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Unusual multiple large abscesses of the liver: interest of the radiological features and the real-time PCR to distinguish between bacterial and amebic etiologies

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We report a rare case of amebiasis generating 19 large liver abscesses. Such a quantity of abscesses is rare, especially when occurring in a young casual traveler without any immunodeficiency disorders. A possible co-infection was excluded. By contrast, the amebic etiology was confirmed by means of serology and real-time PCR.

Keywords: Multiple abscesses, Amebiasis, Computed tomography, *Entamoeba histolytica*, PCR

Case Report

We report the case of a 36-year-old Caucasian man with febrile diarrhea who presented with a loss of appetite and a severe asthenia. His past medical history was insignificant, and he was up to date with his vaccines. As military personnel in the French army, he had frequently traveled to the French West Indies, French Polynesia, and Senegal during the last 10 years. In the month preceding his hospitalization, he went on a trip to Dakar, Senegal, and then to Martinique in the French West Indies. During these trips he did not take his anti-malarial medical treatment correctly (chemoprophylaxis atovaquone–proguanil). He presented with a fever of 38–40 C, chills, sweats, and myalgia, which were not relieved by taking paracetamol, 4 days after his return to mainland France. The lab report excluded malaria. Parasitological examination of stools and bacterial coprocultures remained negative.

Despite the empirical use of antibiotics (amoxicillin–clavulanic acid), the patient’s symptoms became

more severe with hepatomegaly and tender abdomen, particularly in the right upper quadrant. A biological inflammatory syndrome (neutrophil granulocytes=14.38 g/l; CRP=419 mg/l) and a hepatic cytolysis with moderate cholestasis were noticed (AST=91 UI/l; ALT=44 UI/l; ALP=108 UI/l; gamma-GT=66 UI/l). Ceftriaxone was thereafter added to his treatment. The ultrasound scan showed multiple liver hypoechoic lesions with echoic walls, and a hypertrophy of the right liver (length × depth=20 × 12 cm) (Fig. 1). An abdominal computed tomography (CT) confirmed the presence of several large hypodense rounded masses (Fig. 2). The inside aspect of the masses seemed to be made up of a heterogeneous liquid, and their edges were irregular. The presence of at least 19 abscesses was observed. The largest lesion measured 96 mm and was located between the V and VI hepatic segments. The smallest one was 40 mm wide. There was no contrast enhancement after injection of intravenous contrast agent. Pan colitis and radiological cholestasis were also observed.

The serology targeting amebiasis was positive: hemagglutination positive at 1:1280 dilution (IHA

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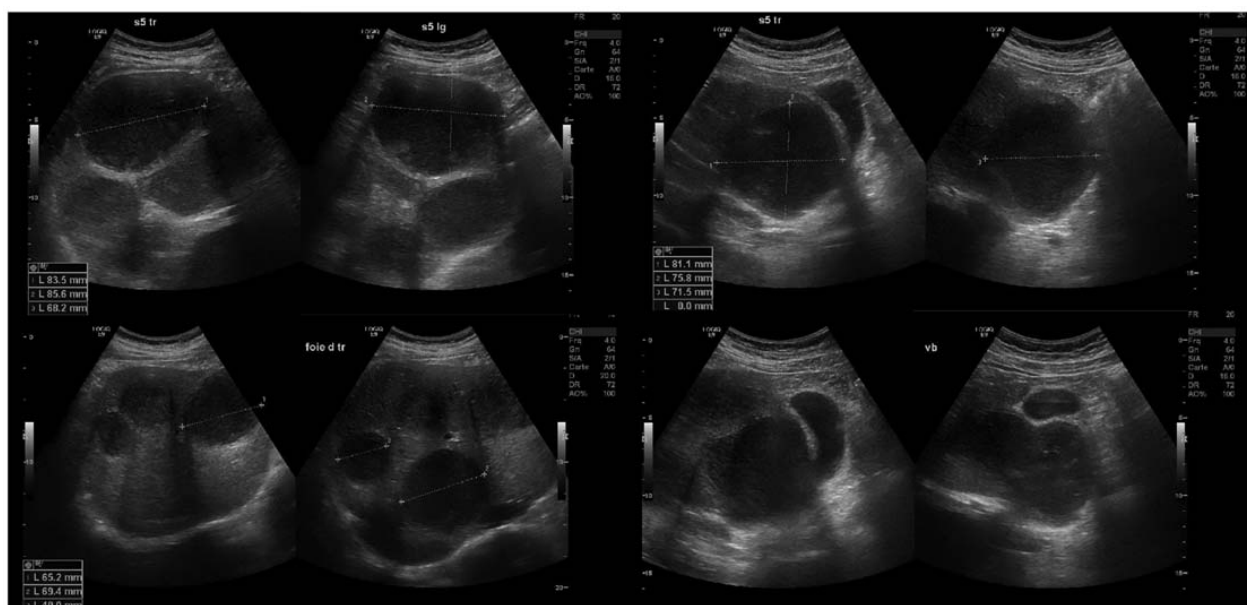


Figure 1 Abdominal ultrasonography showing multiple rounded hypoechoic lesions.

AmoebiasisTM, Fumouze Diagnostics), and indirect immunofluorescence at 1:800 (Amoeba-Spot IFTM, BioMérieux). Concomitantly, all the blood cultures remained sterile. Thereby, an anti-amebic therapy was initiated, based on metronidazole administration at 500 mg tid for 21 days, followed by a luminal amebicide (tiliquinol/tilbroquinol). Within 48 hours the patient had no more fever and his diarrhea had nearly stopped. Neutrophil granulocytes and CRP rate decreased in less than 1 week, to 9.33 g/l and 54 mg/l respectively.

As the patient was still complaining of persistent abdominal pains, a puncture of the painful abscess under the hepatic capsule was performed under CT-guidance (Fig. 3). Standard cultures and the pan-bacterial real-time PCR, targeting the 16S rDNA which were performed directly on the aspirated liquid were all negative. In contrast, the PCR specific for *Entamoeba histolytica* (16S-like SSU rDNA) was

positive ($C_t=27$ cycles; standard deviation=0.104). Briefly, the DNA was extracted using QIAmp DNA mini kit[®] spin columns (QiagenTM). The method performed for amplification and detection was a real-time PCR using a TaqMan 7500[®] Fast Real-Time PCR System (Applied BiosystemsTM). The amplification reactions were performed using 5 μ l of a DNA sample in a volume of 20 μ l of a mixture that contained a TaqMan Fast Universal PCR Master Mix[®] (2 \times) (Applied Biosystems), internal positive control (Applied Biosystems), and the primers for *E. histolytica*: Eh-196F (5'-AAA TGG CCA ATT CAT TCA ATG A-3') and Eh-294R (5'-CAT TGG TTA CTT GTT AAA CAC TGT GTG-3'), and the probe Eh-245 (6FAM)-AGG ATG CCA CGA CAA-(NFQ). The thermal cycling conditions were chosen according to TaqMan[®] Fast protocol (Applied Biosystems) and it consisted of 20 seconds at 95 $^{\circ}$ C followed by 50 cycles of 3 seconds at 95 $^{\circ}$ C, and

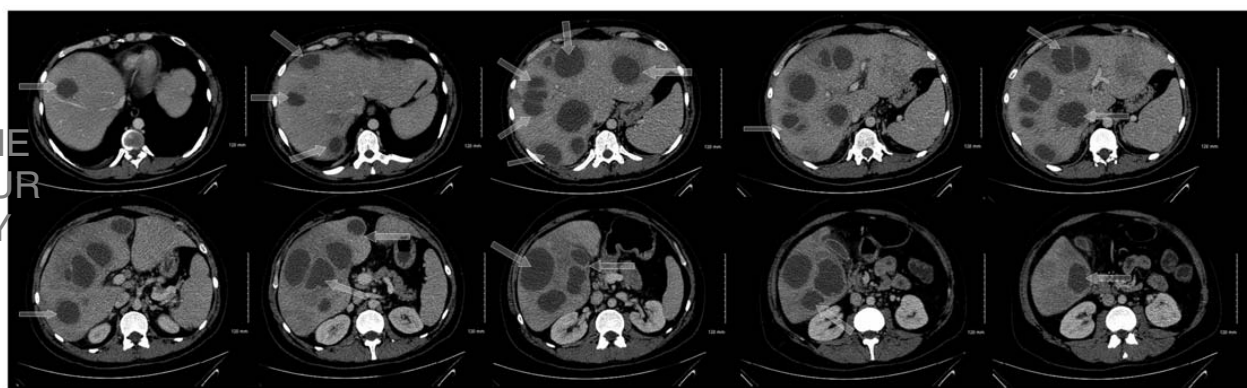


Figure 2 Abdominal-computed tomography (CT) portal-phase showing large hypodense abscesses in all the hepatic segments (helical acquisition; dose length product=3494 mGy/cm; IV injection of 140 ml iohexol, Omnipaque 350[®]). The 19 abscesses are individually labeled by large arrows. Edges were irregular, and the content appeared fluid and heterogeneous.

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Figure 3 Computed tomography (CT)-guided aspiration of one of the more accessible abscess showing a sterile brown-purulent liquid. The punctured abscess was located under the capsule in the hepatic segment VI, and initially measured $78 \times 54 \times 80$ mm. (A) The aspiration was performed laterally by the means of a trocar (large yellow arrow \Rightarrow). (B) The color of the 250 ml harvested fluid appeared macroscopically similar to milk chocolate. (C) The light microscopic observation showed only purulent material with a few degranulated leukocytes (\rightarrow) and some altered red blood cells (\rightarrow), but no viable *Entamoeba histolytica* trophozoites (magnification $\times 400$).

30 seconds at 60°C. The detection and the data analysis were performed with TaqMan Fast 7500 software (Applied Biosystems) version 1.4.0.

The 6-month follow-up was satisfactory. Neither clinical relapse nor subsequent biological perturbations were noticed. There was a substantial decrease in the size of the liver abscesses. However, the number of lesions persisted.

Discussion

Amebiasis is a tropical food- and water-borne parasitosis due to the human-specific protozoan *E. histolytica*.¹ Its worldwide prevalence is estimated at 500 million infected individuals, especially those living in developing countries with low socio-economic level and poor hygienic status.^{1,2} Potential complications are caused by the trophozoite stage that generates ulceration of the colon and leads to bowel perforations. This first step of invasion could be followed by the hematogeneous dissemination of trophozoites toward organs where abscesses are constituted. Thus, liver abscesses are by far the most common extra-intestinal manifestation of invasive amebiasis.^{3,4}

Although hepatic ultrasound scan is widely used,⁵ an abdominal-CT enables a better result allowing more than a 95% detection rate of smaller abscesses and any other associated complications.⁶ Amebic abscesses are usually single and are normally located in the right hepatic lobe.^{1,7} Multiples lesions sometimes occur, but they are infrequently reported. By contrast, a great number of liver abscesses are commonly associated with bacterial etiologies. In 2004, 577 medical records were retrospectively reviewed in order to identify features distinguishing amebic liver abscesses from pyogenic ones.⁸ Multivariate analysis found that multiple abscesses are more likely to be associated with pyogenic origin (adjusted odds ratio=5.34 [2.86–9.96], $P<0.001$). Owing to the very unusual tomodensitometric presentation exhibiting 19 large abscesses, the initial hypothesis of a bacterial co-infection was thereafter obviously raised for our patient. Nevertheless, the bio-clinical investigations did not provide evidence of any bacterial involvement: no germs were isolated,⁹ and the initial use of antibiotics (i.e. amoxicillin-clavulanic acid, then ceftriaxone) had no effect on the fever or the pain. To our knowledge, such a number of large lesions as a result of an amebic infection have rarely been reported, especially in the case of a young short trip traveler. An interesting reported case referred to 25 amebic liver abscesses in a Belgian woman.¹⁰ However, as she used to live permanently in an endemic area of amebiasis, she was thereby continuously exposed to iterative infections. Excepting this isolated case, observations of multiple

amebic liver abscesses have globally remained rare in all the cohorts, and were then limited to a maximum of five or six abscesses, whereas the solitary abscesses represented 65–90% of all these cases.^{7,11–18} Besides, in all these studies, multiples abscesses preferentially occurred in concomitant immunocompromised patients, e.g. HIV-infection,^{19,20} or in cases of advanced amebiasis, e.g. in subjects exposed to recurrent amebic infections.³ In our situation, the patient wasn't known to be immunocompromised, and had only short stays in highly endemic areas of amebiasis.

The present case demonstrates that percutaneous puncture-aspiration enabled the disruption of pain, which was mainly due to abdominal tension induced by the numerous large abscesses. Furthermore, the percutaneous drainage had also a diagnostic role, since the real-time PCR done on the liquid was important in confirming that this was a case of active invasive amebiasis.

According to some authors, image-guided needle aspiration has an evacuation role, and should be considered when there is no clinical improvement after 3 days of taking metronidazole.^{21–23} It is interesting to note that residual abscesses may persist in 5–28% of cases and remain so for up to 13 years, and this in spite of taking an effective treatment.^{24,25} In such situations, there is neither fever nor abdominal pain nor jaundice.^{4,26}

Conclusion

Exceptionally numerous large abscesses could be seen during hepatic amebiasis. Faced with such an unusual abdominal-CT scan findings, the use of amebic serology may be of great interest. Furthermore, the real-time PCR test is now considered a smart diagnostic tool. In complicated amebiasis, the percutaneous puncture-aspiration should be considered as an interesting complement to conventional anti-amebic chemotherapy.

Consent

Written informed consent was obtained from the patient for publication of this report and any accompanying images.

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Data have been collected as part of the routine work of University Hospital of Tours.

Transparency Declarations and Conflict of Interest

All authors declare no financial conflict of interest. No funder has played any decision-making role in the research.

All authors have brought their substantial contributions to research design, or the acquisition, analysis, or interpretation of data. They all have been involved in drafting the paper or revising it critically. All authors declare to have given their agreement for the final submission. They confirm that the manuscript is not under editorial consideration in another journal.

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