
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Approximately 90% of AFH patients have been reported to have t(2;22)(q33;q12) with resultant *EWSR1-CREB1* gene fusion.[1] Other translocations described in AFH include t(12;22)(q13;12) and t(12;16)(q13;p11) which lead to *EWSR1-ATF1* and *FUS-ATF1* fusions, respectively.[5] AFH diagnosis was confirmed by molecular testing in both of our cases. However, it is important to note that *EWSR1-CREB1* and *EWSR1-ATF1* fusions are not specific to AFH. One or both fusions have been associated with clear cell sarcoma-like tumour of the gastrointestinal tract (both), conventional clear cell sarcoma (both), primary pulmonary myxoid sarcoma (*EWSR1-CREB1*), hyalinising clear cell carcinoma of the salivary gland (*EWSR1-ATF1*), and myoepithelial tumour of soft tissue (*EWSR1-ATF1*).[6]

To our knowledge there is only one other similar AFH case reported (the findings are summarised in Table 1). The lesion demonstrated morphological and immunohistochemical features that were initially thought to be consistent with synovial sarcoma (spindle and epithelioid cells with diffuse TLE-1 immunoexpression). AFH diagnosis was eventually confirmed by the detection of *ESWR1* gene rearrangement by FISH.[3]

The diagnosis of AFH can be challenging in the setting of atypical morphology. Both of our cases lacked the characteristic histological findings. Neither showed evidence of pseudoangiomatous spaces, while peritumoral lymphoplasmacytic infiltrate was only identified in one case. Ancillary studies are routinely used in such cases. However, pathologists should be acquainted with the limitations of the immunohistochemical markers and molecular studies.

TLE-1 immunohistochemistry was initially thought to be helpful in distinguishing synovial sarcoma from other histology mimics.[7] However, its expression has been described in other entities, such as clear cell sarcoma, carcinosarcoma, schwannoma, solitary fibrous tumour, epithelioid sarcoma, haemangiopericytoma, and endometrial stromal sarcoma.[3,7] This report underlines the non-specificity of TLE-1 antibody and its immunoreactivity in AFH. Confirmation of signature gene fusions by reverse transcription polymerase chain reaction (RT-PCR) and/or FISH is often helpful, particularly in the setting of atypical morphology. Further studies to look at the prevalence of TLE-1 positivity in larger series of AFH may be important.

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## Use of a rapid faeces multiplex PCR assay for diagnosis of amoebic liver abscess

Sir,

*Entamoeba histolytica* is an intestinal, protozoan parasite endemic to non-industrialised parts of Latin America, Africa and the Indian subcontinent. It causes an estimated 40,000–100,000 deaths each year, primarily from amoebic colitis and amoebic liver abscess (ALA).[1] Although morphologically indistinguishable, strains of *E. histolytica* causing ALA often differ genetically from those causing colitis.[2,3] Colonic carriage can also be asymptomatic, as is always the case for other apathogenic species such as *E. dispar* and *E. moshkovskii*. Therefore, clinical history of colitis or detection of *Entamoeba* in stool do not provide reliable clues for the diagnosis of ALA.

To date, microscopic examination of abscess fluid and serological testing have been the mainstays of diagnosis of ALA. Both techniques have important deficiencies. Examination of liver abscess pus only gives a microscopic diagnosis in $\leq$20% of cases as amoebae are typically found in the periphery of abscesses rather than in the aspirated pus more centrally.[4] Serology may be falsely negative early in the course of ALA, has comparatively poor specificity in areas of high seroprevalence (due to persistence of antibodies following recovery from invasive amoebiasis) and, because of centralised testing in Australia, often yields an unacceptably delayed result.[4,5]

Targeted polymerase chain reaction (PCR)-based assays on abscess pus have been found to be both sensitive and specific for the diagnosis of *E. histolytica*-associated ALA but are only available in research settings and are relatively laborious.[6,7] Antigen detection methods have been used both in stool and liver abscess pus but are not commercially available and are significantly less sensitive and specific than the aforementioned molecular assays.[8] Therefore, there

remains an important need for new, rapid and accurate microbiological methods for diagnosing ALA.

In March 2020, a male from India, aged in his 30s, presented to Royal Darwin Hospital with fever, upper abdominal pain and mild diarrhoea. Examination revealed tachycardia, a temperature of 38.1°C and right upper quadrant tenderness without guarding. Initial blood testing showed an elevated C-reactive protein (408.2 mg/L), a raised neutrophil count ($19.7\times10^9$/L) and a raised alkaline phosphatase (317 U/L). A computed tomography (CT) scan of the abdomen showed two hypodense hepatic lesions in segments 7 and 8, respectively, the largest of which measured 100×58×99 mm, with appearances suggestive of liver abscesses (Fig. 1). Urine, faeces and blood culture were negative for bacterial and fungal growth and no ova, cysts or parasites were seen in two stool specimens. Serum was sent to an offsite reference laboratory for *E. histolytica* serology but results were not available during this patient's admission. A total of approximately 200 mL of non-odorous 'anchovy sauce' pus was aspirated from both liver lesions on day one of admission (Fig. 1). This contained amorphous material and many neutrophils, but no bacterial, fungal or parasitic organisms were seen on microscopy and there was no growth after appropriate incubation. Given the strong suspicion of ALA and the expected delay in the results of serology, we performed a Biofire FilmArray Gastrointestinal multiplex PCR panel (BioMerieux, France), designed for detection of a range of different stool pathogens, including *E. histolytica*, on 200 µL of the liver abscess pus. This gave a positive result for *E. histolytica* within one hour. Treatment was rationalised to high dose metronidazole (and subsequent paromomycin), as per Australian Therapeutic Guidelines,[9] with a rapid clinical response. The *E. histolytica* indirect haemagglutination titre was subsequently reported at ≥1:2560, 20 days after initial diagnosis. Prolonged turnaround times for send-away tests from our hospital in the tropical north of Australia are not unusual but, in this case, air courier service disruption during the COVID-19 outbreak probably exacerbated the time delay.

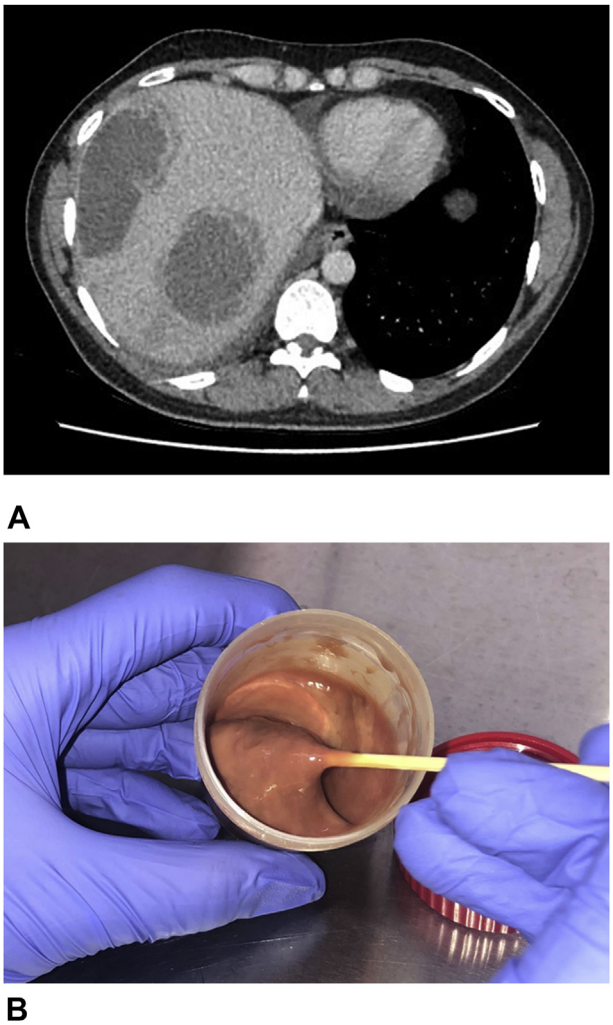


**Fig. 1** (A) Post-contrast computed tomography (CT) scan of the upper abdomen showing two large hepatic abscesses in segments 7 and 8, respectively. (B) Macroscopic appearance of the aspirated pus.

In our patient, analysis of liver abscess pus using a commercially available, multiplex, nested PCR assay designed for stool, provided a rapid result and allowed immediate rationalisation of therapy targeting a single pathogen. To our knowledge, this is the third published use of a stool multiplex PCR assay and the second of the Biofire assay specifically for diagnosis of ALA using abscess pus. Weitzel *et al.* used the Rida Gene Stool Panel (R-Biopharm, Germany) and the Biofire panel on liver abscess pus for rapid diagnosis of ALA in a 34-year-old man from Chile with a 50 mm left lobe liver lesion. Both assays confirmed the presence of *E. histolytica* and subsequent serology was consistent with this diagnosis.[7] Bernet Sánchez *et al.* used the Allplex Gastrointestinal Panel 4 Assay (Seegene, South Korea) in the same manner to diagnose a 55-year-old immunosuppressed female with multiple large ALAs, again with consistent serological results.[10] In both of these cases, the PCR panels were performed prior to initiation of metronidazole, which has been shown previously to improve detection rates of targeted PCR panels on liver abscess pus.[8]

There are several commercially available multiplex PCR panels containing primers for *E. histolytica* and their use for analysis of liver abscess pus has many theoretical advantages over conventional methods for the diagnosis of ALA: simplicity, widespread laboratory availability, rapid turnaround time and the ability to concomitantly exclude several other potential microbiological causes of liver abscess. One would assume that the sensitivity and specificity of nested PCR assays, such as the Biofire panel, would be much greater than standard microscopy and serology. Nevertheless, their use on liver abscess pus remains unvalidated and their false positivity and negativity rates are unknown. We plan to continue using and prospectively validating the Biofire Gastrointestinal multiplex panel on liver abscess pus in patients with suspected amoebic liver abscess and encourage others to consider using one of the commercially available multiplex assays to do the same.

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## A unique case of *TFEB* amplified renal cell carcinoma

Sir,

*TFEB* amplified renal cell carcinoma is a recently described entity that consists of various morphological phenotypes of high grade tumours with a characteristic amplification of chromosome 6p21.2.[1–3] Of the cases reported in the literature to date, the common morphological feature is World Health Organization/International Society of Urological Pathology (WHO/ISUP) grade 3 or 4 nucleoli with an aggressive clinical course. Herein, we describe a unique case of *TFEB* amplified renal cell carcinoma with low nucleolar grade.

The patient was a 64-year-old female with past medical history of hypertension, diabetes mellitus type 2, and nephrotic syndrome secondary to minimal change disease. A computed tomography (CT) imaging study was performed showing a right upper pole mass measuring 4.5 cm in greatest dimension. The patient was referred to urology where she was scheduled for a right partial nephrectomy.

On gross examination of the kidney, a grey-tan haemorrhagic mass measuring 5.0 cm in greatest dimension was identified. The mass was grossly confined to the kidney and was well circumscribed. On microscopic examination, the tumour cells were nested with clear cytoplasm and nuclei with WHO/ISUP nucleolar grade 2 (Fig. 1). The tumour invaded the renal sinus fat and was staged as pT3apNX. In addition, an incidental leiomyoma in the renal pelvis was identified.

Immunohistochemistry (IHC) showed positivity for CK7 in a membranous pattern, and was negative for Melan-A, RCC, carbonic anhydrase IX, P504s, C-kit, and D2-40 (Fig. 1). Florescence *in situ* hybridisation (FISH) for clinical purposes was performed with probes for *TFEB* rearrangement, *TFE3* rearrangement and *TFEB* amplification. The tumour was positive for a *TFEB* amplification with greater than 9 copies per cell in 100% of cells examined without evidence of a *TFEB* rearrangement or a *TFE3* rearrangement, confirming the diagnosis of *TFEB* amplified renal cell carcinoma.

The patient had post-operative follow-up 5 months after initial resection, with post-operative imaging studies that were negative for recurrence. We present this case to add to the small case cohort in the literature. To our knowledge this is the first documented case of low WHO/ISUP nucleolar grade in this entity with short term follow-up showing a less aggressive clinical course.

*TFEB* amplifications in renal tumours were first described by Peckova *et al.* in 2014 with a case series on aggressive translocation associated renal cell neoplasms with secondary *TFEB* amplification.[1] Argani *et al.* first described the entity *TFEB* amplified renal cell carcinoma in a case series of four cases in 2016.[2] Along with multiple other cases series recently published, the data collectively show these tumours to have various morphological appearances, immunohistochemical staining for Melan-A with patchy CK7 and HMB45 expression, and overall aggressive clinical course in an older patient population comparatively to translocation tumours involving *TFEB* or *TFE3*.[1–4] Since the initial description of this tumour, approximately 57 cases have been reported in the literature.[3]

*TFEB* is part of the microphthalmia-associated transcription factor (MiTF) family of proteins that has been implicated as an oncogenic driver involved in MiTF translocation renal cell carcinomas.[2] These tumours show translocations in the *TFE3* and *TFEB* genes leading to renal neoplasia in a younger age population than the more common renal neoplasias such as clear cell renal cell carcinoma or papillary renal cell carcinoma.[5] *TFEB* amplification is a more recently described entity with the discovery of renal neoplasms having genomic amplification at chromosome 6p21.1 locus. Within this locus on chromosome 6 there are also the *VEGFA* and *CCND3* genes which also have been shown to be amplified in some of these renal neoplasms.[3,6] This may be important in future studies of these neoplasms as targeted anti-VEGF therapy may be a future treatment option. Small case series that have looked at outcomes with targeted therapy have been inconclusive and further studies are needed to establish whether this is a viable therapy option. These additional gene amplifications have also been implicated in the aggressive behaviour of these neoplasms.

Interestingly, the level of amplification of the *TFEB* locus does not correlate with outcome, and even low amplifications of this region portend a poor prognosis.[3,6] In addition, the morphology of the amplified tumours can vary significantly and they are not morphologically distinguishable from other