ORIGINAL RESEARCH

Prognostic Value of Anti-*Chlamydia Trachomatis* IgG in Breast Cancer and the Modification Effects of Pro-Inflammatory Cytokines: A 13-Year Prospective Cohort Study

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Purpose: *Chlamydia trachomatis* (*C. trachomatis*) is associated with several gynecological tumors; yet its prognostic role in breast cancer remains unclear. Thus, we investigated the prognostic role of anti-*C. trachomatis* immunoglobulin G (IgG) in breast cancer patients and the modification effects of pro-inflammatory cytokines.

Methods: The serum levels of *C. trachomatis* IgG and four pro-inflammatory cytokines were measured. Cox regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), including product terms to assess the modification effects of pro-inflammatory cytokines on the association between *C. trachomatis* IgG and breast cancer prognosis.

Results: From 2008 to 2018, 1121 breast cancer patients were recruited and followed up until December 31, 2021, with a median follow-up time of 63.91 months (interquartile range: 39.16–90.08 months). Patients positive for *C. trachomatis* IgG showed HRs of 1.09 (95% CI, 0.67–1.78) for overall survival (OS) and 1.24 (0.87–1.78) for progression-free survival (PFS), compared to those who were negative. These associations became statistically significant in women aged 50 years or younger (HR=1.43, 95% CI=0.79–2.58 for OS; HR=1.79, 95% CI=1.16–2.77 for PFS). Positive *C. trachomatis* IgG serology was associated with adverse prognostic effects among patients with higher levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-8, and IL-1β), but with favorable prognostic effects for those with low levels. These interactions were particularly significant in those aged 50 years or younger.

Conclusion: In breast cancer patients younger than 50 years of age or with higher levels of pro-inflammatory cytokines, *C. trachomatis* infection appeared to have a negative prognostic impact. These findings highlight the significance of *C. trachomatis* in predicting prognosis and personalized therapy for breast cancer patients.

Keywords: *Chlamydia trachomatis*, breast cancer, prognosis, pro-inflammatory cytokines

Introduction

Breast cancer is the most prevalent malignancy among women worldwide and is an important public health issue.[1,2] In 2020, breast cancer in women has surpassed lung cancer as the leading cause of cancer incidence worldwide, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is the fifth leading cause of cancer death globally, with 685,000 deaths.[3] The burden of disease due to breast cancer remains substantial around the world.[4] Therefore, improving the prognosis of breast cancer is of great importance for public health.

A variety of risk factors have been found to be associated with breast cancer prognosis, including demographic characteristics, clinicopathological features, therapeutic methods, and lifestyles.[5–7] Current evidence suggests associations between chronic infections with viral or bacterial pathogens and the progression of various cancers, including breast cancer.[8,9] Predominantly, *Chlamydia trachomatis* (*C. trachomatis*) has emerged as a pathogen of interest, given its established association with cervical and ovarian cancers.[10–13] Importantly, both breast and ovarian cancers exhibit hormone sensitivity, particularly to estrogen. The infectivity of *C. trachomatis*, known to be modulated by hormonal variations and heightened by estrogen, posits a hormonal nexus potentially influencing breast cancer. Despite these associations, the impact of *C. trachomatis* on the prognosis of breast cancer remains unknown. This existing gap in our understanding calls for more in-depth research to clarify the potential influence of *C. trachomatis* on breast cancer prognosis.

In addition, *C. trachomatis* infection may trigger immune responses, notably the production of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α).[14,15] Persistent *C. trachomatis* infection leads to sustained cytokine secretion, contributing to a chronic inflammatory state, which in turn plays a significant role in tumor metastasis and invasion.[16–18] Therefore, the present study aimed to evaluate the association of *C. trachomatis* infection with overall survival (OS) and progression-free survival (PFS) in women with breast cancer, with specific emphasis on the modification effects of pro-inflammatory cytokines, which could provide evidence for tailoring treatment plans and prognostic evaluation in breast cancer patients.

Materials and Methods

Study Population

A total of 1121 women with breast cancer were recruited from October 2008 to January 2018 derived from a subset of the Guangzhou Breast Cancer Study (GZBCS) cohort.[19] Briefly, patients who were pathologically diagnosed with primary invasive breast cancer from the First and the Second Affiliated Hospitals of Sun Yat-sen University in Guangzhou, China were enrolled. Women with metastasized breast cancer or previous history of other cancers were excluded. Blood samples were collected immediately after the patients were admitted to the hospitals or after the interview and stored at −80°C before detection. Of them, 1755 patients had serum samples collected at diagnosis. We excluded patients with missing data on the following variables: clinical stage, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, or follow-up. Additionally, 204 patients with poor serum quality were excluded. This study was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University. Informed consent was obtained from each participant before the interview.

Data Collection

The baseline information was collected using a structured questionnaire. Trained and qualified investigators conducted face-to-face interviews with patients in the hospital wards. The collected information included the general demographic characteristics, family history of breast cancer, menstrual history, and reproductive history. Clinicopathological characteristics were extracted from medical records. Immunohistochemistry test was used to determine the ER, PR, and HER2 status. The definitions of ER, PR, and HER2 statuses have been described in detail in a previous study.[20]

Follow-Up

Patients were followed up by telephone or out-patient visits every 3 months during the first-year post-diagnosis, semiannually in the second and third year, and annually thereafter. The endpoints of this study were OS and PFS. OS was defined as the duration from the date of diagnosis to the date of death from any cause. PFS was defined as the interval from diagnosis to the occurrence of disease progression, which includes recurrence, metastasis, or death. The survival status of patients was ascertained on the latest follow-up date or December 31, 2021.

Serological Tests

Immunoglobulin G (IgG) antibody against *C. trachomatis* and total IgG were measured using commercial enzyme-linked immunosorbent assay kits (Savyon diagnostics, Israel and Cusabio Biotech Co, China, respectively). The

5366

Journal of Inflammation Research 2024:17

tests were performed in strict accordance with the manufacturers' instructions. To ensure consistency in the optical density (OD) values across the different test plates, a reference sample provided with each kit was included in each 96-well plate. Seropositivity for *C. trachomatis* IgG was defined by a cut-off index (COI) greater than 1.1. The COI was calculated based on the ratio of the OD value from each sample and to the OD value of the cut-off control in the corresponding plate. In addition, serum concentrations of IL-6, TNF-α, IL-8, and IL-1β were measured using a commercially available cytokine panel from Bio-Rad Laboratories (Cat. No. M500KCAF0Y), following the provided instructions. Analysis was performed on the Luminex xMAP 200 platform (Luminex Corporation, Austin, TX, USA). Standard curves for these measurements were generated using the known concentrations of each cytokine. The data were collected and processed with Bio-Plex Manager 6.0 (Bio-Rad Laboratories).

Statistical Analysis

Seropositivity to IgG against *C. trachomatis* was assessed and total IgG levels were quantified in terms of concentration (g/L). To compare the distribution differences in general demographic and clinicopathological characteristics between the *C. trachomatis* positive and negative groups, continuous variables were expressed as medians and interquartile ranges (IQR). These differences were evaluated using Mann–Whitney U-test. Categorical variables were presented by frequency and constituent ratio, with the χ^2 test applied to compare differences between groups. The levels of pro-inflammatory cytokines were categorized into tertiles for analysis. Univariate and multivariate Cox proportional hazards regression models were used to explore the association of general demographic and clinicopathological characteristics, *C. trachomatis*, and pro-inflammatory cytokines with breast cancer prognosis. Hazard ratios (HRs) and their 95% confidence intervals (CIs) were calculated. Variables adjusted in the multivariable Cox proportional hazards regression models were determined based on previous studies and the factors known to affect breast cancer prognosis. These included age at diagnosis, menopausal status, education, ER status, HER2 status, clinical stage, and total IgG levels. The association between *C. trachomatis* infection and breast cancer prognosis was analyzed using pro-inflammatory cytokine levels as stratification factors. Stratified analyses were also performed based on clinical pathological characteristics. Multiplicative interactions were analyzed by including product term into the multivariate Cox regression analysis. All statistical analyses were performed using R 3.6.1, and a two-sided P-value of less than 0.05 was considered statistically significant.

Results

Demographic and clinicopathological characteristics and the associations with breast cancer prognosis and *C. trachomatis* infection.

A total of 1121 patients with breast cancer were included in this study, 182 (16.2%) of whom were *C. trachomatis* IgG positivity. Table 1 shows that the median age at diagnosis was 48 years (interquartile range: 42–57 years), and 63.7% of women were 41–60 years old. More than half of the subjects were premenopausal (57.9%) and nearly half of them had an education level of junior middle school or below (40.9%). In terms of clinicopathological characteristics, most patients were diagnosed with ER+ (76.8%), PR+ (67.4%), HER2- (58.0%), and early clinical stage (stage I/II: 79.1%). *C. trachomatis* IgG positive breast cancer patients were more likely to be premenopausal, age 41–60 years old at diagnosis, ER-positive, and PR-positive compared to negative ones (Table 1).

Univariate Cox regression analysis showed that age at diagnosis, menopausal status, education level, ER status, PR status, HER2 status, tumor size, lymph node metastasis, distant metastasis, and TNM stage were significantly associated with breast cancer prognosis (Supplementary Table 1).

Prognostic Effects of *C. trachomatis* on Breast Cancer

Of the 1121 subjects, 150 died, and 241 showed disease progression during the follow-up period (median follow-up time: 63.91 months, interquartile range: 39.16–90.08 months). Five-year OS rate and PFS rate were 88.3% and 80.1%, respectively. Table 2 presents the results of univariate and multivariate analyses of the association between *C. trachomatis* IgG levels and breast cancer prognosis. No association of *C. trachomatis* IgG positivity with the risks

Table 1 Demographic and Clinicopathological Characteristics by *C. trachomatis* Infection Status

Variables	Total (%) (N=1121)	*C. trachomatis* IgG (%)		
		Negative (N=939)	Positive (N=182)	P
Age (years)				
≤ 40	244 (21.8)	209 (22.3)	35 (19.2)	**0.035**
41–60	714 (63.7)	584 (62.2)	130 (71.4)	
≥ 61	163 (14.5)	146 (15.5)	17 (9.3)	
Continuous (Median, IQR)	(48, 42–57)	(49, 41–57)	(47, 42–54)	0.176
Menopausal Status				
Pre-Menopausal	649 (59.4)	532 (58.1)	117 (66.1)	**0.047**
Post-Menopausal	444 (40.6)	384 (41.9)	60 (33.9)	
Missing	28	23	5	
Education Levels				
Junior Middle School or Below	458 (43.7)	373 (42.8)	85 (48.0)	0.189
Senior Middle School	321 (30.6)	265 (30.4)	56 (31.6)	
College or Above	269 (25.7)	233 (26.8)	36 (20.3)	
Missing	73	68	5	
Age at Menarche (Years)				
≤ 12.0	136 (12.5)	109 (12.0)	27 (15.1)	0.248
> 12.0	954 (87.5)	802 (88.0)	152 (84.9)	
Missing	31	28	3	
Marital Status				
Never Married	32 (2.9)	24 (2.61)	8 (4.49)	0.097
Married/Cohabiting	1007 (91.7)	851 (92.5)	156 (87.6)	
Divorced/Widowed/Separated	59 (5.4)	45 (4.89)	14 (7.87)	
Missing	23	19	4	
BMI (kg/m^2)				
< 24.0	727 (67.1)	611 (67.5)	116 (64.8)	0.722
24.0 ~ 27.9	284 (26.2)	235 (26.0)	49 (27.4)	
≥ 28.0	73 (6.7)	59 (6.52)	14 (7.82)	
Missing	37	34	3	
Parity				
0	63 (5.7)	49 (5.34)	14 (7.82)	0.193
≥ 1	1033 (94.3)	868 (94.7)	165 (92.2)	
Missing	25	22	3	
Breastfeeding				
Never	149 (14.6)	126 (14.7)	23 (14.1)	0.849
Ever	872 (85.4)	732 (85.3)	140 (85.9)	
Missing	100	81	19	
Breast Cancer History				
No	986 (90.8)	826 (91.4)	160 (87.9)	0.141
Yes	100 (9.2)	78 (8.63)	22 (12.1)	
Missing	35	35	0	
ER				
Negative	260 (23.2)	228 (24.3)	32 (17.6)	**0.050**
Positive	861 (76.8)	711 (75.7)	150 (82.4)	
PR				
Negative	365 (32.6)	324 (34.5)	41 (22.5)	**0.002**
Positive	756 (67.4)	615 (65.5)	141 (77.5)	

(Continued)

Table 1 (Continued).

Variables	Total (%) (N=1121)	C. trachomatis IgG (%)		
		Negative (N=939)	Positive (N=182)	P
HER2				
Negative	650 (58.0)	534 (56.9)	116 (63.7)	0.165
Equivocal	241 (21.5)	204 (21.7)	37 (20.3)	
Positive	230 (20.5)	201 (21.4)	29 (15.9)	
Clinical Stage				
I	319 (28.5)	269 (28.6)	50 (27.5)	0.575
II	567 (50.6)	477 (50.8)	90 (49.5)	
III	180 (16.1)	145 (15.4)	35 (19.2)	
IV	55 (4.9)	48 (5.1)	7 (3.8)	
Tumor Size (cm)				
< 2.0cm	472 (42.3)	399 (42.7)	73 (40.3)	0.559
≥ 2.0cm	644 (57.7)	536 (57.3)	108 (59.7)	
Missing	5	4	1	
Nodal status				
No	623 (55.9)	521 (55.8)	102 (56.4)	0.899
Yes	491 (44.1)	412 (44.2)	79 (43.6)	
Missing	7	6	1	
Metastasis				
No	1066 (95.1)	891 (94.9)	175 (96.2)	0.469
Yes	55 (4.9)	48 (5.1)	7 (3.8)	

Notes: Bold characters indicate statistically significant result.
Abbreviations: *C. trachomatis, Chlamydia trachomatis*; IgG, immunoglobulin G; IQR, interquartile range; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Table 2 Association Between *C. trachomatis* IgG Levels and Breast Cancer Prognosis

C. trachomatis IgG	Total (%)	Events (%)	HR (95% CI)[a]	HR (95% CI)[b]	HR (95% CI)[c]
			OS		
Negative	939 (83.8)	128 (85.3)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Positive	182 (16.2)	22 (14.7)	0.86 (0.55–1.36)	1.09 (0.68–1.76)	1.09 (0.67–1.78)
			PFS		
Negative	939 (83.8)	201 (83.4)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Positive	182 (16.2)	40 (16.6)	1.03 (0.73–1.45)	1.24 (0.87–1.77)	1.24 (0.87–1.78)

Notes: [a]Unadjusted. [b]Adjusted for age at diagnosis, menopause status, education, ER status, HER2 status, and clinical stage. [c]Additionally adjusted for total amount of IgG.
Abbreviations: *C. trachomatis, Chlamydia trachomatis*; IgG, immunoglobulin G; HR, Hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression-free survival.

of death and disease progression (adjusted HR=1.09, 95% CI: 0.67–1.78 for OS; adjusted HR=1.24, 95% CI: 0.87–1.78 for PFS) was found.

Furthermore, stratified analyses according to age at diagnosis revealed that compared with *C. trachomatis* IgG negativity, the positivity was related to a significantly poorer PFS among patients aged 50 years or younger (HR=1.79, 95% CI: 1.16–2.77), while it was associated with a better PFS among those aged over 50 years (HR=0.62, 95% CI: 0.31–1.25); the interaction was significant ($P_{interaction}$=0.013, Supplementary Table 2). For OS, a similar pattern was observed and the HRs and 95% CIs were 1.43 (0.79–2.58) and 0.59 (0.23–1.50) in ≤ 50 and >50 age groups, respectively, though the interaction did not reach a significance ($P_{interaction}$=0.169, Supplementary Table 2).

Table 3 Modification Effects of Pro-Inflammatory Cytokines on the Association of *C. trachomatis* with Breast Cancer OS and PFS

Cytokines (pg/mL)	*C. trachomatis* IgG	OS		PFS	
		Events/Total	HR (95% CI)[a]	Events/Total	HR (95% CI)[a]
IL-6					
≤3.18	Negative	60/405	1.00 (reference)	92/405	1.00 (reference)
	Positive	3/72	**0.23 (0.07–0.76)**	10/72	0.52 (0.27–1.03)
>3.18	Negative	23/193	1.00 (reference)	36/193	1.00 (reference)
	Positive	10/44	**6.46 (2.47–16.88)**	13/44	**2.96 (1.45–6.02)**
P interaction			**< 0.001**		**0.001**
TNF-α					
≤76.55	Negative	63/444	1.00 (reference)	93/444	1.00 (reference)
	Positive	8/85	0.80 (0.37–1.74)	15/85	0.93 (0.52–1.63)
>76.55	Negative	26/225	1.00 (reference)	46/225	1.00 (reference)
	Positive	6/40	1.16 (0.43–3.15)	9/40	1.17 (0.53–2.54)
P interaction			0.422		0.460
IL-8					
≤18.68	Negative	63/449	1.00 (reference)	94/449	1.00 (reference)
	Positive	6/78	0.64 (0.27–1.52)	12/78	0.79 (0.43–1.47)
>18.68	Negative	26/218	1.00 (reference)	44/218	1.00 (reference)
	Positive	8/45	1.80 (0.75–4.35)	12/45	1.65 (0.81–3.33)
P interaction			0.074		0.101
IL-1β					
≤1.18	Negative	65/453	1.00 (reference)	99/453	1.00 (reference)
	Positive	7/75	0.91 (0.40–2.04)	12/75	0.94 (0.50–1.74)
>1.18	Negative	23/212	1.00 (reference)	39/212	1.00 (reference)
	Positive	7/50	1.21 (0.49–2.98)	12/50	1.29 (0.65–2.55)
P interaction			0.466		0.347

Notes: Bold characters indicate statistically significant result. [a]adjusted for age at diagnosis, menopause status, education, ER status, HER2 status, clinical stage, and total amount of IgG.
Abbreviations: *C. trachomatis, Chlamydia trachomatis*; IgG, immunoglobulin G; HR, Hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression-free survival.

Modification effects of pro-inflammatory cytokines on the relationship between *C. trachomatis* and breast cancer OS and PFS.

We then assessed the differential associations between *C. trachomatis* and breast cancer prognosis stratified by cytokine levels. The results showed that *C. trachomatis* IgG positivity obviously elevated the risk of death and disease progression among breast cancer patients with a higher level of IL-6 (HR=6.46, 95% CI: 2.47–16.88 for OS; HR=2.96, 95% CI: 1.45–6.02 for PFS), while there were decreased risks among patients with a lower level of IL-6 (HR=0.23, 95% CI: 0.07–0.76 for OS; HR=0.52, 95% CI: 0.27–1.03 for PFS), and the interactions were significant (*P* interaction < 0.001 for OS; *P* interaction=0.001 for PFS), as shown in Table 3. For IL-8, the effects of *C. trachomatis* infection on breast cancer prognosis were similar to those of IL-6, and the interactive effect tended to be significant (*P* interaction=0.074 for OS; *P* interaction=0.101 for PFS). For TNF-α and IL-1β, however, the interactions were not significant.

We further divided the participants into two subgroups by age (≤ 50 and > 50 years) to examine the interactions between *C. trachomatis* and the cytokines on the prognosis (Supplementary Tables 3 and 4). Significant interactions between the four cytokines (IL-6, TNF-α, IL-8, and IL-1β) and *C. trachomatis* on the prognosis were observed in patients with aged 50 years or younger, whereas these interactive effects were absent (TNF-α, IL-8, and IL-1β) or weak (IL-6) in patients with aged over 50 years.

Discussion

In this study, we found that *C. trachomatis* IgG positivity was associated with increased risks of death and progression in breast cancer patients aged ≤ 50 years or with higher levels of the four pro-inflammatory cytokines (IL-6, TNF-α, IL-8, and IL-1β).

C. trachomatis, the most prevalent pathogen infecting the genital tract in women worldwide,[21] is involved in persistent long-term chronic infections of the reproductive tract in humans, which can be clinically insidious.[22] This infection, a common sexually transmitted infection (STI), can ascend from the cervix to the uterus and fallopian tubes, leading to complications such as pelvic inflammatory disease, chronic pelvic pain, infertility, and ectopic pregnancy.[23,24] Animal studies have demonstrated that murine *C. trachomatis* in mice can spread from the reproductive tract to the gastrointestinal tract through systemic routes other than oral or rectal-anal contact, suggesting that *C. trachomatis* might gain access to the bloodstream through the rich submucosal vessels in the endometrial tissue for dissemination.[25,26]

As a Gram-negative obligate intracellular pathogen, *C. trachomatis* replicates in a specialized membrane compartment, utilizes a large arsenal of secreted effectors to survive in the host, and strictly relied on the host for many of their metabolic requirements.[27] After infecting host cells, *C. trachomatis* generates an environment conducive to malignant transformation by interfering with host chromatin, DNA double-strand break repair, and cell-cycle regulation.[28] Furthermore, *C. trachomatis* alters metabolic program of the host by aerobic glycolysis and an accumulation of certain metabolites (such as acetyl-coenzyme A, glutamate and malate), fostering rapid tumor cell proliferation.[29] Additionally, *C. trachomatis* has been implicated in inhibiting apoptosis in infected tumor cells, further supporting its role in tumor proliferation.[30–35] These in vitro findings align with our observation that *C. trachomatis* infection correlates with increased risks of death and progression in breast cancer patients to some extent.

Previous studies have shown that estrogen enhances the attachment and infectivity of chlamydiae,[34,36–38] potentially leading to the accumulation of *C. trachomatis* in estrogen-rich breast tissues. Guseva et al found that estrogen-responsive MCF-7 cells were more susceptible to *C. trachomatis* than estrogen-negative breast epithelial clone HCC-1806 cells;[34] Bose et al reported that treatment of HeLa 229 cultures with a synthetic estrogen analogue prior to infection with *C. trachomatis* enhanced chlamydial inclusion formation by 50% to 60%.[36] Given that younger women had a higher level of estrogen than the elders, *C. trachomatis* was more likely to adhere to and infect breast cancer cells in younger women, resulting in a greater risk of recurrence, metastasis or death in younger breast cancer patients. Therefore, the observed stronger association between *C. trachomatis* infection and progression-free survival in breast cancer patients aged 50 years or younger may be attributed to these findings.

Interestingly, our study revealed that the impact of *C. trachomatis* IgG on the risk of death and progression was more profound in breast cancer patients with higher levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-8, and IL-1β). Higher levels of pro-inflammatory cytokines, indicative of chronic inflammation, are associated with promoting the process of epithelial-mesenchymal transition (EMT),[39] leading to the loss of cell polarity and adhesion capacity in epithelial cells and subsequently promoting cell migration and invasion.[40] Meanwhile, *C. trachomatis* can promote EMT.[41,42] Thereafter, *C. trachomatis* and pro-inflammatory cytokines can jointly facilitate EMT, resulting in an apparent interaction on the prognosis of breast cancer. Nevertheless, the exact mechanism remained to be explored.

Our study has several potential limitations. First, information on the treatment related to the outcome was not collected, which may potentially confound the results. As the treatment was determined based on clinicopathological characteristics, adjusting these characteristics in the analysis was able to control the confounding effects of the treatment to a large extent. Second, only patients with serum samples were included, which may be difficult to avoid the selection bias. However, the distribution of clinicopathological features of the subjects in this study was similar to those in the same cohort we reported previously.[43] Third, participants were recruited from Guangdong Province, China, potentially introducing geographical limitations that could limit the generalizability of the findings. Nevertheless, upon reviewing the existing literature, it is observed that our findings align with cytological experiments, suggesting that the geographic limitation does not undermine the validity of our results. Furthermore, there is no evidence indicating that the association between *Chlamydia trachomatis* infection and breast cancer prognosis varies by ethnicity or region. Nonetheless, to enhance the generalizability of our findings, future studies should aim to validate these results in more diverse

populations, including different ethnic and regional groups. Finally, considering that the concentrations of pro-inflammatory cytokines were also measured with serum samples collected at the time of diagnosis, we were unable to infer the temporal sequence between *C. trachomatis* and inflammation in this study, which meant that *C. trachomatis* may lead to an increase of the cytokines or the cytokines may reactivate chronic infection of *C. trachomatis*. Thus, relevant biological experiments were needed to clarify this issue.

In conclusion, our study revealed the adverse prognostic roles of *C. trachomatis* infection in breast cancer patients with higher levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-8, and IL-1β) or those aged $\leq$ 50 years. Taking measures to control *C. trachomatis* infection may potentially reduce the risks of death and disease progression in younger patients with breast cancer or those with higher levels of pro-inflammatory cytokines. Further studies are needed to explore the underlying mechanisms.

Abbreviations

C. trachomatis, Chlamydia trachomatis; CI, confidence interval; COI, cut-off index; ELISA, enzyme-linked immuno-sorbent assay; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; GZBCS, Guangzhou Breast Cancer Study; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IgG, immunoglobulin G; IL, interleukin; IQR, interquartile range; OD, optical density; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor; STI, sexually transmitted infection; TNF-α, tumor necrosis factor alpha.

Data Sharing Statement

All data produced in the present study are available upon reasonable request to the corresponding author.

Ethics Approval and Informed Consent

All participants provided informed consent prior to study inclusion. This study was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University (number of Institutional Review Board approval:2012-8) and performed in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Heer E, Harper A, Escandor N, Sung H, McCormack V, Fidler-Benaoudia MM. Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *Lancet Glob Health*. 2020;8(8):e1027–e1037. doi:10.1016/S2214-109X(20)30215-1
2. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer*. 2019;11:151–164. doi:10.2147/BCTT.S176070

3. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660

4. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J*. 2021;134(7):783–791. doi:10.1097/CM9.0000000000001474

5. Liang ZZ, Zhang YX, Lin Y, et al. Joint effects of multiple sleep characteristics on breast cancer progression by menopausal status. *Sleep Med*. 2019;54:153–158. doi:10.1016/j.sleep.2018.10.025

6. Trayes KP, Cokenakes SEH. Breast cancer treatment. *Am Fam Physician*. 2021;104(2):171–178.

7. Wang R, Zhu Y, Liu X, Liao X, He J, Niu L. The clinicopathological features and survival outcomes of patients with different metastatic sites in stage IV breast cancer. *BMC Cancer*. 2019;19(1):1091. doi:10.1186/s12885-019-6311-z

8. Samaras V, Rafailidis PI, Mourtzoukou EG, Peppas G, Falagas ME. Chronic bacterial and parasitic infections and cancer: a review. *J Infect Dev Ctries*. 2010;4(05):267–281. doi:10.3855/jidc.819

9. Oh JK, Weiderpass E. Infection and cancer: global distribution and burden of diseases. *Ann Glob Health*. 2014;80(5):384–392. doi:10.1016/j.aogh.2014.09.013

10. Madeleine MM, Anttila T, Schwartz SM, et al. Risk of cervical cancer associated with Chlamydia trachomatis antibodies by histology, HPV type and HPV cofactors. *Int J Cancer*. 2007;120(3):650–655. doi:10.1002/ijc.22325

11. Koskela P, Anttila T, Bjørge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer*. 2000;85(1):35–39. doi:10.1002/(sici)1097-0215(20000101)85:1<35::aid-ijc6>3.0.co;2-a

12. Fortner RT, Terry KL, Bender N, et al. Sexually transmitted infections and risk of epithelial ovarian cancer: results from the nurses' health studies. *Br J Cancer*. 2019;120(8):855–860. doi:10.1038/s41416-019-0422-9

13. Stone KM, Zaidi A, Rosero-Bixby L, et al. Sexual behavior, sexually transmitted diseases, and risk of cervical cancer. *Epidemiology*. 1995;6 (4):409–414. doi:10.1097/00001648-199507000-00014

14. Xiang W, Yu N, Lei A, et al. Insights into host cell cytokines in chlamydia infection. *Front Immunol*. 2021;12:639834. doi:10.3389/fimmu.2021.639834

15. Mpiga P, Mansour S, Morisset R, Beaulieu R, Ravaoarinoro M. Sustained interleukin-6 and interleukin-8 expression following infection with chlamydia trachomatis serovar L2 in a HeLa/THP-1 cell co-culture model. *Scand J Immunol*. 2006;63:199–207. doi:10.1111/j.1365-3083.2006.01734.x

16. Sun Z, Li Y, Chen H, et al. Chlamydia trachomatis glycogen synthase promotes MAPK-mediated proinflammatory cytokine production via TLR2/TLR4 in THP-1 cells. *Life Sci*. 2021;271:119181. doi:10.1016/j.lfs.2021.119181

17. Faris R, Andersen SE, McCullough A, Gourronc F, Klingelhutz AJ, Weber MM. Chlamydia trachomatis serovars drive differential production of proinflammatory cytokines and chemokines depending on the type of cell infected. *Front Cell Infect Microbiol*. 2019;9:399. doi:10.3389/fcimb.2019.00399

18. De Filippis A, Buommino E, Domenico MD, Feola A, Brunetti-Pierri R, Rizzo A. Chlamydia trachomatis induces an upregulation of molecular biomarkers podoplanin, Wilms' tumour gene 1, osteopontin and inflammatory cytokines in human mesothelial cells. *Microbiology*. 2017;163 (5):654–663. doi:10.1099/mic.0.000465

19. Ye H, Tang LY, Liang ZZ, et al. Effects of infection-induced fever and the Interaction with IL6 rs1800796 polymorphism on the prognosis of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2022;31(11):2030–2037. doi:10.1158/1055-9965.EPI-22-0498

20. He JR, Tang LY, Yu DD, et al. Epstein-Barr virus and breast cancer: serological study in a high-incidence area of nasopharyngeal carcinoma. *Cancer Lett*. 2011;309(2):128–136. doi:10.1016/j.canlet.2011.05.012

21. Wahid M, Dar SA, Jawed A, et al. Microbes in gynecologic cancers: causes or consequences and therapeutic potential. *Semin Cancer Biol*. 2021;86 (Pt 2):1179–1189. doi:10.1016/j.semcancer.2021.07.013

22. Yang X, Siddique A, Khan AA, et al. Chlamydia trachomatis infection: their potential implication in the etiology of cervical cancer. *J Cancer*. 2021;12(16):4891–4900. doi:10.7150/jca.58582

23. Liu C, Hufnagel K, O'Connell CM, et al. Reduced endometrial ascension and enhanced reinfection associated with igg antibodies to specific chlamydia trachomatis proteins in women at risk for chlamydia. *J Infect Dis*. 2021. doi:10.1093/infdis/jiab496

24. Lewis J, White PJ. Estimating local chlamydia incidence and prevalence using surveillance data. *Epidemiology*. 2017;28(4):492–502. doi:10.1097/EDE.0000000000000655

25. Zhang Q, Huang Y, Gong S, et al. In vivo and ex vivo imaging reveals a long-lasting chlamydial infection in the mouse gastrointestinal tract following genital tract inoculation. *Infect Immun*. 2015;83(9):3568–3577. doi:10.1128/IAI.00673-15

26. Rank RG, Yeruva L, Andrews-Polymenis HL. Hidden in plain sight: chlamydial gastrointestinal infection and its relevance to persistence in human genital infection. *Infect Immun*. 2014;82(4):1362–1371. doi:10.1128/IAI.01244-13

27. Elwell C, Mirrashidi K, Engel J. Chlamydia cell biology and pathogenesis. *Nat Rev Microbiol*. 2016;14(6):385–400. doi:10.1038/nrmicro.2016.30

28. Chumduri C, Gurumurthy RK, Zadora PK, Mi Y, Meyer TF. Chlamydia infection promotes host DNA damage and proliferation but impairs the DNA damage response. *Cell Host Microbe*. 2013;13(6):746–758. doi:10.1016/j.chom.2013.05.010

29. Rother M, Teixeira da Costa AR, Zietlow R, Meyer TF, Rudel T. Modulation of host cell metabolism by chlamydia trachomatis. *Microbiol Spectr*. 2019;7(3). doi:10.1128/microbiolspec.BAI-0012-2019

30. Huang X, Tan J, Chen X, et al. Akt phosphorylation influences persistent chlamydial infection and chlamydia-induced golgi fragmentation without involving Rab14. *Front Cell Infect Microbiol*. 2021;11:675890. doi:10.3389/fcimb.2021.675890

31. Dzakah EE, Huang L, Xue Y, et al. Host cell response and distinct gene expression profiles at different stages of Chlamydia trachomatis infection reveals stage-specific biomarkers of infection. *BMC Microbiol*. 2021;21(1):3. doi:10.1186/s12866-020-02061-6

32. Wen Y, Chen H, Luo F, et al. Chlamydia trachomatis plasmid protein pORF5 up-regulates ZFAS1 to promote host cell survival via MAPK/p38 pathway. *Front Microbiol*. 2020;11:593295. doi:10.3389/fmicb.2020.593295

33. Sixt BS, Núñez-Otero C, Kepp O, Valdivia RH, Kroemer G. Chlamydia trachomatis fails to protect its growth niche against pro-apoptotic insults. *Cell Death Differ*. 2019;26(8):1485–1500. doi:10.1038/s41418-018-0224-2

34. Guseva NV, Dessus-Babus SC, Whittimore JD, Moore CG, Wyrick PB. Characterization of estrogen-responsive epithelial cell lines and their infectivity by genital Chlamydia trachomatis. *Microbes Infect*. 2005;7(15):1469–1481. doi:10.1016/j.micinf.2005.05.004

35. Thomas M, Lawrence A, Kroon S, et al. Chlamydial clinical isolates show subtle differences in persistence phenotypes and growth in vitro. *Access Microbiol*. 2021;3(3):000204. doi:10.1099/acmi.0.000204

36. Bose SK, Goswami PC. Enhancement of adherence and growth of Chlamydia trachomatis by estrogen treatment of HeLa cells. *Infect Immun*. 1986;53(3):646–650. doi:10.1128/iai.53.3.646-650.1986

37. Rank RG, Sanders MM, Kidd AT. Influence of the estrous cycle on the development of upper genital tract pathology as a result of chlamydial infection in the Guinea pig model of pelvic inflammatory disease. *Am J Pathol*. 1993;142(4):1291–1296.

38. Pasley JN, Rank RG, Hough AJ Jr, Cohen C, Barron AL. Effects of various doses of estradiol on chlamydial genital infection in ovariectomized guinea pigs. *Sex Transm Dis*. 1985;12(1):8–13. doi:10.1097/00007435-198501000-00003

39. Baram T, Rubinstein-Achiasaf L, Ben-Yaakov H, Ben-Baruch A. Inflammation-driven breast tumor cell plasticity: stemness/EMT, therapy resistance and dormancy. *Front Oncol*. 2020;10:614468. doi:10.3389/fonc.2020.614468

40. Igietseme JU, Omosun Y, Nagy T, et al. Molecular pathogenesis of chlamydia disease complications: epithelial-mesenchymal transition and fibrosis. *Infect Immun*. 2018;86(1). doi:10.1128/IAI.00585-17

41. Rajic J, Inic-Kanada A, Stein E, et al. Chlamydia trachomatis infection is associated with e-cadherin promoter methylation, downregulation of E-cadherin expression, and increased expression of fibronectin and alpha-SMA-implications for epithelial-mesenchymal transition. *Front Cell Infect Microbiol*. 2017;7:253. doi:10.3389/fcimb.2017.00253

42. Zadora PK, Chumduri C, Imami K, et al. Integrated phosphoproteome and transcriptome analysis reveals chlamydia-induced epithelial-to-mesenchymal transition in host cells. *Cell Rep*. 2019;26(5):1286–1302e1288. doi:10.1016/j.celrep.2019.01.006

43. Zhang YX, Liang ZZ, Li YQ, et al. Association between weight change and breast cancer prognosis. *Breast Cancer Res Treat*. 2022;193(3):677–684. doi:10.1007/s10549-022-06592-6