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Original Article

Seroprevalence and risk factors for Toxoplasma gondii infection in women with breast tumors in Eastern China

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Abstract: Objective: To investigate the seroprevalence and risk factors of *Toxoplasma gondii* (*T. gondii*) infection in females with breast tumors in eastern China. Methods: This case-control study enrolled 610 breast tumor patients and 610 healthy controls. Serum anti-*T. gondii* IgG and IgM antibodies were measured using ELISA. Multivariate logistic regression analysis was used to analyze relevant risk factors. Results: The overall *T. gondii* seroprevalence was higher in breast tumor patients than that in controls (18.85% vs. 9.67%, P=0.001), with elevated IgG (P=0.001) and IgM (P=0.011) levels. Consumption of undercooked seafood (OR=3.00, *P*=0.001) and rural living environment (OR=1.65, P=0.035) were independent risk factors. There was no significant difference in the *T. gondii* seroprevalence between patients with malignant and benign breast tumors (P=0.430). However, the elevated infection rate was associated with tumor invasiveness characteristics, including a tumor-infiltrating lymphocyte ratio >20%, high Ki67 expression (up to 30%), and HER2 amplification (all P<0.05). The constructed predictive model demonstrated good discriminative power for *T. gondii* infection (AUC=0.804, 95% CI: 0.76-0.85) and good calibration (MAE=0.010). Decision curve analysis confirmed that the model provided significant net clinical benefit within the threshold probability range of 0-0.8. Conclusion: *T. gondii* infection is highly prevalent in female patients with breast tumors and is correlated with specific environmental exposures and clinicopathologic features of tumor invasiveness. This robust predictive model can be used accurately and reliably for individualized risk assessment of *T. gondii* infection in women with breast tumors. It can assist clinicians in developing targeted screening and intervention plans, maximizing clinical benefits while reducing unnecessary diagnostic and treatment procedures.

Keywords: *Toxoplasma gondii*, seroprevalence, breast tumor, Eastern China

Introduction

Breast cancer (BC) is one of the most common malignant tumors in women. According to cancer statistics released in 2022, there were nearly 2.26 million new cases of BC in women and approximately 685,000 deaths [1]. With the continuous advancement of clinical treatment strategies, the prognosis of BC has been significantly improved [2, 3]. However, antitumor therapy can cause serious complications from bacterial, viral, and parasitic infections [4, 5]. *Toxoplasma gondii* (*T. gondii*) is one of the severe opportunistic parasites that can cause cancer under immunosuppression [6, 7]. The occurrence of breast tumors is the result of the combined effects of genetic susceptibility, pathogen infection, and physical and chemical factors [8]. Increasing research evidence suggests that *T. gondii* infection may be involved in the pathogenesis of human malignant tumors [9]. In recent years, studies have reported a possible association between *T. gondii* infection and various malignant tumors [10], including glioma [11], oral cancer [12], and BC [13]. Furthermore, Kalantari et al. and Pourahmad et al. detected *T. gondii* messenger RNA in paraffin-embedded BC specimens, providing direct evidence of infection with this parasite in breast tumor tissue [13, 14]. Related studies have found that the incidence of *T. gondii* infection may be higher in BC patients than in other cancer patients [15-17].

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T. gondii can transform into bradyzoites-containing cysts, which can survive for a long time in the host [15]. Under normal physiologic conditions, the body's cellular immune response, especially T lymphocytes and macrophages, can strictly limit the proliferation of *T. gondii* [18]. However, in cancer patients, immunosuppression leads to a decrease in $CD4^+$ T cell levels, making it easier for *T. gondii* cysts to colonize and proliferate in breast tissue [16, 19]. Studies have confirmed that *Toxoplasma gondii* can participate in key cellular processes such as inflammatory signaling pathways, programmed cell death, and autophagy, all of which are closely related to tumor biology [20]. Therefore, further clarifying the interaction between *T. gondii* infection and breast tumors is expected to provide new insight into the study of malignant progression of breast tumors. Over the past 30 years, epidemiologic studies on *T. gondii* infection in the human population have been extensive. Cats are the only definitive host of *T. gondii* [21]. Humans can be infected with *T. gondii* by ingesting oocysts excreted by felines or cysts in other mammalian tissues [22]. Moreover, contact with cats, consumption of undercooked meat, and fecal exposure are the most common risk factors for toxoplasmosis [18, 22-24]. Studies by Zhou et al. and Yu et al. have found that *T. gondii* infection is relatively common in people with malignant tumors, and that blood transfusions and antitumor chemotherapy promote the spread of *T. gondii* [12, 25]. However, epidemiologic data on *T. gondii* infection in women with breast tumors, especially women with benign fibroadenomas of the breast, in eastern China are still relatively scarce, and the risk factors for infection in this population are still unclear. Based on this, this study aimed to investigate the *T. gondii* infection rate in women with breast tumors and to screen for risk factors leading to transmission and infection.

Materials and methods

From August 2021 to August 2025, this study recruited 610 volunteers diagnosed with primary breast tumors from the Affiliated Hospital of Qingdao University, China. None of the participants had received immunoglobulin therapy before blood collection. An equal number of age- and residence-matched healthy individuals were recruited as a control group. All diagnostic procedures and the use of clinical data from volunteers in this study followed the guidelines of the Declaration of Helsinki and were approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (Approval No.: QYFY WZLL30943).

Sample collection

Venous blood (4 ml) was collected from each participant and allowed to stand at room temperature for 2 hours to allow serum separation. The serum was then separated by centrifugation at 1000 g for 10 minutes and frozen at -80°C for 1 hour for later use.

Sociodemographic and clinical data

A structured questionnaire developed in previous studies by Yu et al. [25] was used to collect sociodemographic characteristics and lifestyle data of the participants. Clinical data, including primary breast disease information, were retrieved from the medical record system.

Serological assay

Serum anti-*T. gondii* IgG and IgM antibodies were detected using ELISA (Kanghua Bio, Inc., China), following a modified experimental protocol by Yu et al. [25]. In brief, serum samples were diluted 1:400 and added to 96-well plates. Incubation was performed at 37°C for 20 minutes to complete antigen-antibody binding. After incubation, the plates were washed three times with distilled water to remove unbound reactants. 50 μL of horseradish peroxidase-labeled detection enzyme solution was added to each well, and after washing five times to remove excess enzyme, 50 μL of colorimetric solution A and 30 μL of colorimetric solution B were added to each well sequentially. Incubation was performed at 37°C in the dark for 10 minutes. Absorbance was measured at 450 nm using an Infinite F200 fully automated ELISA reader (Australia). Baseline correction was performed using blank wells. A positive cutoff value was defined as 2.1 times the average optical density of the negative control group; values exceeding this threshold were considered antibody-positive. To reduce experimental bias, samples from the case and control groups were randomly placed in different ELISA plates.

Table 1. Combined IgG and IgM anti-*T. gondii* antibody seroprevalence in patients with breast tumor and healthy controls

Seroreaction	Patients with a breast tumor (n=610)		Healthy controls (n=610)		Patients with a breast tumor vs. Healthy controls	
	No. positive	%	No. positive	%	χ^2	*P*
IgG	112	18.36	58	9.51	19.930	0.001
IgM	32	5.25	15	2.46	6.385	0.011
IgG^+/IgM^+	24	3.93	14	2.29	2.716	0.099
IgG^+/IgM^-	88	14.42	44	7.21	16.446	0.001
IgG^-/IgM^+	8	1.31	1	1.64	5.485	0.038[a]
Total	115	18.85	59	9.67	21.021	0.001

[a]Fisher's exact test.

Statistical analysis

Data analysis was performed using SPSS 19.0 statistical software. The association between categorical variables and anti-*T. gondii* antibodies was assessed by the chi-square test or Fisher's exact test. Variables with P≤0.25 in univariate analysis were included in the multivariate logistic regression model to screen for independent influencing factors for *T. gondii* infection. This study employed a purposive variable screening method, using P=0.25 as the inclusion criterion for variables entering the multivariate model to avoid exclusion of variables with potential confounding effects and interactions during the univariate screening [26]. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, with P<0.05 considered significant.

Based on pooled case-control data from breast tumor patients and healthy controls, a *T. gondii* infection prediction model was constructed using the predictive factors retained in the final multivariate logistic regression model. The predictive performance of the model for *T. gondii* infection risk was evaluated from three dimensions. Receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to assess the model's discrimination; a calibration curve was plotted using 1000 bootstrap resampling and the mean absolute error (MAE) was calculated to assess the degree of agreement between the model's predicted infection probability and the actual infection probability, i.e., the calibration degree; and decision curve analysis (DCA) was used to calculate the net benefit at different threshold probabilities to evaluate the model's clinical application value. Furthermore, an interaction term was introduced into the regression model to analyze whether tumor type (benign vs. malignant) alters the association strength between risk factors and *T. gondii* infection.

Results

Comparison of T. gondii seroprevalence

The seroprevalence of *T. gondii* antibodies was significantly higher in women of both breast tumor groups than in controls (18.85% [115/610] vs. 9.67% [59/610], *P*=0.001) - and the sensitivity rate of anti-*T. gondii* IgG antibodies in breast tumor was nearly twice as high as that of controls (18% vs. 9.51%, *P*=1). The detection rate of IgM antibodies (indicating recent or active infection) was also higher in patients (5.25% vs. 2.46%, *P*=0.011). See **Table 1**.

Comparison of baseline characteristics and univariate analysis of risk factors

The baseline sociodemographic characteristics and lifestyle habits of the study participants are summarized in **Table 2**. Multivariate analysis showed that the *T. gondii* seroprevalence played a key role in the environmental and lifestyle factors. The infection rate was significantly higher among people living in rural areas, engaged in farming, frequently exposed to soil, and consuming undercooked seafood (all P<0.05). Regarding clinical treatment history, those with a history of blood transfusion had a significantly higher infection risk (27.04% vs. 15.96%, P=0.002). The infection rate in patients with a history of chemotherapy (21.55%, 39/181) was slightly higher than that of patients without a history of chemotherapy (17.71%,

Table 2. Seroprevalence of *T. gondii* infection in women with breast tumor and control subjects in eastern China

Characteristic	Women with breast cancer tumor (n=610)				Healthy controls (n=610)			
	Prevalence of *T. gondii* infection				Prevalence of *T. gondii* infection			
	No. tested	No. positive	χ^2	*P*	No. tested	No. positive	χ^2	*P*
Age (years)								
≤40	80	24 (30.00)	8.327	0.040	38	1 (2.63)	12.163	0.007
41-50	162	30 (18.52)			172	10 (5.81)		
51-60	204	31 (15.19)			192	16 (8.33)		
>61	164	30 (18.29)			208	31 (14.90)		
Residence area								
Rural	182	51 (28.02)	14.256	0.001	158	30 (18.99)	21.178	0.001
Urban	428	64 (14.95)			452	29 (6.42)		
Contact with cats								
Yes	186	41 (22.04)	1.781	0.182	129	30 (23.26)	34.551	0.001
No	424	74 (17.45)			481	29 (6.03)		
Contact with dogs								
Yes	84	19 (22.62)	0.903	0.342	158	15 (9.49)	0.008	0.930
No	526	96 (18.25)			452	44 (9.73)		
Contact with pigs								
Yes	127	25 (19.69)	0.073	0.787	135	10 (7.41)	1.018	0.313
No	483	90 (18.63)			475	49 (10.32)		
Consumption of raw/undercooked meat								
Yes	271	53 (19.56)	0.158	0.691	247	30 (12.15)	2.907	0.088
No	339	62 (18.29)			363	29 (7.99)		
Consumption of raw vegetables								
Yes	333	70 (21.02)	3.186	0.133	138	10 (7.25)	1.201	0.273
No	277	45 (16.26)			472	49 (10.38)		
Exposure to soil								
Yes	94	25 (26.60)	4.355	0.037	92	21 (22.82)	21.456	0.001
No	516	90 (17.44)			518	38 (7.33)		
Source of drinking water								
Tap	203	40 (19.70)	0.144	0.704	130	8 (6.15)	2.341	0.126
River	407	75 (18.43)			480	51 (10.63)		
Occupation								
Farmer	299	68 (22.74)	5.801	0.016	146	8 (5.48)	3.862	0.049
Worker	311	47 (15.11)			464	51 (10.99)		
History of smoking								
Yes	344	67 (19.48)	0.201	0.654	252	26 (10.32)	0.205	0.651
No	266	48 (18.05)			358	33 (9.22)		
History of drinking								
Yes	317	62 (19.56)	0.215	0.643	360	36 (10.00)	0.108	0.742
No	293	53 (18.09)			250	23 (9.20)		
Consumption of undercooked seafood								
Yes	287	79 (27.53)	26.655	0.001	195	26 (13.33)	4.398	0.036
No	323	36 (11.15)			415	33 (7.95)		
History of chemotherapy								
Yes	181	39 (21.55)	2.254	0.269				
No	429	76 (17.71)						
History of blood transfusion								
Yes	159	43 (27.04)	9.433	0.002				
No	451	72 (15.96)						

Table 3. Multivariable analysis of women with breast tumor and healthy controls and the association of characteristics with *T. gondii* infection

Characteristic		Adjusted odds ratio[a]	95% CI[b]	*P*[c]
Consumption of undercooked seafood	Yes *vs.* No	3.00	1.90-4.73	0.001
Occupation	Farmer *vs.* Worker	0.76	0.47-1.23	0.273
History of blood transfusion	Yes *vs.* No	1.44	0.88-2.33	0.152
Residence area	Rural *vs.* Urban	1.65	1.04-2.63	0.035
Exposure to soil	Yes *vs.* No	1.19	0.68-2.09	0.541
Consumption of raw vegetables	Yes *vs.* No	1.49	0.96-2.31	0.073
Contact with cats	Yes *vs.* No	1.01	0.63-1.62	0.968

[a]Adjusted by age; [b]Confidence interval; [c]Multivariate logistic regression analysis.

76/429), but the difference was not significant (P=0.269).

Multivariate analysis of independent risk factors

To further screen for independent risk factors for *T. gondii* infection, variables with a *P*-value ≤0.25 by univariate analysis (residence area, occupation, soil exposure, consumption of undercooked seafood, raw vegetables, history of contact with cats, and history of blood transfusion) were included in a multivariate logistic regression model. The results showed that consuming undercooked seafood (OR=3.00; 95% CI: 1.90-4.73; P=0.001) and living in a rural area (OR=1.65; 95% CI: 1.04-2.63; *P*=0.035) were independent risk factors for *T. gondii* infection in this population (**Table 3**).

Model performance and validation

Based on the independent predictive factors identified through multivariate analysis, a predictive model for *T. gondii* infection was constructed by integrating all case-control datasets from breast tumor patients and healthy controls. The model demonstrated excellent discriminative performance, with an AUC of 0.804 (95% CI: 0.76-0.85), a sensitivity of 0.774, and a specificity of 0.715. Calibration was assessed using a 1000-times bootstrap resampling method. The results showed a high degree of agreement between the model's predicted and observed risks (MAE=0.010), indicating good calibration consistency and no overfitting. Moreover, DCA found that within the threshold probability range of 0-0.8, the clinical benefit of this model was superior to the full intervention or no intervention approach, and it could assist in clinical decision-making (**Figure 1**).

Subgroup analysis and interaction of risk factors by tumor malignancy

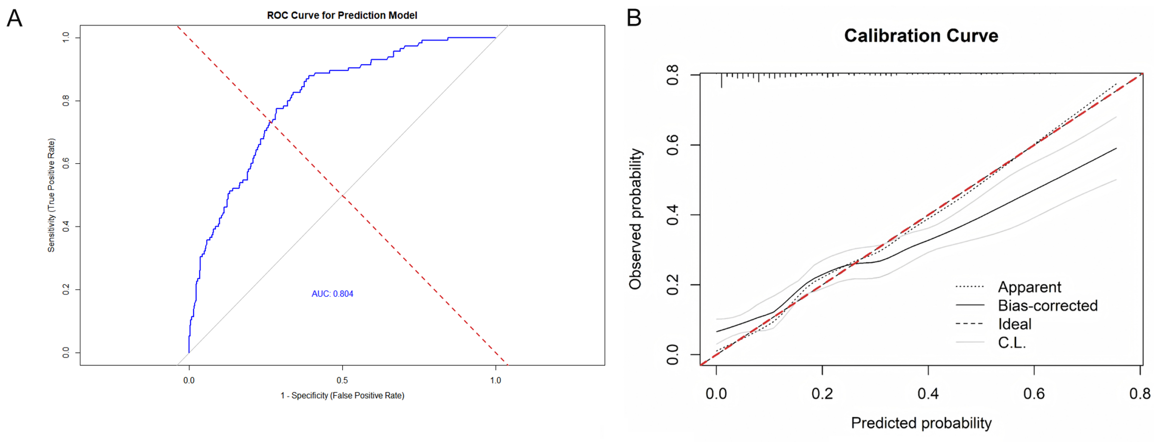
Further interaction analysis was conducted to explore whether the association between identified risk factors and *T. gondii* infection was affected by tumor type (benign vs. malignant). As shown in **Table 4**, consuming raw seafood increased the odds ratio for infection (OR=3.36, 95% CI: 2.04-5.67), but this association did not reach signficance (P=0.06), only showing an increasing risk trend. There was no significant difference in the effect of each risk factor on the risk of *T. gondii* infection in patients with benign and malignant tumors (all P>0.05), suggesting that regardless of tumor benignity or malignancy, known risk factor characteristics are not significantly associated with infection risk.

Distribution of T. gondii infection across histologic subtypes

The distribution of *T. gondii* seroprevalence in different breast tumor subtypes is reported in **Table 5**. There was no significant difference in the infection rate between malignant (20.00%, 66/330) and benign tumors (17.50%, 49/280) (P=0.430), but there were differences in infection status among different histologic subtypes (P=0.001~P=0.015). Intraductal papilloma had the highest seroprevalence (35.71%) (P=0.001), followed by invasive ductal carcinoma (24.49%) (P=0.001), and ductal carcinoma in situ (21.05%) (P=0.112).

Correlation between T. gondii infection and clinicopathologic markers

This study further analyzed the correlation between *T. gondii* infection and routine clinico-



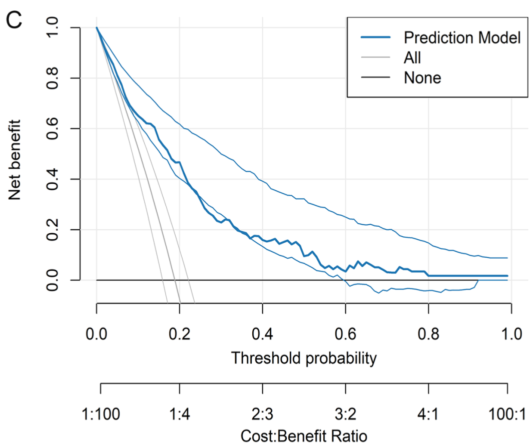


Figure 1. Performance evaluation of the predictive model for *T. gondii* infection risk: (A) Receiver operating characteristic (ROC) curve, (B) Calibration Curve, and (C) Decision curve analysis (DCA).

Table 4. Interaction analysis of risk factors for *T. gondii* infection stratified by breast tumor type

Risk Factor	Overall OR (95% CI)	P^a
Dietary Habits		
Raw seafood (Yes vs. No)	3.36 (2.04-5.67)	0.060
Raw vegetables (Yes vs. No)	1.50 (0.92-2.47)	0.782
Environmental & Lifestyle		
Residence (Urban vs. Rural)	0.58 (0.34-0.97)	0.601
Soil contact (Yes vs. No)	1.36 (0.73-2.49)	0.261
Cat ownership/contact (Yes vs. No)	1.07 (0.63-1.79)	0.421
Medical History		
Blood transfusion (Yes vs. No)	1.66 (0.95-2.90)	0.703

[a]*P* values represent the interaction between each risk factor and tumor type (benign vs. malignant).

pathologic markers in 330 BC patients (**Table 6**). The study found an association between *T. gondii* infection and tumor invasiveness. Patients with a higher percentage of tumor-infiltrating lymphocytes (TILs, >20%), high Ki67 expression (≥30%), and human epidermal growth factor receptor 2 (HER2) amplification had a higher *T. gondii* seroprevalence rate. The infection status was not significantly correlated with tumor stage, histological grade, p53 gene mutation status (wild-type/mutant), estrogen receptor, progesterone receptor and androgen receptor expression level.

Discussion

T. gondii is a pathogen that primarily infects humans and can be transmitted through contaminated water or undercooked meat, especially prevalent in endemic areas [27-29]. Infection with *T. gondii* in immunocompetent individuals is usually self-limiting, generally causing only mild to moderate symptoms such as fever, jaundice, and abdominal pain [30]. However, in immunocompromised patients such as those with *HIV*, cancer, or organ transplant recipients, *T. gondii*

Table 5. Distribution of the seroprevalence of *T. gondii* antibodies according to the histologic type of breast tumor

	No. tested	No. positive (%)	χ^2	P
Fibroadenoma	252	39 (15.48)	5.962	0.015
Intraductal papilloma	28	10 (35.71)	18.824	0.001[a]
Ductal carcinoma in situ	19	4 (21.05)	2.648	0.112[a]
Invasive ductal carcinoma	196	48 (24.49)	28.289	0.001
Medullary carcinoma	33	6 (18.18)	2.495	0.132[a]
Metaplastic carcinoma	39	4 (10.26)	0.014	0.784[a]
Invasive lobular carcinoma	24	3 (12.50)	0.209	0.721[a]
Mucinous breast carcinoma	19	1 (5.26)	0.415	0.519[a]

[a]Fisher's exact was test.

Table 6. Correction between clinical pathology markers and incidence of *T. gondii* in women with breast cancer (n=330)

Characteristic	Prevalence of *T. gondii* infection			
	No. tested	No. positive	χ^2	*P*
Tumor stage				
I	56	8 (14.29)	1.376	0.241
II+III	274	58 (21.17)		
Tumor NOS grade				
I+II	211	44 (20.85)	0.266	0.606
III	119	22 (18.49)		
Tumor Infiltrating Lymphocytes				
<20%	196	21 (10.71)	26.012	0.001
≥20%	134	45 (33.58)		
Ki67 expression				
<30%	145	21 (14.48)	4.921	0.027
≥30%	185	45 (24.32)		
P53 mutation				
Mutation type	77	19 (24.68)	1.372	0.241
Wide type	253	47 (18.58)		
ER expression				
<2+	163	35 (21.47)	0.436	0.509
≥2+	167	31 (18.56)		
PR expression				
<2+	171	34 (19.88)	0.003	0.956
≥2+	159	32 (20.13)		
AR expression				
<2+	185	34 (18.38)	0.692	0.405
≥2+	145	32 (22.07)		
HER2				
Wildtype	252	40 (15.87)	11.349	0.001
Amplification	78	26 (33.33)		

can cause serious and potentially life-threatening complications, including acute organ failure and chronic infection [31, 32]. Studies have shown that the infection rate of *T. gondii* in organ transplant recipients is significantly higher than in immunocompetent individuals [31]. Several studies have proposed an association between *T. gondii* infection and various cancers [14, 33, 34], and there are also reports of a possible correlation with BC [35, 36]. However, data on *T. gondii* infection in women with benign or malignant breast tumors in eastern China remained unclear.

The results of this study showed that the seroprevalence of *T. gondii* antibodies in female patients with breast tumor was significantly higher than that of a healthy control group. BC patients usually need to receive immunosuppressive drug therapy or antitumor chemotherapy, and their bodies are prone to a state of immunodeficiency, making them more susceptible to opportunistic pathogens such as *T. gondii*. A study by Haghbin et al. indicated that nearly 46% of female patients with breast tumor tested positive for *T. gondii*: serologically, a higher infection rate than in this study. This difference may stem from variation in race, age distribution, dietary habits, and environmental exposure among the study population, all of which may affect the transmission of *T.* gondii [36]. Furthermore, the seroprevalence of anti-*T. gondii* IgM antibodies was also higher in women with breast tumor than controls. Anti-*T. gondii* IgM antibodies are often used as a diagnostic criterion for acute *T. gondii* infection. Therefore, clinicians need to pay attention to the problem of acute *T. gondii* infection in women with breast tumor. This study found that the *T. gond*ii seroprevalence was highest in people under 40 years of age; while the infection rate of *T. gondii* in healthy individuals increased with age. This trend is mainly due to the increasing probability of exposure to *T. gondii* as people age, coupled with factors such as occupation. However, among cancer patients, the incidence of *T. gondii* infection is higher in younger people, possibly because young cancer patients often have weakened immune systems, making them more susceptible to *T. gondii* [12].

From a behavioral perspective, consuming undercooked seafood and living in rural areas were independent risk factors for *T. gondii* infection in women with breast tumor. The common transmission routes of *T. gondii* are contaminated water sources, animal contact, and consumption of undercooked meat [12]. Research confirms that seafood such as oysters, clams, and mussels can carry *T. gondii*, becoming important transmission vectors [25, 37]. Coastal and estuarine areas are more economically developed, and the proportion of residents keeping pets is higher [12], and pets are an important source of *T. gondii* infection. Animal feces can flow into the ocean through sewage systems, causing water sources to be contaminated by the excrement of infected animals [25]. Seafood carries *T. gondii* through contact with contaminated water. Consuming undercooked or raw seafood, especially shellfish such as oysters, can lead to parasitic invasion of the human body [25]. This study confirms that people who like to eat undercooked seafood are more susceptible to *T. gondii* infection, but the public generally lacks awareness of this. Therefore, it is urgent to strengthen public health education and popularize preventive measures for *T. gondii* infection, especially for people who like to eat half-cooked seafood. At the same time, patients with BC need to be closely monitored for early signs of *T. gondii* infection and take effective preventive intervention measures.

In addition to clarifying the seropositivity rate and risk factors for *T. gondii* infection in breast tumors, this study also constructed and verified a stable quantitative prediction model. The AUC of the model was 0.804, which had good accuracy in identifying high-risk groups for *T. gondii* infection; the MAE was <0.010, which confirmed that the model's prediction probability had very high reliability. Furthermore, DCA demonstrated that the model provided significant net clinical benefit within a wide threshold probability range of 0-0.8.

Patients with breast tumor often have impaired immune function due to the disease itself or anti-tumor treatment, making them vulnerable to opportunistic infections such as *T. gondii*. Clinicians can use this predictive model for individualized infection risk assessment, enabling targeted serologic screening and intervention for high-risk groups. This can reduce the testing frequency and psychological burden on low-risk individuals and optimize the clinical management of opportunistic infections in cancer patients.

This study also revealed a noteworthy finding. Cancer patients with high expression of TILs and Ki67 were more frequently infected with *T. gondii*. TILs are a subset of lymphocytes that primarily participate in immune regulation, suppressing excessive immune responses and mediating immune tolerance [38, 39]. Recent studies have suggested that TILs reduce the body's immune response to pathogen infection [40], leading to a decreased ability of the body to clear pathogens such as *T. gondii*. However, it remains unclear whether *T. gondii* infection upregulates the expression of *TILs*, or whether high expression of these lymphocytes increases the risk of *T. gondii* infection. High expression of TILs induces a state of immune tolerance, weakening the body's ability to resist pathogen invasion [41]. In immunocompromised individuals or patients with tumor, this state of immune tolerance makes *T. gondii* more likely to proliferate and remain latent for a long time [42]. On the other hand, *T. gondii* infection might induce an immunosuppressive microenvironment, and high expression of TILs further exacerbates this state [35, 43]. Therefore, high expression of TILs may explain why these patients are more susceptible to *T. gondii* infection, and there is a potential interaction between the two. This study clearly confirms that high expression of TILs in female patients with BC is associated with *T. gondii* infection, but the specific mechanism of their interaction remains unclear, and the causal relationship between *T. gondii* infection and high expression of TILs needs further investigation. Ki67 is a protein associated with cell proliferation, and its expression level is often used as a marker for assessing cell cycle activity and can also be used to determine the proliferative activity of lymphoma. High Ki67 expression indicates accelerated cell turnover and proliferation rate [44], which can create a more suitable microenvironment for the invasion and proliferation of *T. gondii*. At the same time, the number of mitotic cells in tumor tissues with high Ki67 expression increases, providing more infection targets for *T. gondii*. Chronic *T. gondii* infection can upregulate Ki67 expression on naïve CD4 positive T cells, hindering the body from producing specific T cell immune responses against pathogens and neoantigens [45]. Therefore, under the state of *T. gondii* infection, pathogen proteins can induce host cells to form a microenvironment conducive to the survival and spread of *T. gondii*. Chemotherapy also increases the risk of *T. gondii* infection [46]. Tumor patients with high Ki67 expression and amplification of *HER2* often need to receive immunosuppressive therapy or antitumor chemotherapy, which will further damage the body's immune barrier, making it easier for *T. gondii* to invade cells and escape host immune surveillance [15, 47]. This study had several limitations. First, the sample size was relatively limited, making it difficult to represent comprehensively the overall status o*f T. gondi*i infection among Chinese women with breast tumor Second, the lack of *T. gondii* nucleic acid testing means that false-positive interference from ELISA cannot be ruled out, and anti- *T. gondii* IgM antibodies can persist for months or even longer, not necessarily indicating recent acute infection, and the impact of false positives due to testing methods cannot be assessed at this time. Third, the incomplete recording of the subjects' past treatment history makes it impossible to clarify the impact of immunosuppressive therapy and anti- *T. gondii* drugs on the *T. gondii* antibody status in women with breast tumor.

Conclusion

This study confirmed the serious *T. gondii* infection situation in women with breast tumor. Consuming undercooked seafood and living in rural areas are independent risk factors for *T. gondii* infection. Clinicians need to be highly vigilant about the potential risk of *T. gondii* infection in women with breast tumor, and the epidemiologic characteristics of *T. gondii* in this population require further in-depth research.

Disclosure of conflict of interest

None.

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