Mémoire de Maîtrise en médecine

Echinococcus multilocularis infection in solid organ transplant recipients

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Contents

Abstract

Alveolar echinococcosis (AE) is a zoonosis caused by the ingestion of eggs of the tapeworm *Echinococcus multilocularis*, causing a severe infection most often localized in the liver. Its behavior is similar to that of a malignant tumor as it invades surrounding tissues and can metastasize to distant organs. If left untreated, the mortality of AE can be as high as 90% after 10 years. In the immunosuppressed host, a higher incidence of AE has been reported. Additionally, AE seems to have a faster evolution, with more severe manifestations. However, there are very few data on the epidemiology and clinical manifestations of AE specifically in solid-organ transplant (SOT) recipients.

In this multicentric case series, we retrospectively collected *de novo* cases of AE in SOT recipients by searching the STCS database in Switzerland and the FrancEchino registry in France for cases from 01/2008 to 08/2018. We collected data about the clinical presentation, diagnosis, treatment and outcome at each center using a standardized collection form.

A total of 7 patients were identified (kidney=5, heart=1, lung=1), 5 in France and 2 in Switzerland. Six patients presented with liver AE and one with lung AE. AE was asymptomatic at diagnosis in 4 patients and presented with abdominal pain in 2 of them. One had undocumented symptoms. The median time between transplantation and diagnosis was 66 months (ranging from 12 to 240). Two patients had no liver lesions 26 and 43 months prior to diagnosis, respectively. Diagnosis was done by serology in all cases (Western-blot was positive in all 7 cases, Em2+ was positive in 1/3, hydatic fluid antigen ELISA in 4/4 and indirect hemagglutination in 3/3). Imaging was atypical in 2 cases, with a pseudo-tumoral appearance in one case. Biopsies confirmed AE in 3 cases but led to an erroneous diagnosis in one case. Four of the 7 patients were operated (all incomplete resections) and 2 died following the operation. Albendazole was started in all surviving patients and was well tolerated by all patients (tolerance undocumented in one case). AE remained stable in 3 of the 5 cases and progressed in 1 case. The evolution is undocumented in one case. One patient died of cause unrelated to AE.

The incidence of AE seems to be higher and its evolution faster in SOT recipients than in the general population. Our data also suggest that diagnosis of AE in this population is more challenging, with atypical imaging and sometimes misleading biopsies. In this series, post-operative mortality was high, perhaps suggesting that a more conservative approach is needed in this immunocompromised population.

Alveolar echinococcosis

Epidemiology

AE is a parasitic infection found in the northern hemisphere and is endemic in Central Europe, Northern America, North Asia and Eastern Europe. In Europe, estimates for the annual incidence per 100'000 individuals vary between 0.03 [1] and 0.26 [2]. Annual incidence per 100'000 persons has high as 7-98 has however been recorded in St-Lawrence island [3], mostly due to a high parasite density and the proximity of its inhabitants to dogs and foxes. AE seems to be spreading to other countries and its incidence also seems to be increasing, but it is not clear how progresses in diagnostic tools is responsible for this [4].

Studies assessing the epidemiology of AE by screening serology studies have estimated the prevalence in humans to be about 0.2% in [5], 0.1% in [6] and 0.01% in [7]. Interestingly, most patients that had positive ELISA by screening did not develop an active AE and had normal radiology or images compatible with so- called "abortive" lesions. This suggests a low susceptibility of human beings to *E. multilocularis* infection. It has been estimated that only one person in ten exposed to *E. multilocularis* will develop AE [5]. The disease seems to develop in hosts with a predominant Th2 response, with a high level of IgE and IgG production [8, 9]. Abortive lesions are most often found in hosts with predominant Th1 response [9]. Cytokines profile also seems to be different: levels of IL-10 are lower in patients with abortive lesions than in patients with progressive lesions [10]. 13% of the cases of AE in [11] are found in family clusters, bringing further evidence to a possible genetic susceptibility [9].

Prevalence of AE has also been measured in its intermediate and definitive hosts. The prevalence of AE in foxes may highly vary at a regional level, with local prevalence based on necropsy ranging from 1% to 75% in some parts of Germany [3]. Moreover, the parasite population also increases with the fox population, and as suggested by Schweiger et al [2] may cause a higher incidence of AE in humans. A 4-fold increase of fox population was indeed recorded during the late 80s and early 90s and was followed by a 3-fold increase of the incidence of AE in humans for the 2001-5 period in Switzerland. The ten years interval roughly corresponds to the estimated latency time of AE. Additionally, *E. multilocularis* was also found in urban environment, in Zürich [12], raising the question of the risk of transmission outside of rural areas.

Most patients are diagnosed in their fifties, with an identical prevalence in men and women. Several risk factors for AE have clearly been identified: being a farmer (OR 4.7) or living in rural areas (OR 7), gardening, in particular growing leafy

vegetables or roots (OR 2.5), owning a dog (OR 4.2, 18.0 if it is a hunting dog), to cite a few. A complete risk score, with eleven independent items has been developed in [13]. The most important and robust risk factor remains to live in an endemic area (RR 80) [14].

Cycle

Humans are an accidental host in the sylvatic infection cycle of *E. multilocularis*. The cycle is summarized in Figure 1. The definitive hosts are wild carnivores, such as red fox in Switzerland, although domesticated animals such as dogs and cats can also be infected. The adult worm stays in the small intestine of its definitive host and releases gravid proglottids (the mature reproductive part of the worm) containing eggs in the feces of its host.

The egg is composed of an oncosphere (the first stage of the larva) surrounded by an embryophore and a vitelline layer, which is lost when the egg is released. The embryophore makes *Echinococcus* eggs particularly resistant: they can survive months once deposited in the environment and resist to temperature below 0°C and to some commercially available disinfectants. However, desiccation is quickly fatal, killing the eggs in a matter of days. Likewise, the eggs are sensitive to high temperatures and will rapidly die if exposed to temperatures in the range of 60-80°C[15].

Small rodents (typically, the common vole) act as intermediate hosts. When they ingest eggs, the action of the digestive enzymes helps release the oncosphere from its protective membranes. The oncosphere then infiltrates the bowel walls and enters either the lymphatic or the blood circulation. From there, it first reaches the liver, although the brain, the kidneys, the lungs or other organs may also be primarily infected. Upon reaching the infection site, the oncosphere transforms into the secondary larval stage, the metacestode. Metacestodes form vesicles filled by protoscolices and a viscous liquid. The wall of the vesicles is composed of an outer acellular layer and a layer of germinal totipotent cells. Ultimately, when the rodent is consumed by a predator, the protoscolices are freed and infect the predator's bowel, ending the cycle.

Sylvatic cycle actually accounts for most of human infections, although it is still possible to be contaminated by domestic animals [17]. Indeed, owning a dog or cat may increase the risk of contamination [18]. Unlike rodents, humans are a dead-end in the parasitic cycle as very few protoscolices are produced by the metacestodes.

In humans, alveolar echinococcosis lesions can attain sizes of up to 20cm, composed of multiple vesicles. The center of the lesion is usually necrotic, due to a reduced access to nutrients. A key difference between alveolar echinococcosis and the hydatic disease lies in the growth of the lesion. While hydatic cysts remain well delimited, alveolar echinococcosis exhibits an infiltrative growth and the possibility of distant metastases. Germinal cells or small vesicles shed in the blood stream or lymphatic circulation are thought to be the cause of the metastases [15].
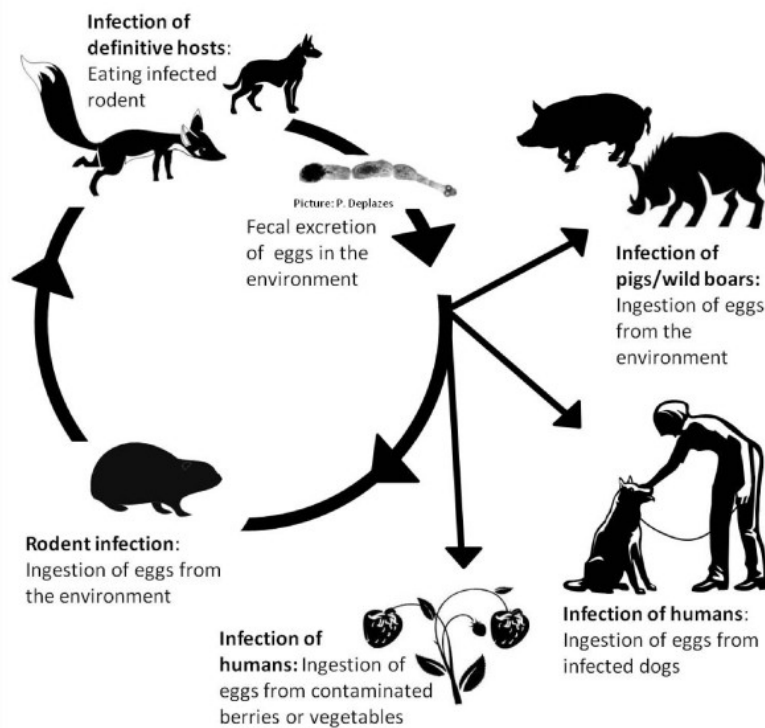
Figure 1: Cycle of *E. multilocularis*, reproduced from [16]

Alveolar echinococcosis in humans

Clinically, AE shares some characteristics with malignant tumors: the parasitic mass grows without symptoms for a latency period of about 10-15 years.   The   first symptoms are often non-specific and at the time of diagnosis, AE may have metastasized to distant organs. The only curative treatment is a complete resection of the lesion, which may not be possible if the disease has excessively spread. In case of partial resection, a lifelong treatment of parasitostatic drugs will be needed, typically albendazole. AE initially had a poor prognosis, with 70% and 90% death rate at 5 and 10 years, respectively [3]. Introduction of benzimidazole drastically improved the situation, with a ten-year survival rate of about 90% as of 2005 and a lifespan shortened by 3 years [19].

Clinical manifestations

After the infection, AE remains asymptomatic for an estimated period of 10-15 years [15]. Most patients are symptomatic at diagnosis: 73% in [20] and 71% in [11]. The other cases are either diagnosed during mass screening or are incidental findings discovered during imaging for another disease. The usual presenting symptoms   are nonspecific but are suggestive of gastrointestinal or hepatic disease: abdominal pain, jaundice sometimes with fever and anemia [15]. AE can also present with unspecific symptoms such as an isolated asthenia, a chronic cough or an allergic angioedema [11], reflecting the invasive nature of AE. Indeed, for up to one third of the patients [11, 15], the disease already affects organs adjacent to the liver and for

3

10% of patients, AE has already metastasized at diagnosis [15]. The most frequent locations for metastases are the brain, the lungs and the spleen. Infiltrative growth can damage the diaphragm, the kidney, the adrenal glands, the lungs or the pleura. Extra-hepatic lesions without liver involvement is rare, accounting for about 4% of cases [11]. In most cases, the disease starts in the right lobe of the liver [3].

Diagnostic tools

According to the WHO-Informal Working Group on Echinococcosis [3], a confirmed AE case is defined as a patient with a clinical and epidemiological history, imaging and serology compatible with AE, plus a confirmation by histopathology or PCR. This classification reflects the different diagnostic tools available to the clinician: anamnestic and epidemiological findings, the morphology of the lesions, immunodiagnostic tools and to a lesser extent, pathology and molecular tools [3]. A positive serology is not sufficient to establish a diagnosis of AE, since antibodies can be detected in patients whose immune system cleared the infection.

Imaging techniques are a cornerstone for the diagnosis of AE and for the PNM staging of the disease [21]. Unlike cancer, neither the PNM stage nor the presence of metastases seem to correlate with the prognosis [11]. This is best explained by the efficacy of albendazole to slow AE. US is the most available imaging modality, useful in the screening and follow-up, while Doppler is useful to study a potential invasion of the surrounding vessels [22]. It is however not as sensitive as CT or MRI to detect small lesions, calcifications, and to evaluate the extension of the lesions. However, contrast US using micro-bubbles seems to be promising to assess the activity of the disease [23]. CT is more sensitive than US, especially at assessing the size, extension and number of lesions and at detecting the almost pathognomonic calcifications pattern in the liver [3, 22, 23]. MRI is the best modality to study the soft tissue structure of the lesions (necrotic center, fibrous outer layer, parasitic vesicles, etc, ...) [23]. The pathognomonic "honeycomb" pattern, due to the multiple small vesicles, can sometimes be observed with MRI [22, 23]. Finally, MRI seems to be superior to CT to assess the morphology of the invasion of the surrounding tissues, making it particularly interesting for pre-operative evaluation [22]. PET-CT is also useful, particularly when checking for the presence of metastases and in the follow-up of the disease [24], but should not be used as an initial diagnostic tool as it lacks specificity [23].

Immunodiagnosis is another cornerstone technique at the disposal of clinicians to diagnose AE. Several tools have been developed over time and are summarized in Table 1. One of the issue with AE serologies is their important cross-reactivity with *E. granulosus*, other parasitic disease and even cancer [25, 26]. Some of those serologies use crude antigens, usually offering a good sensitivity, some others use purified or recombinant antigens, improving the specificity at the cost of a lower sensitivity. Finally, whole larval antigens are also used in Western blots, offering good results. Indirect hemagglutination assay (IHA) uses *E. granulosus* antigens, but can also be used in AE. Em2 and Em2+ (Em2+ is a serology that associates purified Em2 antigen and recombinant II/3-10 protein [27]), Em18 and Em4 are actually all degradation products of a larger protein, Em10 [28]. Also, some of those serologies have proven to be not only useful in the diagnosis, but also in the follow-

Serology	Sensitivity	Specificity	Remarks
IHA [25]	74-94%	ND	Uses crude antigens.
EgHF [27]	97%	61%	Crude antigen ELISA based on hydatic fluid.
Em2+ [27]	97.1%	99%	Two-antigens ELISA: purified Em2 and recombinant II/3-10. Used for follow-up : fall in antibody titers may indicate a complete resection [29].
Em2 [27]	89%	100%	
Em18 [26]	97%	74%	Not commercially available. Titers reflect PNM staging. May be used for follow-up [29].
Western Blot [25]	96%	88-100%	Several patterns are specific for AE and can be used to differentiate the forms of echinococcosis

Table 1: AE serologies

up of the disease, in conjunction with imaging techniques. This is typically the case for Em18 and Em2+ [29].

Finally, biopsies can also be helpful for the diagnosis of AE, particularly when both serologies and imaging have unclear results. However, diagnosis puncture are best avoided as they carry the risk of parasitic mass dissemination [15]. Histopathology may highlight the periparasitic granuloma and the parasitic vacuoles, while real-time PCR may be used to assess the viability of the parasites and PCR detect the presence of specific nucleic acids [15].

Treatment

Treatment of AE follows the same principles as the treatment of cancer: complete resection should be attempted whenever possible, as it is curative. When the lesion if inoperable, a lifelong chemotherapy is indicated.

PNM staging is a good help in choosing which treatment should be used. P1N0M0 or P2N0M0 stages should undergo surgery, as there is good chances of a complete resection [30], it is however possible for only about 30% of patients [31]. Despite a complete resection, those patients should still follow 2 years of benzimidazole treatment due to the risk of having remaining viable parasitic tissue after the surgery. For inoperable patients, a lifelong treatment of benzimidazoles is indicated. Partial resection of the parasitic mass has not shown any benefits and should be avoided [30]. However, palliative surgery or percutaneous interventions may be necessary due to secondary manifestations of AE: liver abscess, bile duct obstruction, etc, ... Liver transplantation may also be indicated in some specific cases, when resection is not possible or in case of hepatic failure. However, absence of any extra-hepatic le- sion is mandatory before considering liver transplantation, due to the risk of relapse in patients with an immunosuppressive treatment [9, 30, 32].

Currently, the chemotherapy of choice for AE is albendazole, with the usual posology of 400mg twice daily with a fat-rich meal. An alternative is another molecule of the same class, mebendazole, taken three times daily and usually given when albendazole is not tolerated. Benzimidazoles may have serious hepatic and hematologic (neutropenia) adverse effects and may also cause alopecia [3]. Liver function and haemogram should be performed to monitor toxicity every two weeks at the beginning of the treatment, with the possibility of making the controls less frequent when the treatment is well tolerated [3, 30]. However, benzimidazoles are

only parasitostatic, justifying the lifelong treatment [33]. Despite this, abortive lesions in previously inoperable patients treated by chemotherapy were reported [24]. Such patients would benefit from an interruption of the treatment, the difficulty being to assess the viability of the parasitic lesions. Standard imaging techniques are useless for this purpose [24], although certain MRI patterns have been shown to correlate with glucose uptake in PET-CT [34]. Serologies, in particular Em2+ and Em18 [29] and PET-CT with delayed acquisition [24, 35] have been shown to be useful as surrogate markers, particularly when used together and can be used to safely interrupt benzimidazoles treatment in some patients [24, 36].

Follow-up should include monitoring of the benzimidazole concentration in the blood, at least initially and every time the posology is changed [30]. Imaging should also be conducted regularly to ensure regression of the lesion. US may be done frequently while and MRI/CT is advised every 2-3 years [30].

Alveolar echinococcosis in immunocompromised patients

Illustrating the importance of the immune system to control and eradicate AE, up to one fourth of the patients currently diagnosed with AE were immunocompromised [11]. Moreover, clinical manifestations, diagnosis and even radiologic manifestations have been shown to be modified in SOT recipients [32, 37, 38, 39, 40]. AE seems to develop faster, have a higher incidence, to sometimes have atypical imaging mimicking cancer [32, 40] or with abscess-like appearance [32]. Serologies also seem to be less reliable for those patients. AE is more often a fortuitous discovery, some- times even discovered while hepatic surgery is performed [32, 37, 40]. Cases of AE were also reported in AIDS patients [41, 42], also showing a fast development of the lesions and falsely negative serologies.

Conventional treatment with surgery and benzimidazole has been shown to work well for those patients, although albendazole was found to have more frequent side effects in immunosuppressed patients [32]. This was however not the case in two others studies in liver transplant patients [43, 44], despite the fear of interaction between albendazole and immunosuppressive drugs.

To date, no study has been done specifically on AE in SOT recipients. The only available data are case reports and studies of broader scope on AE in immuno-compromised patients. With this work, we aimed to determine the clinical manifestations, the epidemiology and the specificity of the management of AE in SOT recipients. As demonstrated in previous studies and case reports [32, 37, 38, 39, 40], we expect to find a higher incidence as well as a faster evolution of AE in SOT recip- ients. We also expect to find the same diagnosis challenges as reported in previous papers: less reliable serologies and less typical imaging. Studies have had contra- dicting results about albendazole tolerance in SOT recipients, making hypothesis about this particular point is thus difficult.

Methods

Study design

This is a multicentric retrospective case series grouping cases of AE in France and Switzerland. To identify patients, we searched the STCS (Swiss Transplant Co- hort Study) database with keywords "ecchino", "echino", "bandwurm", "zoonose", "parasite infection", "echinococcus" and "other parasitic infections". In France, the search was performed on the FrancEchino registry, looking for patients with the keywords "graft" and "transplant". Only solid-organ transplant recipients were se- lected: patients that underwent bone marrow transplantation were excluded. We also excluded patients that had liver transplantation in the context of AE. Patients were only included if the diagnosis of AE was made between start of 2008 (creation of the STCS, to have reliable data to estimate the incidence) and 08/2018 (end of the data collection period).

Information about cases was then retrieved from the patients file by mean of a standardized form. In Switzerland, a few cases with cystic echinococcosis had to be filtered out at this point as they had been selected by the echinococcosis keyword. The form includes information about the transplantation, the immunosuppressive drugs and if present, the last serologies and imaging that showed the absence of AE. The clinical presentation, the results of the radiologic examinations and of the laboratory works, the treatment and the outcome of AE were also documented.

The STCS cohort and the FrancEchino registry

The FrancEchino network aims to group all AE cases in France in a national registry, the FrancEchino registry [45]. The registry is managed by the National Reference Center for Alveolar Echinococcosis, in Besançon. Cases have been registered retro- spectively for the 1982 to 1996 period and prospectively since then. The registry included 691 patients as of 2017. Since there is no legal obligation to declare AE cases, the FrancEchino network detects new cases by monitoring the prescription of albendazole by pharmacies and by cooperation of hospitals in endemic areas (hospi- tals of the FrancEchino network). They also work in cooperation with the pathology laboratories.

The STCS includes all solid organ transplant recipients in Switzerland since 2008, which represents more than 5000 patients at the end of 2018. The database includes clinical information and biological samples that are prospectively collected at baseline, 6 months and every year by transplant physicians in all 6 transplant centers of Switzerland [46]. This follow-up of patients has been made mandatory by law since 2007. Collected information notably include all infectious disease episodes

occurring post-transplantation.

Statistical analysis

We intended to compare the data for the SOT recipients population to the data for the general population. However, statistical analysis would not be significant with such a small number of patients. This is therefore a descriptive study of a small case series.

Results

Patients population

A total of 7 patients were identified: two in Switzerland and five in France. Five of them were kidney transplant recipients, two of which had to be transplanted a second time (for causes unrelated to AE). The other two patients were lungs and heart transplant recipients, respectively. The calculated incidence in the STCS cohort was 5 cases per 100'000 patient-year, while it was not possible to calculate the incidence in the FrancEchino network. Median age at diagnosis was 57 years old (range 46-66). There were 5 women and 2 men in this population of patients. Immunosuppressive treatment was documented for 5 patients, with exact doses available in four of them (Table 2).

Clinical presentation

AE was a fortuitous radiologic discovery in otherwise asymptomatic patients in 5 cases and presented with abdominal pain for the other two patients. The primary lesion was hepatic in 6 cases and pulmonary in one case. In this latter case, patient suffered of hepatorenal polycystic disease, making it difficult to assess the presence of hepatic AE. In the two symptomatic cases, AE had extended into the pancreas in one case and in the stomach and diaphragm in another case. The lesions were otherwise localized in the liver. The two patients with a $N > 0$ staging were both symptomatic at diagnosis.

ID	Prednisone	MMF[1]	Cyclosporine	Tacrolimus	Δt[2]
1	5mg	1500mg	-	1mg	43
2	5mg	-	-	3mg	12
3	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	240
5	7.5mg	2000mg	175mg	-	117
6	?	Unk. pos.	-	Unk. pos	57
7	20mg	2000mg	150mg	-	75

[1] Mycophenolate mofetil

[2] Δt is the interval in month between the transplantation and the diagnosis of AE

Table 2: Immunosuppressive treatment (daily doses)

ID	Transplant organ	Primary lesion	PNM stage	Extension
1	Lungs	Liver	P1N0M0	No
2	Kidney[1]	Liver	P1N1M1	Diaphragm and stomach
3	Kidney	Liver	ND	ND
4	Kidney[1]	Liver	P1N0M0	No
5	Heart	Liver	P4N1M0	Head of pancreas
6	Kidney	Lungs	PXN0M1	No
7	Kidney	Liver	P1N0M0	No

[1] Were transplanted twice (for reasons unrelated to AE)

Table 3: Characterization of the lesions

ID	Western blot	Em2+	Others
1	Positive for *E. multi.*	Negative	Positive EgHF
2	Positive (no details)	-	-
3	Positive, band 7 & 26-28[1]	Positive	Positive EgHF
4	Positive, band 7 & 26-28[1]	-	Positive IHA
5	Positive, band 16 & 26-28[2]	Negative	Positive *E. gran.* ELISA
6	Positive, band 7 & 26-28[1]	-	Positive IHA & *E. gran.* ELISA
7	Positive, band 7 & 26-28[1]	-	Positive IHA

[1] Those bands are shared by *E. multilocularis* and *E. granulosus*
[2] Band 16 is more specific of *E. multilocularis*

Table 4: Serologies

Diagnosis

Median time between the transplantation and the diagnosis is 66 months (range 12-240). Imaging showed the absence of liver lesions in two patients, 26 and 43 months prior to diagnosis, respectively. For the latter patient, a serology performed 50 months prior to diagnosis was also negative.

Diagnosis of AE was faster in the symptomatic cases than in the asymptomatic cases (0 vs 7.5 months, range [1 30], the duration being the interval between the initial radiologic finding and the diagnosis). The pulmonary lesion was mistaken for tuberculosis based on a wrongly interpreted biopsy and anti-tuberculosis treatment was initiated, with important interactions with the immunosuppressive drugs. The results of the serologies are summarized in Table 4. Western blots were positive in all cases, with detailed results available in 6 of them. They allowed a discrimination between *E. multilocularis* and *E. granulosus* in 2/6 cases (33%). The Em2+ serology was positive in only 1/3 case (33%). Crude antigen serologies (EgHF, IHA and *E. granulosus* ELISA) were always positive when tested.

MRI were performed on all patients. In two cases, the imaging was atypical for AE : it had a pseudo-tumoral appearance in one and was similar to a metastasis in the other. Histopathological examinations were conducted in four cases. They were positive in two cases, inconclusive in one and led to the wrong diagnosis in one case (but ultimately hinted at the correct diagnosis after a second look).

Patient ID	Δt^1	Imaging[2]	Serologies[2]	Outcome
2	43	MRI (31 & 43): stable	Unk. res.[3] (19 & 42)	Stabilization
3	42	MRI (30 & 42): progression	-	Progression
6	18	CT (6 & 12): stable	Titers lowering[3] (18)	Stabilization
7	48	MRI (18): stable	-	Stabilization
		PET-CT (54): stable		

[1] Duration of the follow-up in months
[2] With the timing of the examination in parentheses (in months)
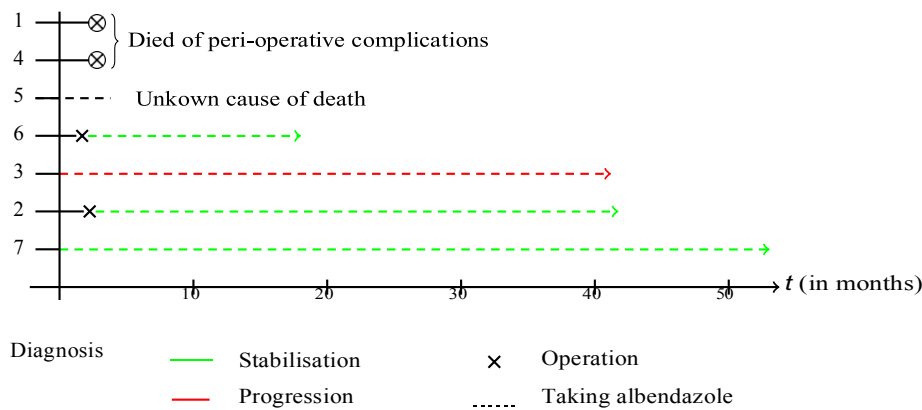[3] Exact serology unknown

Table 5: Follow up

Figure 2: Evolution

Treatment and outcome

The outcome and treatment of the 7 patients of this case series are summarized in Figure 2. Four of the seven patients were operated on. However, complete resection was not possible in any of them. Two of those patients died of complications of the operation. One additional patient died five months after the diagnosis. The exact cause of the death is unknown. All surviving patients started a treatment of albendazole at 400mg *bid*, which was well tolerated. In particular, no significant liver function tests perturbation was reported and the doses of albendazole did not have to be changed for any of the patient.

Information about the follow-up was available for four of the surviving patients and is summarized in table 5. It included MRIs, serologies, PET and CT. AE was stable for three patients and progressed in one patient. However, complete information on the follow-up was scarce, with a median follow-up duration of 42 months.

11

Discussion

This multicentric case series on AE in transplant patients is the largest to date. Despite low numbers, it somewhat concurs with previous studies and case reports about a potential increased incidence [32] and faster evolution of AE [32, 40] in SOT recipients. This study also highlights the diagnostic challenges, particularly regarding some cases of falsely positive and falsely negative imaging and serologies. Finally, we observed that albendazole seems to be well-tolerated and effective even in SOT recipients and that peri-operative mortality seems to be higher in this population, although the low number of cases does not allow to extract any firm conclusion.

We estimated the incidence in Switzerland to about 5 new cases per 100000 person-year in SOT recipients, while the incidence in the general population (in endemic areas) ranges between 0.03 and 0.27 new cases per 100000 person-year. Incidence in SOT recipients would thus be between 10 - 100 higher than that of the general population, although these numbers are approximate and should be taken with caution given the low number of patients with AE in the STCS.

The clinical presentation of AE in our series is different to that of the general population: while only 2/7 (28%) of SOT-recipients were symptomatic at diagnosis, 75% experience symptoms in the general population. Discovery of AE was thus more often fortuitous in the SOT recipients population. A more common asymptomatic presentation in immunocompromised patients was also reported in another study [32], probably reflecting a closer follow-up of SOT recipients with more access to imaging techniques. The distribution of the PNM stages presents a more biphasic distribution than in general population [11], with 3 patients with stage I AE and 3 with stage IV. About the same proportion of low PNM stages was also observed in the study by Chauchet et al [32] (41% vs 23% in the general population), and again, this is probably a consequence of an earlier (and fortuitous) detection of AE. The biphasic distribution is probably caused by the important immunosuppressive regimen administered to SOT recipients: it likely enhances the progression of AE, which develops more quickly from a low stage disease to a symptomatic and high- grade disease. The short median interval between transplantation and diagnosis (66 months vs 84 for SOT recipients in [32]) and the proof of absence of AE for two patients 26 and 43 months prior to diagnosis bring further evidence of a faster evolution than the usual 10-15 years of latency.

As already demonstrated in previous studies and case reports [32, 37, 39, 40], diagnosis of AE is more challenging in the setting of immunosuppression. In two case reports [37, 40], the diagnosis was made during surgery for a suspicion of hep- atic tumor. In patient 6 in our series, an initial misdiagnosis of tuberculosis was made after a biopsy was incorrectly interpreted, and anti-tuberculous drugs were

prescribed, with important interaction with immunosuppressive drugs. These cases illustrate the difficulty of diagnosing a rare disease with atypical presentation. Incidental findings in asymptomatic patients and atypical imaging of AE probably account for the diagnostic delays (median of 7.5 months vs 11 in [32]) in immuno-compromised patients. Serologies are also affected by the immunosuppression. The usually effective Em2+ seems to lose sensitivity, while the Western-blot, usually specific and able to differentiate alveolar and hydatic echinococcosis, seems to lose its specificity. Specie-specific antigens, as used in the Em2+ serology, indeed seem to be less immunogenic than the antigens common for all the Echinococcus genus, losing some of their diagnostic value. We however reach the same conclusion as the study of Chauchet et al [32]: serologies using whole larval antigens or *Echinococcus* antigens are more reliable in this setting. In the context of hepatic lesion in SOT recipients with risk factors, AE should thus be included in the differential diagnosis and ruled-out, ideally by Western-blot. Interestingly, immunosuppression regimens that include Tacrolimus were linked to faster evolution of AE, although it is impossible to formally prove in such a small group of patients. With the importance of cellular immunity to stop or slow-down AE, it is expected that drugs inhibiting T lymphocytes would speed the disease.

Regarding the therapeutic approach to AE, we observed two important issues. First, surgery was complicated in SOT recipients, with a peri-operative mortality of 50%. These figures can be due to particularly difficult surgeries in these two patients, but may also reflect the frail conditions of transplant recipients. In addi- tion, no complete resections could be obtained with those patients, despite the low PNM stages in the majority of them and despite available pre-operative imaging. Similar problems were observed in a cohort of liver transplant recipients due to AE [44]: surgeons noticed during the operation that not all parasitic tissue would be removable. Second, long-term use of albendazole seems to have been well supported, without any major interaction with the immunosuppressive drugs. Previous study had contradicting conclusions about albendazole tolerance: adverse effects were frequently reported in the immunosuppressed patients in the study by Chauchet [32]. Albendazole was however well tolerated after liver transplantation in the context of AE [43, 44]. Similarly to previous studies [32], albendazole was effective, allowing a stabilization of the disease in 75% of patients.

A discontinuation of albendazole therapy has been proposed in selected immuno-competent patients, 2 years after radical surgery or in case of absence of parasite viability assessed by PET-CT and specific serology (Em2+) [24, 36]. The decision to stop albendazole is even more challenging in SOT recipients. Firstly, Em2+ appears to be less useful in this setting (loss of sensitivity due to the immunosuppression in our series and in the study by Chauchet et al [32]). *E. granulosus* IgG ELISA has however been cited has a potential alternative to Em2 for the follow-up of SOT recipients [43]. Secondly, the performance of PET-CT for assessing the activity of AE has not been extensively evaluated in immunocompromised patients. Metabolic uptake around the parasitic lesion is thought to be mainly caused by cell mediated immunity and therefore, we may wonder whether the absence of uptake is due to the efficacy of albendazole or to immunosuppression.

Our study has several limitations. AE being a rare disease, we were able to

study a limited number of patients in the setting of solid-organ transplantation and, therefore, conclusions in our study should be taken with caution. The lack of a comprehensive transplant cohort (such as the STCS) in France prevented the evaluation of the whole incidence of AE in the 2008-2018 period for both countries. A collaborative registry of AE in SOT recipients grouping all endemic countries would be ideal, as it would reach a larger population pool than national registries Finally, we were not be able to obtain more epidemiological data on those patients, particularly about risk factors and compare them to those found in the general population. The biggest studies about AE were realized either retrospectively or thanks to registry like the FrancEchino. Unfortunately, such registry doesn't exist at the European level. A collaborative registry grouping all endemic countries would be ideal, as it would reach a bigger population pool than national registries.

Conclusion

This case series confirms previous findings about a higher incidence of AE in SOT recipients. Diagnosis is difficult with usual serologies unreliable, imaging sometimes atypical and asymptomatic patients concurring to cause a diagnosis delay. In the presence of hepatic lesions in SOT patient with risk factors (*e.g.* living in endemic area), it seems reasonable to search for AE with Western blot or indirect hemagglutination. The high perioperative mortality and the difficulty to completely resect the lesions in our cases suggest that a conservative treatment should be favored in SOT recipients. The efficacy of albendazole in this population and the absence of interactions with immunosuppressive drugs are further arguments in favor of this type therapeutic strategy.

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