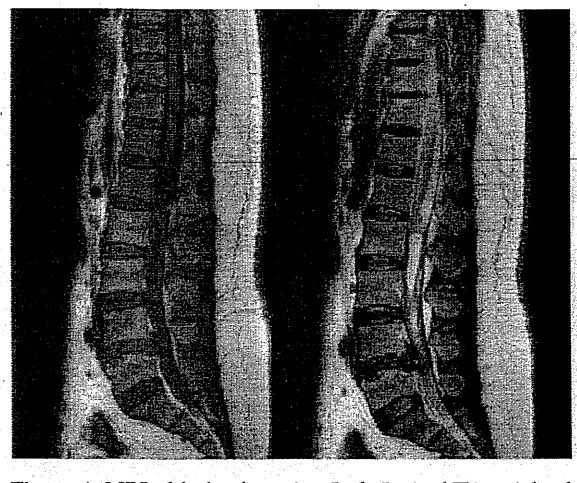

Figure 1 MRI of the lumbar spine. Left: Sagittal T1-weighted image before and after gadolinium administration disclosed the presence of two separate teardrop-shaped cystic structures beginning at level L1 and extended down to L4 with displacement of the roots peripherally. Right: Post-contrast images demonstrated there is peripherally an avid ring of enhancement along the cysts.

She recovered from the surgery uneventfully, and at a 3-month follow-up visit she complained of mild residual left leg numbness and weakness in the legs after prolonged standing. She had subjective decrease in light touch sensation on the left lower leg compared with the right and strength was slightly diminished on the left compared with the right leg that had normal strength. To further evaluate where the infection was acquired from, we analyzed cytochrome *c* oxidase 1 (*cox1*) of mitochondrial DNA (mtDNA) using the formalin-fixed and paraffin-embedded histological specimen prepared from the patient and stored in the pathology department in tissue blocks.[1] Comparing with the GenBank database, the sequence was completely identical to the *cox1* sequence of *T solium* from Korea and China (data not shown).[1]

Discussion

NCC is a neurologic infestation caused by the larval form of *T solium*. *Taenia solium* has a complex life cycle that requires two hosts. Humans are the only known definitive hosts for the adult cestode, whereas pigs are the natural intermediate host and humans may become accidental intermediate hosts for the larval form or cysticercus.[2] Humans acquire the intestinal tapeworm *T solium* by eating raw pork. They acquire NCC by ingesting *T solium* eggs through fecal oral contamination. In the United States, NCC has become an increasingly important emerging infection. This has largely been driven by the influx of immigrants from endemic regions.[3] Despite an increasing number of NCC cases overall, the number of spinal NCC cases remains very low.[4] The incidence of spinal NCC among

Figure 2 Photomicrograph showing fragments of degenerated wall of the cysticercal cyst. Hematoxylin and eosin, original magnification ×40 (upper) and ×200 (lower).

travelers is extremely rare. To our knowledge, there has been only one case of spinal NCC reported in the world literature that potentially developed after travel. Sheehan and colleagues[5] described a case of intramedullary spinal cysticercosis in a 16-year-old American woman who traveled to Mexico 10 years before the presentation. This patient lived just outside Washington, DC. She adhered to a Kosher diet and denied consuming pork.

For our patient, we analyzed *cox1* gene of mtDNA for the identification of the haplotype of the unstained histopathological specimens.[1] The *cox1* sequence data revealed that it was completely the same as the haplotype of Korea and China[1] in Asian genotype.[6] Since this patient has never visited Korea and China and the haplotype of *T solium* in Thailand differs from Korea and China, so far as we know it is most likely that she acquired the infection in Laos during one of her previous trips. It suggests that the haplotype of Korea and China may be distributed widely in Asia including Laos. It is unlikely that she acquired the spinal cysticercosis during her most recent trip, because the symptoms had begun before her recent trip and the parasite had already degenerated into the tissue specimen. Probably,

she had a chronic infection that became progressively symptomatic prompting her recent presentation to the hospital. This approach to use unstained pathological specimens can become a powerful tool to assess where the patient became infected, especially in the case of patients who traveled to multiple endemic countries or who had never visited such regions but got accidental infections in developed countries from some others who were either visitors from endemic areas or residents after traveling to such endemic areas.[1,7,8]

NCC can be divided into parenchymal, leptomeningeal, intraventricular, and spinal cysticercosis according to the location of involvement.[9] Most often the brain is affected and is involved in 60% to 92% of all patients with cysticercosis.[10] Spinal NCC is rare compared with intracranial NCC involving the brain, basal cisterns, and ventricles. In 1963, Canelas and colleagues[11] reported a 2.7% incidence of spinal NCC in 296 cases of NCC. Since that time, others have suggested that the incidence of spinal NCC is up to 5%;[5] however, an incidence of <1% to 3% is most often reported among more recent case series.[3,12]

A differential diagnosis of the spinal cystic lesions includes spinal tumors, epidermoid tumors, echinococcosis, arachnoid/colloid cysts, and meningoceles. Accurate diagnosis of NCC is based on neuroimaging studies, laboratory analysis of the cerebrospinal fluid, and antibody detection in the serum. A set of diagnostic criteria has been proposed to help clinicians and health workers with the diagnosis of NCC.[13] One of the absolute or gold standard criteria for the diagnosis of NCC is histological demonstration of the parasite in biopsy or operation material. Histologically, encystment of cysticercus larva is seen. The cyst is comprised of the outer layer, covered by hair-like projections. The cyst fluid is clear as long as the parasite remains alive; an invagination in its wall corresponds to the scolex of the parasite. Only a minor inflammatory reaction is seen if the cyst walls remain intact and the organism is viable. After the death of the parasite, the cyst wall and surrounding neural parenchyma are infiltrated by intense inflammatory reaction.[14]

MRI is generally better than computed tomography scanning for the diagnosis of NCC, particularly in patients with skull base lesions, brainstem cysts, intraventricular cysts, and spinal lesions. Nevertheless, an important shortcoming in the accuracy of MRI for the diagnosis of NCC is the detection of small calcifications.[2] The entire neuraxis should be evaluated to find additional lesions.[15] Immunodiagnostic tests of serum samples have been widely used to exclude or confirm the diagnosis of NCC in patients with neurological signs but in whom neuroimaging findings are inconclusive. The ELISA and immunoblots are most commonly used.[7]

Therapy must be individualized according to the level of disease activity, location, and number of parasites within the central nervous system. Given the rarity of spinal involvement, treatment recommendations were based on the published literature. According to the treatment guidelines, treatment of spinal cysticercosis is primarily surgical.[16] Nonetheless, there are anecdotal reports of successful use of albendazole and steroids without surgery.[17] Parenchymal NCC is considered to be most responsive to pharmacological intervention.[4] Surgical treatment is required in cases of spinal NCC in which patients experience severe and progressive neurological dysfunction regardless of whether medical therapy has been attempted.[4] The drugs of choice for the antiparasitic treatment are albendazole and praziquantel. Since the inflammation is a conspicuous accompaniment in many forms of NCC, corticosteroids are also concurrently used as therapy for meningitis, cysticercal encephalitis, and angiitis.

Conclusions

We described a rare case of isolated intradural-extramedullary cysticercosis treated successfully with surgical treatment. Spinal cysticercosis is not commonly seen in developed countries and should be considered in the differential diagnosis in high-risk populations with new symptoms suggestive of a spinal mass lesion. Timely diagnosis and treatment can lead to a successful outcome in patients with spinal cysticercosis. Unstained histopathological specimens are strongly recommended to be applied for confirmation of the haplotype of mtDNA which may indicate where the infection was acquired from.[1,7,8]

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Declaration of Interests

The authors state that they have no conflicts of interest to declare.

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Short communication

A possible nuclear DNA marker to differentiate the two geographic genotypes of *Taenia solium* tapeworms

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ABSTRACT

Cysticercosis caused by infection with embryonated eggs of the pork tapeworm *Taenia solium* is an important cause of neurological disease worldwide. Based on the phylogenetic analysis of mitochondrial DNA, the pathogen has been divided into two geographic clades, corresponding to Afro-American and Asian genotypes. In this study the genotyping of *T. solium* was carried out by using the nuclear DNA sequences of the immunodiagnostic antigen genes *Ag1V1* and *Ag2*. The two geographic genotypes were supported by the *Ag2* sequences, especially showing unique substitutions in each of the genotypes. It seems likely that the *Ag2* may be a novel nuclear DNA marker to distinguish the two geographic genotypes of *T. solium*.

The larval stage of the pork tapeworm *Taenia solium* is responsible for cysticercosis. Humans are accidentally infected with *T. solium* by ingestion of embryonated eggs excreted with feces of symptomatic and asymptomatic carriers harboring the adult tapeworm in the intestinal tract. The hatched embryos migrate throughout the body of humans and swine, invade mostly skeletal muscle and encyst to form larval cysticerci. The larvae can also reach subcutaneous tissue, eyes and the central nervous system, resulting in neurocysticercosis (NCC). Among human tapeworms, *T. solium* is the most important as the pathogen of emergent or re-emergent zoonosis because the NCC causes focal neurological deficits and seizures in endemic countries [1,2]. The diagnosis of cysticercosis has been done by clinical criteria, computed tomography (CT), nuclear magnetic resonance (NMR) imaging and serology [3,4]. Glycoproteins in the cyst fluid of *T. solium* have been widely used as crude antigens for serodiagnosis [5,6]. Recombinant antigens have been used for the diagnosis of cysticercosis. We demonstrated that the chimeric recombinant protein Ag1V1/Ag2 is a superior antigen for immunodiagnosis in humans and animals [7–11]. Each of the *Ag1V1* and *Ag2* is a gene encoding a low molecular weight backbone protein of the cystic glycoproteins. Our previous phylogenetic analysis of mitochondrial DNA (mtDNA) revealed that *T. solium* individuals can be divided into two geographic clades, corresponding to Afro-American and Asian genotypes [12]. During the analysis process of Western blotting, we also noticed that the banding profiles of crude glycoproteins differ among cyst fluids from geographically different origins [13,14]. In the present study, we evaluated the usefulness of *Ag1V1* and *Ag2* as a nuclear DNA marker to characterize the local isolates of *T. solium*.

A total of 7 geographic samples of *T. solium* cysticerci collected from Indonesia (Papua, former Irian Jaya), India, China, Brazil, Tanzania, Cameroon and South Africa in 1996 through 2001 were examined for this study. All the samples were obtained from muscles of domesticated pigs [14] and were preserved in 70% ethanol until DNA extraction. Genomic DNA was extracted from a single cysticercus by using a spin column kit (DNeasy tissue kit, QIAGEN). Each of *Ag1V1* and *Ag2* genes was amplified by polymerase chain reaction (PCR). Two sets of PCR primers were designed from the cDNA sequences of *Ag1V1* and *Ag2*. The primer pair Ag1V1F (5′-CTC GCT CTC ACT GTA TTC GT-3′) and Ag1V1R (5′-TTG ACA AGT TAA GCA GTT TT-3′) allowed us to amplify the genomic sequence of *Ag1V1*. The amplicons of *Ag2* were obtained by using the primer pair Ag2F (5′-CTC GCT CTC AGT GTT TTC GT-3′) and Ag2R (5′-TTG ACA AGT TAA GCA GCT TC-3′). PCR was carried out in a 50 μl reaction mixture containing 1 μl of template DNA (approximately 100 ng), each dNTP at 200 μM each primer, 1U of DNA polymerase (PrimeSTAR, TaKaRa Biomedicals) and the manufacturer-supplied reaction buffer. For PCR amplification, we employed 30 thermal cycles (94 °C for 30 s, 50 °C for 5 s and 72 °C for 30 s) for both genes. Prior to DNA sequencing, each amplified product was purified by using a PCR clean-up kit (NucleoSpin, Macherey-Nagel). The Bigdye terminator cycle sequencing kit and the ABI PRISM 377 genetic analyzer (Applied Biosystems) were used as recommended by the manufacturer. DNAs were directly sequenced by using PCR primers. In

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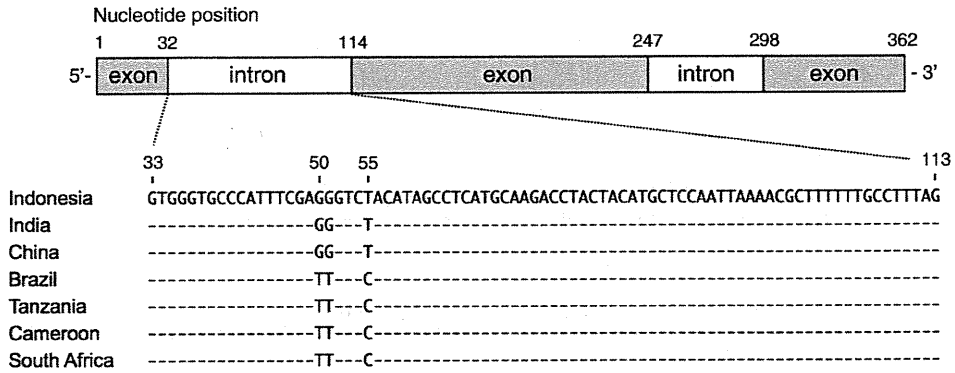

Fig. 1. The exon–intron structure of genomic PCR products for *Ag2* and their multiple alignment showing nucleotide substitutions. The Asian isolates of *T. solium* are originated from Indonesia, India and China, and the Afro-American isolates from Brazil, Tanzania, Cameroon and South Africa.

the case of low amounts of PCR products, the amplicons were subcloned into pGEM-T plasmid vector (Promega) by using an adenine tailing kit (Qiagen). Insert DNAs in the plasmid were read by T7 promoter primer (5′-ATT ATG CTG AGT GAT ATC CC-3′).

As demonstrated by Sato et al. [14], the diagnostic antigen genes *Ag1V1* and *Ag2* are universally present in *T. solium* isolates from Asia, Africa and America. In this study we could amplify the 379 bp fragments of *Ag1V1* and the 362 bp fragments of *Ag2* from all of the isolates examined. After sequencing, we found 3 haplotypes in *Ag1V1* and 2 haplotypes in *Ag2*. Both of the genomic sequences included 2 introns. The nucleotide sequences of *Ag1V1* (DDBJ/EMBL/GenBank accession nos. AB263426–AB263432) contained 2 substitution sites in the second exon and 1 substitution site in the first intron. All of them were transitional changes from thymine to cytosine bases. The two point mutations, which were observed at position 183 in the Brazilian isolate and at position 223 in the Chinese isolate, caused amino acid changes. Although these mutations had no geographic characteristics, more studies on the genetic polymorphisms of *T. solium* in Brazil and China are necessary to determine whether *Ag1V1* is usable as a genetic marker at local level.

As contrasted with *Ag1V1*, the sequences of *Ag2* (AB263419–AB263425) were geographically informative in classifying the isolates. The exon sequences were completely identical among the isolates examined. However, there were 3 substitutions in the first intron. As shown in Fig. 1, transversional substitutions (G and T) occurred at positions 50 and 51, and a transitional substitution (T and C) further occurred at position 55. All of the point mutations were highly correlated with the Afro-American and Asian genotypes of *T. solium*, which were defined from the sequences of mtDNA [12].

The observation of parasitic material is the most important step for diagnosis in parasitology. However, morphological identification is usually difficult in the case that specimens are just a piece of the entire material [15]. The development of DNA markers for *T. solium* worldwide allows us to design a useful tool for diagnosis even if all the morphological characteristics are lost. Based on the sequences of mtDNA, PCR-based techniques such as base excision sequence scanning thymine-base reader analysis, multiplex PCR and DNA sequencing have been used for the molecular identification of adult tapeworms and metacestodes, particularly in differentiating the Afro-American and Asian genotypes of *T. solium* [10,14–16]. However, nuclear DNA markers are still required for the phylogeographic studies of *T. solium* because the maternally inherited haploid mtDNA is unsuitable to use as an ideal marker for population genetics. As shown in this study, the nuclear *Ag2* gene may serve as an alternative DNA marker to determine the geographic genotypes of *T. solium* specimens.

The identification of the genotypes is an important issue on travel medicine programs. In some cases the endemic areas of *T. solium* have natural resources that attract outer people who can become worm carriers for non-endemic areas [9]. Tracking geographic areas where taeniasis/cysticercosis patients became infected may be achieved by examining the genetic polymorphism of *T. solium* [17]. Recently, the coexistence of the Afro-American and Asian genotypes has been found in Madagascar [18]. The nuclear DNA marker of *Ag2* may be useful to detect cross-hybridization events between the two genotypes.

In conclusion, we found a possible nuclear DNA marker to differentiate the geographic genotypes of *T. solium*. More nuclear markers are needed to examine the population genetic structures of *T. solium* worldwide. A panel of the genetic markers will depict the evolutionary tracks of the parasite and humans.

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Case report

An ocular cysticercosis in Bali, Indonesia caused by *Taenia solium* Asian genotype ☆

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ABSTRACT

An ocular cysticercosis case of a nine-year-old Balinese girl in Indonesia is reported. She presented with redness and pain in the left eye and showed a cysticercus in the anterior chamber in December 2010. Morphological feature of the cysticercus removed from the anterior chamber indicated that it was an immature cysticercus of *Taenia* species with no hooklets. However, mitochondrial DNA analysis using a piece of histopathological specimen revealed it a cysticercus of *Taenia solium* Asian genotype. Serology by immunoblot and ELISA highly specific to cysticercosis was negative.

1. Introduction

Cysticercosis due to *Taenia solium* is one of the neglected tropical diseases with echinococcosis, rabies, brucellosis, anthrax, leptospirosis and several others [1]. Resurgence of cysticercosis is one of the most serious public health problems not only in developing but also developed countries [2,3]. Although cysticercosis is one of the most important life-threatening cestode zoonoses worldwide, it is endemic mainly in remote or rural areas of developing countries where local people consume pork without any adequate meat inspections in the closed communities [4,5]. Recent trends in international tourism into remote or rural areas, expansion of global business and increase of the number of transmigrants from rural to urban areas as well as increase of immigrants and refugees, however, have drastically increased the cases of taeniasis and cysticercosis even in USA and other developed countries and even in Jewish or Muslim societies [3,6,7]. Among cysticercosis, ocular cysticercosis is not so common as neurocysticercosis (NCC) or subcutaneous cysticercosis (SCC) in Asia and most ocular cysticercosis cases are simultaneously found as disseminated cysticercosis, mainly NCC and/or SCC [8–10].

We report an OCC case of a 9-year-old Balinese girl in Indonesia, confirmed by mitochondrial DNA analysis. This study was carried out after obtaining the recommendation from the Ethical Committee at Udayana University and Asahikawa Medical University.

2. Case report

A 9-year-old Balinese girl who lived in Karangasem District, east part of Bali was presented with a two-week history of redness and tearing in her left eye accompanied with pain on 1st December 2010 (Fig. 1a). On examination it was found that the visual acuity of the right eye was 6/6 but that of the left eye was lower (6/10). Slit-lamp examination showed the presence of a fibrinous form resembling a larva in the anterior chamber of the left eye. Funduscopic examination of the left eye was within normal limits. Examination of the right eye showed no abnormalities. There was no history of

☆ Nucleotide sequences determined in this report was deposited into DDBJ/EMBL/GenBank databases under the accession number of AB631045.

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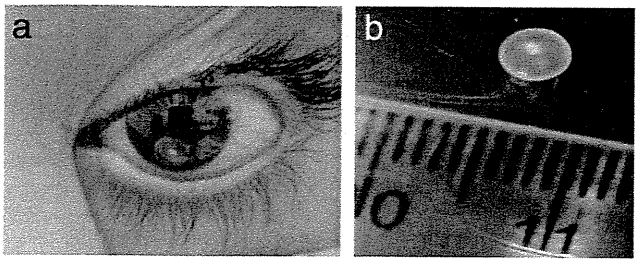

Fig. 1. An intact cysticercus of *T. solium* in the anterior chamber (a) and removed from the patient (b).

trauma, hypertension, diabetes or any neurological disorder. The larva aspirated/extracted from the anterior chamber on 13th December 2010 had the size of approximately 5×3 mm, round, white, and transparent with a white dot in the center (Fig. 1b). The size of this cysticercus was much smaller than well-developed or mature cysticercus of *T. solium*. Histopathological examination of the specimen revealed the cyst wall and the scolex with suckers and short neck region but no hooklets in the scolex, the morphological marker of *T. solium* in humans (Fig. 2). During and after the surgical treatment, the patient was given atrophine sulfate eye drop one drop 2 times daily, antibiotic and corticosteroid eye drop (tobramycin 0.3%, dexamethasone 0.1%) one drop every 4 hours, and methylprednisolon tablet 24 mg daily to prevent secondary infections and anterior uveitis. Blood samples were taken from the patient 1 week and 9 months follow-up after surgery with approval from her parents. Serologic tests carried out by ELISA and immunoblot using native antigen (purified glycoproteins) and recombinant chimeric antigen for cysticercosis [11] were negative (data not shown). Her eye functions at 9 month follow up were in the normal range.

Mitochondrial DNA (mtDNA) examination was carried out using the small piece of paraffin-embedded histopathological specimen. A few sheets of histological section were processed to extract parasite DNA using DNeasy tissue kit (Qiagen, Germany) according to the manufacturer's instruction. Short fragments of cytochrome *c* oxidase subunit I (*cox1*) gene were amplified by PCR and directly sequenced as previously reported [12], with additional outer primers Tsol-Trp-F (5′-TTT TCA AAA CAT CAA GTG AC-3′) and Tsol-Thr-R (5′-CCA TCA CAG ATT TAC AAA ATC TA-3′). Complete *cox1* gene sequence (1620 bp) was determined and compared with available sequences in GenBank database. Blast search showed the sequence was 99.9% identical to the *cox1* gene sequence of *T. solium* collected in Papua, Indonesia (accession no. AB066488).

Microscopy of the patient's stools using modified Kato-Katz method was negative for *Taenia* eggs. The stool sample was also applied for multiplex PCR [13] and copro-LAMP [14] but there was no positive result (data not shown).

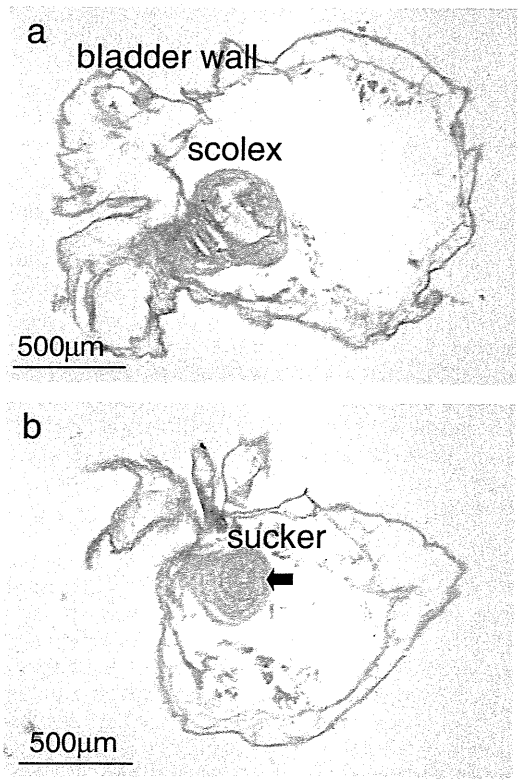

Fig. 2. An immature cysticercus of *T. solium* (Fig. 1b) stained by PAS showing scolex (a and b), sucker (b) and short neck but no hooklet (a and b).

3. Discussion

In this OCC case, the cyst was located in the anterior chamber of the left eye. Location of cysts in OCC is most often in the subretinal (35%) and least in the orbit (1%), other vitreous locations (22%), conjunctiva (22%), and anterior segment (5%) [8–10]. Patients with orbital cysticercosis are more frequently found than OCC. *T. solium* cysticercus can settle in practically all parts of the eye. OCC shows various symptoms, determined by its location, size of the cyst, host's immune status, and level of inflammatory reaction [8–10]. The viable cyst evokes minimal inflammatory response, but a fully matured cysticercus can cause compression on the surrounding structures [10].

OCC and orbital cysticercosis is very rare in Indonesia. So far we know, there is no other case report on OCC from Indonesia [15]. The most favored sites for OCC are vitreous and subretinal spaces, whereas the most common type of orbital cysticercosis is extraocular muscle form. OCC and orbital cysticercosis show various clinical symptoms such as proptosis, restriction of ocular motility, subconjunctival cyst, acquired ptosis, atypical optic neuritis, papilledema, lid nodule, subretinal cyst and intra vitreous cyst [8–10]. There are few reports describing cysticercus in the anterior chamber [16,17]. In this case, serological results even 9 month follow-up in September 2011 were negative. She had no problem with her visual acuity, no more complain about her eyes, and no headache. She could study well in the school and we, therefore, are expecting that the girl had a solitary cysticercus in the eye.

Although the gold standard to identify if a cysticercus in human body is due to *T. solium* cysticercus or not is to confirm rostellar hooklets on the scolex by histopathological examination [18], we could not confirm any hooklets from this cysticercus and the size of it was smaller than that of well-developed, mature cysticercus. Therefore, it was concluded to be an immature cysticercus [10,18]. Based on mtDNA analysis, this cysticercus was confirmed to be an Asian genotype of *T. solium* and had the almost identical sequence to that of *T. solium* from Papua [19]. Just after we confirmed this OCC case, we did a field survey in the same village in January 2011 and 9 month follow-up in September 2011 and confirmed two taeniasis carriers of *T. solium* and one pig full of cysticerci based on a real time serology carried out in the village (Swastika et al., unpublished).

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LIVER TRANSPLANTATION 17:855-865, 2011

ORIGINAL ARTICLE

Should Possible Recurrence of Disease Contraindicate Liver Transplantation in Patients With End-Stage Alveolar Echinococcosis? A 20-Year Follow-Up Study

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Liver transplantation (LT) is currently contraindicated in patients with residual or metastatic alveolar echinococcosis (AE) lesions. We evaluated the long-term course of such patients who underwent LT and were subsequently treated with benzimidazoles. Clinical, imaging, serological, and therapeutic data were collected from 5 patients with residual/recurrent AE lesions who survived for more than 15 years. Since 2004, [^{18}F]-2-fluoro-2-deoxyglucose (FDG)–positron emission tomography (PET) images were available, and the levels of serum antibodies (Abs) against *Echinococcus multilocularis*–recombinant antigens were evaluated. Median survival time after LT was 21 years. These patients were from a prospective cohort of 23 patients with AE who underwent LT: 5 of 8 patients with residual/recurrent AE and 4 of 9 patients without residual/recurrent AE were alive in September 2009. High doses of immunosuppressive drugs, the late introduction of therapy with benzimidazoles, its withdrawal due to side effects, and nonadherence to this therapy adversely affected the prognosis. Anti-Em2^{plus} and anti-rEm18 Ab levels and standard FDG-PET enabled the efficacy of therapy on the growth of EA lesions to be assessed. However, meaningful variations in Ab levels were observed below diagnostic cutoff values; and in monitoring AE lesions, images of FDG uptake taken 3 hours after its injection were more sensitive than images obtained 1 hour after its injection. In conclusion, benzimidazoles can control residual/recurrent AE lesions after LT. Using anti-rEm18 or anti-Em2^{plus} Ab levels and the delayed acquisition of FDG-PET images can improve the functional assessment of disease activity. The

Abbreviations: Ab, antibody; ABZ, albendazole; AE, alveolar echinococcosis; AgB, antigen B; CT, computed tomography; Em2^{plus}, affinity purified Em2 and recombinant EmII/3-10/Em18 antigens; FDG, [^{18}F]-2-fluoro-2-deoxyglucose; IVC, inferior vena cava; LSHV, left suprahepatic vein; LT, liver transplantation; MBZ, mebendazole; MRI, magnetic resonance imaging; MSHV, median suprahepatic vein; OD, optical density; PET, positron emission tomography; PNM, parasitic lesions/invasion of neighboring organs/metastasis; rAgB, recombinant antigen B; rEm18, recombinant Em18; RSHV, right suprahepatic vein; US, ultrasound; WHO-IWGE, World Health Organization Informal Working Group on Echinococcosis.

The authors declare that in accordance with French and Swiss law, no donor organs were obtained from executed prisoners or other institutionalized persons.

The authors have no personal, professional, or financial conflicts of interest with respect to this article. In particular, there is no relationship between GlaxoSmithKline, which currently provides albendazole, and any of the authors.

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potential recurrence of disease, especially in patients with residual or metastatic AE lesions, should not be regarded as a contraindication to LT when AE is considered to be lethal in the short term. *Liver Transpl 17:855-865, 2011.* © 2011 AASLD.

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Liver transplantation (LT) is rarely advocated as a cure for parasitic diseases. Alveolar echinococcosis (AE), which is caused by the growth of the *Echinococcus* (*E.*) *multilocularis* metacestode, is an uncommon disease with features comparable to those of slow-growing hepatocellular carcinoma.[1] AE is lethal in the absence of appropriate management. During the past 30 years, progress has been made in the management of patients with AE, and their prognosis has improved appreciably.[2] Long-term parasitostatic treatments with the benzimidazoles mebendazole (MBZ) and albendazole (ABZ) have been associated with improvements in survival.[3] The surgical removal of parasitic lesions has a favorable influence on the prospects of a cure, but this can be performed for only 30% to 40% of patients.[3] LT was proposed in the 1980s for the management of patients with symptomatic and otherwise incurable AE in whom the parasitic lesions extended to the hepatic hilum (with severe cholangitis and/or secondary biliary cirrhosis resulting) or to the hepatic veins and vena cava.[4] However, it soon became apparent that the high rate of AE recurrence, which was favored by immunosuppression,[5] was related to unresectable intra-abdominal parasitic foci (growing locally and/or invading the liver allograft) or to distant metastases of such foci (mainly in the lungs and brain).[6,7] Before 1990, patients with AE who underwent LT were not given antiparasitic drugs after LT because of the experience with benzimidazoles and their interference with immunosuppressants. Benzimidazoles were administered to most patients only as a secondary option.[4,6] Subsequently, the World Health Organization Informal Working Group on Echinococcosis (WHO-IWGE) recommended the following: (1) LT should be undertaken only as a salvage therapy for patients with AE and life-threatening complications, (2) all patients with AE should be treated with benzimidazoles as early as possible after LT, and (3) drug therapy should not be discontinued in patients with residual parasitic foci.[3]

The long-term results of therapy with benzimidazoles for residual/recurrent AE after palliative LT have not been reported. Some patients with AE are still alive more than 20 years after LT and after more than 15 years of treatment with benzimidazoles (usually ABZ in combination with immunosuppressants). Until recently, the results of neither imaging techniques nor serological follow-up provided a justification for the withdrawal of ABZ therapy. Within the past few years, new methods for assessing the viability of the metacestode have become available: [^{18}F]-2-fluoro-2-deoxyglucose (FDG)–positron emission tomography (PET) combined with computed tomography (CT)[8,9] and new serological tests based on the detection of antibodies against Em2 and recombinant EmII/3-10 antigens EmII/3-10/Em18 (Em2^{plus})[10] and Em18 recombinant antigens from *E. multilocularis*[11-13] enable improved evaluations of the response to chemotherapy and, in some cases, provide evidence of curative efficacy.[14] We report here the course of 5 patients with residual/recurrent AE lesions after LT; these patients were from a cohort of 23 patients with AE who underwent LT at 2 referral centers specializing in both LT and AE. The course for each of these 5 patients exceeded 15 years. In addition to the prospective follow-up of these patients from the time of their recruitment by one of the centers, FDG-PET/CT evaluations were carried out when this technique was introduced into the hospitals, and specific antibodies (Abs) were studied with 3 recombinant antigens of *E. multilocularis* and *E. granulosus*.

PATIENTS AND METHODS

Patients and Follow-Up

Since 1986, 23 patients with AE underwent LT in the Jura geographical area, in which the disease is endemic (22 in Besançon, France and 1 in Lausanne, Switzerland). Each diagnosis was based on the WHO-IWGE criteria.[3] Patients were staged with the parasitic lesions/invasion of neighboring organs/metastasis (PNM) classification of AE lesions.[15] All patients with AE were followed prospectively after LT: every 3 months for the first 5 years and then every 6 months. Each follow-up evaluation included a full blood count, routine tests of liver function, measurements of specific *E. granulosus* and *E. multilocularis* Abs with enzyme-linked immunosorbent assays against crude extracts from *Echinococcus* sp. and the Em2 purified antigen from *E. multilocularis*,[16] and an abdominal ultrasound (US) examination. CT scanning was performed at yearly intervals. Since 2004, all patients in the cohort who were being followed prospectively underwent at least 1 FDG-PET/CT scan. The FDG-PET/CT acquisition protocol followed the recommended guidelines: images were acquired 1 hour after the injection of FDG.[8,9,14] Measurements of blood levels of ABZ sulfoxide 4 hours after the morning dose of ABZ[3,17] (trough blood levels) and magnetic resonance imaging (MRI) examinations were performed as indicated and especially when new residual lesions, increases in the size of existing recurrences/metastases, or both were revealed by US and/or CT. Additional follow-up visits were arranged if changes in the clinical symptoms or extent of the disease occurred. Blood levels of immunosuppressive drugs were

TABLE 1. Pre-LT Data for Five Patients With AE and Residual/Recurrent Lesions

Patient Number (Sex)	Age (Years)	Location of AE Lesions in the Liver	Extrahepatic Location of AE Lesions	PNM Staging*	Indication for LT	Year of LT
1 (male)	38	Central part	Suprahepatic IVC, diaphragm, and right adrenal gland	4, 1, 0	Chronic AE-related Budd-Chiari syndrome	1987
2 (male)	54	Central part, hilum, and RSHV	Pedicle, celiac trunk, diaphragm, and suprahepatic IVC	4, 1, 0	Cholangitis	1988
3 (male)	48	Segments VI and VII	Pedicle, right prerenal fascia, right adrenal gland, diaphragm, retrohepatic IVC, and 1 lung metastasis	4, 1, 1	Cholangitis	1988
4 (male)	64	Hilum	Pedicle, retrohepatic IVC, right prerenal fascia, diaphragm, and peritoneum	4, 1, 0	Cholangitis and biliary cirrhosis	1991
5 (female)	23	Segments I-IV and VIII, hilum, LSHV, and MSHV	Diaphragm and IVC	4, 1, 0	Severe jaundice (2 months of pregnancy)	1990

*International staging system for AE lesions from Kern et al.[15] (2006).

measured twice weekly for the first month, then monthly until month 6, and every 3 months thereafter; they were also measured if any suspicion of rejection was raised by clinical or laboratory findings.

With the informed consent of the patients, serum samples were systematically taken and stored at −80°C before LT and at each visit after LT (since the beginning of follow-up in Besançon and since 2003 in Lausanne). Formal approval for the protocol was obtained from the Comité de Protection des Personnes en Recherche Biomédicale de Franche-Comté in 1997. The protocol was included as part of a research program on the immunology of AE. Initial observations showed an early increase in Ab levels in the *E. granulosus* antigen/Ab system in patients with a recurrence of AE after LT.[6] Subsequently, new specific recombinant antigens became available. Accordingly, in the month of October 2009, we measured retrospectively and in a blinded fashion anti-$Em2^{plus}$ Abs (Bordier Affinity Products, Crissier, Switzerland)[10] in Besançon and anti-rEm18[12] and anti-recombinant antigen B (rAgB) Abs[18] in Asahikawa, Japan by enzyme-linked immunosorbent assays with frozen sera obtained throughout the entire period of follow-up. For technical reasons, only 1 frozen serum sample was available for patient 4 for the period of 1999-2008. The results were expressed as optical densities (ODs) at 405 nm. The diagnostic cutoff values were 0.445, 0.116, and 0.170 OD units for $Em2^{plus}$, rEm18, and rAgB enzyme-linked immunosorbent assays, respectively.

The inclusion criteria for the present study were as follows: parasitic foci of *E. multilocularis*, LT for AE, and prospective follow-up for more than 15 years. Two situations were considered relevant: (1) the progression of residual lesions/AE (ie, the growth in the liver allograft or other organs of AE lesions known to be present when LT was performed) and (2) true recurrent lesions/AE [ie, the growth of *E. multilocularis* foci in locations (the liver allograft or other organs) in which no identifiable parasitic lesions were present when LT was performed].

RESULTS

Clinical and Morphological Follow-Up

Among the 23 patients who underwent LT, 6 died in the perioperative period and could not be evaluated for the recurrence of disease. Among the remaining 17 patients, 9 had no residual or recurrent AE. Four of these patients were alive in September 2009; the causes of death for the other 5 patients (mean survival = 8.0 years) were chronic rejection of the liver allograft (2), gastrointestinal hemorrhaging (2) [unknown origin (1) and associated ischemic cholangitis (1)], and dementia (1). Eight patients had residual or recurrent AE; 5 of these had been followed for more than 15 years and were included in this study. Four members of this group of 8 patients (1 female and 3 males) were alive in September 2009 (3 in Besançon and 1 in Lausanne). One of these 8 patients died in April 2009. The causes of death for 4 of these 8 patients (mean survival = 8.4 years) were the progression of metastatic AE to the brain (1), septic shock (2) [associated ischemic cholangitis (1) and associated bacterial infection of metastatic AE in a lung (1)], and a combination of progressive AE and complications of prostate cancer (1: patient 4 of this series). The clinical characteristics and PNM staging at the time of LT are summarized in Table 1. The median survival after LT was 21 years

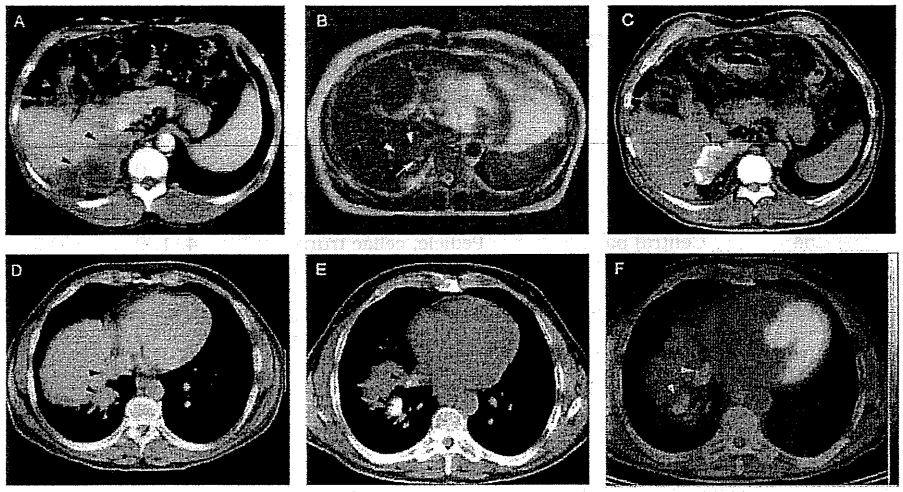

Figure 1. Residual parasitic lesions after LT for AE in (A-C) patient 1 and (D-F) patient 3. (A) Abdominal CT scan: invasion of the liver by diaphragmatic parasitic remnants (arrowheads) 2.5 years after LT. (B) MRI image (T2-weighted) of recurrence in the liver (arrowheads): pathognomonic honeycomb-like vesicles (arrow) 8 years after LT. (C) Abdominal CT scan: calcifications of the recurrent lesions (arrowheads) 22 years after LT. (D) Thoracic CT scan: paradiaphragmatic lung metastases (arrowheads) 7 years after LT. (E) Thoracic CT scan: calcifications of the lung metastases (arrowheads) 20 years after LT. (F) Combined FDG-PET/CT scan (acquired 1 hour after the FDG injection): no FDG uptake by the lung lesions 20 years after LT.

(range = 18-22 years), and the median duration of treatment with benzimidazoles was 19 years (range = 10-20 years). None of the patients received benzimidazoles immediately after LT, but all of them were subsequently treated with benzimidazoles.

Residual lesions (especially retrohepatic lesions that involved hepatic veins and the vena cava and small diaphragmatic foci that were unresectable) were left in situ by the surgeon in 4 cases (patients 1-4); metastatic lesions in a lung were already present before LT in 1 patient (patient 3; Fig. 1).

The progression of residual lesions was observed in all 4 patients before the introduction of therapy with benzimidazoles. The main clinical events and the antiparasitic and immunosuppressive treatments are summarized in Table 2.

The recurrence of AE in the liver allografts and other organs was revealed by US examinations and was confirmed by CT, MRI, or both during follow-up in 3 patients (patients 2, 4, and 5). The recurrence of AE remained asymptomatic in all patients except patient 5, who in 2003, 4 years after the diagnosis of recurrent disease, presented with relapsing syncope, dyspnea, and signs and symptoms of vena caval obstruction. Table 2 shows the times when new AE foci appeared and their locations. Recurrent disease in the liver allograft was revealed in patient 4 during his US examination, which first showed hemangioma-like hyperechogenic lesions. These lesions varied from 0.4 to 10.0 mm in diameter and had pathognomonic features according to MRI. After several months of evolution, the lesions exhibited hypoechogenicities at US examination and hypodensities on the CT scan (Fig. 2). An initial focus of recurrent disease in the liver allograft was diagnosed 6 years after LT, and a second focus was diagnosed 10 years after LT. In patient 5, LT was considered to be curative. However, recurrent AE lesions in the liver allograft (6.0 cm in diameter) and metastatic AE of a lung were diagnosed during follow-up 8 years later; other recurrent lesions in the liver allograft (segment IV, 2.0 × 1.2 cm^2) and in the apical segment of the inferior right pulmonary lobe (4.0 mm in diameter) were diagnosed 15 years after LT. Additional surgery was performed in these 2 patients. In patient 4, the initial focus of recurrence in the liver allograft was resected. In patient 5, a complex surgical procedure was undertaken in August 2004: resection of the recurrent parasitic mass and hepato-atrial anastomosis (Fig. 2). Recurrent AE lesions also occurred in organs distant from the residual foci left at the time of LT. Figure 2 shows images of recurrent disease in the spleen of patient 2.

For patients 1 to 3, the regression of lesions was apparent on sequential US and CT scans; specifically, this was shown by the stabilization or reduction of the size of the lesions and their progressive calcification after the initiation of treatment with benzimidazoles (Figs. 1 and 2). The progression of lesions led to chronic cholestasis in patient 4, who also developed kidney and prostate cancer and eventually died in 2009 18 years after LT. Lesion stabilization occurred in patient 5 10 years after the introduction of ABZ therapy.

Individual Characteristics of the Immunosuppressive Treatments

The immunosuppressive regimens administered to the 5 patients are presented in Table 2. All patients

TABLE 2. Post-LT Data for Five Patients With AE and Residual/Recurrent Parasitic Foci

Patient Number	Location of Residual AE at the Time of LT	Location of New AE Foci (Years Post-LT)	Immunosuppressive Treatment		Post-LT Parasitostatic Treatment		Evolution of Residual AE	Post-LT Survival/ Follow-Up Duration (Years)
			Basal	Acute Rejection	Drug (Time of Introduction)	Duration (Years)		
1	Retrohepatic	No	Cyclosporine, azathioprine, and prednisone (5 months) Cyclosporine and prednisone (10 years) Cyclosporine (from 1997 onward)*	No	MBZ (1989-1996) and ABZ (from 1996 onward)	7 and 13	Stabilization and increased calcification	22
2	Retrohepatic and suprahepatic IVC	Spleen (2.4)	Cyclosporine, azathioprine, and prednisone (3 years) Cyclosporine and prednisone (9 years) Cyclosporine (from 1999 onward)*	No	ABZ (from 1991 onward)	19	Stabilization and increased calcification	21
3	Pericaval and lung	No	Prophylactic anti-CD3 cure and prednisone (day 1 to day 14) Cyclosporine, azathioprine, and prednisone (2 years) Cyclosporine and prednisone (9 years) Cyclosporine (from 1998 onward)*	No	ABZ (from 1991 onward)	19	Stabilization and increased calcification	21
4	Diaphragmatic AE	Graft (6 and 10)	Cyclosporine, azathioprine, and prednisone Tacrolimus and mycophenolate mofetil†	Yes/ corticosteroid bolus (n = 1)	ABZ (1995-1999) and ABZ (2001-2009)	4 and 8	Progression and death in 2009	18
5	Apparently none 15)	Graft (8 and and lung (8 and 15)	Cyclosporine and prednisone (2 years) Cyclosporine (1992-2006) Tacrolimus (from 2006 onward)*	Yes/ corticosteroid bolus (n = 1)	ABZ (from 1999 onward)	10	Progression	20

*The dosages at the end of 2009 were 100 mg of cyclosporine per day for patients 1 to 3 and 3.5 mg of tacrolimus per day for patient 5.
†The last dosages in 2009 were 1 mg of tacrolimus per day and 1 g of mycophenolate mofetil 2 times per day.

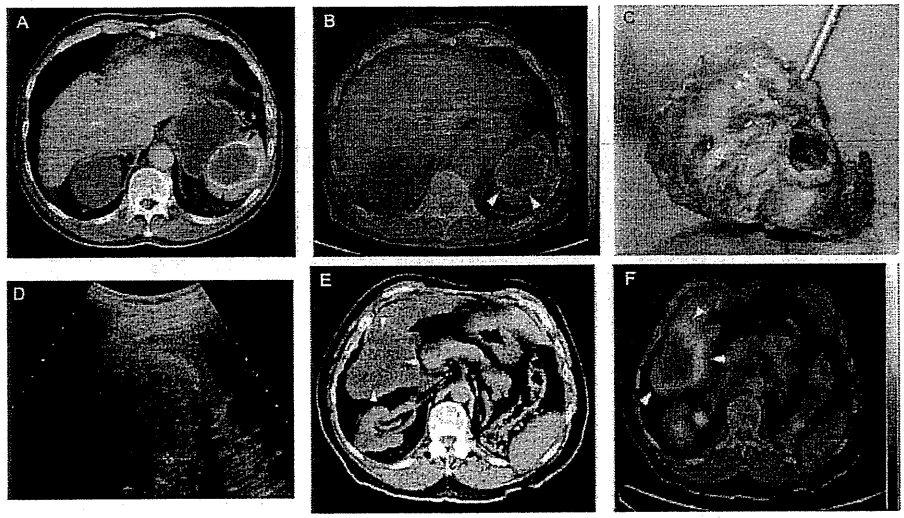

Figure 2. Recurrence of AE in the liver and spleen after LT for AE in (A,B) patient 2, (C) patient 5, and (D-F) patient 4. (A) Abdominal CT scan: calcification at the periphery of a large splenic AE lesion (arrowheads) 15 years after its discovery. (B) Combined FDG-PET/CT scan: no FDG uptake by the spleen lesion (arrowheads) and no AE lesions in the liver 20 years after LT. (C) Macroscopic image of a recurrent AE lesion during surgery 14 years after LT. (D) US examination: hyperechogenic AE recurrence (cross) in the liver 6 years after LT. (E) Unenhanced abdominal CT scan: hypodense recurrent lesion in the right lobe of the liver (arrowheads) 6 years after LT. (F) Combined FDG-PET/CT scan: high perilesional FDG uptake by the liver (arrowheads) 16 years after LT.

except for patients 4 and 5 received cyclosporine for long-term immunosuppression after cyclosporine/prednisolone combination therapy for 2 (patient 5), 9 (patients 2 and 3), or 10 years (patient 1). Patient 3 was included in a therapeutic trial of anti-CD3 monoclonal Abs from days 1 to 14 after LT. Immediately after LT, patients 4 and 5 suffered acute rejection and received methylprednisolone by a bolus injection; their long-term immunosuppressive regimens included tacrolimus. Low doses of tacrolimus were combined with mycophenolate mofetil in patient 4. When recurrent AE lesions were observed in the liver allograft and a lung of patient 5, the dosage of ABZ was increased, and the dosage of tacrolimus was reduced; thus, the trough blood levels tended to be 5 μmol/L on average rather than the recommended level of 7 to 9 μmol/L. These changes were associated with no significant effects on the tolerance of the liver allograft. In all the patients, the tolerance of the liver allograft appeared to be satisfactory over a period of 15 years, during which a limited immunosuppressant regimen was administered (Table 2). There was no evidence of chronic rejection in patient 4.

Individual Characteristics of the Treatment With Benzimidazoles

Treatment with benzimidazoles was introduced when the progression of residual or recurrent lesions was diagnosed [ie, from 3 (patient 2) to 10 years (patient 5) after LT]. Between 1990 and 1995, ABZ was administered in repeated 28-day cycles, during which the average dosage was 10 mg/kg/day (ABZ was taken during a fat-rich meal); the duration of the washout phases between cycles was 14 days as recommended elsewhere.[3] Later, after a discussion within the WHO-IWGE therapy network, ABZ was administered continuously to all patients. In addition, patient 1 received MBZ, which was introduced 2 years after LT at a daily dosage of 4.5 g/day and was withdrawn and replaced by ABZ 7 years later (Table 2). In patient 4, ABZ therapy was interrupted in 1999 (after it was administered for 4 years) when serum levels of liver enzymes became elevated; ABZ therapy (600 mg/day) was reintroduced in 2001 after another lesion of recurrent AE was found in the liver allograft, and it was well tolerated until his death in 2009.

Doses were adjusted to achieve trough plasma levels of ABZ sulfoxide of 1.5 to 2.5 μmol/L. Measurements of ABZ sulfoxide indicated that the coadministration of ABZ with immunosuppressive drugs did not influence significantly the pharmacokinetics of ABZ. Whenever they were measured, the trough levels were consistently found to be within the accepted therapeutic range in patients 1 to 4 (the data are not shown here and will be published separately). In patient 5, who received ABZ at the dosage of 600 mg/day when new recurrent lesions were diagnosed in July 2006, the trough level of ABZ sulfoxide was 1.8 μmol/L; the daily dosage of ABZ was increased to 800 mg/day, and subsequently, the ABZ sulfoxide levels were found to be 4.7, 2.9, and 2.8 μmol/L in 2006. The lesions stabilized satisfactorily, and no further side effects occurred. Two years later, however, when

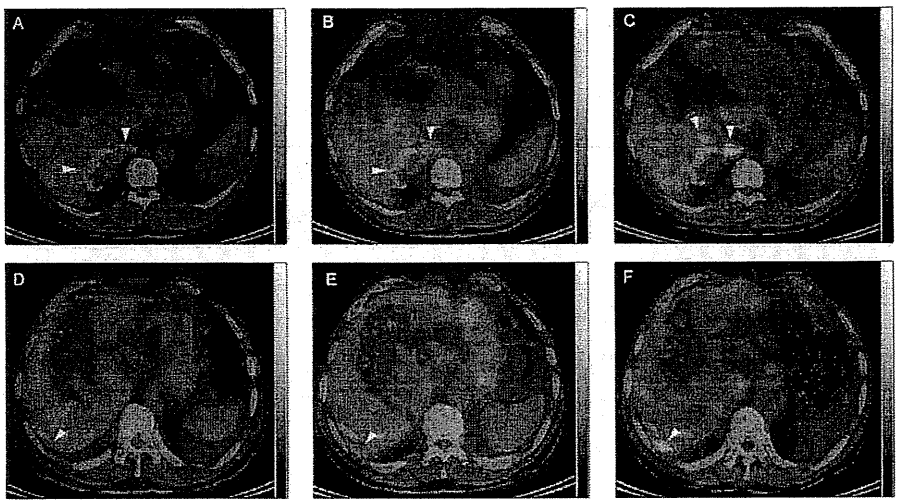

Figure 3. Follow-up of patients with residual/recurrent AE after LT with FDG-PET/CT in (A-C) patient 1 and (D-F) patient 3. (A) Follow-up FDG-PET/CT scan 19 years after LT: no FDG uptake by AE lesions. (B) Follow-up FDG-PET/CT scan 22 years after LT: no abnormal uptake 1 hour after the injection. (C) Two foci of perilesional FDG uptake in the liver 3 hours after the injection (arrowheads). (D) Follow-up FDG-PET/CT scan 17 years after LT: no FDG uptake 1 hour after the injection and a decision to withdraw ABZ. (E) Follow-up scan 2 years after ABZ withdrawal and 19 years after LT: no FDG uptake 1 hour after the injection. (F) Perilesional FDG uptake in the liver (arrowheads) 3 hours after the injection and a decision to reintroduce ABZ.

FDG-PET/CT provided evidence of new disease activity, ABZ sulfoxide was found to be undetectable in the blood, and this suggested nonadherence to treatment possibly due to minor side effects (abdominal discomfort). Lesion stabilization was attained in 2009 after the patient had been persuaded to regularly take ABZ at a dosage of 800 mg/day. In these patients, no significant side effects led to the interruption of drug therapy.

FDG-PET/CT Follow-Up

Initial FDG-PET/CT evaluations, which were conducted for patients 1 and 3 in 2004 and for patient 2 in 2005, did not show any abnormal FDG uptake by either residual or recurrent AE lesions (Figs. 1 and 3). In contrast, a PET/CT evaluation, which was conducted in 2004 for patient 4, revealed clear periparasitic uptake (Fig. 2). In patient 5, the initial PET/CT evaluation in 2006 indicated periparasitic FDG uptake by a hepatic lesion located in segment IV, but there was no abnormal uptake by metastatic pulmonary lesions. In patients 1 to 3, standard FDG-PET/CT scans remained negative from 2004/2005 to 2009. In patient 5, an FDG-PET/CT evaluation in July 2007 indicated persistent periparasitic activity of the residual hepatic lesion; the last examination in August 2009 still showed abnormal uptake of FDG, although it was decreased.

Serological Follow-Up

After LT, the development of recurrent disease was suggested by increases in the levels of Abs against crude *E. granulosus* antigens and, subsequently, in the levels of Abs against crude *E. multilocularis* and Em2 antigens, as reported previously[5] (data not shown). The time course of anti-rAgB, anti-Em2^{plus}, and anti-rEm18 Abs is shown in Fig. 4. In all patients, the levels of anti-Em2^{plus} and anti-rEm18 Abs increased early, before or at the time that imaging evidence indicated the growth of residual lesions and/or the recurrence of lesions. Ab levels reached maximal values just after the introduction of therapy with benzimidazoles and, subsequently, decreased progressively in patients 1 to 3, in whom the course of the disease was favorable; anti-rEm18 and anti-Em2^{plus} Abs reached diagnostic levels only in patients 1 and 3 after 15.8 and 8.9 years of benzimidazole therapy, respectively (median values). Conversely, the long-term persistence of elevated levels of anti-rEm18 Abs was observed in patient 4, who had progressive lesions, although the levels of anti-Em2^{plus} Abs in this case decreased rapidly to subthreshold values after the introduction of ABZ therapy. In patient 5, the levels of both anti-Em2^{plus} and anti-rEm18 Abs increased when FDG-PET/CT revealed recurrent disease activity, and the dosage of ABZ was inadequate; the levels decreased when the dosage of ABZ was increased. However, the levels of anti-Em2^{plus} Abs remained subthreshold despite disease progression. The levels of anti-rAgB Abs consistently remained subthreshold in patients 2, 4, and 5; they increased above the threshold in patient 1 when the retrohepatic residual lesion increased in size 30 months after LT. The time course of anti-rAgB Abs paralleled that of anti-Em2^{plus} Abs. In patient 3, the levels of anti-rAgB

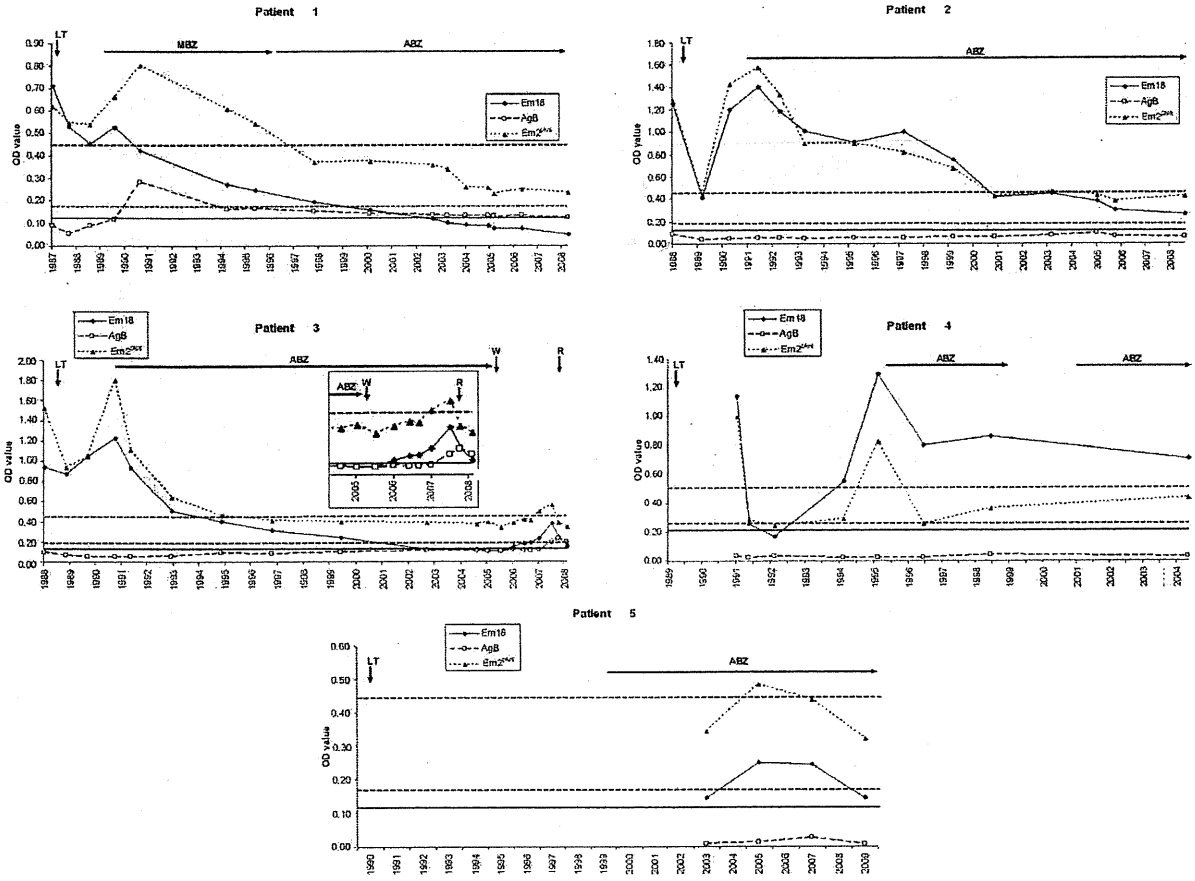

Figure 4. Time course of *E. multilocularis*–specific Abs against recombinant Em18 (Em18), recombinant AgB (AgB), and $Em2^{plus}$, in OD Units in 5 patients with residual/recurrent AE after LT. Details of the course of specific Abs during an attempt at ABZ withdrawal are provided for patient 3 (W indicates withdrawal, and R indicates reintroduction). The diagnostic thresholds are indicated by solid lines for anti-Em18 Abs, by dashed lines for anti-AgB Abs, and by dotted lines for anti-$Em2^{plus}$ Abs.

Abs did not increase at the time of the initial clinical recurrence of disease; however, their time course paralleled those of anti-$Em2^{plus}$ and anti-rEm18Abs after the withdrawal of ABZ treatment (Fig. 4).

Therapeutic Decisions Based on Serology and PET Imaging

Because the serological findings had been negative for more than 12 years and no abnormal uptake of FDG at 1 hour had been found on PET/CT scans (Figs. 3 and 4), a decision to withdraw ABZ therapy was made for patient 3 in June 2005. Follow-up examinations 6, 12, and 15 months after drug discontinuation showed rebounding increases in the levels of $Em2^{plus}$ and rEm18 Abs, although all Ab levels were subthreshold, and there was no periparasitic uptake of FDG at 1 hour on PET images. Because of the progressive increases in the levels of specific Abs during the subsequent year, PET/CT images were also acquired 3 hours after the injection of FDG in an attempt to optimize the sensitivity of FDG-PET/CT. An appreciable uptake of the radioisotope was then observed on 2 successive PET/CT scans (Fig. 3). Accordingly, treatment with ABZ was reintroduced. After peak levels were reached around the time of the reintroduction of ABZ therapy, all Ab levels decreased to the previous baseline values (Fig. 4). This patient is still being treated with ABZ and is asymptomatic. All FDG-PET/CT scans subsequently undertaken for the follow-up of patients with AE have included delayed image acquisition. FDG uptake was also demonstrated 3 hours after its injection in patient 1 (Fig. 3). In 2009, the delayed uptake of FDG was still found in patients 1 and 3 even though standard FDG-PET/CT scans yielded normal images. Thus, no decision to withdraw ABZ treatment was made for these patients.

DISCUSSION

Our study of this patient series constitutes the first report of the long-term prospective follow-up of patients who were treated for AE by palliative LT in

the 1980s. It provides evidence that this management option, despite the presence of residual and/or recurrent parasitic lesions, has allowed patients with AE (considered lethal in the short term) to survive for 20 years. Even though potent immunosuppressive therapy and nonadherence to an ABZ treatment may enhance the development of parasitic lesions, long-term treatment with ABZ seems to control the growth of residual *E. multilocularis*, and no evidence of resistance to this drug has been found. Moreover, this study emphasizes the usefulness of new tools (particularly FDG-PET/CT scans and specific serology) for the follow-up of such patients. The combination of these 2 new follow-up aids appears to be very useful in detecting recurrent disease and indicating at an early stage the efficacy of ABZ therapy; however, none of the new methodologies can reliably assess the viability of the metacestode. The delayed acquisition of PET-CT images 3 hours after the injection of the radioisotope is currently the most sensitive method for assessing the metabolic activity of lesions due to AE; specific serology kinetics, especially for anti-rEm18 Abs, seems to be promising, but the threshold levels used for the diagnosis of AE do not seem to be relevant for follow-up.

In the mid 1980s, LT was performed for the first time for incurable AE[4,19]; the patients had severe diseases (eg, chronic Budd-Chiari syndrome and hepatic hilar invasion) that led to recurrent and life-threatening biliary bacterial and/or fungal infections.[20] It was challenging to propose this management option for patients in such poor physical condition (eg, malnutrition and chronic infections). When LT was undertaken as a palliative procedure in patients with severe AE, it was associated with technical difficulties and the probability of parasitic remnants not being removed, despite the total removal of the native liver.[7] After LT, the possibility of accelerated AE progression as a result of antirejection therapy could not be fully anticipated. LT teams were concerned about potential interference between benzimidazoles and immunosuppressants because benzimidazole compounds (eg, levamisole) were known to act as immune-stimulating agents. In addition, in the period of 1986-1990, there was still uncertainty about the efficacy of benzimidazoles in the management of AE and about the potential risks of benzimidazole-induced lethal side effects, such as drug-induced hepatitis and leukopenia; accordingly, benzimidazoles were considered to be unacceptable for use in patients who had undergone LT.[3] Therefore, the first patients with AE to undergo LT did not receive benzimidazoles after LT. However, the observation of the increased growth potential of *E. multilocularis* after LT[5,21] led us to report in 2004 the European experience of LT in patients with AE (45 in all).[7] This experience indicated that LT was curative in only 50% of cases. After LT, there was an accelerated progression of residual lesions that was presumably related to immunosuppression; 5 deaths were attributable to recurrent AE in the liver allograft, brain, and/or lungs. WHO-IWGE recommendations in 1996[3] and 2010[22] proposed that LT be contraindicated in patients with residual lesions and/or metastatic disease. Since the mid 1990s, the number of patients who have undergone LT for AE has decreased substantially, especially in European countries in which the disease is endemic; only isolated examples of LT for AE in China, Turkey, and the United States have been published.[23-25] Our prospective study shows that patient survival rates after LT were similar for patients who had residual foci and were subsequently treated with ABZ (whether or not the lesions were recognized by the surgeons at LT) and for patients who were considered to have undergone curative LT. These findings strongly suggest the potential benefit of LT as a salvage therapy in patients with an advanced stage of AE, and they also suggest that LT should be offered to such patients as long as appropriate therapy with ABZ is ensured subsequently.

Replacing sequential cycles of ABZ treatment with continuous ABZ treatment and monitoring the blood levels of ABZ sulfoxide to facilitate increases in the dosage of ABZ when indicated may have contributed to the efficient control of parasitic growth in most of our patients, despite the delayed introduction of ABZ in this series. Treatment with ABZ was initiated later in the 2 patients in whom the disease was progressing. The early introduction of ABZ therapy at the appropriate dosage after LT should improve the overall prognosis.[22] In patient 4, the progression of lesions was associated with a prolonged interruption of ABZ therapy due to the possibility of side effects (increased serum aminotransferase levels). In patient 5, low plasma levels of ABZ sulfoxide might have accounted for the treatment failure. The poor response to treatment was associated initially with the low bioavailability of the drug, which was corrected by an increase in the dosage, and then with nonadherence to treatment. Thus, the regular monitoring of drug levels appears to be essential not only to enable the dosage of the drug to be adjusted but also to permit the assessment of a patient's adherence to therapy. The improved adherence of patient 5 to ABZ therapy is currently associated with her clinical improvement.

The levels of immunosuppression were higher within the first years after LT in the 2 patients whose AE lesions progressed versus those without progressive lesions; in these 2 patients, acute liver allograft rejection and the adoption of tacrolimus for long-term immunosuppression appeared to be common risk factors. In patient 3, the rapid growth of metastatic lung disease and recurrent disease in the liver may have been enhanced by the OKT3/prednisolone treatment that this patient received. This reasoning is entirely compatible with current concepts of the immunopathology of AE, which may be considered an opportunistic infection.[26] In contrast, the disease tends to be prevented by immune stimulation.[27] Improved control of the metacestode may be achieved if minimal doses of immunosuppressants are maintained. In our experience, this policy did not impair allograft survival: liver allografts were well tolerated for 2 decades in

these patients with residual/recurrent AE lesions. In patient 5, the withdrawal of cyclosporine therapy and the administration of low doses of tacrolimus were associated with the stabilization of AE lesions without the tolerance of the liver allograft being compromised. Paradoxically, a role for the parasite itself cannot be totally ruled out in the unexpectedly satisfactory tolerance of liver allografts in these patients. *E. multilocularis* and a number of other parasites induce a state of tolerance that may prevent liver allograft rejection.[26,28] The survival of heart and liver allografts is significantly prolonged in rats after a secondary infection with *E. multilocularis*.[29]

Despite important interindividual variations, correlations between serological findings based on the use of crude extracts of *E. granulosus* or *E. multilocularis* and changes in the status of the disease are acceptable for assessing the efficacy of surgical resection of AE lesions. Such serological findings are not reliable for assessing the efficacy of ABZ therapy. This point also applies to Em2-related Abs because the carbohydrate component of the laminated layer of the metacestode can persist for an appreciable time in inactive parasitic tissue.[30] Our data on anti-rAgB Abs suggest that the higher sensitivity of Ab levels based on the use of crude extracts of *E. granulosus* in predicting recurrent disease, which we have observed previously, is not related to the presence of proteins that are highly specific for *E. granulosus* but is likely due to the polyclonal nature of the Abs detected with crude extracts. Reports of immunocompetent patients with AE have suggested that levels of Abs against *E. multilocularis*–specific recombinant antigens are useful for evaluating the efficacy of ABZ therapy.[31,32] In our immunosuppressed patients, Ab levels based on the use of recombinant EmII/3-10/Em18–containing Em2^{plus} and rEm18 paralleled the clinical evolution of the disease and the results of standard FDG-PET/CT evaluations. Levels of anti-Em2^{plus} and anti-rEm18 Abs exhibited similar kinetics; variations in Ab kinetics were more informative than absolute Ab levels with respect to recommended diagnostic thresholds. This finding suggests that such thresholds are not useful when serology is used to monitor follow-up, especially in patients who have undergone long-term immunosuppression.

Until recently, it was impossible to obtain information on metacestode viability and/or disease activity or on a patient's prognosis with conventional radiological techniques. In most of our patients after several years of ABZ therapy (with or without disease progression), US, CT, and MRI follow-up indicated the stabilization of AE remnants; this included calcifications, which are considered to be evidence of parasitic death. The introduction of first FDG-PET and then FDG-PET/CT has enhanced the acquisition of data on parasite viability and the efficacy of treatment with ABZ in patients with AE, at least indirectly.[8,9,14] German and Swiss reports have shown that sequential FDG-PET/CT evaluations in patients with AE can be misleading and cannot predict an absence of recurrent disease after the withdrawal of ABZ therapy.[9,14] Patients with residual/recurrent AE after LT have not been studied previously. We have confirmed that the absence of FDG uptake 1 hour after its injection on repeated PET/CT scans of the liver does not exclude the possible reactivation of the disease after the withdrawal of ABZ therapy. In addition, we suggest that the delayed acquisition of liver images 3 hours after the injection of FDG can increase the sensitivity of the metabolic assessment of disease activity.[33] It now appears that a prospective study of the interruption of ABZ treatment in AE patients with this technical modification of FDG-PET/CT is clearly indicated.

In conclusion, this follow-up of AE patients who underwent LT more than 20 years ago confirms that long-term survival after LT with an acceptable quality of life may be expected for patients with residual/recurrent AE. Key factors for controlling the growth of *E. multilocularis* in these patients are the initiation of treatment with ABZ as early as possible after LT, the combination of its continuous administration with the careful monitoring of its blood levels, and the maintenance of the immunosuppressive treatment at its minimal levels. The combination of sequential measurements of anti-rEm18 Abs and FDG-PET/CT may suggest an uncontrolled, early recurrence of AE and enable the efficacy of ABZ therapy to be assessed. The addition of delayed PET/CT images of hepatic lesions after the injection of FDG could provide a better indication for the possible withdrawal of ABZ therapy; consequently, this should be introduced into the routine follow-up of patients with AE. Thus, possible disease recurrence should not be a contraindication to LT in patients with end-stage AE.

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