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Article Title:

Treatment of larva migrans syndrome with long-term administration of albendazole

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Running title:

Long-term albendazole treatment for larva migrans

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Abstract

Background: Larva migrans syndrome is a food-borne parasitic disease in humans, caused by accidental ingestion of eggs or larvae of ascarid nematodes, namely, *Toxocara canis*, *Toxocara cati*, or *Ascaris suum*, the roundworms commonly found in the intestines of dogs, cats and pigs respectively. When a patient is diagnosed as having larva migrans syndrome, oral-administration of albendazole is recommended, however, the regimen remains controversial worldwide. In Japan, the duration of albendazole administration is longer than those of European and North American countries. The purpose of this study was to assess the efficacy and safety of long-term administration treatment of albendazole for larva migrans syndrome.

Methods: From 2004 to 2014, our laboratory was involved in the diagnosis of 758 larva migrans syndrome cases, of which 299 cases could be followed up after the treatment. We analyzed these 299 follow-up cases on the ELISA results before and after the treatment as well as on anthelmintic used, dose and duration of medication, clinical findings, and side effects, recorded on a consultation sheet provided by the attending physicians. We have 288 cases as the subjects of this study.

Results: Albendazole represented a 78.0% efficacy rate. The side effects represented 15.0% in using albendazole alone cases; however, the side effects were mild to moderate and there were no severe cases reported.

Conclusions: The long-term administration treatment of albendazole is safe and effective for larva migrans syndrome.

Keywords

albendazole; larva migrans syndrome; *Ascaris suum*; *Toxocara canis*; *Toxocara cati*

Introduction

Larva migrans syndrome (LMS) is an important food-borne parasitic disease caused by *Toxocara canis*, *Toxocara cati*, or *Ascaris suum*, the ascarid roundworms commonly found in the intestines of dogs, cats and pigs respectively.[1-4] Humans acquire infections by accidental ingestion of embryonated eggs or infective larvae of these parasites. The source of infections is contacting with contaminated soil where infected dogs and cats defecated, or taking contaminated foods, which include raw or undercooked meat as well as unwashed fruit or vegetables.[2,5] The magnitude of infection depends on the number of ingested larvae or embryonated eggs.[6,7] Once the embryonated eggs or larvae are ingested, larvae penetrate through the intestinal wall and migrate via circulatory system to the liver, lungs, central nerves system, or eyes causing larva migrans syndrome (LMS). LMS can be classified into four major clinical presentations based on the involved organs: visceral larva migrans (VLM), neural larva migrans (NLM), ocular larva migrans (OLM), and asymptomatic type (referred to as 'covert type' in some literature).[3,7-9]

As for the treatment of LMS, it is widely accepted that albendazole is the first choice, though the regimen particularly the optimal duration has not been standardized yet.[7,10,11-13] When an individual is diagnosed positive for LMS, most institutions in Europe and the US recommend treatment with oral-administration of albendazole at 400mg twice a day for five days for both adult and pediatric.[4,5] However, the regimen showed only 32% clinical cured rate in a previous study.[14] On the other hand, in Japan, the recommended treatment for LMS has been 10~15 mg/kg/day taken orally for one cycle of four weeks following by two weeks of drug free and an additional one cycle of four weeks, which is based on the regimen for echinococcosis.[15]

In this study, we assessed the efficacy and safety of long-term administration treatment of albendazole for larva migrans syndrome with an aim to establish a standardized effective treatment regimen.

Methods

Ethical clearance

This retrospective clinical study was approved by the Research Ethics Committee of Faculty of Medicine, University of Miyazaki, under the title of "Surveillance of parasitic diseases in Japan" (permission # 2014-087). Our study strictly adhered to the Ethical Guideline for Clinical study released from the Ministry of Health, Labour and Welfare, Japan.

Patients analyzed in the present study

From 2004 to 2014, our laboratory conducted serological tests of 4,934 cases, of which 758 were judged positive for ascarid infections (see below). Among them, we had 299 follow-up cases with recovery criteria described herewith, there were 288 cases as the subjects of the present study (Figure 1). Upon antibody tests, both at initial diagnosis and follow-up study, we asked attending physicians for providing us with a consultation sheet describing age, sex, place of accommodation, ethnic background, chief complaint, medical history, administered drugs and its dose and duration, dietary history, overseas travelling history, medical imaging findings, and laboratory data. Side effects, if any, were also supposed to be recorded on the consultation sheet.

Based on the information, the regimen data were categorized into four groups. Patients treated with only albendazole were grouped as albendazole group. For patients treated with anthelmintic other than albendazole and switched to albendazole or vice versa, these patients were grouped as albendazole plus other anthelmintic group. Patients treated with anthelmintic other than albendazole were grouped as other anthelmintic group. Patients received no anthelmintic treatment were grouped as no anthelmintic treatments group.

Along with the symptoms, we also examined side effects recorded on consultation sheets to assess the safety of the anthelmintic used. Although we did not set standardized format for recording side effects on the consultation sheet, attending physicians who had adverse events with albendazole treatment frequently contacted us in e-mails and/or phone calls because of the lack of the treatment experience (note that nematode infections are rare in Japan). Therefore we consider that we could collect substantial portion, if not all, of the adverse side effects during the treatment.

Enzyme-linked immunosorbent assay (ELISA)

Sera and body fluid samples, such as pleural effusion and ascites, sent by the attending physicians to our laboratory, were tested for specific antibodies in enzyme-linked immunosorbent assay (ELISA). Antigen preparations used in ELISA were *Ascaris* soluble worm antigen preparation (*As*-SWAP) and excretory/secretory (ES) antigen of *T. canis* larvae (*Tc*-ES) [16]. In our standard procedure, samples were first tested for the binding to *As*-SWAP and *Tc*-ES, and in non-visceral types (see below), samples were further tested in ELISA with ES antigen of *A. suum* larvae (As-ES), or in Western blotting (LDBIO Diagnostics, Lyon, France) for the confirmation of the diagnosis.

In ELISA, wells of microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 2 μg/ml of *As*-SWAP or 1 μg/ml of *Tc*- or *As*-ES in 0.05 M carbonate-bicarbonate buffer (pH 9.6). Wells were washed with PBST (PBS containing 0.05% Tween 20), blocked with 1% casein (Nakarai Tesque, Kyoto, Japan), and incubated with diluted sera (1: 900 and 1: 2,700) for 1 hour at 37°C. After washing with PBST, binding of antibodies was detected with horseradish peroxidase conjugated rabbit anti-human IgG (Dako, Glostrup, Denmark). For colour development, ABTS (KPL, Gaithersburg, MD, USA) was added and incubated at room temperature for 30 minutes in the dark. Optical density at 405nm was read in a Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA). Based on negative control serum data, we set a cut-off point at 0.200 for 1: 900 diluted sera, pleural effusion, and ascites. For body fluids such as CSF and vitreous humour fluid, optical density more than 0.1 at 1: 30 dilution were judged positive.

Diagnosis of LMS and the criteria for recovered and unrecovered

At initial diagnosis, the positive cases were determined by at least one of the followings: (1) positive reactivity of serum or body fluid samples to *Toxocara* spp. or *A. suum* antigens; (2) clinical information from the applications recorded by physicians in charge along with any available medical imaging techniques such as ultrasound, computed tomography, and magnetic resonance imaging indicated lesions in lungs/liver with positive reactivity of serum to support diagnosis of ascarid infections.

Follow-up study was carried out three to four months after the initial diagnosis in most cases. When follow-up patients became antibody negative, or showed decrease in serum anti-parasite antibody concentration (more than 30% of reduction in optical densities at 1 to 2,700 dilution in ELISA) and with one of the following findings, we considered them as ‘recovered’: peripheral blood eosinophilia returned to

within the normal range; disappearance of symptoms and/or abnormal medical imaging. In addition, when previously positive local body fluid samples turned negative or reduced more than 30% of reduction in optical densities at 1 to 900 dilution in ELISA, we judged these patients as 'recovered'. Figure 1 showed the flow chart for the evaluation of the efficacy of albendazole.

Disease types

In this study, we analyzed the clinical manifestations and divided patients into five disease types based on clinical findings provided in the consultation sheet:[16] Visceral larva migrans (VLM) were cases with typical eosinophilic pneumonia, multiple nodular lesions in liver or lungs with eosinophilia, or pleural eosinophilia showing symptoms like cough, dyspnea or chest pain. Ocular larva migrans (OLM) were cases of visual disturbance with uveitis and/or retinal nodular lesions. Neural larva migrans (NLM) were cases associated with neurological disorders showing symptoms like paresthesia, muscle weakness, or urination disorder. Asymptomatic were cases with no specific symptoms or objective findings other than simple peripheral blood hyper-eosinophilia. Miscellaneous were symptomatic cases other than VLM, OLM or NLM. This type included cardiovascular involvement such as myocarditis or pericarditis, cutaneous involvement such as skin rash, and gastrointestinal involvement such as diarrhea or abdominal pain.

Statistical tests

All the statistical tests (Fisher's exact test and chi-squared test) were performed with a significance threshold of $p < 0.05$.

Results

Outcome of the treatment

Among 299 follow up LMS cases, 11 cases were excluded from the study due to the optical densities were not reduced even the clinical symptoms improved as shown in Figure 1. We have 288 cases for the present study. There were 246 patients treated with albendazole which represented 85.4%; and 16 cases (5.6%) were treated with albendazole plus other anthelmintic; 19 cases (6.6%) were treated with other anthelmintic; and seven cases (2.4%) received no anthelmintic treatments.

In 246 cases which were treated with albendazole alone, 192 cases recovered and 54 did not, representing recovery rate of 78.0%. In 16 cases with the treatment with albendazole plus other anthelmintic, eight cases recovered and eight cases failed to recover, which represented 50% of recovery rate. In cases which patients were not treated with albendazole, 11 cases recovered and eight cases unrecovered, which represented 57.9% recovery rate. In those cases using other anthelmintic, ten cases were administered ivermectin, showing the recovery rate of 70% (Table 1).

Age and sex distribution

We examined age and sex distribution of recovered and unrecovered patients to see if age or sex could have affected the efficacy of albendazole. The male to female ratio was 2.15 (131:61) in recovered group and 2.86 (40:14) in unrecovered group. The average age was 49.0 (range 8 to 82 years old) in male and 47.1 (range 20 to 85 years old) in female in the recovered group. The average age was 54.5 (range 30 to 84 years old) in male and 55.7 (range 25 to 76 years old) in female in the unrecovered group. In both groups, the peak for male patients was in their 50s whereas in female patients was pretty much evenly spread out among the age groups of 20s, 30s, 50s, and 60s. Male patients were more than female patients in every age group except in 20s (Figure 2). There were no significant differences in the age and sex distribution between recovered and unrecovered groups.

Disease types

We then investigated the disease types among recovered and unrecovered groups, suspecting that some disease type might have resistant to the treatment. In the recovered group, there were 91 cases of VLM, 23 cases of OLM, 30 cases of NLM, 34 cases of Asymptomatic, and 13 cases of Miscellaneous (n=191).

There was one case that was not included due to lack of medical information on the consultation sheet. In the unrecovered group, there were 22 cases of VLM, 11 cases of OLM, five cases of NLM, 13 cases of Asymptomatic, and three cases of Miscellaneous (n=54). Again, there were no significant differences in the efficacy of albendazole treatment among the disease types.

Duration of medication

The recommended treatment in Japan is orally administration of albendazole at 10~15 mg/kg/day for a cycle of four weeks followed by two weeks of drug free interval and repeat another cycle of four weeks of medication. However, the length of treatments varied among follow up cases from a few days to 17 weeks with most cases allocated in four weeks and eight weeks; there were some cases without indication. The recovery rates of different medication groups were not different significantly, reaching approximately 80% in each groups (Table 2). It appears that at least a portion of LMS caused by ascarid roundworms is self-limiting.

Side effects

We analyzed the side effects from using albendazole alone cases. There were a total of 37 cases out of 246 cases reported side effects representing 15.0%. Among them, there were 25 cases from recovered group with the following symptoms: 21 cases of liver dysfunction, two cases of depilation, one case of vomiting, and one case of skin rash. There were 12 cases from unrecovered group with the following symptoms: 11 cases of liver dysfunction, and one case of nausea (Table 3). Thus, the occurrence rate for recovered group was 13.0% (25 /192); the occurrence rate for unrecovered group was 22.2% (12/54).

Then we examined when side-effects appeared by comparing groups with different medication duration. We found that patients treated less than four weeks or treated for five to seven weeks had higher side effects rate compared to other groups (Table 4). It should be noted that patients treated for eight weeks or more had less side effects rate. These findings indicate that the appearance of side effects does not simply depend on the duration of medication.

There were four cases in each of the recovered and the unrecovered groups that used albendazole and when the liver dysfunction side effect appeared, they switched to other anthelmintic mainly ivermectin. There were three liver dysfunction cases worth mentioning: (1) one case which was treated for four weeks

and when liver dysfunction occurred the treatment stopped for two weeks and resumed treatment for two weeks; (2) one case was treated for one week and reduced dosage for three weeks; (3) one case ignored the side effect and continued treatment for a total of ten weeks. All these three patients were recovered and once albendazole treatment stopped, liver dysfunction was reversed.

Discussion

Albendazole is a broad spectrum anthelmintic effective to nematode infections in general,[17] and has been used for the treatment of larva migrans syndrome (LMS) caused by ascarid roundworms as well. However the regimen of albendazole for ascarid LMS treatment still remains controversial. Literatures and institutions recommend 400 mg twice a day for five days without specifying optimum duration,[3, 4,13] though in neurotoxocariasis, albendazole is used for a period of at least three weeks, which often needed to be repeated.[18] In cases with cardiac involvement, various regimens have been employed, such as 800 mg/day for two weeks, 50 mg/kg/day for 28 days, 600 mg/day for 14 days, or 1000 mg/day for four weeks (reviewed by Kuenzli E *et al.*).[19] Importantly, Sturchler *et al.* reported that the standard five days regimen of albendazole showed only 32% cure rate.[14]

In the present study, we showed that albendazole had overall recovery rate of 78.0% against LMS caused by ascarid nematodes when used for four or eight weeks, which was much higher than the five days regimen.[14] Recovery rate was 78.9% and 81.3% for four weeks- and eight weeks-administration, respectively. Furthermore, the present study also indicates that the long-term albendazole administration was effective equally to all disease types including OLM. This is in the accordance to the previous studies that revealed that OLM required a long-term administration treatment.[20-23] To the best of the author's knowledge, this is the first research that has included a large number of subjects to evaluate albendazole for ascarid infections.

As for side effects, we found that the occurrence rate for side effects was 15.0%, which peaked at four weeks of treatment. The most frequently observed side effect was mild liver dysfunction that returned normal once the patients stopped taking the medicine. Other side effects, such as nausea, vomiting, skin eruption, and depilation, were also reversible which indicating that the side effects from long-term administration of albendazole were well acceptable. Previous studies also indicated albendazole could be well tolerated even with the long-term administration treatment,[14,20-24] agreeing with the overview evaluating albendazole as an anthelmintic agent by United States National Library of Medicine.[25]

In case of patients with chronic liver dysfunction who are not suitable for albendazole treatment, ivermectin could be another choice, because its effectiveness was 70% in this study. However, there were studies suggested that ivermectin appeared to have weak efficacy and should not be used for ascarid infections especially for OLM as that it was only 40% effective in reducing clinical symptoms and no

significant decrease in the blood eosinophil count.[9,26] Since there were only seven recovered cases in our data which consisted three of VLM, one of OLM, and three of NLM cases, further study is obviously required to evaluate ivermectin for LMS.

In conclusion, albendazole is safe and effective for treatment of larva migrans syndrome caused by ascarid infections. We recommend albendazole for 10~15 mg/kg/day for four weeks or even up to eight weeks and evaluate with post-treatment serological tests.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgment

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Table 1 Outcome of treatment

Medication	Recovered	Unrecovered	Recovery rate
Albendazole	192	54	78.0%
Albendazole+Other Anthelmintic	8	8	50.0%
Other Anthelmintic	11	8	57.9%
(Ivermectin)	7	3	70.0%

Table 2 Recovery rate in different administration duration

Duration	Cases	%
< 4 weeks	35/46	76.1%
4 weeks	60/76	78.9%
5 - 7 weeks	18/22	81.8%
8 weeks	61/75	81.3%
> 8 weeks	14/17	82.4%
not described	4/10	40.0%
Total	192/246	78.0%

Table 3 Symptoms of side effects

Symptom	Recovered	Unrecovered	
	Cases	Cases	Total
Liver dysfunction	21	11	32
Nausea and vomiting	1	1	2
Skin rash	1	0	1
Depilation	2	0	2
Total	25	12	37

Table 4 Onset of side effects in different administration duration

Duration	Cases	%
< 4 weeks	11/46	23.9%
4 weeks	14/76	18.4%
5 - 7 weeks	7/22	31.8%
8 weeks	2/75	2.7%
> 8 weeks	2/17	11.8%
not described	1/10	10.0%
Total	37/246	15.0%

Figure legends

Figure 1 Flow chart of the process to evaluate the efficacy of albendazole.

Figure 2 Age and sex distribution in male and female patients in recovered and unrecovered groups.

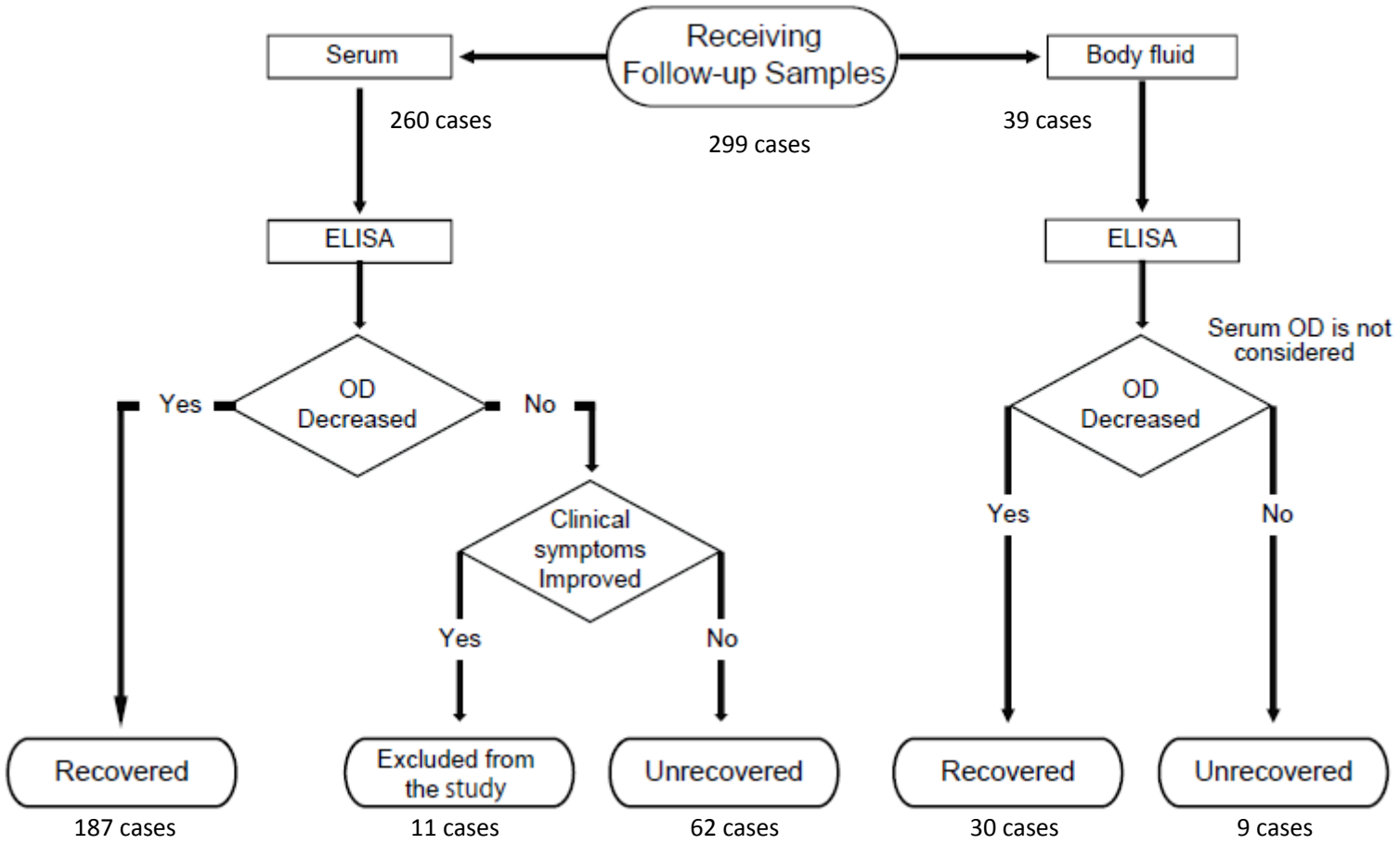

Fig. 1 Flow chart of the process to evaluate the efficacy of albendazole.

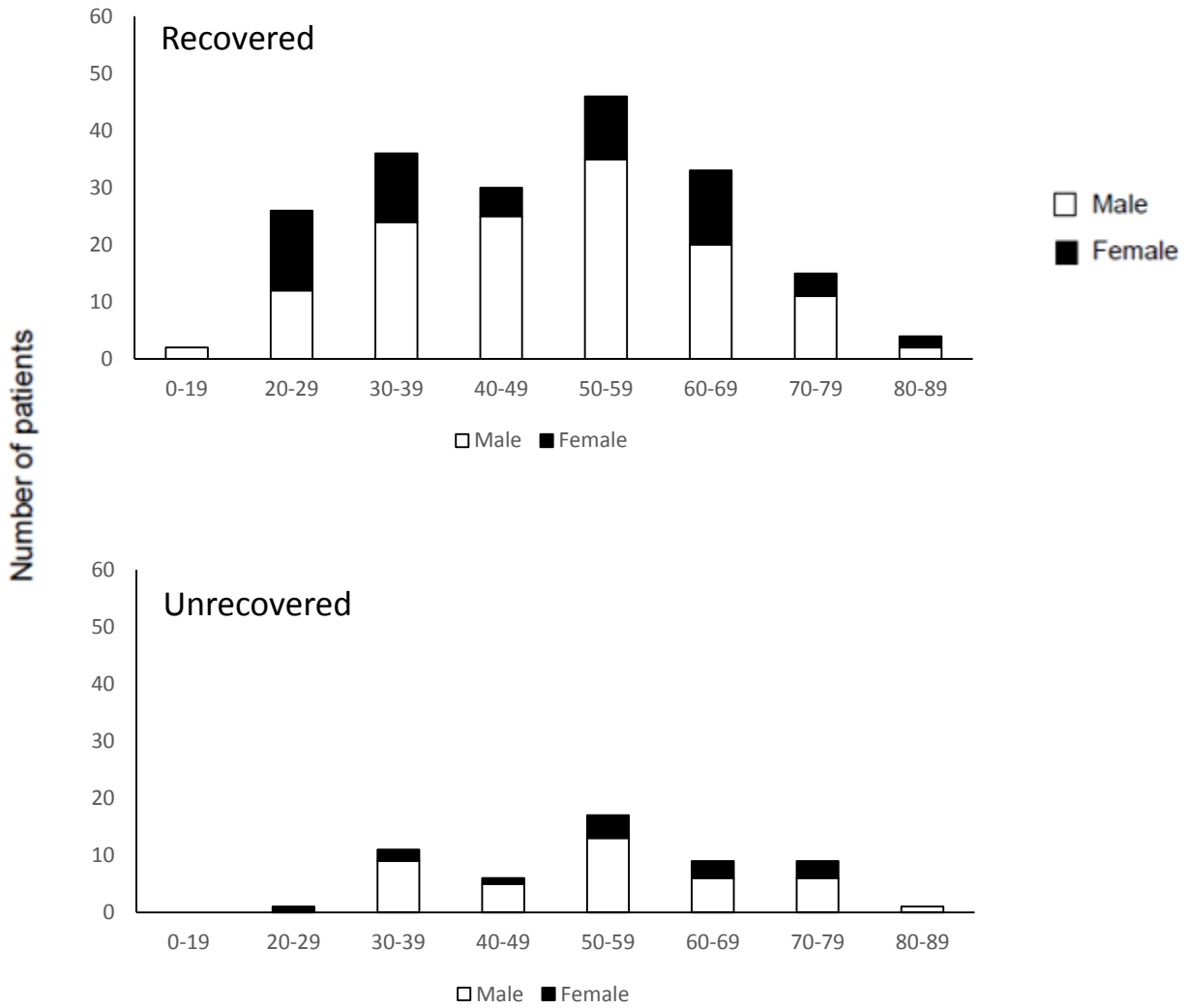

Fig. 2 Age and sex distribution in male and female patients in recovered and unrecovered groups.