ORIGINAL ARTICLE

Arch AHS

Archives of Allied Health Sciences 2025; 37(1): 1-12.

# Determination of serum IgG level for *Strongyloides stercoralis* in cholangiocarcinoma

**Autchasai Siriprayong[1,2,†], Supakan Amontailak[1,2,†], Phattharaphon Wongphutorn[2,3], Paiboon Sithithaworn[2,3], Kanjanaporn Taenseesaeng[1], Warakorn Pengpala[1], Gungsadan Wilaipan[1], Porntip Pinlaor[1,2], Anchalee Techasen [1,2*]**

[1] Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[2] Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.
[3] Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[†] These authors contributed equally

**KEYWORDS**
*Stronglyloides stercoralis*; Cholangiocarcinoma; IgG detection.

**ABSTRACT**

*Strongyloides stercoralis* is a helminth parasite that is predominantly endemic to the northeastern region of Thailand. Infection in people typically does not result in any noticeable symptoms. However, immunocompromised or immunodeficient patients, such as those with cancer, AIDS, or undergoing chemotherapy, are at risk of developing severe and potentially life-threatening forms of this parasite infection. Indirect enzyme-linked immunosorbent assay (ELISA) employing IgG antibodies in serum are highly sensitive for diagnosing *S. stercoralis* infection. The objective of this study was to assess the serum IgG levels of *S. stercoralis* in cholangiocarcinoma (CCA) patients and to analyze the correlation between IgG levels and clinical pathology, chemotherapy treatment status, and laboratory findings. The study found that among the 107 individuals with CCA, 34 (31.78%) tested positive for *S. stercoralis*, while 73 (68.22%) tested negative based on their serum IgG levels. Nevertheless, no statistically significant correlation was found between any of the analyzed factors, including the status of receiving chemotherapy treatment. This study indicated no significant impact of IgG antibody levels of *S. stercoralis* on the survival time of CCA patients. Additionally, no link was observed between IgG antibody levels and the severity of CCA in patients. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Nevertheless, this study focuses on measuring the concentration of antibodies rather than antigens. Further investigation of the antigen levels is necessary to validate the entirety of the data.

***Corresponding author:** Anchalee Techasen, PhD. Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Email address: anchte@kku.ac.th
Received: 17 January 2024/ Revised: 21 February 2024/ Accepted: 17 April 2024

## Introduction

*Strongyloid stercoralis* belongs to the roundworm type, Nematoda phylum. Its life cycle is complete with two cycle sets including free-living cycle and parasitic cycle which enable auto-infection without a discharge[1]. Strongyloidiasis has been a major burden to public health caused by *S. stercoralis* infection in tropical and sub-tropical areas including Africa, Latin-America, Australia and Asia[2]. The highest incidence of infection belonged to the Southeast Asia where Thailand exhibited 15.9 – 28.9% of prevalence[3,4]. Khon Kaen, a province in the Northeastern part of Thailand, demonstrated a prevalence rate of 12.9% utilising the formalin-ethyl acetate concentration technique (FECT)[5,6]. Most patients were probably asymptomatic or had mild gastrointestinal symptom. Immunocompromised patients, such as those with cancer, AIDS, alcoholism, malnutrition, kidney disease, and undergoing chemotherapy, are at a higher risk of developing severe conditions. Autoinfection can lead to the dissemination of the parasite to many organs, such as the lung, trachea, heart, liver, kidney, spinal cord, and brain. This can result in inflammation, organ dysfunction, bloodstream infection, and ultimately death.

Serodiagnosis of *S. stercoralis* infection provided high sensitivity, such as indirect enzyme-linked immunosorbent assay (ELISA) using sera and extracted antigen from *S. ratti* larvae as alternative to *S. stercoralis* larvae. *S. stercoralis* larvae were difficult in antigen extraction, carrying high risk of accidental infection and ineffective cost[7]. Previous report revealed similarity between *S. ratti* and *S. stercoralis* antigen as well as specificity and sensitivity of diagnosis[8]. It was also discovered that rodent could be a source of *S. ratti* antigen production for immunological diagnosis associated strongyloidiasis in human[9]. IgG was reported to be the most common antibody in response to the antigenic surfaces of infective stage of *S. stercoralis* (Filariform larvae; L3). IgG associated with hyper-infected protection in immunocompromised host with chronic and mild symptomatic strongyloidiasis which was considered as chronic infection indicator[10].

The failure to diagnose and treat *S. stercoralis* infection may lead to the development of chronic inflammatory bowel illness, which in turn increases the risk of gastrointestinal cancer. A case of colorectal cancer with *S. stercoralis* infection was documented in a patient from Peru. Chemotherapy administration was discussed for risks and benefits after *S. stercoralis* was detected with cancer signs[11]. Individuals with immunodeficiency or those undergoing chemo-therapy are at risk of developing a life-threatening hyper-infection, which may be facilitated by chemotherapy-induced development of parasite larvae. Immunocompromised individuals, such as those undergoing chemotherapy, are at risk of developing a life-threatening hyperinfection syndrome caused by an increasing of matured parasite and consequent organ invasion[12]. Therefore, discreet decision of chemotherapy administration is highly necessary.

Cholangiocarcinoma (CCA) originates from abnormal growth of biliary mucosa cells[13]. Northeastern of Thailand was endemic area and Khon Kaen province accounted 84.6 and 36.8 per 100,000 people in respective male and female[14]. The incidence tended to decrease to 14.6 per 100,000 people by 2017[15]. CCA still be a major concern because most patients were asymptomatic in the first stage, then became noticeably symptomatic, such as weight loss, jaundice, body itch, dark urine, pale-colored stool and infected biliary system[16]. *Opisthorchis viverrini* was the causative agent of CCA in Thailand. By consuming raw fish carrying infective larvae, up to 7-time risk of CCA was reported compared to uninfected people[17]. Primary sclerosing cholangitis, chronic hepatitis virus infection, and cirrhosis were also found relating to CCA[13].

The objective of this study was to assess the serum IgG levels of *S. stercoralis* in CCA patients and to analyze the correlation between IgG levels

and clinical pathology, chemotherapy treatment status, and laboratory findings.

## Materials and methods

### *Patient samples*

Serum was obtained from patients diagnosed with CCA (n=107) and normal group (n=54). The CCA sera were collected from patients who underwent surgery at Srinagarind Hospital, Khon Kaen University, Thailand. Samples was kept at Cholangiocarcinoma Research Institute (CARI), Khon Kaen University, Thailand. Normal sera were collected from healthy volunteer from Ban Wha subdistrict, Khon Kaen, Thailand. The inclusion criteria consist of those who have had normal results from ultrasonography and have normal blood and liver function tests. All human specimens and the protocols in this study were approved by the Human Ethics Committee of Khon Kaen University, based on the ethics of human specimen experimentation of the National Research Council of Thailand (HE611196), and informed consent was obtained from each subject.

### *S. ratti antigen extraction*

*S. ratti* (Filariform larva stage) was infected through epithelial injection mice. After one week, feces were collected from mice and cultured by using filter paper culture method. *S. ratti* (Filariform larva stage) were concentrated and washed by normal saline. The filariform larva was kept at -20 ºC before antigen extraction. Crude soluble antigen extract was added into phosphate buffer with anti-protease and kept in -70 ºC for 30 minutes. Then the solution was defrosted four times and the parasite was separated by using sonication and kept at -4 ºC overnight. Next, the solution was centrifuged at 15,000 g at -4 ºC for 30 minutes. The supernatant was estimated by using Bradford protein and kept at -20 ºC. This study was conducted under the approval of animal ethic by the Institutional Animal Ethical Committee, Khon Kaen University (IACUC-KKU-99/62). The procedure was performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals of the National Research Council of Thailand.

### *Determination of anti- S. stercoralis IgG level*

Indirect-ELISA was used for anti-*S. stercoralis* IgG level determination. The crude antigen of *S. ratti* was diluted to 2.5 µg/µL in coating buffer (pH 9.6) and added to 96 well MaxiSorp flat bottom plate for a duplicate in each sample 100 µl/well then incubated overnight. The plate was washed using a washing buffer and discarded. Blocking non-specific binding with 3% skim milk in 1X PBS+0.5% tween20 for two hours at room temperature. Sample was diluted in 3% skim milk with 1X PBS+0.5% tween20 and incubated for one hour at 37°C. Then the plate was washed by using a washing buffer followed by adding horseradish peroxidase-conjugated secondary antibodies and incubated for one hour at 37 ºC. The plate was washed again and added with the orthophenylene diamine hydrochloride (OPD) substrate and incubated in dark and stopped the reaction by 4M $H_2SO_4$ 50 µl/well. The activity was observed by using an ELISA reader at the optical density (OD) of 492 nm. The OD value was calculated by using standard curve.

### *Standard curve for evaluating IgG unit*

The standard IgG unit curve was performed from pooled positive serum as the following dilutions: 1:1000, 1:3000, 1:9000, 1:27000, 1:81000, and 1:243000. The negative serum was diluted into 1:4000 and blank were also included. The sample absorbance was measured utilizing a microplate reader (EZ Read 2000 Microplate Reader) at 492 nm against a standard graph for pooled positive serum. The result of IgG unit expressed the level of *S. stercoralis* infection.

### *Statistical analysis*

The IgG unit level was calculated from OD result and compared to the standard curve. The cutoff values for IgG unit in serum by ELISA were determined by receiver-operating characteristic (ROC) analysis based on analysis of 40 proven-

positive and 40 proven-negative sera samples for *S. stercoralis*. Indicative performance of parameter could be distinguished by area under the curve (AUC). The cutoff was calculated for highest sensitivity and specificity which was described in our previous study[8]. The criteria for classification of *S. stercoralis* positive and negative by serum ELISA were based on the determined cutoff value. It was considered as *S. stercoralis* positive if the IgG unit ≥ 132 and negative if < 132. The correlation between of *S. stercoralis* infection and clinical pathologic parameters was investigated. The clinical pathologic parameters were analyzed using the Chi-square test, and Mann-Whitney U test. Kaplan-Meier survival analysis was used to determine the overall survival. A statistical significance was considered if *p*-value < 0.05.

## Results

**The level of *S. stercoralis* antibody in CCA sera**

Complete data of CCA serum samples (n=107) were collected from patients diagnosed with CCA. The mean age of the patients was 60.37 years. Out of the total 107 patients, 66 (61.68%) were male and 41 (38.32%) were female. Among the 76 patients diagnosed with CCA who had available chemotherapy data, 45 of them (59.21%) had chemotherapy, as shown in table 1. For data analysis, the reference value of laboratory results from Srinagarind Hospital, Khon Kaen University, Thailand was used.

CCA serum samples were tested for the presence of anti-IgG antibody in response to *S. stercoralis* infection. A cut-off of 132 units was used to determine the detection of the IgG based on our previous study [8]. The findings demonstrated that out of 107 samples, 34 (31.78%) tested positive, while 73 (68.22%) tested negative. The statistical analysis revealed that there was no significant correlation between IgG levels and the clinical, pathological, and laboratory data, as shown in table 1.

**Table 1** Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy

| Variables | *S. stercoralis* | | | *p*-value |
|---|---|---|---|---|
| | N | Positive (34) | Negative (73) | |
| Age (year) | | | | |
| <60 | 54 | 17 (50.0) | 37 (50.7) | 1.000 |
| ≥60 | 53 | 17 (50.0) | 36 (49.3) | |
| Gender | | | | |
| Female | 41 | 9 (26.5) | 32 (43.8) | 0.081 |
| Male | 66 | 25 (73.5) | 41 (56.2) | |
| Size (cm) | | | | |
| <5 | 65 | 25 (73.5) | 40 (54.6) | 0.089 |
| ≥5 | 42 | 9 (26.5) | 33 (45.2) | |
| Location of tumor | | | | |
| Extrahepatic CCA | 27 | 9 (26.5) | 18 (24.7) | 0.841 |
| Intrahepatic CCA | 80 | 25 (73.5) | 55 (75.3) | |

**Table 1** Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

| Variables | *S. stercoralis* | | | *p*-value |
|---|---|---|---|---|
| | N | Positive (34) | Negative (73) | |
| Histological type | | | | |
| Non-papillary | 45 | 15 (44.1) | 30 (41.1) | 0.768 |
| Papillary | 62 | 19 (55.9) | 43 (58.9) | |
| T stage | | | | |
| 0 | 3 | 1 (3.1) | 2 (2.9) | |
| 1 | 23 | 5 (15.6) | 18 (25.7) | |
| 2 | 29 | 14 (43.8) | 25 (35.7) | 0.676 |
| 3 | 28 | 8 (25.0) | 20 (28.6) | |
| 4 | 9 | 4 (12.5) | 5 (7.1) | |
| Lymph node metastasis | | | | |
| No | 45 | 13 (41.9) | 32 (48.5) | 0.546 |
| Yes | 52 | 18 (58.1) | 34 (51.5) | |
| Distance metastasis | | | | |
| No | 103 | 32 (94.1) | 71 (97.3) | 0.425 |
| Yes | 4 | 2 (5.9) | 2 (2.7) | |
| Stage | | | | |
| 0 | 3 | 1 (2.9) | 2 (2.7) | |
| 1 | 20 | 4 (11.8) | 16 (21.9) | |
| 2 | 16 | 6 (17.6) | 10 (13.7) | 0.600 |
| 3 | 60 | 19 (55.9) | 41 (56.2) | |
| 4 | 8 | 4 (11.8) | 4 (5.5) | |
| OV antibody | | | | |
| Negative | 31 | 8 (23.5) | 23 (31.5) | 0.397 |
| Positive | 76 | 26 (76.5) | 50 (68.5) | |
| Chemotherapy | | | | |
| No | 31 | 9 (34.6) | 22 (44.0) | 0.470 |
| Yes | 45 | 17 (65.4) | 28 (56.0) | |
| **Liver function test** | | | | |
| Total protein (g/dL) | | | | |
| Normal (6.6-8.7) | 70 | 20 (60.0) | 50 (70.4) | |
| Low (< 6.6) | 25 | 12 (36.4) | 13 (18.3) | 0.076 |
| High (> 8.7) | 9 | 1 (3.0) | 8 (11.3) | |
| Albumin (g/dL) | | | | |
| Normal (3.5-5.2) | 71 | 19 (59.4) | 52 (73.2) | |
| Low (< 3.5) | 29 | 13 (40.6) | 16 (22.5) | 0.104 |
| High (> 5.2) | 3 | 0 (0.0) | 3 (4.2) | |

**Table 1** Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

| Variables | *S. stercoralis* | | | *p*-value |
|---|---|---|---|---|
| | N | Positive (34) | Negative (73) | |
| Globulin (g/dL) | | | | |
| Normal (2.6-3.4) | 33 | 9 (28.1) | 24 (34.3) | |
| Low (< 2.6) | 15 | 8 (25.0) | 7 (10.0) | 0.162 |
| High (> 3.4) | 54 | 15 (46.9) | 39 (55.7) | |
| Direct bilirubin (mg/dL) | | | | |
| Normal (0.3-1.2) | 67 | 21 (63.6) | 46 (65.7) | 0.829 |
| High (> 1.2) | 36 | 12 (36.4) | 24 (34.3) | |
| Alkaline phosphatase (U/L) | | | | |
| Normal (40-129) | 37 | 12 (36.4) | 25 (35.2) | |
| Low (< 40) | 8 | 3 (9.1) | 5 (7.0) | 0.948 |
| High (> 129) | 59 | 18 (54.5) | 41 (57.7) | |
| **Complete Blood Count** | | | | |
| Hemoglobin (g/dL) | | | | |
| Normal (13.0-16.7) | 46 | 15 (44.1) | 31 (42.5) | |
| Low (< 13.0) | 57 | 18 (52.9) | 39 (53.4) | 0.951 |
| High (> 16.7) | 4 | 1 (2.9) | 3 (4.1) | |
| Hematocrit (%) | | | | |
| Normal (40.5-50.8) | 50 | 15 (44.1) | 35 (47.9) | |
| Low (< 40.5) | 5 | 18 (55.9) | 35 (47.9) | 0.415 |
| High (> 50.8) | 3 | 0 (0.0) | 3 (4.1) | |
| WBC count ($10^3$/uL) | | | | |
| Normal (4.6-10.60) | 66 | 20 (60.6) | 46 (63.0) | |
| Low (< 4.6) | 4 | 1 (3.0) | 3 (4.1) | 0.928 |
| High (> 10.60) | 36 | 12 (36.4) | 24 (32.9) | |
| Lymphocyte (%) | | | | |
| Normal (20.1 - 44.5) | 55 | 19 (55.9) | 36 (49.3) | |
| Low (< 20.1) | 46 | 15 (44.1) | 31 (42.5) | 0.282 |
| High (> 44.5) | 6 | 0 (0.0) | 6 (8.2) | |
| Monocyte (%) | | | | |
| Normal (3.4 - 9.8) | 87 | 31 (91.2) | 56 (76.7) | |
| Low (< 3.4) | 8 | 2 (5.9) | 6 (8.2) | 0.178 |
| High (> 9.8) | 12 | 1 (2.9) | 11 (15.1) | |
| Eosinophil (%) | | | | |
| Normal (0.7 - 9.2) | 66 | 16 (47.1) | 50 (68.5) | |
| Low (< 0.7) | 23 | 10 (29.4) | 13 (17.8) | 0.102 |
| High (> 9.2) | 18 | 8 (23.5) | 10 (13.7) | |

**Table 1** Correlation between *S.stercoralis* infection, clinicopathological data, laboratory results and chemotherapy (Cont.)

| Variables | *S. stercoralis* | | | *p*-value |
|---|---|---|---|---|
| | N | Positive (34) | Negative (73) | |
| Basophil (%) | | | | |
| Normal (0.0-2.6) | 103 | 34 (100.0) | 69 (94.5) | 0.164 |
| High (> 2.6) | 4 | 0 (0.0) | 4 (5.5) | |

**Note:** The reference range used in categorizing laboratory results derived from Srinagarind Hospital laboratory.

**Abbreviation:** WBC, White Blood Cell; OV antibody, antibody to *Opisthorchis viverrini*.

**Survival analysis and clinicopathological data of *S. stercoralis* infection in CCA patients**

The survival analysis of *S. stercoralis* infection in CCA patients showed that there was no correlation between *S. stercoralis* infection and survival time (*p*-value = 0.792). The mean survival rates for patients with positive and negative *S. stercoralis* infection were 879.2 and 906.3 days, respectively, as shown in figure 1.

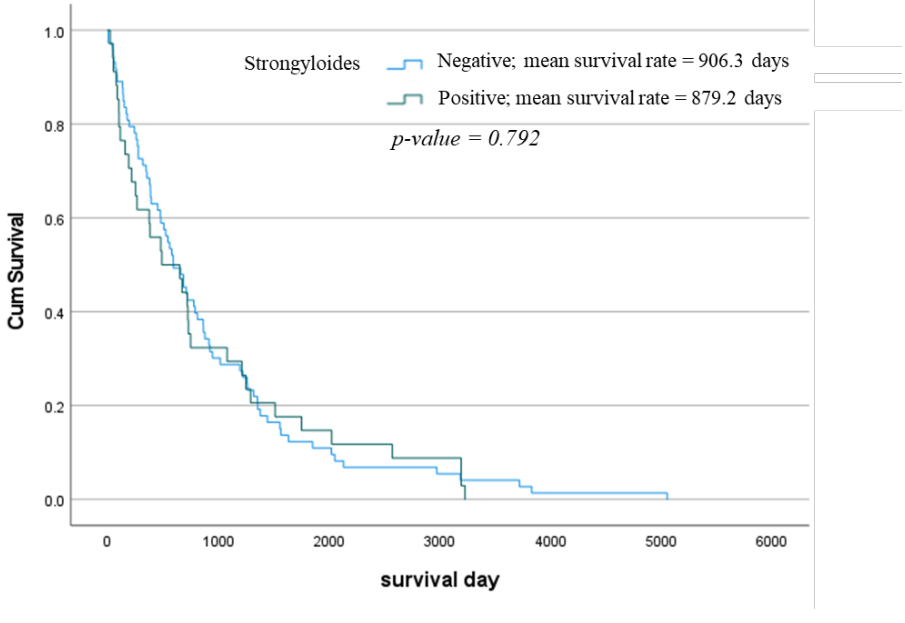


**Figure 1** Comparison of *S. stercoralis* infection in CCA patients with and without a history of *S. stercoralis* infection (*p*-value = 0.792).

The relationship between CCA patients who underwent chemotherapy and their history of *S. stercoralis* infection was examined. The analysis revealed no significant link between *S. stercoralis* infection and the response to treatment, as indicated in table 1. The investigation of *S. stercoralis* infection in CCA patients revealed that there was no significant difference in survival rates between patients with and without *S. stercoralis* infection who received chemotherapy (*p*-value = 0.109). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 731.8 and 1152.9 days, respectively, as shown in figure 2. Moreover, the study found no

significant difference in the 5-year survival rate of patients with CCA who received chemotherapy, regardless of whether they had a *S. stercoralis* infection or not (*p*-value = 0.173). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 578.1 and 829.3 days, respectively, as shown in figure 3A. The 3-year survival rate of CCA patients who undergo chemotherapy showed no significant difference between patients with *S. stercoralis* infection and those without *S. stercoralis* infection (*p*-value = 0.399). The mean survival rates for patients with and without a history of *S. stercoralis* infection were 403.0 and 602.6 days, respectively, as shown in figure 3B.

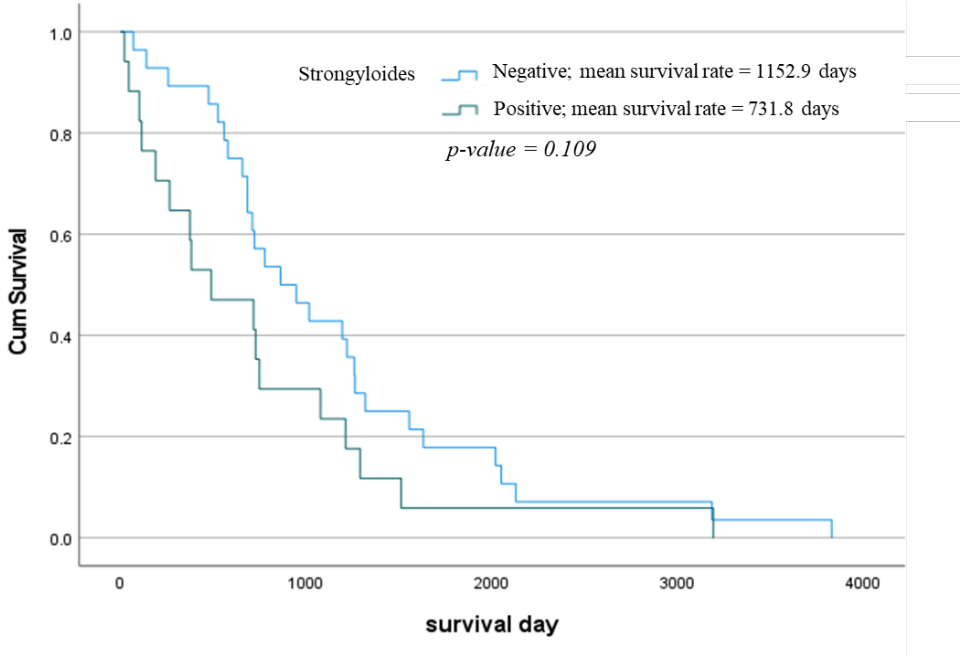


**Figure 2** Comparison of *S. stercoralis* infection in CCA patients who receive chemotherapy (*p*-value = 0.109).

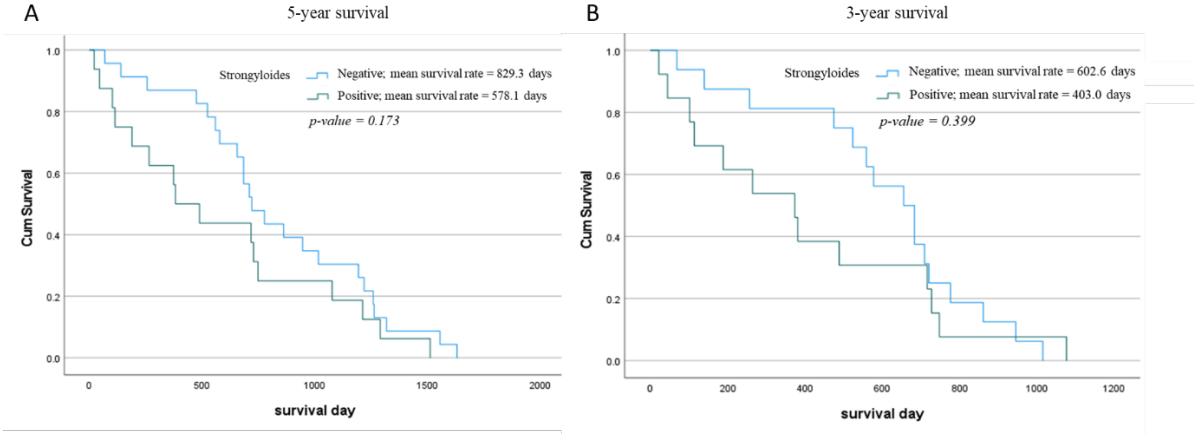


**Figure 3** Comparison of *S. stercoralis* infection in CCA patients.
(A) receiving chemotherapy at 5-year survival (*p*-value = 0.173)
(B) receiving chemotherapy at 3-year survival (*p*-value = 0.399)

**Comparison of anti-IgG levels in response to *S. stercoralis* infection between normal and CCA sera**

IgG unit levels between CCA group and normal group were investigated and compared. CCA patients were classified by using *S. stercoralis* infection status based on and *O. viverrini* infection status. Of 107 CCA patients, positive *S. stercoralis* infection was found in 34 cases (31.78%), while negative *S. stercoralis* infection was found in 73 cases (68.22%). In CCA patients with positive *S. stercoralis* infection, twenty-six patients (34.21%) had *O. viverrini* infection and fifty patients (68.5%) had no *O. viverrini* infection. In the healthy volunteer group, positive *S. stercoralis* infection was found in 5 cases (9.25%), while negative *S. stercoralis* infection was found in 49 cases (90.75%).

Analysis of IgG unit of *S. stercoralis* infection in all groups was performed by Mann-Whitney U test and presented as mean ± SD as shown in figure 4. The mean IgG unit values of normal group were 351.8 ± 123.4. The CCA patients, with evidence of *S. stercoralis* infection, had IgG unit values of 660.8 ± 574.1. There was no significant difference between the CCA and the normal group (*p*-value = 0.225). The IgG unit values of *S. stercoralis* in CCA patients with *O. viverrini* infection (CCA OV+) and normal group exhibited significant difference (*p*-value = 0.042). Moreover, the IgG unit values of CCA OV+ and CCA OV- were highly significantly different (*p*-value < 0.001), providing mean IgG values by 778.2 ± 607.3 and 279.5 ± 119.1, respectively. No significant difference was observed in IgG unit values of the CCA OV- and the normal group (*p*-value = 0.284). The findings indicated that the IgG levels of *S. stercoralis* infection were significantly different in CCA with OV infection status but there was insignificant difference when comparing to normal. Focusing on the subgroup of CCA, the CCA OV+ had high IgG levels compared to the normal group.

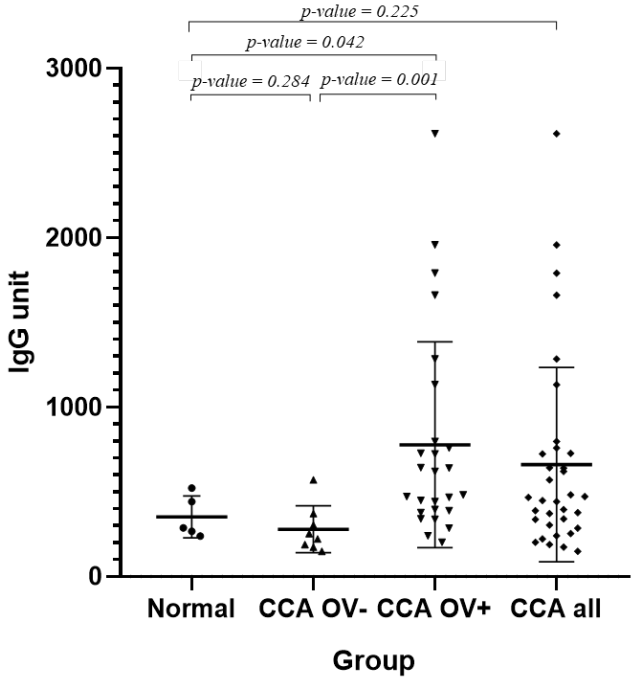


**Figure 4** Comparison IgG unit in each group.
**Note:** The data were presented as mean ± SD. Mann-Whitney U test was used in data analysis between cholangiocarcinoma patient and healthy people.
**Abbreviations:** CCA, cholangiocarcinoma patient; OV+, with the presence of *O. viverrini* infection; OV-, without the presence of *O. viverrini* infection.

## Discussion

Serodiagnosis of *S. stercoralis* infection provided high sensitivity, for instance, indirect enzyme-linked immunosorbent assays (ELISA) using extracted antigen from *S. ratti* larvae to detect antibody in serum. The *S. ratti* antigen used as a substitute for *S. stercoralis* due to the high cost, challenging extraction process, and potential hazard to workers if infected with *S. stercoralis*[6]. Previous report found the similarity of antigen among *S. ratti* and *S. stercoralis*, and also provided similar sensitivity and specificity in detection[7]. IgG was primarily responded to surface of filariform larvae (L3), infective stage, during *S. stercoralis* infection[9]. Thus, IgG in responsive to *S. stercoralis* infection was inspected from CCA patient serum to elucidate the association with clinicopathology, laboratory results, chemotherapy treatment status and history of *O. viverrini* infection for CCA severity analysis and survival rate of patient. In our study, the IgG levels of *S. stercoralis* were significantly elevated in individuals with CCA and *S. stercoralis* infection, particularly in the CCA OV+ group compared to the normal group. Additionally, within the CCA group, the IgG levels were higher in the CCA OV+ group compared to the CCA OV- group. An analysis of the correlation between IgG levels and clinical pathology, chemotherapy treatment status, and laboratory data revealed no observed correlation between *S. stercoralis* infection and CCA in any of the investigated parameters. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Another research report examined the association between *S. stercoralis* infection and patients with gastrointestinal cancer. The findings demonstrated a significantly higher occurrence of *S. stercoralis* infection in patients with gastrointestinal cancer compared to the control group ($p$-value < 0.05). Gastrointestinal cancer patients have a 6.7-fold chance of *S. stercoralis* infection more than the control[18]. Zueter et al[18] evaluated the correlation between the detection of *S. stercoralis* infection by real time-PCR and ELISA in cancer patients receiving chemotherapy, with or without steroid treatment, at a hospital in Malaysia. The result showed that the prevalence of *S. stercoralis* infection by using parasite-specific IgG test and IgG4 by ELISA test in cancer patients with immunocompromised were higher than the healthy control group ($p$-value < 0.05)[19].

## Conclusion

This study indicated no significant impact of IgG antibody levels of *S. stercoralis* on the survival time of CCA patients. Additionally, no link was observed between IgG antibody levels and the severity of CCA in patients. It implies that there is no requirement for further testing on the past presence of *S. stercoralis* infection before starting chemotherapy in CCA patients. Nevertheless, this study focuses on measuring the concentration of antibodies rather than antigens. Directly detecting the presence of antigens in an infected patient could be more relevant and provide insights into the severity of CCA. It is also recommended to detect IgG4 antibodies because studies have shown that IgG4 is more specific than IgG, although it has lower sensitivity[19].

### Take home messages

Detection of *S. stercoralis* infection prior to embark chemotherapy in CCA patients might not be demanded because of no significant correlation among patient's survival, CCA severity, and IgG levels of *S. stercoralis*. Detection of antigens and IgG4 could be additional evidence to fulfill this study.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgements

We express our gratitude to the Cholangiocarcinoma Research Institute for their assistance in sample collection, as well as to the lab members of Prof. Paiboon Sithithaworn for providing the necessary analytical equipment. This work was supported by Fluke Free Thailand through Cholangiocarcinoma Research Institute, Khon Kaen University, Thailand.

## References

1. Kassalik M, Mönkemüller K. *Strongyloides stercoralis* hyperinfection syndrome and disseminated disease. Gastroenterol Hepatol (N Y) 2011; 7(11): 766-8.
2. Becker SL, Piraisoody N, Kramme S, Marti H, Silué KD, Panning M, et al. Real-time PCR for detection of *Strongyloides stercoralis* in human stool samples from Côte d'Ivoire: diagnostic accuracy, inter-laboratory comparison and patterns of hookworm co-infection. Acta Trop 2015; 150: 210-7.
3. Jongsuksuntigul P, Intapan PM, Wongsaroj T, Nilpan S, Singthong S, Veerakul S, et al. Prevalence of *Strongyloides stercoralis* infection in northeastern Thailand (agar plate culture detection). J Med Assoc Thai 2003; 86(8): 737-41.
4. Nontasut P, Muennoo C, Sa-nguankiat S, Fongsri S, Vichit A. Prevalence of strongyloides in Northern Thailand and treatment with ivermectin vs albendazole. Southeast Asian J Trop Med Public Health 2005; 36(2): 442-4.
5. Beknazarova M, Whiley H, Ross K. Strongyloidiasis: A Disease of Socioeconomic Disadvantage. Int J Environ Res Public Health 2016; 13(5).
6. Prasongdee TK, Laoraksawong P, Kanarkard W, Kraiklang R, Sathapornworachai K, Naonongwai S, et al. An eleven-year retrospective hospital-based study of epidemiological data regarding human strongyloidiasis in northeast Thailand. BMC Infect Dis 2017; 17(1): 627.
7. Rodrigues RM, de Oliveira MC, Sopelete MC, Silva DA, Campos DM, Taketomi EA, et al. IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using Strongyloides ratti saline extract as heterologous antigen. Parasitol Res 2007; 101(5): 1209-14.
8. Eamudomkarn C, Sithithaworn P, Sithithaworn J, Kaewkes S, Sripa B, Itoh M. Comparative evaluation of Strongyloides ratti and *S. stercoralis* larval antigen for diagnosis of strongyloidiasis in an endemic area of opisthorchiasis. Parasitol Res 2015; 114(7): 2543-51.
9. Grove DI, Blair AJ. Diagnosis of human strongyloidiasis by immunofluorescence, using *Strongyloides ratti* and *S. stercoralis* larvae. Am J Trop Med Hyg 1981; 30(2): 344-9.
10. Genta RM, Weil GJ. Antibodies to *Strongyloides stercoralis* larval surface antigens in chronic strongyloidiasis. Lab Invest 1982; 47(1): 87-90.
11. Sava M, Huynh T, Frugoli A, Kong L, Salehpour M, Barrows B. Colorectal Cancer Related to Chronic *Strongyloides stercoralis* Infection. Case Rep Gastrointest Med 2020; 2020: 8886460.
12. Mishra S, Patnayak R, Panda SS, Jena A. *Strongyloides stercoralis* hyperinfection in a patient with acute lymphoblastic leukemia. J Postgrad Med 2021; 67(2): 113-4.
13. Plentz RR, Malek NP. Clinical presentation, risk factors and staging systems of cholangiocarcinoma. Best Pract Res Clin Gastroenterol 2015; 29(2): 245-52.
14. Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. Cancer Epidemiol Biomarkers Prev 1993; 2(6): 537-44.
15. Treeprasertsuk S, Poovorawan K, Soonthornworasiri N, Chaiteerakij R, Thanapirom K, Mairiang P, et al. A significant cancer burden

and high mortality of intrahepatic cholangiocarcinoma in Thailand: a nationwide database study. BMC Gastroenterol 2017; 17(1): 3.

16. Altaee MY, Johnson PJ, Farrant JM, Williams R. Etiologic and clinical characteristics of peripheral and hilar cholangiocarcinoma. Cancer 1991; 68(9): 2051-5.
17. Kamsa-ard S, Kamsa-ard S, Luvira V, Suwanrungruang K, Vatanasapt P, Wiangnon S. Risk Factors for Cholangiocarcinoma in Thailand: A Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev 2018; 19(3): 605-14.
18. Machado ER, Teixeira EM, Gonçalves-Pires Mdo R, Loureiro ZM, Araújo RA, Costa-Cruz JM. Parasitological and immunological diagnosis of *Strongyloides stercoralis* in patients with gastrointestinal cancer. Scand J Infect Dis 2008; 40(2): 154-8.
19. Zueter AM, Mohamed Z, Abdullah AD, Mohamad N, Arifin N, Othman N, et al. Detection of *Strongyloides stercoralis* infection among cancer patients in a major hospital in Kelantan, Malaysia. Singapore Med J 2014; 55(7): 367-71.