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RESEARCH ARTICLE

Case reports of *Strongyloides stercoralis* infection in three patients with haematological malignancies

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ABSTRACT

Strongyloidiasis is a parasitic nematode infection mainly caused by *Strongyloides stercoralis*. Immunocompromised conditions, particularly cancer patients treated with chemotherapy and corticosteroids, have a significant risk of developing *Strongyloides* hyperinfection. The lack of a gold standard laboratory method to rule out this infection and the insensitivity of microscopic stool examination due to low and intermittent larvae output in stool contribute to the low detection rate of this infection. We present three cases of strongyloidiasis in adults with haematological malignancies and significant eosinophilia in the early course of their cancer. Two patients were diagnosed with a combination of serological and molecular tests, and one was diagnosed serologically. Ivermectin at 200 mcg/kg/day for two days was commenced for all patients; unfortunately, one patient succumbed.

Keywords: *Strongyloides stercoralis*; hematological malignancies; real-time PCR; ELISA; SsRapid®.

INTRODUCTION

Strongyloides stercoralis infection occurs globally, with prevalence estimated at 8.1%, corresponding to 613.9 million people, mainly in tropical and subtropical regions (Buonfrate *et al.*, 2020). Host cell-mediated immunity is believed to be crucial in regulating *Strongyloides* autoinfection (Carvalho & Da Fonseca, 2004). Chronic strongyloidiasis is often asymptomatic in immunocompetent individuals. However, if an infected person is immunocompromised, autoinfection may dominate and become overwhelming. Strongyloidiasis accounted for 60 – 85 % of mortality rates amongst infected immunosuppressed patients and contributed to 16.7 % of mortality in infected patients requiring hospitalization (Siddiqui & Berk, 2001; Iriemenam *et al.*, 2010).

Among malignancies associated with severe strongyloidiasis, 90% were haematological, i.e. leukaemia and lymphoma (Schaffel *et al.*, 2001). Corticosteroids are employed in the treatment regime of many of these patients and have been identified as an important factor in accelerating *Strongyloides* infection into hyperinfection (Keiser & Nutman, 2004). Fatal hyperinfection of *S. stercoralis* following allogeneic hematopoietic stem cell transplantation (HSCT), a common treatment option in this patient group, has also been reported (Wirk & Wingard, 2009). Thus, detecting *Strongyloides* infection should be improved in these patients since they are at risk of hyperinfection and fatal outcomes. Here, we present three cases of strongyloidiasis in adults with haematological malignancies who resided in Kelantan, an area where soil-transmitted helminths, including *Strongyloides*, is endemic. They were among the participants in our recent study on the prevalence of cancer patients treated with chemotherapy and corticosteroids at a university teaching hospital on the east coast of Malaysia (not yet published).

ETHICAL APPROVAL

Ethical approval was obtained from the USM Human Research Ethics Committee No. USM/JEPem/20050254.

CASE REPORTS

Case 1: A 55-year-old Malay male with underlying hypertension, diabetes mellitus, right nephrolithiasis, and gout arthritis. He had multiple visits to the emergency department for intolerable pain in the right loin and lower abdomen. Subsequently, he was incidentally noted to have thrombocytopenia with platelet 35 x 10^9/L. The haemoglobin and white blood cells were normal at 13.1 x 10^9/L and 5.6 x 10^9/L, respectively. Full workups were done, and eventually, he was diagnosed with Hypoplastic Myelodysplastic Syndrome (MDS) in 2018 and has been on routine follow-up by the oncology team at our centre. He was started on long-term eltrombopag (tablet Revolade), with the dose stepped up frequently during follow-up sessions due to poor response. In July 2021, the chemotherapy regime was changed to cyclosporine. He was also on multiple prednisolone courses.

During one of his follow-ups post-MDS diagnosis, the highest eosinophil count was documented (in August 2018) at 0.97 x 10^9/L (normal range is 0.02 - 0.5 x 10^9/L), with a full blood picture reported

as bi-cytopenia with significant dysplastic features and eosinophilia. The eosinophil count declined 14 months later due to the worsening of his condition. In September 2021 the patient was admitted for symptomatic anaemia secondary to MDS and complicated with neutropenic sepsis, and the chemotherapy regime was changed to Azacitidine. Multiple antibiotic courses were given during the febrile episodes, including tazocin, meropenem, ciprofloxacin, fluconazole, caspofungin, and voriconazole. The blood culture grew *Klebsiella pneumoniae* Extended Spectrum β-*Lactamase* (ESBL), and the antibiotic regime was tailored accordingly; however, no fungal growth was detected. No stool sample was obtained during the admission since the patient was constipated. The interview and written consent for this study occurred two days prior to the patient's discharge. Thus, his serum sample was tested for *Strongyloides* infection using two serological methods after the discharge. They comprised a lateral flow prototype test (SsRapid®), which showed a strong positive result (Figure 1A), and a commercial anti-*Strongyloides* IgG enzyme-linked immunosorbent assay (Euroimmun, Lubeck, Germany), which also showed a high positive result. During his follow-up in September 2021, the patient was given tablet ivermectin 15 mg (200 mcg/kg body weight) daily for two days. A longer course of Ivermectin was not given due to the absence of strong evidence of hyperinfection since the patient could not provide stool samples for *Strongyloides* larvae/DNA analysis. The patient was readmitted at the end of September 2021 and succumbed to death within 24 hours of admission due to severe sepsis secondary to pneumonia with underlying MDS.

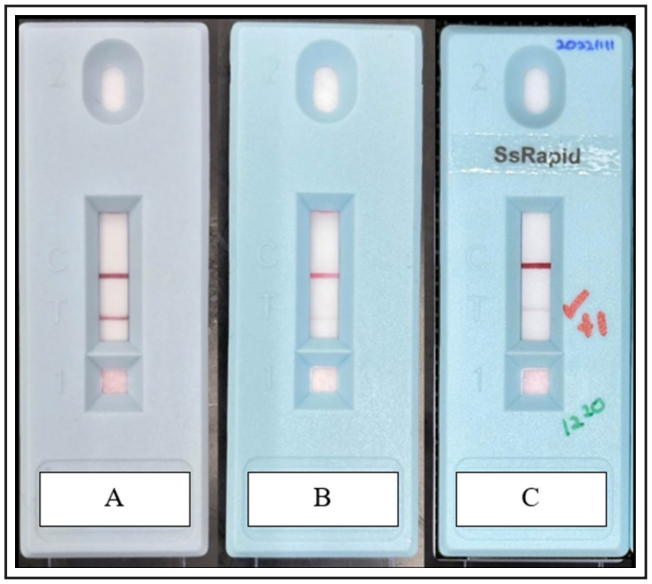
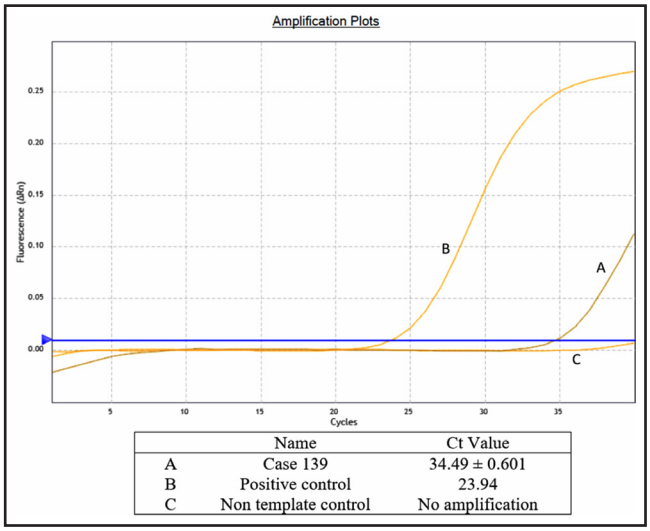


Figure 1. Results of SsRapid® tests of the three patients. Cassette A showed a strong positive result with test line intensity of +4 (Case 1), while cassettes B and C showed weak positive results with tests line intensity of +1 (Cases 2 and 3, respectively). Variations in cassettes casing colour is due to lighting differences during image capture.

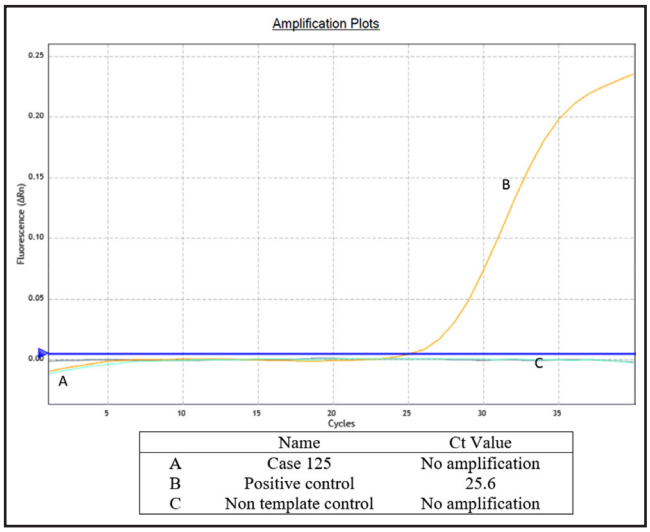
Case 2: A 65-year-old Malay male with underlying hypertension and dyslipidaemia diagnosed with Diffuse Large B-cell Lymphoma (DLBCL). He initially presented with abdominal fullness and constitutional symptoms for five months, and a computerized tomography (CT) abdomen pelvis scan in August 2021 suggested high-grade Lymphoma. CT scan of the abdomen revealed splenomegaly, lymphadenopathy, and a left abdominal mass. The diagnosis was confirmed by tissue biopsy of the mass on the left hypochondriac region. The bone marrow trephine biopsy indicated a reactive marrow and was negative for primary tumour infiltration. The highest documented eosinophil count was 0.59 x 10^9/L in September 2021 and subsequently fluctuated within the normal range after a month. Chemotherapy with R-CHOP protocol was started in October 2021, together with steroid administration. Both serum and stool samples were sent for *Strongyloides* investigation during the first chemotherapy cycle. SsRapid® test was weakly positive (Figure 1B), and the Euroimmun IgG ELISA showed a borderline result. A stool sample examined using direct microscopy was negative for ova/cyst/larva. Real-time PCR of the stool sample for *S. stercoralis* was positive, with a CT value of 34.49 ± 0.601 (Figure 2). Tablet ivermectin 12 mg daily for two days was given in March 2022. The patient was well upon discharge and continued the haemato-oncology follow-ups.



	Name	Ct Value
A	Case 139	34.49 ± 0.601
B	Positive control	23.94
C	Non template control	No amplification

Figure 2. Real-time PCR amplification plot for detection of *S. stercoralis* in stool sample of Case 2 showing a positive result.

Case 3: A 63-year-old Chinese man with underlying diabetes mellitus, hypertension, left nephrolithiasis, and an old cerebrovascular accident was diagnosed with B-cell lymphoma in October 2021. The lymphoma diagnosis was confirmed by a biopsy of his left anterior chest swelling. The highest eosinophil count was 0.68 x 10^9/L in October 2021, and his full blood picture showed monocytosis with mild eosinophilia. Tablet prednisolone 0.5 mg/kg was started in November 2021, and chemotherapy with R-CHOP was started in December 2021. SsRapid® test using his serum taken during the third chemotherapy cycle showed a weak positive result (Figure 1C). Stool direct microscopic examination, Euroimmun IgG ELISA, and stool real-time PCR (Figure 3) were negative. He was prescribed ivermectin tablet 9 mg daily for two days in February 2022.



	Name	Ct Value
A	Case 125	No amplification
B	Positive control	25.6
C	Non template control	No amplification

Figure 3: Real-time PCR amplification plot for detection of *S. stercoralis* in stool sample of Case 3 showing a negative result.

Table 1. Summary of the parasitological, serological and real-time PCR results of the three patients

Test	Case 1	Case 2	Case 3
Microscopy	NA	No ova, cyst, larva	No ova, cyst, larva
SsRapid®	Strong Positive (+4)	Weak Positive (+1)	Weak Positive (+1)
Stool real-time PCR	NA	Positive#	Negative
IgG ELISA (Euroimmun, Germany)	Strong Positive	Borderline*	Negative

NA = not available.
# Triplicate tubes, average CT 34.49 ± 0.601.
* Results were interpreted based on sample optical density (OD) ratio (OD/cut-off OD). A sample ratio less than 0.8 is considered a negative result; between 0.8 and 1.0 was borderline, and a ratio greater or equal 1.1 was positive.

Table 1. summarizes the results of the parasitological, serological and stool real-time PCR of samples of the three cases.

DISCUSSION

The neglect of *Strongyloides* infection is contributed by the lack of gold standard laboratory method and the insensitivity of microscopic stool examination due to low and intermittent larvae output in stool. *S. stercoralis* infection seldom rings a bell with most clinicians in Malaysia when treating immunocompromised patients despite the country's tropical climate and its location in a *Strongyloides*-endemic region. Screening for the infection is not routinely done for this group of patients, and anti-helminths are not included in the treatment regime for febrile neutropenic patients. The three patients were from urban areas, however Kelantan is still considered one of the less developed states in Peninsular Malaysia. We have previously reported that *Strongyloides* larvae were detected in water used to wash vegetables and herbs sold at a central wet market in Kota Bharu, Kelantan (Zeehaida *et al*., 2011).

Most *Strongyloides*-infected patients are thought to be asymptomatic. However, according to Gomez-Hinojosa *et al.* (2020), episodic diarrhoea is more common, may alternate with constipation and can mimic irritable bowel syndrome. Assessment of the disease solely based on symptoms is challenging; moreover, the symptoms are usually masked by the underlying disease.

The usage of corticosteroids and the decline of a patient's immunity due to cancer and chemotherapy may significantly increase the risk for fatal outcomes of strongyloidiasis. Corticosteroids are known immunosuppressive drugs and are believed to increase the *Strongyloides* larval moulting rate and trigger an overload of its numbers, thus leading to progression from chronic strongyloidiasis to hyperinfection (Keiser & Nutman, 2004). The transformation of previously clinically undetectable infection into overwhelming dissemination can take less than ten days in a high-dose corticosteroid treatment (Ghosh & Ghosh, 2007).

Since *Strongyloides* autoinfection often leads to eosinophilia, the infection should be suspected in a patient with unexplained eosinophilia, especially if the patient has resided in an endemic area (Czeresnia & Weiss, 2022). Eosinophilia may appear as the only indicator for screening for *Strongyloides* infection, although it may be mild in chronic *Strongyloides* infection (Requena-Mındez *et al.*, 2013). Absolute eosinophils count (AEC) > 0.5 – 1.5 x 10^9/L refers to mild eosinophilia, AEC >1.5 – 5.0 x 10^9/L moderate eosinophilia and AEC >5.0 x 10^9/L severe eosinophilia (Leru, 2019). The decrease in circulating eosinophil count may be due to corticosteroid usage (Keiser & Nutman, 2004; Snydman *et al.,* 2009). The three patients we described had intermittent eosinophilia in the early course of the malignant disease, particularly before corticosteroid commencement, and the eosinophilia declined after chemotherapy/corticosteroid and with the disease progression.

Although routine stool microscopic examination is available in most diagnostic laboratories, low and intermittent larvae output often lead to missed detection of *S. stercoralis* (Siddiqui & Berk, 2001). The stool samples in Cases 2 and 3 were negative for ova, cyst, or larva. If a stool sample is the only sample available or microscopy the only test available, the diagnosis of strongyloidiasis will not come to light, as seen in Case 1. Compared to a blood sample, a stool sample may not be available since cancer patients on chemotherapy may experience constipation. Combining serum and stool tests is thus a good sampling approach in these cases.

SsRapid® detects IgG4 antibodies against *S. stercoralis* rNIE. The rapid test's diagnostic performance was reported to be 97% sensitive and 94.5% specific (Noordin *et al*., 2022), and another study showed a diagnostic sensitivity of 95% (Anuar *et al.*, 2023). A serum absorption study validated that the rapid test is highly specific (Noordin *et al*., 2021). In a study using samples from a highly endemic population of sub-Saharan migrants in Italy, the test sensitivity was 86.3% compared to the fecal test comprising faecal concentration, agar plate culture and/or real-time PCR (Tamarozzi *et al*., 2022). Among 778 school children in a low endemic area in Ecuador, the rapid test sensitivity and specificity were 79.4% and 93.6%, respectively and showed a high negative predictive value; the sensitivity increased to 90.9% when combined with PCR or Baermann concentration technique with little effect on the specificity (Tamarozzi *et al*., 2023). In a recent study in Thailand, SsRapid® showed a sensitivity of 93.9% compared to fecal tests (stool concentration and culture) (Wongphutorn *et al.*, 2024). Thus, SsRapid® showed high diagnostic sensitivity and specificity in studies performed in several countries. Meanwhile, the Euroimmun IgG ELISA uses native *Strongyloides* antigen and has been reported to have a diagnostic sensitivity and specificity of 95% in one study (Warnecke, 2019), and 90.6% sensitivity and 87.7% specificity in another study (Buonfrate *et al*., 2021).

In Case 1, the serum sample tested using both serological methods were strongly positive. The patient was admitted for febrile neutropenia, a potentially life-threatening condition for cancer patients treated with chemotherapy and faced a risk of infection. In hindsight, this patient might have had *Strongyloides* hyperinfection, which contributed to the fatal outcome. Usually, *Strongyloides* larvae can be readily seen in stool of patients with hyperinfection. Unfortunately, the patient could not produce stool due to his constipation; thus, we did not have concrete evidence that showed he had a hyperinfection. A sputum sample was sent for bacterial culture and sensitivity early on admission; however, it was not examined for *S. stercoralis*. Consequently, he was only given a two-day course of ivermectin based on the diagnosis of chronic *Strongyloides* infection. The final patient outcome might have been different if a timely diagnosis of a hyperinfection was made, and adequate treatment instituted.

The immune response among individuals is known to be highly variable. Nevertheless, in immunocompetent individuals, serodiagnosis is generally reliable. However, false negative results may occur with samples from immunosuppressed individuals. Thus, a seronegative result of an immunocompromised patient does not mean the patient is not infected.

In Case 3, although the rapid test showed a weak positive result and the molecular and IgG-ELISA tests were negative, the patients was still diagnosed as having *Strongyloides* infection and treated accordingly. The rapid test is a qualitative assay and any distinct test line, irrespective of its intensity, is interpreted as a positive result. Furthermore, since the patient was immunosuppresed, it is not unexpected to see a weak positive test line. The likely reason the IgG ELISA (Euroimmun) was negative is because it is an IgG test.

In chronic *Strongyloides* infection specific IgG4 antibody level (as detected by SsRapid®) is more elevated than specific IgG due to the constant antigenic stimulation of the autoinfective larvae (Arifin *et al*., 2018; Osman *et al*., 2022). Since the initial specific IgG4 level was elevated, it was still detectable despite the host's weak immune response. During early infection, specific IgG antibody level is more elevated than IgG4 (Arifin *et al*., 2018; Anuar *et al*., 2023), thus, for immunosuppressed patients, performing both IgG and IgG4 assays may be useful.

With regard to the negative real-time PCR in Case 3, it is not surprising due to the intermittent nature of *Strongyloides* larvae shedding in the stool. PCR is sensitive if larvae are present in the stool, however, it may be negative if the sample is taken on days there is no larvae output. Ideally, stool samples for PCR or microscopy should be taken in multiple days, however this is often not possible. Thus, negative real-time PCR on a single stool sample does not confirm a true negative case.

To date, there is no specific national recommendation for *Strongyloides* screening as part of the infectious disease screening of patients with underlying hematological malignancies. We hope local data such as the present report will contribute to such recommendation to be made in the future. According to the Centers for Disease Control and Prevention, USA (CDC, 2019), the treatment for strongyloidiasis is indicated in symptomatic and asymptomatic persons. Treatment options for strongyloidiasis are limited to oral formulations, with ivermectin, albendazole, and thiabendazole being the drugs most used. First-line therapy for acute and chronic strongyloidiasis is Ivermectin 200 µg/kg in a single dose for 1–2 days. If a hyperinfection is suspected, a fourteen-day course of ivermectin is needed. It includes evidence of stool/sputum larvae clearance post-treatment. However, ivermectin is not available in most health institutions in Malaysia. Thus, we concur with Bisoffi *et al.* (2013) that ivermectin should be made available in countries/areas with endemic strongyloidiasis.

CONCLUSION

Future research must address gaps in diagnosing and managing *Strongyloides* infection in cancer patients, especially those on chemotherapy and corticosteroids. Eosinophilia in these patients at any point, particularly before corticosteroid therapy, should warrant further investigations of *S. stercoralis* infection. Combining serological and molecular tests provides a more robust approach to detecting the infection, and urgent action might be needed in cases of positive serology results to prevent the fatal outcome of a hyperinfection.

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Competing interests

The authors declares that they have no competing interests.

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