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Hardman, N, Young, N, Hobson, R et al. (4 more authors) (2020) Prolonged survival after disseminated Rhinocladiella infection treated with surgical excision and posaconazole. Transplant Infectious Disease, 22 (2). e13264. ISSN 1398-2273

https://doi.org/10.1111/tid.13264

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Article type : Case Report

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Title: Prolonged survival after disseminated *Rhinocladiella* infection treated with surgical excision and posaconazole.

Running title: Disseminated Rhinocladiella infection

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Acknowledgments
We acknowledge the permission of the patient to publish this report.

Abstract
Cerebral abscess due to pigmented moulds are a rare but usually fatal infection occasionally seen in transplant recipients. A 67 year old male of Iraqi origin underwent a deceased donation renal transplant for renal failure and 2 months later was diagnosed

with an abscess in the left posterior frontal lobe of his brain. Subsequent biopsy proved this to be due to the mould *Rhinocladiella mackenziei.* Further interventions included two operations to aspirate the lesion, voriconazole, then liposomal amphotericin B, then a combination of posaconazole and flucytosine which he continued for over four years. He also suffered from right ankle pain and was diagnosed with septic arthritis; *R. mackenziei* was isolated from pus aspirated from the ankle joint. He responded well to the treatment and has had little loss of function, and on CT the cerebral lesion has stabilised. Beta-D-glucan, initially at very high levels proved useful to monitor response over the 5 years and the latest sample was negative

(38 pg/mL). This case is notable for the first disseminated case of this infection, its favourable outcome on a novel antifungal combination and a new approach to monitoring the course of disease.

Keywords: Rhinocladiella, brain abscess, excision, fungal, Beta-D-glucan, disseminated

Introduction

Rhinocladiella mackenziei is a dematiaceous fungus and a rare cause of cerebral phaeohyphomycosis. It is thought that cerebral *R. mackenziei* infections are a result of either haematogenous spread, possibly from pulmonary sites or direct spread from accidental introduction of spores at contiguous sites[1]. With one exception (from India), all cases have either been in patients residing in, or originating from the Middle East, however the organism has yet to be recovered from the environment. To date, including this case there have been 33 reported cases of cerebral *R. mackenziei* infection in the literature[2,3]. Where the outcome was known, mortality was reported in over 95%[2]. The majority of infections have occurred in immunocompetent individuals[4] however, infections in patients with diabetes, haematological malignancies, and renal transplantation have also been documented. In a review of cerebral phaeohyphomycosis cases 2 of 13 *Rhinocladiella* cases were in renal transplant recipients[5], and one in a haemodialysis patient. *Rhinocladiella* infections are neurotropic, and to date, the brain is the only site of infection that has been reported.

Here we report, to the best of our knowledge, the first successfully treated case of disseminated *R.mackenziei* infection.

Case report

A 67 year old HIV-negative male of Iraqi origin with end stage renal failure secondary to urolithiasis underwent deceased donation renal transplant in August 2014. Induction immunosuppression consisted of alemtuzumab 30mg s/c and, methylprednisolone 500mg IV. Prior to transplant a chest X-ray was normal. After transplantation he was maintained on tacrolimus monotherapy[6] target range 9-14ng/ml trough level for the first 3 months post transplant, followed by target range 5-9ng/ml thereafter.

On 27 September 2014 he presented with a fever and non-specific right flank pain. Examination findings were non-diagnostic. His white blood cell (WBC) count was 5.99 × 10^9/L (reference range 4.0-11 × 10^9/L) with a normal neutrophil count (5.54 × 10^9/L, reference range 2.0-7.5 × 10^9/L) and lymphopenia (0.1 × 10^9/L, reference range 1.0-4.5), which had been persistent since his immunosuppression induction, and is an expected effect of alemtuzumab, with lymphocyte reconstitution to normal levels occurring between 7 and 9 months post renal transplant. C-reactive protein was elevated, however, at 33 mg/L (reference <10 mg/L). Trans-thoracic echocardiogram (TTE) revealed mild-moderate aortic stenosis and a tricuspid mass, which had been present in 2011 and may have represented a persistent vegetation (haemodialysis had been complicated by an episode of *Staphylococcus aureus* infective endocarditis in October 2011). On this occasion *Enterococcus faecalis* was isolated from three pairs of blood cultures. A putative diagnosis of infective endocarditis was made and he was started on intravenous amoxicillin 2 g four-hourly. A trans-oesophageal echocardiogram (TOE) carried out on 7 October 2014 did not confirm the presence of any vegetations. However, on 10 October 2014 an abdominal ultrasound demonstrated the presence of two echo poor areas in the spleen (2.0 cm and 2.6 cm), which were considered likely to represent splenic abscesses. The amoxicillin was continued for 6 weeks.

On 18th October 2014 the patient developed a right sided facial weakness and expressive dysphasia. Though able to mobilise independently, his gait was now unsteady and he had developed a right pronator drift. There was no headache or fever and the patient remained haemodynamically stable. A contrast-enhanced computed tomography (CT) scan on 21 of October 2014 (day 0) demonstrated a 2.5 cm ring-enhancing lesion with a low attenuation centre and a large volume of surrounding vasogenic oedema in the

left posterior frontal lobe, which was considered most likely to represent an abscess (Figure 1a).

On day 1 the patient was referred to neurosurgery and image-guided aspiration of the abscess was undertaken. Gram-film of the black pus aspirated showed no bacteria but profuse septate fungal hyphae, and fungal hyphae were also seen in a potassium hydroxide-calcofluor preparation. In view of the detection of septate fungal hyphae the patient was commenced on iv voriconazole 6 mg/kg bd for the initial two doses, then 4mg/kg 12 hourly. His tacrolimus was discontinued and he was commenced on prednisolone 20 mg/day as sole continuing immunosuppression.

A darkly pigmented filamentous fungus grew on Sabouraud dextrose agar (30°C and 35°C) after 3 days of incubation which produced terminal condia on poorly defined phialides. The patient's serum was tested for galactomannan and beta-D-glucan (Fungitell). Galactomannan was negative with an index value of 0.068. The beta-D-glucan level was 1727pg/mL (normal range <60pg/mL).

Resident in the UK since 1976, the patient had visited Iraq in 2003 as well as Jordan in 2008 and Dubai in 2014. In view of his exposure to the Middle East, the presence of a solitary intracerebral abscess and the growth of a slow-growing, pigmented fungus a presumptive diagnosis of *Rhinocladiella mackenziei* cerebral phaeohyphomycosis was made. On day 6 the organism was identified as *R. mackenziei* following fungal DNA amplification and sequencing part of the large ribosomal subunit using primers NL1 primers (5' GCATATCAATAAGCGGAGGAAAG-3') AND NL4 (5'-GGTCCGTGTTTCAAGACGG-3')[7]. Voriconazole was discontinued due to concerns about its effect on liver function, when the Alkaline phosphatase level rose to 965 iu/L (reference range 70-300) after 7 days of voriconazole; levels were not done. The patient was commenced on posaconazole gastro-resistant tablets (300 mg bd for 2 doses then 300 mg daily) and liposomal amphotericin B 5 mg/kg/day. Susceptibility testing was performed by the PHE Mycology Reference Laboratory (MRL) in Bristol by Etest and results were available on day 8. The minimum inhibitory concentrations observed at 192 and 216 hours and MRL interpretations were as follows: - amphotericin B 32 mg/L

(resistant), itraconazole <0.03 mg/L (susceptible), voriconazole 0.06 mg/L (susceptible), posaconazole <0.03 mg/L (susceptible) and flucytosine 0.125 mg/L (susceptible).

During the patient's admission it was noted that he had been suffering with pain in his right ankle for five weeks. An MRI scan, showed appearances that were suspicious of septic arthritis, with surrounding myositis. On day 1 a small amount of pus was aspirated from the joint. The sample was negative by bacterial culture and 16S PCR. However, on 11 November 2014 a slow growing darkly pigmented mould was isolated and, confirmed to be *R. mackenziei.* On day 2 an attempt was made to aspirate the presumed splenic abscesses. The fluid was negative for both bacteria and fungi by Gram-film, culture, 16 and 18S PCR. A contrast-enhanced CT head scan performed 5 days after the initial scan showed the continuing presence of a ring-enhanced frontal lesion, which had slightly reduced in size, although the area of surrounding oedema had enlarged. On day 8 the serum posaconazole concentration was 2.15 mg/L (for clinical efficacy the target is ≥1.25 mg/L). On day 9 the patient was converted from oral to IV posaconazole 300 mg/day and liposomal amphotericin B was stopped.

Repeat CT head scan on day 13 revealed a 2.5 cm lesion with a marginal decrease in surrounding oedema. The patient was returned to theatre for a repeat image guided aspiration of the lesion. The aspirated pus was negative on Gram stain but on day 23 *R. mackenziei* was isolated. On day 17 iv posaconazole was discontinued (after a total of 8 doses) and the patient re-started oral posaconazole gastro-resistant tablets 300 mg/day. The posaconazole level on the evening of day 17 was 2.26 mg/L (reference range >1.25 mg/L). The patient was discharged home on day 25.

Despite almost two months of posaconazole, with good levels, there was a limited effect on the fungus as repeat outpatient CT head imaging on day 41 revealed an enlarged ring enhancing mass lesion maximal diameter of 2.9 cm. With the abscess close to the internal capsule, excision carried a significant risk of hemiparesis and speech disturbance. The patient had previously declined excision, but now was prepared to undergo the procedure. Intra-operatively a necrotic cavity was found however, in contrast to mature bacterial abscesses there was no clearly defined wall. Due to the eloquence of

this area of the brain maximal debulking was carried out but complete excision was not possible. IV flucytosine was added prior to surgery. The patient had no post operative motor or sensory loss and on day 57, he was converted from iv to oral flucytosine 1.5 g 6-hourly and oral posaconazole was continued. Pre-dose flucytosine levels were maintained between 25-50 mg/L. Once stabilised, flucyosine and posaconazole levels were monitored monthly.

By day 59, the patient was considered suitable for hospital discharge. Blood cultures obtained during the admission were negative after 4 weeks incubation. Whilst mobility and speech continued to improve, the patient reported right ankle pain, especially worse on palpation. An MRI scan of the ankle revealed osteomyelitis and abscess in the distal anterior fibula measuring approximately 2.3 x 2.5 x 2.6 cm (Fig. 2). There was also marked abnormality in the talus and extensive ankle soft tissue oedema. On day 111 he underwent excisional biopsy and insertion of cement spacer (containing voriconazole) to the right distal fibula. Fungal hyphae were seen in four fibula pus samples (microscopic appearance). *R. mackenziei & E. faecalis* were isolated on culture. Following the biopsy and insertion of cement spacer he was able to mobilise and put weight on his foot with the use of a stick. Oral posaconazole and flucytosine were continued and oral amoxicillin 1g 8-hourly was added and continued for 4.5 months. By day 173 the ring enhancing lesion in the posterior left frontal lobe had decreased slightly in size as compared to CT from day 111 (Fig. 1b).

At the time of writing, at almost five years since diagnosis, the patient had continued to improve in terms of mobility and speech. Head CT imaging showed the lesion was stable, not increasing in size but with increased calcification. Beta-D-glucan testing showed initially very high levels of this fungal antigen, which have declined steadily during the treatment period (Fig. 3) and in July 2019 (day 1372) was 38pg/ml (reference range <60mgl/L). In February 2019 (day 1190) the patients antifungal therapy (flucytosine and posaconazole) was stopped due to the response and possibility that the posaconazole was causing some leg pain. At the most recent follow up for his renal transplant (November 2019, 9 months after stopping antifungal therapy) the patient remained relatively well, continues on prednisolone 7.5mg PO od as his only immunosuppression, and had a creatinine level of 79 umol/L with eGFR >90ml/min (2

variable Modification of Diet in Renal Disease Study). He was normotensive with no proteinuria.

Discussion

We report here the first case of disseminated *R.mackenziei* infection and one of the few cases of prolonged survival. The ankle and brain lesions became apparent from symptomology at approximately the same time. Thus whilst this is speculation we consider that the organism was acquired by inhalation and then spread haematogenously to two sites, the brain and ankle concomitantly. This is consistent with the current view of how this kind of infection is acquired[1]

Cerebral phaeohyphomycosis usually presents with one or more of the following: limb weakness, hemiparesis, hemisenory loss, seizure and behavioural change. Fever and headache are uncommon presenting features. Radiographically, cerebral infection typically appears as a single ring-enhancing lesion. The presence of multiple lesions is most commonly associated with an immunocompromised status[1]. Isolation of the fungus from biopsy samples or aspirated pus from brain lesions is required for definitive diagnosis.

With the exception of a few isolated cases, combined antifungal chemotherapy and surgical debridement appear to be essential for successful outcome.

Revanker et al. reviewed 101 cases of culture-proven cerebral phaeohyphomycosis reported in the literature from 1966 to 2002; and determined that excision of brain lesions may provide better results than simple aspiration or partial excision[5]. In this case due to the eloquence of the brain where the abscess was located aspiration was used on two occasions before an excision was attempted. Most mature bacterial abscesses develop a thick capsule after 10 - 14 days and therefore become easier to excise over time, however we found in this fungal case that attempted excision was not possible as no capsule was present. Maximal safe debulking was therefore used and is likely to have contributed to the successful management of this patient. Al-Abdely et al. (2005)[8] report evidence of relapse of excised *R. mackenziei* on itraconazole, following temporary improvement with voriconazole. The patient subsequently responded to posaconazole therapy albeit with significant neurological compromise. Furthermore, the superiority of posaconazole over amphotericin B and itraconazole for the treatment of cerebral *R.*

mackenziei infection has been demonstrated in a murine model[9]. There has been a case of documented treatment failure with posaconazole, however on that occasion the cerebral lesion was aspirated rather than excised and posaconazole monotherapy was used rather than combination therapy[10]. Posaconazole is not known to penetrate into the CSF or cerebral tissue well. A case of *Rhinocladiella* cerebral abscess recently reported in a patient with Crohn's disease and successfully treated with posaconazole showed that levels of posaconazole in the CSF were low (<10% of plasma levels), and levels in brain tissues removed during varied though some showed slightly higher levels of posaconazole all were less than the plasma level[3]. The MICs were not reported in that study, but if cerebral tissue posaconazole levels our case were similar to those found by Barde et al (2019)[3] ie 0.12 to 0.55 mg/L this would still be in excess of the posaconazole MIC (<0.03mg/L). Similar to our case, success was attributed to a combination of antifungals and surgical removal of the abscess. Use of beta-D-glucan to monitor the progress of treatment in this patient is novel for this fungus, but this approach has been reported in some other dematiacous fungal infections[11], and this case shows its value in long term monitoring in a group of diseases where lack of diagnostics has been highlighted[12].

A review of cerebral phaeohyphomycosis by Li & Sybren de Hoog[1] identified that combination antifungal therapy was more frequently successful than monotherapy, a view reiterated in a more recent review[12]. This is a condition with very high mortality rates, and as such we opted for combination therapy of posaconazole plus flucytosine in addition to surgical excision. We advise therapeutic drug monitoring to ensure appropriate levels are reached and where there is relapse after initial response, antifungal susceptibility testing should be done and repeated to check for resistance that could have developed on therapy.

Author contributions

NH: Concept/design, drafting manuscript, NY: critical review of article, RH: Concept/design, critical review of article, JS: Critical review of article, MWS: Critical review of article, drafting of manuscript (renal aspects) ST: Critical review of article, drafting of manuscript (neurosurgery aspects), RB: Concept/design, drafting manuscript

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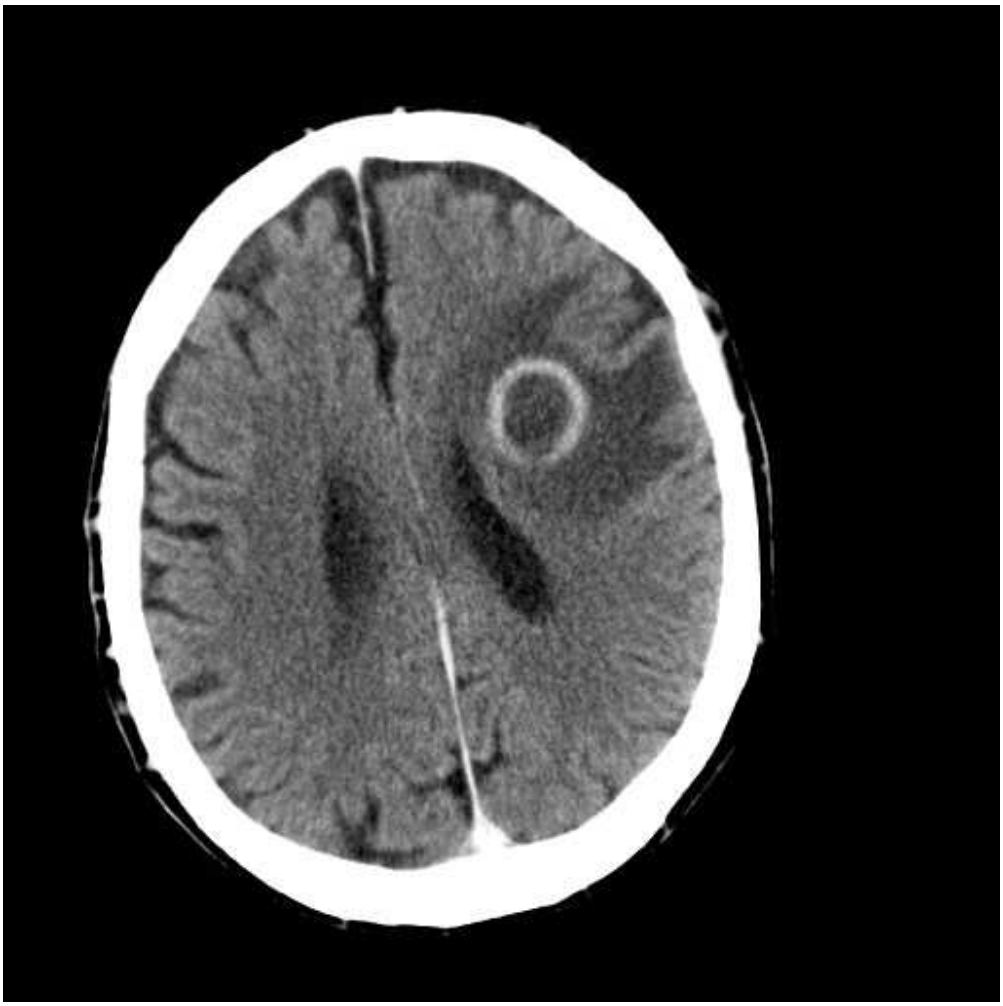
Figure legends

Figure 1: Contrast enhanced CT head at a) day 0 and b) 6 months.

Figure 2 MRI of the right ankle

Figure 3 Time course of serum Beta D glucan, white stars are brain isolations of *R mackenziei*, black star is ankle isolation.



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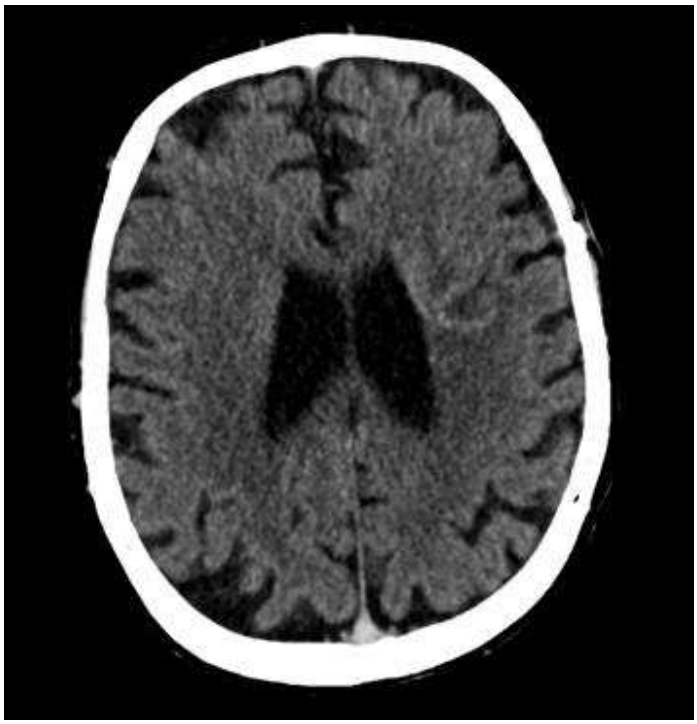

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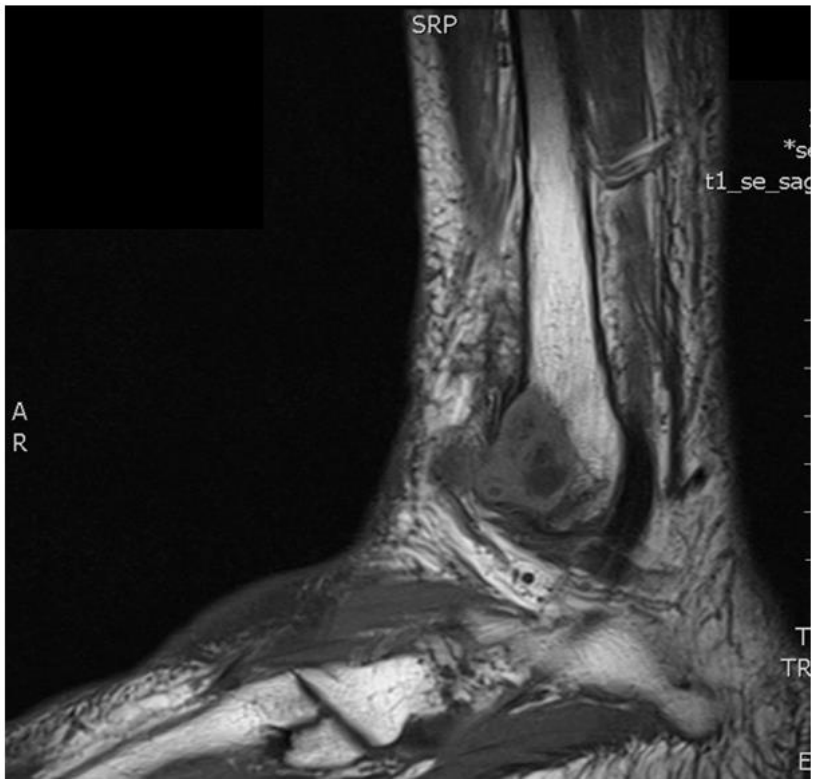

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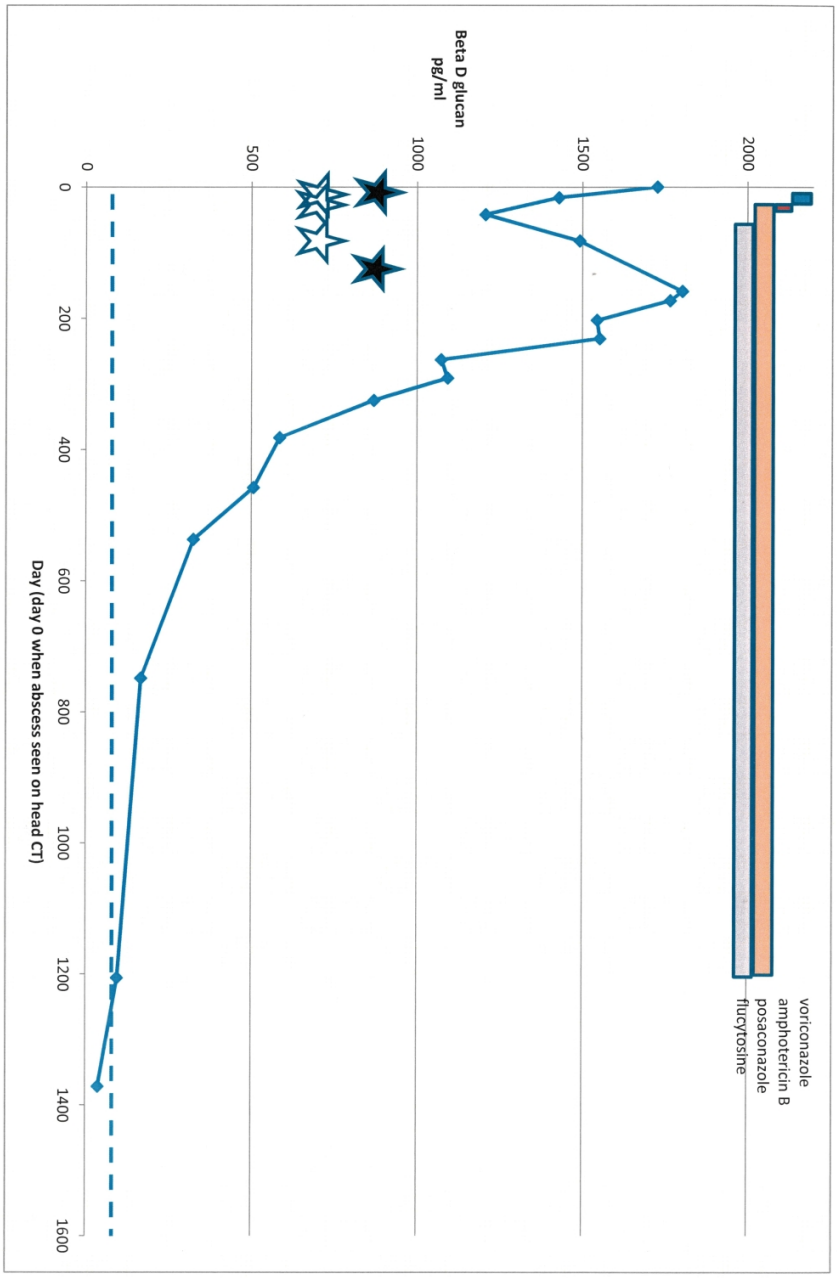

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