 2016). The studies were analyzed and discussed. Any potential disagreement in the process was solved by reaching a consensus before proceeding to the next steps.

Risk assessment tool

The Risk of Bias assessment for Non-randomized Studies (RoBANS) was used (Park et al., 2011). This tool has six domains: participant selection (selection bias by inadequately choosing the participants), confounding variables (selection bias by inadequately confirming and considering confounding variables), intervention/exposure measurement (performance bias by inadequately measuring intervention/exposure), outcome assessment blinding (detection bias by inadequately blinding the outcome assessment), incomplete outcome data (attrition bias by inadequately handling incomplete outcome data), and selective outcome reporting (reporting bias by selective outcome reporting). Lastly, each domain has only three possible answers for the risk of bias: low, unclear, and high.

Meta-analysis

The meta-analyses were conducted with R software (version 3.6.3) at α=0.05. Sex (female and male) was related to low and high CRT expression. Also, sex (female vs. male), the presence of metastatic lymph nodes, and clinical stage (I-II vs. III-IV) were the outcomes used in the meta-analysis for high CRT levels. The odds ratio was the selected effect measure, the results were interpreted with the random effects model, and the inverse variance method was used for the meta-analysis. In the case of sparse data, the Peto method (a variant of the inverse variance method) was used. Trim-and-fill detected the publication bias related to the small-study effect in the meta-analysis. The heterogeneity level was high for I^2 >50%.

Quality of evidence assessment

The quality of the meta-analysis results was assessed with the Grades of Recommendation, Assessment, Development, and Evaluation Working Group (GRADE Working Group) (Atkins et al., 2004).

Results

Search results

Figure 1 shows the flowchart detailing the database

search, screened abstracts and titles, and full-text articles included in the systematic review. The database search identified 49 articles in PUBMED, 104 in SCOPUS, one in COCHRANE, 53 in LILACS, 958 in SCIENCE DIRECT, 67 in WEB OF SCIENCE, and 160 in EMBASE, totaling 1232 articles. Then, 214 duplicates were removed, leaving 1018 studies in the first selection. Initially, the titles and abstracts were read, which excluded 1352 articles, and 53 articles remained for the next step. Subsequently, the articles were fully read, and 39 were excluded for not meeting the inclusion criteria. Thus, 14 articles were selected for data extraction in our study.

Synthesis of results

The data in the articles were extracted by reading the full texts and distributing the extracted information into a table containing the author and year of publication, study population, the age of patients in the sample, the TNM stage (T=tumor size; N= the presence of metastatic lymph node; M= the presence of metastasis), sample size, methodology, and the primary outcomes from each study included in the systematic review (Table 1) (Chiang et al., 2013; Peng et al., 2010; Prins et al., 2020; Cooney et al., 2017; Yang et al., 2013; Ye et al., 2021; Lee et al., 2012; Kabbage et al., 2013; Vaksman et al., 2013; Du et al., 2007; Liu et al., 2012; Lwin et al., 2010; Chignard et al., 2006; Harada et al., 2017).

All articles included in the present systematic review are observational, reporting CRT expression (high or low) in different carcinomas. Breast and colon cancers were the most common in our study. Ages ranged from pediatric patients (1-15 years old) to older adults (>29 years old). Moreover, the TNM stage reported in the studies highly varied. The most common methodologies used in the included articles were immunohistochemistry, Western blot, and qRT-PCT. Regardless of the carcinoma, these tumors showed higher CRT expression than normal tissues.

Risk of bias assessment for non-randomized studies

This step used the RoBANS criteria tool. Participant selection was biased because some articles did not use paired control groups or had a small sample size or significant differences between the sample sizes of experimental and control groups. Additionally, all articles selected for the systematic review showed biases related

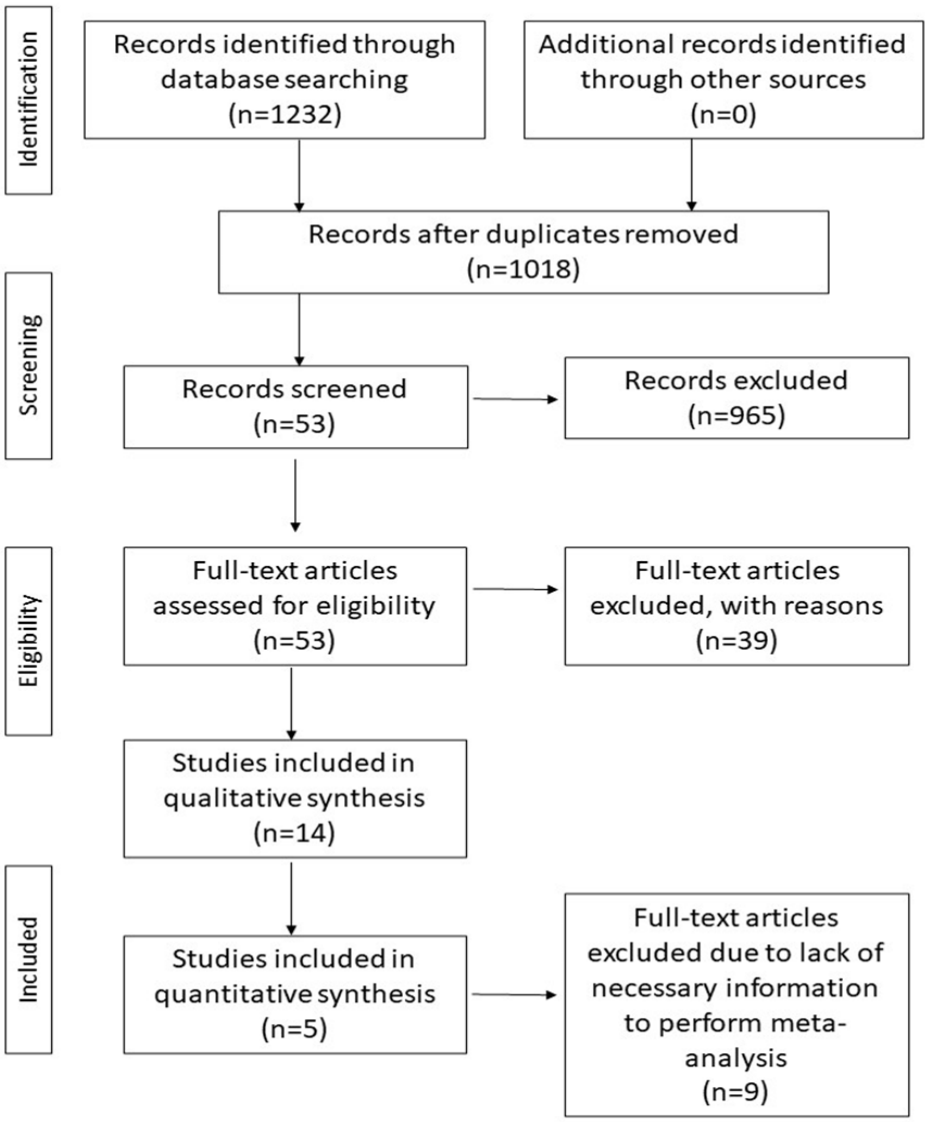

Figure 1. Flowchart Aaccording to the PRISMA Statement

Table 1. Data Extracted from the Articles.

Author	Population	Age	TNM stage	Sample size and characteristics	Outcomes
Cooney et al., 2017 [19]	Sample of patients of the Stanford University - Department of Pathology	45 years and younger	ND	Method: Immunohistochemistry G1: n=9 samples of fibrolamellar hepatocellular carcinoma (FL-HCC) and normal tissue (adjacent areas to the tumor) G2: n=21 hepatocellular carcinoma (HCC) and normal tissue (adjacent areas to the tumor)	G1: 100% showed moderate and strong CRT expression in the tumor and normal tissue. G2: 71% showed moderate and strong CRT expression, 19% weak, and 10% no CRT expression.
Yang et al., 2013 [20]	Shandong Tumor Hospital – China	Low CRT expression: 45 =/- 17.4 High CRT expression: 37 =/- 14.5	ND	Method: Proteomic and immunohistochemistry 12 primary adrenocortical carcinomas (ACC) and normal tissue (adjacent areas to the tumor) G1: n=39 ACC and paired normal adrenocortical tissues G2: n=31 benign adrenocortical adenomas (ACC) G3: n=39 normal adrenocortical tissues	CRT was upregulated in the ACC identified in the proteomic study. Higher CRT expression in ACC than in normal adrenocortical tissues High CRT expression in advanced-stage ACC compared to early-stage ACC (65% vs. 31.6%).
Ye et al., 2021[21]	First Affiliated Hospital of Zhengzhou University	<60: n=9 >/60: n=23	N status: N0: 28 N1/2: 13 Tumor size (cm): <5: 21 >/5: 11 Clinical stage: I-II: 7 III-IV: 25	Method: Immunohistochemistry and qRT-PCR n=32 paired gallbladder cancer and adjacent normal gallbladder tissues	CRT is higher in 25/32 gallbladder cancer tissues than in normal gallbladder and chronic cholecystitis tissues. CRT mRNA expression was higher in patients with a tumor size >/5cm than in patients with a tumor size <5cm. Patients with high CRT expression had a poor outcome and a higher risk of death than those with low CRT expression. CRT expression was not associated with lymph node metastasis, TNM stage, histological grade, or other clinicopathological parameters.
Lee et al., 2012 [22]	Penang General Hospital	36-85 years old	N0: n=13 patients N1: n=13 patients N2: n=12 patients	Method: Proteomic and Western blot 38 pairs of infiltrating ductal carcinoma (IDC) tissues comprised normal and cancerous tissues	Proteomic: CRT was upregulated in three N stages: N0 – 77%, N1 – 92%, and N2 – 83%. Western blot: Higher CRT expression in cancerous tissues than in normal tissues.
Kabbage et al., 2013 [23]	Middle coast of Tunisia	Breast cancer patients: 29-85 years (median=48 years) Healthy volunteer women (control): 26-75 years (median=45 years)	ND	Method: Proteomic, ELISA, and immunohistochemistry n=66 breast tumor tissues and their serum n=65 serum from healthy volunteer women (control) Proteomic and ELISA: n=15 tumors and surrounding histologically normal tissues IHC: n=35 samples of infiltrating ductal breast carcinomas and other types of breast cancer	High CRT levels in epithelial tumor cells compared to normal duct tissues. CRT expression in infiltrating ductal breast carcinomas was restricted in the cytoplasm. High CRT expression in three breast cancer subtypes: 5/7 infiltrating lobular carcinomas, 2/6 medullary carcinomas, and 1/3 ductal in situ carcinomas.
Vaksman O, Davidson B, Tropé C, Reich R., 2013. [24]	Department of Gynecologic Oncology, Norwegian Radium Hospital	39-79 years old (mean=61)	ND	Method: Western blot and qRT-PCR 102 ovarian carcinoma specimens (55 effusions and 47 solid specimens)	RT-PCR showed CRT mRNA expression in 54 of 55 effusions and all solid specimens. CRT mRNA overexpression in solid metastases compared with effusion and primary carcinomas. CRT expression was significantly higher in solid metastases and primary carcinomas than in effusions. Secreted CRT levels were higher in peritoneal than pleural effusions.
Peng et al., 2010. [8]	Cancer Center of Sun Yat-Sen University, Guangzhou, China	<60 years old = 44.1% >/ 60 years old = 55.9%	T3N1M0	Method: Immunohistochemistry 68 samples of colon cancer	CRT was stained in the cytoplasm. CRT expression was lower in colon cancer than in adjacent normal epithelium. 61.8% of colon cancer samples were positive for CRT.

Table 1. Continued

Author	Population	Age	TNM stage	Sample size and characteristics	Outcomes
Du et al., 2007 [25]	Department of Pathology in the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China	ND	ND	Method: Proteomic: n=41 fresh tissues from esophageal squamous cell carcinoma (ESCC) and adjacent normal tissue. Method: Western blot: n=4 cases of cancerous tissues and adjacent normal esophageal tissues. Method: immunohistochemistry: n=89 ESCC samples of paraffin-embedded tissues.	Proteomic: CRT was upregulated in esophageal squamous cell carcinoma. Western blot: CRT was high in all ESCC tissues. IHC: Normal esophageal epithelial cells showed negative cytoplasm immunoreaction in most cases. Tumor cells showed a strong positive immunoreaction in the cytoplasm. High CRT levels occurred in 73.03% of tumors. High CRT levels were most frequent in tumor tissues than in esophageal epithelial tissues. CRT overexpression was correlated to a poor prognosis.
Chiang et al., 2013 [2]	E-Da Hospital	ND	ND	Method: Western blot, immunohistochemistry, qRT-PCR, flow cytometry 110 samples from patients diagnosed with head and neck squamous cell carcinoma.	CRT expression was higher in OSCC than in control (normal tissue). For immunohistochemistry, 96.1% of head and neck squamous cell carcinoma was positive for CRT.
Liu et al., 2012 [26]	Respiratory Department of Xijing Hospital (China), Forty Military Medical University	25 to 75 (median age: 57 years)	ND	Method: ELISA, flow cytometry, Western blot, and immunohistochemistry 80 lung cancer tissues and 10 normal lung tissues	CRT was significantly higher in lung cancer than in healthy patients.
Lwin et al., 2010 [27]	Department of Pathology, Singapore General Hospital	23 to 89 (median age: 55.6 years)	112 patients had lymph node metastasis	Method: qRT-PCR, Western blot, immunohistochemistry, and immunofluorescence staining Tissues from 228 patients diagnosed with breast invasive ductal carcinoma	CRT mRNA expression was higher in MDA-MB-231 than in MCF-7. CRT was located in the cytoplasm of MCF-7 and MDA-MB231 cells, especially in the perinuclear region.
Chignard et al., 2006 [28]	ND	ND	ND	Method: Proteomic Tumor and non-tumor tissues from 7 hepatocellular carcinoma patients Serum from 27 healthy individuals, 33 from chronic hepatitis patients, 28 from cirrhosis patients, and 34 from hepatocellular carcinoma patients.	CRT isoforms were lower in hepatocellular carcinoma than in non-tumor tissues. Statistically significant differences occurred in CRT18 serum levels between hepatocellular carcinoma patients and healthy patients.
Vera et al., 2020 [14]	Hospital Clínico Universidad de Chile	ND	ND	Method: qRT-PCR, Western blot, immunohistochemistry, and immunocytochemistry. 67 ovarian samples	Increased CRT mRNA levels in ovarian cancer samples compared with normal inactive ovaries, benign tumors, and borderline tumors (adjacent areas to the tumor). The semi-quantitative analysis of CRT protein levels revealed an increased rate of this protein in epithelial ovarian cancer samples compared to borderline and benign tumors. There was positive staining in all human ovarian tissues.
Harada et al., 2017	Yamaguchi University Hospital	<65= 41 >65 = 70	I = 26 II = 41 III = 12 IV = 32	Method: Immunohistochemical staining	A significant association occurred between CALR expression and T and N classifications and stage and patient outcome. Higher levels of CALR were also significantly associated with lower overall and event-free survival probability.

Table 2. Risk of Bias Assessment Tool (RoBANS) for the Articles Included in the Systematic Review

Study	Selection of participants	Confounding variables	Exposure measurement	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting
Chignard et al., 2006	Low	Low	Low	High	Low	Low
Du et al., 2007	Unclear	Low	Low	High	Low	Low
Lwin et al., 2010	Unclear	Low	Low	High	Low	Low
Peng et al., 2010	Unclear	Low	Low	High	Low	Low
Lee et al., 2012	Low	Low	Low	High	Low	Low
Liu et al., 2012	High	Low	Low	High	Low	Low
Vera et al., 2020	High	Low	Low	High	Low	Low
Chiang et al., 2013	Low	Low	Low	High	Low	Low
Kabbage et al., 2013	Low	Low	Low	High	Low	Low
Vaksman et al., 2013	Unclear	Low	Low	High	Low	Low
Yang et al., 2013	Low	Low	Low	High	Low	Low
Cooney et al., 2017	Low	Low	Low	High	Low	Low
Harada et al., 2017	Low	Low	Low	High	Low	Low
Ye et al., 2021	Low	Low	Low	High	Low	Low

to blinding assessments (Table 2).

Meta-analysis

In this step, a meta-analysis was performed with the following variables:

• High and low CRT expression in male carcinoma patients;

• High and low CRT expression in female carcinoma patients;

• High CRT levels in male and female carcinoma patients;

• High CRT levels associated with metastatic lymph nodes in carcinoma patients;

• High CRT levels associated with the clinical stage (I-II vs. III-IV) in carcinoma patients.

Differences regarding male (OR=1.79 [0.69; 4.67/p = 0.2337/I^2 = 67.4%) and female (OR = 2.53 [0.89; 7.16/p = 0.0809/I^2 = 70.4%) sexes of carcinoma patients with low and high CRT levels were not significant (Figure 2A and Figure 3A), as well as for high CRT levels compared to male and female sexes (OR = 1.11; [0.65; 1.91]/p = 0.6974/I^2 = 0%) (Figure 4A). The clinical stage (I-II vs. III-IV) did not show significant differences for high CRT levels (OR = 1.64 [0.66; 4.08]/p = 0.2851/

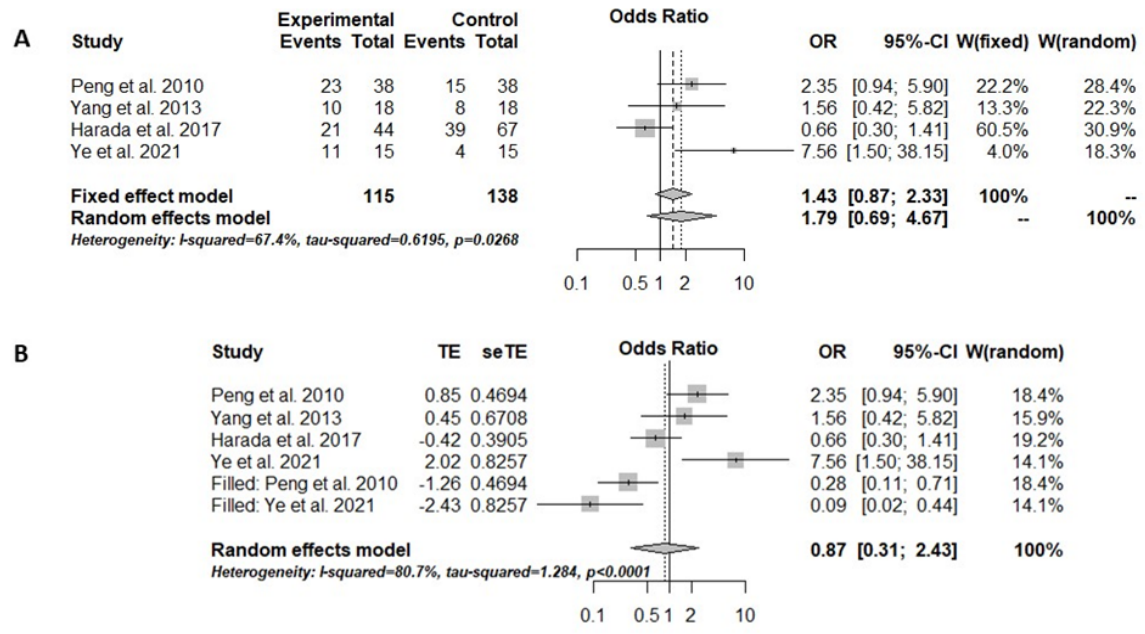

Figure 2. (A) Meta-analysis results for the male sex illustrated in a forest plot. The experimental group included men affected by carcinomas with high and positive CRT levels, and the control group consisted of male carcinoma patients with low and negative CRT levels. (B) Trim-and-fill method results are illustrated in a forest plot, highlighting the meta-analysis and publication biases. OR= odds ratio; CI= confidence interval; W= weight; TE= estimated mean values; seTE= estimated standard deviation values. The heterogeneity levels for both analyses were very high (I-squared >50%).

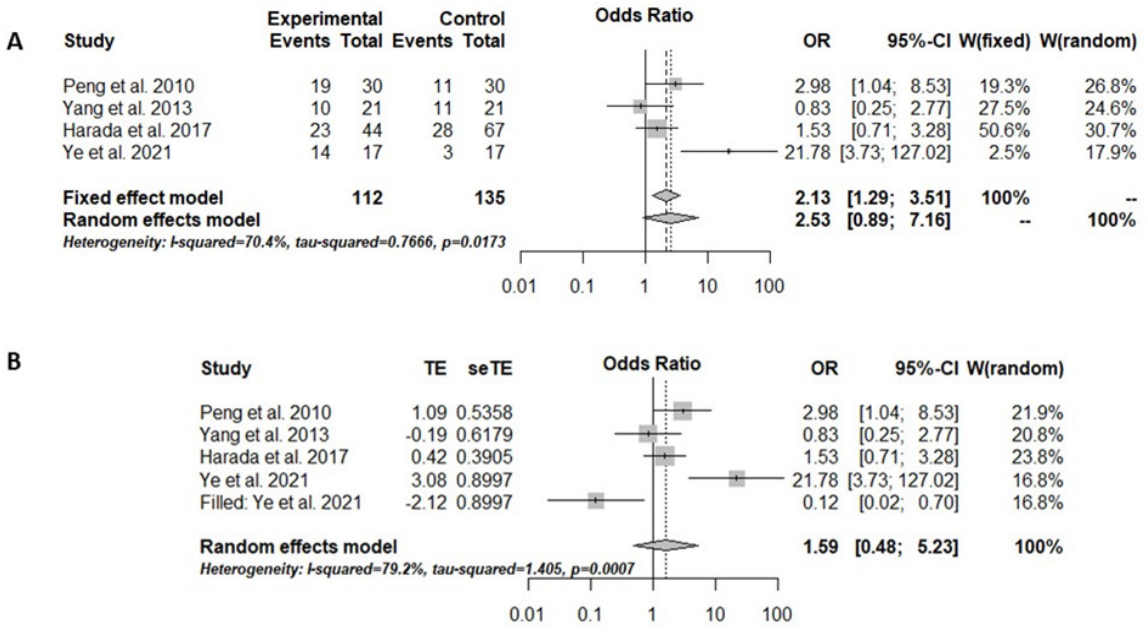

Figure 3. (A) Meta-analysis results for the female sex illustrated in a forest plot. The experimental group included women affected by carcinomas with high and positive CRT levels, and the control group consisted of female carcinoma patients with low and negative CRT levels. (B) Trim-and-fill method results are illustrated in a forest plot, highlighting the meta-analysis and publication biases. OR= odds ratio; CI= confidence interval; W= weight; TE= estimated mean values; seTE= estimated standard deviation values. The heterogeneity levels for both analyses were very high (I-squared >50%).

$I^2 = 37.5\%$) (Figure 5A). Significant results only occurred for high CRT levels associated with the presence of metastatic lymph nodes (OR=2.46 [1.24; 4.88]/p = 0.0098/ $I^2 = 0\%$) (Figure 6A). Furthermore, all evaluated outcomes had biases related to publication and meta-analysis but without significant changes in the understanding of results (Figures 2-6 B). The sole exception was metastatic lymph nodes because the OR value was corrected to 3.06 [1.71; 5.48/p = 0.0002/$I^2 = 0\%$) (Figure 6B).

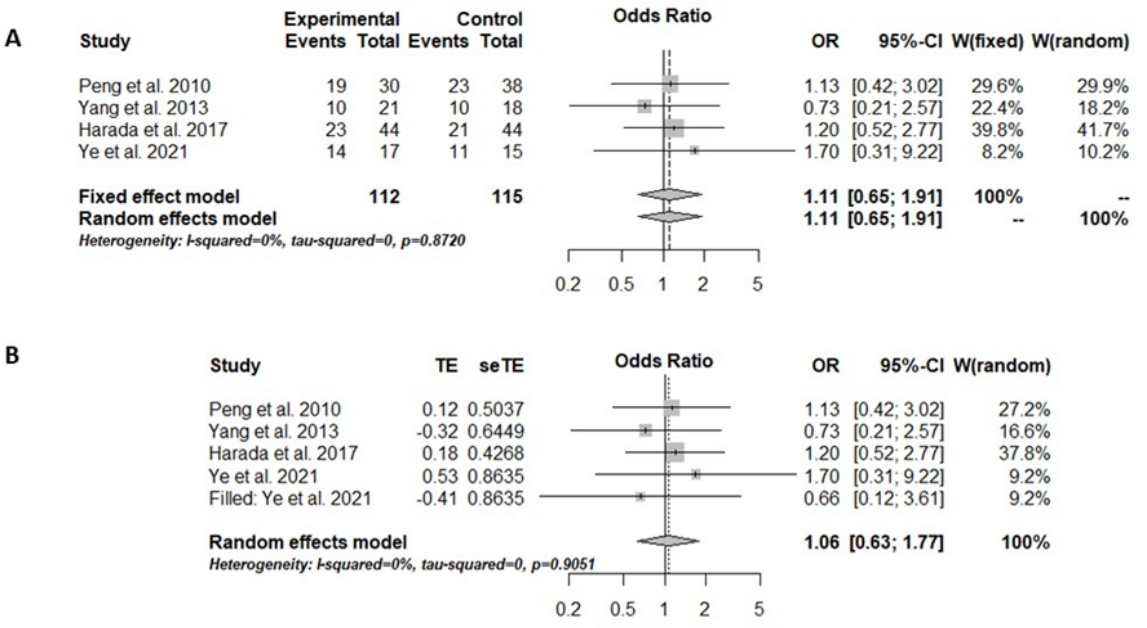

Figure 4. (A) Meta-analysis results for the differences between sexes (female vs. male) for high CRT levels illustrated in a forest plot. The experimental group included female carcinoma patients with high CRT levels, and the control group consisted of male carcinoma patients with high CRT levels. (B) Trim-and-fill method results are illustrated in a forest plot, highlighting the meta-analysis and publication biases. OR= odds ratio; CI= confidence interval; W= weight; TE= estimated mean values; seTE= estimated standard deviation values. The heterogeneity levels for both analyses were sufficient (I-squared <50%).

Table 3. Calreticulin Expression in Human Carcinoma

Patient or population: human carcinoma patients.

Setting: calreticulin expression, human carcinoma.

Intervention: high/positive and low/negative calreticulin expression.

Comparison: sex, metastatic lymph node, and clinical stage.

Outcomes	Illustrative comparative difference (95%CI)		Effect measure [95%CI]	Number of participants (studies)	Quality of evidence (GRADE)	Comments
	Assumed risk/usual care	Corresponding risk/self-management				
Male sex	The mean of the male sex for low/negative calreticulin expression ranged from 4 to 15 for positive events.	The mean of the male sex for high/positive calreticulin expression ranged from 10 to 23 for positive events.	OR = 1.79 [0.69; 4.67]	138	(+)(+)(-)(-) Low	High heterogeneity levels and wide confidence intervals were detected for this outcome.
Female sex	The mean of the female sex for low/negative calreticulin expression ranged from 3 to 28 for positive events.	The mean of the female sex for high/positive calreticulin expression ranged from 10 to 23 for positive events.	OR= 2.53 [0.89; 7.16]	135	(+)(+)(-)(-) Low	High heterogeneity levels and wide confidence intervals were detected for this outcome.
Male vs. female sex (high calreticulin expression)	The mean of the male sex for low/negative calreticulin expression ranged from 10 to 23 for positive events.	The mean of the female sex for high/positive calreticulin expression ranged from 10 to 23 for positive events.	OR= 1.11 [0.65; 1.91]	115	(+)(+)(+)(-) Moderate	A wide confidence interval was detected for this outcome.
Metastatic lymph node (high calreticulin expression)	The mean absence of positive metastatic lymph nodes for calreticulin expression ranged from 5 to 16 for positive events.	The mean presence of positive metastatic lymph nodes for calreticulin expression ranged from 8 to 28 for positive events.	OR= 2.45 [1.24; 4.88]	79	(+)(+)(+)(-) Moderate	A wide confidence interval was detected for this outcome.
Clinical stage (I-II vs. III-IV) (high calreticulin expression)	The mean early clinical stages positive for calreticulin expression ranged from 5 to 19 for positive events.	The mean advanced clinical stages positive for calreticulin expression ranged from 1 to 25 for positive events.	OR= 1.64 [0.66; 4.08]	99	(+)(+)(+)(-) Moderate	A wide confidence interval was detected for this outcome.

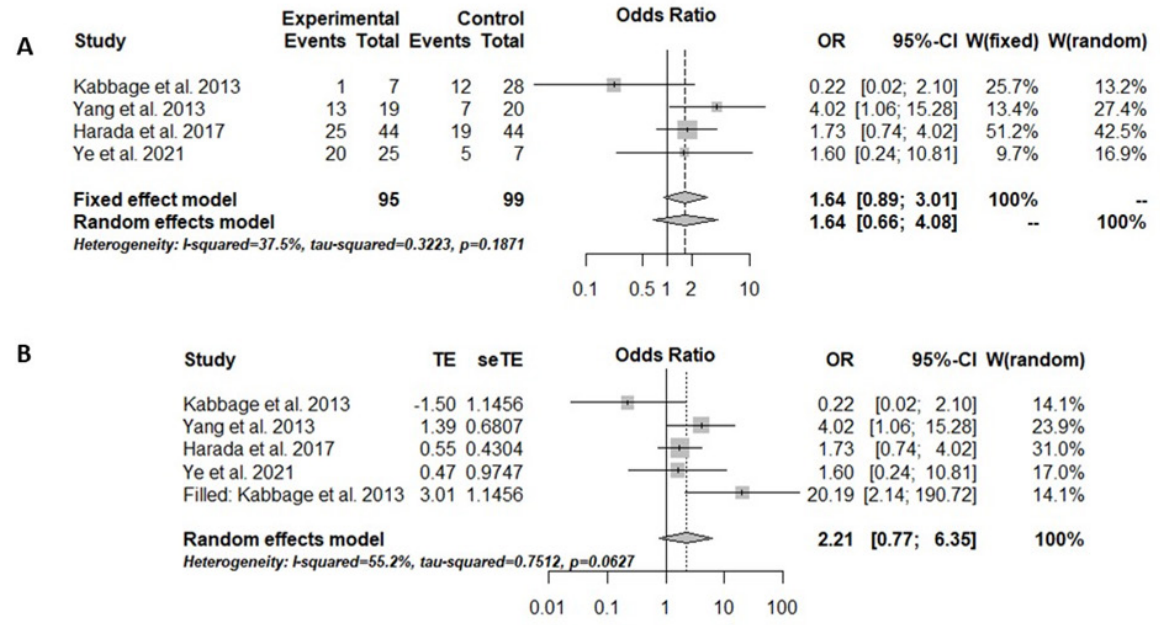

Figure 5. (A) Meta-analysis results from the clinical stage of tumor disease with high CRT levels. The experimental group included advanced clinical stages (III-IV) of carcinoma patients, and the control group consisted of early clinical stages (I-II) of carcinoma patients. (C) Trim-and-fill method results are illustrated in a forest plot for the clinical stage of tumor disease with high CRT levels, highlighting the meta-analysis and publication biases. OR= odds ratio; CI= confidence interval; W= weight; TE= estimated mean values; seTE= estimated standard deviation values. In (A), the heterogeneity level was sufficient (I-squared <50%), but in (B), it was high (I-squared >50%).

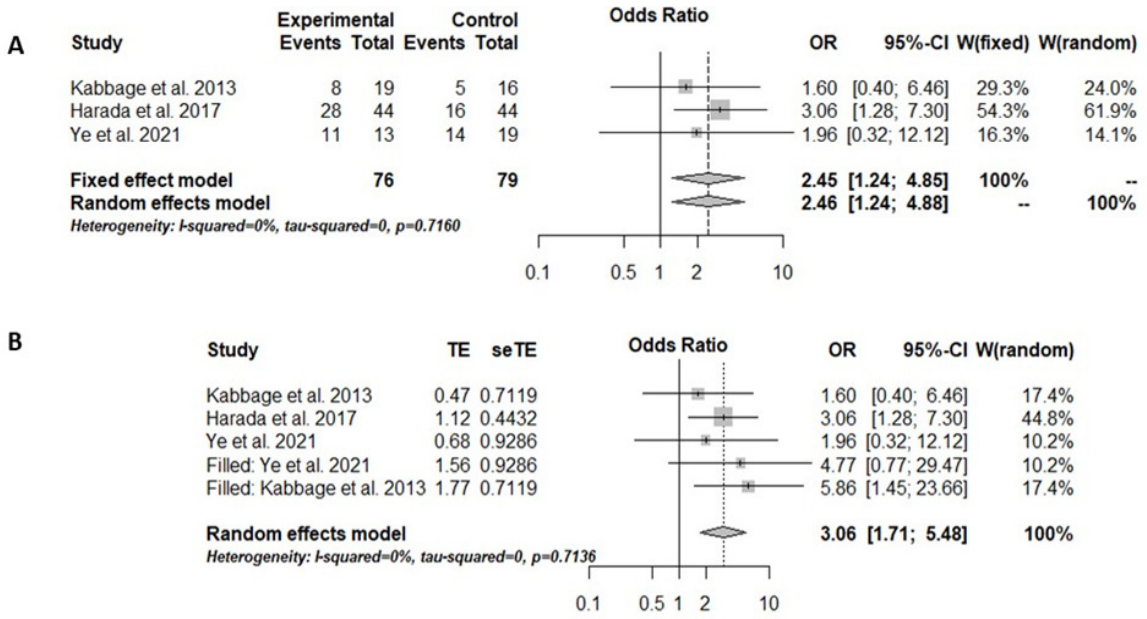

Figure 6. (A) Meta-analysis results for the presence of metastatic lymph nodes with high CRT levels. The experimental group included the presence of metastatic lymph nodes in carcinoma patients, and the control group consisted of the absence of metastatic lymph nodes in carcinoma patients. (B) Trim-and-fill method results are illustrated in a forest plot, highlighting the meta-analysis and publication biases. OR= odds ratio; CI= confidence interval; W= weight; TE= estimated mean values; seTE= estimated standard deviation values. The heterogeneity levels for both analyses were sufficient (I-squared >50%).

Grade results

The main reason for downgrading the quality of evidence was the wide confidence interval noted for all evaluated outcomes. The second reason was the heterogeneity found for male and female sexes with low and high CRT levels (Table 3).

Discussion

In the 21st century, cancer is the primary cause of premature death and a barrier to increasing life expectancy. Cancer incidence is expected to double in 2070 compared to 2020, and the main reasons are population aging and growth and demographic changes (Soerjomataram and Bray, 2018). Despite high investments in cancer research and significant improvements in antineoplastic treatment, the life expectancy of oncologic patients is unlikely to improve (Zeng, 2018). In this context, it is critical to strengthen cancer prevention programs because early diagnosis is essential for improving patient prognosis. Studies that explain the oncogenesis mechanism and use biomarkers for translational clinical patterns are a path to better understanding the clinical course of malignant diseases and developing new therapies to improve expectancy and quality of life (Zeng, 2018). CRT is a biomarker example reported in the literature, which shows pro- and anti-oncogenic activities (Houen, 2019).

CTR related to cancer cells may show high or low expression in different carcinomas and between carcinomas and normal tissues. This difference in neoplasms may be due to overlapping protein functions and the presence of the mutant protein. The present systematic review showed that the overall multifunctional CRT level is higher in carcinomas than in normal tissues. Thus, meta-analyses assessed whether CRT levels represent clinical parameters. In this context, high CRT levels are associated with a higher probability (OR = 2.46 – 3.06) of finding metastatic lymph nodes in carcinoma patients. Intrinsic and immunological cellular mechanisms related to CRT may explain malignant transformation and tumor progression. The role of CRT in calcium homeostasis, antigen presentation, and danger signaling stands out among these mechanisms (Fucikova et al., 2021).

Maintaining calcium homeostasis in the body is relevant for cell function and survival. CRT participates in this mechanism due to its chaperone activity, as it prevents the premature export of misfolded proteins from the endoplasmic reticulum (ER), consequently supporting protein refolding (Rutkevich and Williams, 2011). Changing this CRT function can damage tumor progression and the proper functioning of human body organs, such as the heart. For instance, CRT is essential for cardiac development, leading to cardiac pathology when highly expressed in the adult heart, causing contractility problems and potentially sudden death (Groenendyk et al., 2022). Therefore, the role of CRT should be better evaluated in different tissues and diseases, considering its essential function in numerous cells.

One of CRT's oncogenic activities refers to the mutation in the gene that synthesizes this protein. These mutations alter protein structure, mainly in the C-domain, affecting the KDEL signal and activating JAK2 and MAPK signaling pathways. Furthermore, mutant CRT can modify calcium homeostasis and accumulate unfolded proteins, activating the oncogenic pathway by oxidative stress (generating more reactive oxygen species) and

following genomic instability (Salati et al., 2019).

CRT composes the peptide loading complex (PLC) in the ER membrane, which ensures proper antigen loading in MHC class I molecules (Fucikova et al., 2021). Mutant CRT behavior changes in the presence of antigens, reducing their presentation to MHC class I molecules and favoring immune evasion due to the loss of tumor antigenicity, showing a high CRT expression in carcinomas (Arshad and Cresswell, 2018). However, the low CRT expression in some tumors also relates to the low antigen presentation to MHC class I molecules and is associated with a poor disease prognosis (Cathro et al., 2010; Noblejas-López et al., 2019). A few studies in the present systematic review showed low CRT expression in carcinomas (Peng et al., 2010; Ye et al., 2021; Chignard et al., 2006), which may be associated with accumulated malignant features and advanced disease stages. These results were obtained for colon (Peng et al., 2010), gallbladder (Ye et al., 2021), and liver (Chignard et al., 2006) carcinomas.

CRT is mainly found in the ER, although it may translocate to other cellular components due to cell stress, which leads to a loss of homeostasis in the ER (Lu et al., 2015). When exposed to the cell surface, it emits an "eat me" signal (prophagocytic signal) to antigen-presenting cells, specifically dendritic cells, and later CD8+ T cells, to combat tumor progression (Fucikova et al., 2021). This signaling is among the hallmarks of immunogenic cell death, so CRT acts as a DAMP (damage-associated molecular pattern) (Krysko et al., 2012). Conversely, mutant CRT has an altered structure, is more secreted, and has higher plasma levels than the wild type. This change in the CRT scenario is a danger sign, causing the mutated CRT to prevent the onset of tumor-targeted immunity, which identifies an immunological advantage of CRT mutations to cancer cells (Liu et al., 2020; Fucikova et al., 2021).

Although studies associate high CRT levels with poor prognosis (Cooney et al., 2017; Yang et al., 2013; Ye et al., 2021; Vaksman et al., 2013; Du et al., 2007), the present meta-analysis did not find significant results for advanced clinical stages and differences between sexes. Pancreatic cancer is the most common aggressive cancer in adult men, difficult to diagnose in early stages, and potentially affecting other organs (Goral, 2015). CRT overexpression in this cancer is associated with UICC stage and lymph node metastasis, thus representing an unfavorable prognostic indicator for this neoplasm (Sheng et al., 2014). Likewise, squamous cell carcinoma also prevails in men, presenting high CRT expression with a poor disease prognosis (Du et al., 2007).

Gastric cancer is another prevalent neoplasm in men, with high CRT expression. Chen et al. (2009) concluded that associating CRT overexpression with angiogenesis events facilitates the proliferation of tumor cells and invasion of tumor lymph node metastases, leading to a poor prognosis (Chen et al., 2009). Harada et al. (2017) also observed that a high CRT expression is associated with tumor progression and poor lesion prognosis. Thus, the high CRT expression for this tumor suggests that intracellular functions, such as calcium homeostasis and antigen signaling, are compromised, favoring tumor progression.

Patient selection is crucial in observational studies to eliminate potential biases related to variable measures. Thus, experimental and control groups tend to be the same, except for the evaluated variable (Vandenbroucke et al., 2007). Blinding procedures are also relevant tools to eliminate biases related to estimated treatment effects (Moustgaard et al., 2020). The study design of the articles included in the present systematic review only allowed the blinding of researchers and statisticians, which was not performed in any of the studies. The downgrading of the quality of evidence related to high heterogeneity levels and wide confidence intervals mainly occurred because there was no restriction on tumor type in the meta-analysis and no similarities between control and experimental groups.

CRT expression differs in some carcinomas, although most of them are high. Our data showed that high CRT levels may be a prognostic factor for metastatic lymph nodes in cancer patients; therefore, CRT may represent a relevant biomarker for some carcinomas. Nevertheless, an in-depth study of the possible immune evasion mechanisms is required for a better understanding of CRT involvement in the process of tumor progression.

Author Contribution Statement

MPP, TMF, COB, EMSM, and AB conceptualized and designed the study; MPP and TMF collected the data; MPP, TMF, COB, EMSM, and AB analyzed and interpreted the results; MPP prepared the initial manuscript. All authors reviewed the results and approved the final version of the manuscript..

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Study Registration

The study was registered in the Open Science Framework (OSF) (registration DOI: 10.17605/OSF.IO/9DQWC).

Conflicts of Interest

The authors declare no conflicts of interest.

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