

CLINICAL MYCOLOGY COURSE

CASE 1: NAUSEA, VOMITING, AND HEADACHE IN A KIDNEY-TRANSPLANT PATIENT PRESENTED BY JOHN R. PERFECT, MD



Initial Presentation

A 22-year-old white woman from North Carolina, USA presented with evidence for rejection of a 4-year-old kidney transplant and was treated with high doses of corticosteroids (> 1 mg/kg/day of methylprednisolone and the monoclonal antibody OKT3). During the hospitalization, she was given even more corticosteroids prednisone for development of acute pericarditis.

Case Work-up

Approximately 3 months after treatment of the acute organ rejection and pericarditis while receiving low-dose prednisone (10 mg/day), mycophenolate, and tacrolimus, she presents to an emergency department with a 1-week history of headaches, nausea, and vomiting. Her temperature is 37.2°C (99.0°F). On neurological exam, she had bilateral clonus and papilledema but a normal mental status exam. She had no skin lesions. Her work-up was significant for a normal computed tomography (CT) scan and, as shown in Figure 1, a magnetic resonance imaging (MRI) scan showing mild leptomeningeal enhancement that suggested a basilar meningitis.

CLINICAL MYCOLOGY COURSE



Figure 1. MRI image showing leptomeningeal enhancement consistent with basilar meningitis. Permission pending.

Her complete blood count was normal, and her creatinine level was 4.0 mg/dL (creatinine clearance less than 20 cc/min).

Her initial lumbar puncture results were:

- WBCs-100 nucleated cells/mm³
- Glucose 43 mg/dL (blood glucose level 110 mg/dL)
- Protein 79 mg/dL
- Opening pressure was 400 mm of CSF

Challenge Question 1. What is your initial diagnosis?

- A. *Listeria* meningitis
- B. Cryptococcal meningitis
- C. Tuberculous meningitis
- D. *Histoplasma* meningitis
- E. Pneumococcal meningitis

CLINICAL MYCOLOGY COURSE

Discussion

A. *Listeria meningitis*

This can be considered in this immunocompromised patient and classically may present as a rhombencephalitis. However, the CSF form of *Listeria* typically includes many more nucleated cells in CSF and is similar to a common bacterial meningitis. It is unusual for a patient with this type of meningitis to be afebrile. Furthermore, a 1-week untreated history of central nervous symptoms without severe clinical deterioration would be unlikely with this gram-positive-rod infection.

B. Cryptococcal meningitis¹⁻⁴. Invasive cryptococcal infections account for approximately 8% of all invasive mycoses among transplant recipients. This rate may be even higher in parts of the world that are endemic for this infection, such as the South-eastern part of the United States of America (USA) in which this patient lives. At presentation, generally, there are headaches, and these can be subacute (1-3 weeks) to chronic (> 4 weeks). Interestingly, the disease can occur with a febrile presentation, but frequently, patients do not present with fever on the initial diagnosis. The CSF formula of a mild pleocytosis, hypoglycorrhachia (low CSF glucose) with mildly elevated protein is typical of cryptococcal meningitis in transplant recipients. However, infrequently, patients present with severe CSF pancytopenia as observed in advanced AIDS patients. An elevated intracranial pressure is commonly observed in cases of cryptococcal meningitis. Generally, cryptococcal meningitis occurs in the late transplant period, so this case would fit into this time period (4 years). Finally, cryptococcal meningitis is generally a reactivation disease, and the patient's recent elevation of immunosuppression would support the pathophysiology of possible reactivation of disease. It is important to note that the designation of such disease as cryptococcal meningoencephalitis reflects the proper common CNS parenchymal and subarachnoid space involvement with this fungus, but in this case, we will use the shorter common term of meningitis.

C. Tuberculous (TB) meningitis. Both the symptoms and signs of this case have many features that are consistent with TB meningitis, and this infection could have been reactivated during the high-level immunosuppression for organ rejection. However, it is extremely important when evaluating infections in immunocompromised hosts to understand the local epidemiology. Most transplant recipients during pre-transplant work-up are checked for latent TB and if present, they are pre-emptively treated. Both this surveillance and the very low incidence of TB within the USA make this diagnosis very unlikely and emphasizes the importance of providing an initial risk assessment at the bedside for infectious causes and providing local epidemiological understandings.

D. *Histoplasma meningitis*. This fungal infection in the CNS could look like this presentation. If one looks at the epidemiological risk factors, North Carolina is not classically in the maps of endemicity for this fungus, but it is clear with global warming and resulting climate changes that the geographic maps for endemic mycoses are changing and patients can acquire *Histoplasma* in North Carolina without leaving the state. Thus, old maps for endemic mycoses exposures may be out of date, highlighting that clinicians need to focus on specific epidemiological exposure in their area of practice and on the impact of travel on their patients. Thus, *Histoplasma* is possible by exposure(s), but our patient is not in a very high endemic area. Furthermore, even

CLINICAL MYCOLOGY COURSE

in areas of high endemicity for this fungus, clinical disease with this fungus is not as common in transplant recipients as cryptococcus, and it is even less common that disseminated disease will manifest in the CNS (although it can travel into the CNS). Therefore, in a Bayesian prediction, the pre-probability of this infection being histoplasmosis is low in North Carolina without any travel history and would be possible but less likely than cryptococcus.

E. Pneumococcal meningitis. This is an extremely unlikely diagnosis for a variety of reasons. First, the CSF formula is not consistent with the acute inflammatory reaction typically observed with pneumococcal meningitis. Second, a 1-week history of symptoms with an acute, untreated bacterial meningitis without dire consequences (death) would be rare or even unreported. Third, again one must know the patient population; within the USA, most transplant recipients will have received pneumococcal vaccines during the pre-transplant evaluations. Of course, this vaccine may not have complete pneumococcal protection, but it significantly reduces the incidence of pneumococcal disease in solid-organ-transplant recipients. For many reasons, pneumococcal meningitis or even empiric antibiotics awaiting specific diagnostic tests for treatment are not indicated here.

Correct answer: From our basic assessment of the patient's risks and presentation the most likely diagnosis is cryptococcal meningitis (**B**), but the diagnostic maneuvers continue.

Challenge Question 2. What additional diagnostic tests would be reasonable at this stage?

- A. India ink
- B. Biofire PCR Film Panel for CNS pathogens
- C. Cryptococcal polysaccharides antigen test (CRAG)
- D. Culture for bacteria, mycobacteria and fungi
- E. All of the above

Discussion

A. India ink. This has been used for years as a rapid diagnostic test for cryptococcosis, and in the proper hands, the test is positive in half the cases of transplant recipients and even a greater proportion of AIDS patients. However, it has fallen out of favor in many medical centers for the following reasons: (1) it is observer dependent with poor quality controls; (2) for low burden yeast infections in CSF ($<1 \times 10^3$ CFUs/ml), it will generally be negative; (3) there are simply more accurate and sensitive rapid tests for cryptococcal meningitis such as CrAg by lateral flow assay (LFA) and the Biofire PCR Film assay. It is likely that any institution caring for transplant recipients will use these rapid and accurate testing assays rather than an India ink examination.

B. The Biofire CSF PCR Film assay. This is a rapid multiplex polymerase chain reaction (PCR) test for 14 major CNS pathogens and is frequently used for screening meningitis cases. It can be rapid and accurate, and it includes *Cryptococcus neoformans* as one of the pathogens on its detection list. Regarding cryptococcal meningitis, however, several comments need to be made about this test. First, it can frequently be negative in

CLINICAL MYCOLOGY COURSE

low-burden CSF cryptococcal yeast disease since it is not easy to break open yeasts cells for the release of DNA. Therefore, experts recommend pelleting the CSF before performing the test for *Cryptococcus* detection. Second, this test may be attractive for determining relapse vs Immune Reconstitution Inflammatory Syndrome (IRIS), since a positive PCR assay correlates with viable yeasts in the CSF and a persistent pathogen disease, while a negative PCR may be consistent with dead yeasts and disease caused by the host reaction (IRIS)⁵. Third, the cryptococcal antigen test (CrAg) is likely more sensitive than PCR for all stages of infection.

C. Cryptococcal polysaccharides antigen test (CrAg). This test is an integral part of diagnosing and managing cryptococcal meningitis. The yeast sheds an impressively large polysaccharide molecule from its capsule, which protects it from host damage, but is also easy to detect in biological fluids such as blood and CSF. The CrAg test is the most effective of all fungal diagnostics. The test's sensitivity and specificity are both well over 90%⁶. It can be used in many ways: on serum to rapidly screen high-risk patients; to diagnose CNS disease by positive test at times before CSF culture is positive; via titer levels on the CNS to estimate the burden of yeast in the subarachnoid space. For instance, the higher the titer, then the larger the burden of yeasts in the CNS and thus a worse prognosis. Unfortunately, both serum and CSF titers are not precise for serial measurement usage to determine treatment decision. For instance, at times, CrAg titers will stay positive for long periods of time especially in the serum without apparent consequences. Of course, it is encouraging when CrAg titers decrease and eventually become undetected during and after treatment.

There have been outstanding advances in CrAg testing. Latex agglutination and enzyme-linked immunosorbent-assay (ELISA) have been the gold standards for CrAg detection previously. However, the lateral flow assay (LFA) is comparable to these other methods and requires minimal laboratory time to complete and is cheap. It can be a "point-of-care" test⁷ and is now commonly used in both resource-available and resource-limited health care systems. For clinicians, the titers by LFA are higher (approximately 4-fold) than the other testing methods, so this fact needs to be considered when using titers for prognosis. New LFA tests also control for prozone effects.

D. Culture for bacteria, mycobacteria, and fungi. All CSF from immunocompromised hosts needs to be cultured for these major pathogen groups. It is simply not always possible to precisely predict the infection and co-infections that may occur. For example, TB and *Cryptococcus* in some resource-poor health care systems occur together and with some regularity. Culture also allows for susceptibility testing and possibly better understanding of the yeast genotype. Recent work in transplant recipients suggested that certain genotypes like VNII had a favourable outcome⁸. Viral studies will likely still be primarily PCR-based rather than culture-based in most cases.

E. All of the above. All these tests may be appropriate in this case. The use of the India ink test is becoming less frequently used, but the others are standard important tests when considering cryptococcosis.

Correct answer: E is the most appropriate response although some tests are more essential to management than others.

CLINICAL MYCOLOGY COURSE

Lab Results

Further lab results in this patient were as follows: India ink was positive; serum CrAg was >1:1024 titer, and CSF CrAg was 1:256 titer. In this patient, the CrAg was done by latex agglutination and the Biofire CSF PCR Film assay and LFA CrAg were not yet clinically available. The culture was positive for *Cryptococcus neoformans*.

Most fungal and bacterial media will grow the yeasts if no cycloheximide is added, and colonies will appear in 3-7 days unless this is a heavily antifungal pre-treated patient, in which cultures may take 10-21 days to be positive. -Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can distinguish *Cryptococcus neoformans* from *Cryptococcus gattii* isolates. Quantitative CSF yeast cultures were not performed. Quantitative cultures have been extensively used in research studies to determine the effective-fungicidal activity (EFA) of treatment⁹⁻¹³. They have been well evaluated as a prognostic factor for burden of infection and as a therapeutic guide for dynamically determining the clinical response to therapy. They have not yet been adopted for general clinical practice because of quality-control issues and lack of routine clinical data for their use. Further studies are required to determine if they have therapeutic implications and can be integrated into a coherent management strategy.

The diagnosis is clear. We have a renal transplant recipient with cryptococcal meningitis and prominent renal dysfunction. The next issue is management.

Challenge Question 3. How should this infection be managed?

- A. Immediately, obtain minimum inhibitory concentrations (MICs) to check for antifungal drug resistance of the isolate
- B. Rapidly taper her off all immunosuppressive agents
- C. Perform daily lumbar puncture until CSF opening pressure is normal
- D. Begin high-dose fluconazole therapy
- E. Begin liposomal amphotericin B plus flucytosine as induction therapy for cryptococcal meningitis for 14 days.
- F. Begin single-dose liposomal amphotericin B 10 mg/kg x 1 plus flucytosine and fluconazole for 14 days

Discussion

A. Immediately, obtain minimum inhibitory concentrations (MICs) to check for antifungal drug resistance of the isolate. We are frequently programmed into determining *in vitro* susceptibility on isolated bacterial pathogens; however, it is not clear that it is helpful in initial management of the fungus in cryptococcal meningitis^{14,15}. There are very rare cases of amphotericin B-resistant cryptococcal strains, and those have only been observed in cases involving strains with substantial prior antifungal exposure. Flucytosine is not used alone, and in combination therapy, the usefulness of determination of susceptibility value (MIC) is not clear. Finally, with fluconazole, there is no official breakpoint for drug resistance in *Cryptococcus*, but there has been some data suggesting that MICs of 16 mg/L or greater are associated with failure. This has created the “dreaded” MIC of 16 mg/L, which is frequently found in wild-type cryptococcal strains although the

CLINICAL MYCOLOGY COURSE

epidemiological cut off value has been reported to be 8 mg/L. Fortunately, we do have standard methods for detecting MICs of *Cryptococcus*, but with its known heteroresistance¹⁶ and relative lack of azole resistance in wild-type strains, it is currently difficult to be precise in identifying a resistant strain. It is probably best to store the original isolate to compare MICs with any relapse isolates since strains can develop stable resistance after exposure to azoles. There is at least one antifungal guideline (Infectious Diseases Society of America) that suggests comparing isolates, and those with greater than 3-tube increases in MICs should be considered as azole resistant or becoming azole resistant¹⁷. Any strain with MIC of 64 mg/L or greater should probably be considered high-level fluconazole resistant. MICs do not need to be known immediately for an initial case of cryptococcal meningitis management, except under unique circumstances such as prior azole exposures.

B. Rapidly taper her off all immunosuppressive agents. Immunosuppressive management is the bane of the clinician's decision-making existence in transplant recipients. Of course, immune suppression is a major factor in the disease occurrence. However, a too rapid immune reconstitution can precipitate a deadly IRIS in the CNS, and the retention of function in the transplanted organ is generally essential to survival (less so with renal transplants in which dialysis is possible)^{18,19}. Therefore, immunosuppression reductions need to be carefully and consistently monitored and tapered. With cryptococcal disease, the calcineurin-inhibitors have direct anti-cryptococcal activity² and don't need to be stopped. Immunosuppressive agents should rarely be rapidly reduced or stopped but instead should be tapered in their dosing.

C. Perform daily lumbar puncture until CSF opening pressure is normal. -Increased intracranial pressure can be a major factor in mortality and morbidity, particularly in high- burden CSF yeast infections in AIDS patients²⁰. The pathophysiology of this early increased intracranial pressure involves an outflow obstruction of fluid resorption through the arachnoid villi with obstructing yeasts. Thus, external removal or withdrawal of CSF can improve the obstructive pressure build up and its resultant symptoms²¹. However, the precise management of increased intracranial pressure in a transplant recipient in a generally resourced health care system is less certain. It is likely that it needs to be managed based on symptoms rather than just a specific pressure measurement. In this patient, there were symptoms and signs of increased intracranial pressure (ICP) such as nausea/vomiting and papilledema, so the increased intracranial pressure needed to be addressed. The best strategy may be to first have a discussion with neurosurgical colleagues who have expertise in ICP and then tailor an approach that incorporates the availability of not only repeat lumbar punctures but also lumbar drains and ventricular-peritoneal shunts²². Increased intracranial pressure is important but how and when to precisely manage ICP has a less certain pathway. This patient had a repeat LP but with no improvement in signs and symptoms, although she improved eventually with further antifungal therapy.

D. Begin high-dose fluconazole therapy. The use of initial induction therapy with fluconazole alone now has substantial experience in patients with cryptococcal meningitis, and it is clear that this approach for initial induction therapy is inferior²³ and should be discouraged. If used, the patient must receive high doses of 1200 mg/day or greater^{10,24}. There is now insightful data on how transient fluconazole heteroresistance occurs with the in vivo plasticity of the cryptococcal genome¹⁶. Under stress, the yeast adapts rapidly to produce drug resistance, which can be transient but does reduce fluconazole's antifungal activity. Addition of flucytosine in an all-oral regimen (fluconazole plus flucytosine) is an alternative therapy with better elimination of yeast.

CLINICAL MYCOLOGY COURSE

However, in resource-available health care systems that do transplantation, fluconazole should not be used as monotherapy in an induction regimen for cryptococcal meningitis due to reduced activity and complicating drug-drug interactions with high dose azole therapy.

E. Begin liposomal amphotericin B plus flucytosine as induction therapy for cryptococcal meningitis.

Liposomal amphotericin B and flucytosine are the drugs of choice as a combination regimen for induction therapy and should be favored in transplant recipients with cryptococcal meningitis^{11,12,25-28}. Several large studies support this combination's superior EFA and outcome in AIDS patients, and we should extrapolate these results to immunosuppressed transplant recipients^{9,13}. First, lipid formulations of amphotericin B²⁹⁻³² must be used in transplant recipients because amphotericin B deoxycholate (conventional) has too much toxicity on the kidneys¹⁵. In fact, in this patient with her present renal function, conventional amphotericin B would have likely put her on dialysis or interrupted induction therapy. It is important to note that any stoppage for toxicity during induction therapy may put the patient at increased risk of failure. Therefore, conventional amphotericin B is not favoured over liposomal amphotericin B in most patient populations, and this is particularly important regarding transplant recipients who have calcineurin inhibitors on board. Liposomal amphotericin B doses between 3-4 mg/kg/d have been suggested for induction therapy. Amphotericin B lipid complex (ABLC) at 5 mg/kg/d can be considered as an alternative although it has less experience. Second, flucytosine dosing adjustments must be made and followed. In patients with normal renal function the dose is 100 mg/kg/day, but clinicians should follow algorithms of dosing in response to renal function. In this patient, doses as low as a quarter to an eighth of maximum dose were used with her reduced renal function. Toxicity needs to be monitored with examination for cytopenias and/or drug level measurements if timely results can be obtained to protect against toxicity in the first 2 weeks of induction therapy.

F. Begin single-dose liposomal amphotericin B 10 mg/kg x 1 plus oral flucytosine and fluconazole for 14 days. The recent AMBITION trial examined the use of a single dose of liposomal amphotericin B 10 mg/kg combined with 14 days of flucytosine 100 mg/kg/day and fluconazole (1200 mg/day) vs the 7-day AmB course (with flucytosine and fluconazole) standard of care in resource-limited settings³³. This study, conducted in HIV-infected patients in Africa with cryptococcal meningitis, demonstrated the non-inferiority and potential kidney-sparing effect of the simpler regimen in a resource-limited setting. While the study was not conducted in a transplant setting in the USA, the microbiologic equivalency, and the potentially kidney-sparing benefits of a shorter course of LAmB make this an attractive strategy for a patient with renal issues. However, issues remain whether this is a first line regimen outside of AIDS patients. -For example, its efficacy does not yet approach success in resource-available health care settings; there are concerns that patients might be discharged from hospitals too early since most complications of severity occur in first two weeks of therapy; the high dose fluconazole and flucytosine might cause serious drug adverse events such as drug-drug interactions. In general, until more experience occurs in resource-available health care system and all patient populations, it may be wise to stay with two-week combination therapy for induction therapy. Of note, if this regimen is used, the flucytosine and fluconazole dosage will need to be adjusted based on the creatinine clearance.

Correct answer: E.

CLINICAL MYCOLOGY COURSE

Case Continued

So, this patient received 2 weeks of liposomal amphotericin B 4 mg/kg/day with added saline infusions for kidney protection prior to receiving liposomal amphotericin B and flucytosine at reduced doses for reduced renal functions. Her mycophenolate was tapered off and her tacrolimus was continued with the low-dose prednisone (5 mg/day).

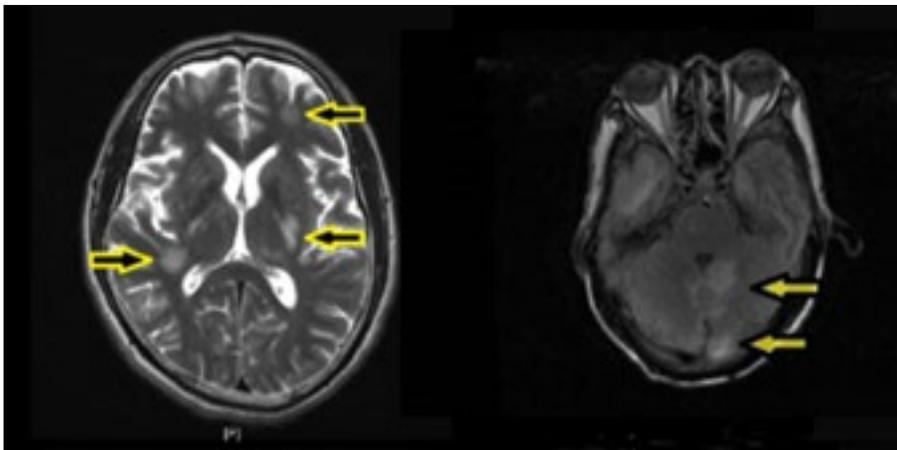
Her LP results at 2 weeks showed the host cell count was 28 mm^3 , and her CrAg titer was 1:256. Opening pressure was 140 mm of CSF.

She was completely asymptomatic. She was started on fluconazole 200 mg/d since her creatinine clearance remained at 20-30 cc/min, and her immunosuppressive agents were continued in the reduced regimen. She was discharged with follow up and although renal function was reduced, she was not placed on dialysis.

Four months after discharge and on fluconazole 200 mg/d, the patient presented to the emergency department with severe, new onset of global headaches. On the initial work-up, her MRI scan showed a diffuse supra- and infra-tentorial leptomeningeal enhancement, as shown in Figure 2. The LP had a normal opening pressure, the initial CSF CrAg titer was 1:16, and the CSF WBC showed 100 host cells/ mm^3 .

Figure 2. Supratentorial (left panel by T1W1 imaging) and infratentorial (right panel by Flair Imaging) lesions consistent with cryptococcal meningitis. Reprinted from Wu G, et al. *Sci Rep.* 2020, 10:9948.

doi:10.1038/s41598-020-67031-4



Challenge Question 4. What would you do next for her management?

- A. Continue fluconazole and add corticosteroids
- B. Restart liposomal amphotericin B and flucytosine for relapsed infection
- C. Stop all immunosuppressants, start pain medications, and add combination antifungal therapy
- D. Start gamma interferon therapy
- E. No change in therapy and manage pain with medications

CLINICAL MYCOLOGY COURSE

Discussion

At this point the final culture results will not be available for an extended time period. In fact, for relapsed patients on antifungal treatment, the fungal cultures can take from 10-21 days for yeasts to grow in the laboratory. Therefore, the clinician must empirically make management decisions without all culture information available. It may be helpful to perform a PCR on a CSF specimen, since a positive PCR may suggest a future culture positivity and thus a relapsed case, but even this test is not a certainty for diagnosis.

A. Continue fluconazole and add corticosteroids. The routine use of corticosteroids during the induction phase of treatment in cryptococcal meningitis in AIDS patients did not improve survival but did cause increased morbidity and reduced antifungal drug killing³⁴. These findings have generally been adapted regarding all risk groups, and corticosteroid therapy is not routinely used during induction therapy. However, the use of corticosteroids in management of cryptococcal IRIS and post-infectious inflammatory response syndrome (PIIRIS) is well established and likely necessary. IRIS can occur in all major risk groups (AIDS, transplant recipients, and non-AIDS, non-transplant patients) and within the CNS space, so this inflammation must be controlled. In this patient, there was evidence of increased inflammation from the admitting data as observed in the MRI scan and an increase in pleocytosis even though the CrAg titer decreased. These decisions to use corticosteroids in management of cryptococcal meningitis are some of the hardest bedside decisions in medicine. With the timing of symptoms, clinical picture, and the lack of data suggesting treatment failure, corticosteroids for management of symptomatic CNS IRIS should be started¹⁸. There is elegant immunological support for altering the immune reaction in cryptococcosis³⁵. However, the specific steroid treatment regimens have not been well studied and are empiric. Two suggested regimens are prednisone 1 mg/kg/day tapered over a month or dexamethasone taper, which has been used in tuberculous meningitis management³⁶.

B. Restart liposomal amphotericin B and flucytosine for relapsed infection. Many clinicians might use this strategy until yeast cultures are finalized. This is not a mistake, but if IRIS is not considered and a dampening of the immune reaction performed, the patient may not improve clinically in a rapid fashion. Therefore, if this strategy is taken, then corticosteroids should probably be added to the antifungal therapeutic regimen.

C. Stop all immunosuppressants, start pain medications, and add combination antifungal therapy. The stoppage of immunosuppressants should not be considered at this point for several reasons. First, there will be risk of the transplanted organ failure through rejection. Second, transplant rejection may already be accelerating since increased organ rejection has been directly associated with the development of cryptococcal IRIS. Third, loss of the immune suppressants might theoretically further exacerbate the CNS IRIS and its potentially deadly inflammation.

D. Start gamma interferon therapy. Gamma interferon treatment remains somewhat of a therapeutic enigma. Surely, appearance of this cytokine is increased in CSF during cryptococcal meningitis and is associated with a better outcome³⁷. Furthermore, two studies in cryptococcal meningitis have shown positive results with

CLINICAL MYCOLOGY COURSE

adjunct recombinant interferon treatments in AIDS patients^{38,39}. However, it remains as an alternative strategy and is rarely used with combination antifungal therapy in the induction phase. It has generally been considered during infection empirically for treatment failure. The issue around this patient with the potential for IRIS is that the immune modulation by the cytokine might make inflammation worse. The use of gamma interferon in cryptococcal meningitis management unfortunately remains as an orphan regimen utilized by experts in unique circumstances.

E. No change in therapy and manage pain with medications. The acuteness and severity of this clinical presentation makes it very difficult for a clinician to know how to best proceed. One should respond with a new therapeutic strategy that attacks the pathophysiology of the pain, rather than just attempt to reduce symptoms with pain meds.

Correct answer: A is the best answer, but for some clinicians, combination therapy may make them more comfortable until CSF culture results return **(B)**.

Case Management

In this particular patient, the initial treating clinicians felt this presentation was a relapse of infection and they restarted liposomal amphotericin B and flucytosine for 2 weeks, but symptoms persisted and at that time, CSF cultures were negative. The patient was then started on a dexamethasone taper, and her symptoms improved dramatically. Unfortunately, the patient rejected her transplanted kidney and was placed on dialysis, and her transplant immunosuppressive agents were stopped. The taper of dexamethasone lasted 6 weeks, and after stopping steroids, in 1 week the symptoms recurred. An MRI scan at that time showed continued leptomeningeal inflammation.

Challenge Question 5. What is the best option for her long-term management?-

- A. Continue fluconazole and add pain medications
- B. Continue fluconazole but add liposomal amphotericin B and flucytosine
- C. Stop fluconazole and add posaconazole
- D. Continue fluconazole and start a more prolonged steroid taper
- E. Stop all antifungal drugs and give pain medications such as non-steroidal-anti-inflammatories

Discussion

A. Continue fluconazole and add pain medications. Pain meds may temporarily help but do not address the continuing inflammation which is the root cause of symptoms.

B. Continue fluconazole but add liposomal amphotericin B and flucytosine. It is always possible that the immunosuppression from the steroids tipped the balance back to the yeast and its ability to start to grow. Thus, a large volume LP should be performed for culture and PCR testing to determine if viable yeasts are still

CLINICAL MYCOLOGY COURSE

present. The empiric use of triple drug therapy is interesting, and although two drug combination is favored in cryptococcal meningitis, the three-drug regimen has some studies supporting its use such as the AMBITION study³³. In this case, it may be reasonable to search for viable organisms diagnostically rather than start an empiric triple drug regimen initially.

C. Stop fluconazole and add posaconazole. The other triazoles have excellent in vitro antifungal activity against *Cryptococcus*, and there are some small open-labelled studies with voriconazole, posaconazole, and isavuconazole in cryptococcosis in which these agents have shown anticryptococcal activity⁴⁰⁻⁴³. In fact, their MICs are much lower than fluconazole. However, it has been very hard to prove that they have any advantage over fluconazole and should probably only be used in unique drug-resistant cases.

D. Continue fluconazole and start a more prolonged steroid taper. It is important to emphasize that the management at present of IRIS is empirical and neither dose nor duration has been precisely studied. In fact, even other immune modulators such as anti-TNF therapies have been tried for treatment of IRIS. Therefore, consideration of another anti-inflammatory regimen for IRIS is always reasonable if there is no evidence that viable yeasts are present.

E. Stop all antifungal drugs and give pain medications such as non-steroidal anti-inflammatories. Long-term management of cryptococcal meningitis has not been well-studied except in a few small studies in AIDS patients^{44,45}. However, when cryptococcal meningitis relapses, it generally does so in the first year after the beginning of infection. Thus, because of this epidemiology and the safety of fluconazole, most experts and guidelines suggest that patients with cryptococcal meningitis receive at least 1 year of antifungal therapy.

Correct answer: D.

Case Outcome

This patient received a lumbar puncture that showed the CSF was sterile with mild pleocytosis, and she was started on another taper of dexamethasone over 4 months. She became asymptomatic and had no other relapse of symptoms. She received fluconazole treatment for 1 year, and 2 years after this infection and 1 year off antifungal agents, she was considered ready to receive another transplanted kidney.

The timing of re-transplanting an organ in a recipient who has had disseminated cryptococcosis is a bedside decision, but personal experience suggests that the patient can be considered for re-transplantation after adequate therapy and time off drug without relapse. Of course, timing of the renal transplant might be dictated by the need for an organ, and there are times in which it might be prudent to provide prophylaxis with an azole during the initial high-risk immunosuppressive time of the early transplant period.

CLINICAL MYCOLOGY COURSE

Summary of Special Issues Regarding Cryptococcosis in Transplant Recipients³

In one of the most comprehensive epidemiological studies of cryptococcosis in transplant recipients in the USA, the TRANSNET Database for fungal infections in solid organ transplants reported on 97 cases of cryptococcosis. The majority of cases were observed in kidney transplants, but cases were also observed in liver, lung, pancreas, and small bowel transplants, but rarely in hematopoietic stem-cell transplants^{46,47}. In transplant recipients, 45% of cases presented with CNS disease and 39% with lung involvement. Approximately 25% of disseminated cases had positive blood cultures but only 4% had cryptococemia without evidence of other organ involvement. Importantly, the median time from transplant to diagnosis of cryptococcosis was 575 days⁴⁶, thus, clearly this infection is not generally an early transplant-period infection, and this has been supported by many reviews. Also, cryptococcosis appears to occur earlier in liver and lung transplant recipients compared to kidney transplant recipients probably due to high intensity immunosuppression with the former. Furthermore, pulmonary disease typically presents later than CNS disease and is associated with lower prednisone dosing. However, if the yeast is in the transplanted donor tissue and placed in an immunocompromised host, then appearance of infection may occur in the early transplant period. For instance, contaminated tissue has been reported in both lung and corneal transplant recipients. The incidence of cryptococcosis has been reported to be up to 2.8% in transplants in some reviews, so it may depend on local factors⁴. The general cumulative incidence of cryptococcosis has been placed at 0.2-0.3% per year in patients receiving high-level immunosuppression with antithymocyte globulin or alemtuzumab⁴⁸. In one review, mortality for disseminated cryptococcosis in transplant recipients at 1 year was 27%⁴⁹. This figure is consistent with that reported for other medically advanced health care systems, but it can approach 40-50% mortality in less-resourced medical centers.

In the diagnostic arena, around 90% of solid organ transplant recipients with meningitis will have positive serum CrAg detected. Serum CrAg titers are detected in 70-80% of patients with pulmonary disease and solid-organ transplant recipients. Furthermore, 35-40% of solid organ transplant recipients may have their pulmonary cryptococcosis detected as an incidental finding during an imaging study.

The importance of gradual reduction of immunosuppressants during management of cryptococcosis is emphasized by the finding that IRIS was independently associated with the discontinuation of calcineurin inhibitors, with an estimated 5-fold increased risk. In fact, receipt of calcineurin inhibitor was associated with lower mortality². On the other hand, the presence of renal failure at baseline is associated with a higher rate of death in cryptococcal meningitis in organ transplant recipients.

CLINICAL MYCOLOGY COURSE

References

1. Singh N, Lortholary O, Alexander BD, et al. Antifungal management practices and evolution of infection in organ transplant recipients with *Cryptococcus neoformans* infection. *Transplantation*. 2005;80(8):1033-1039. doi:10.1097/01.tp.0000173774.74388.49
2. Singh N, Alexander BD, Lortholary O, et al. *Cryptococcus neoformans* in organ transplant recipients: impact of calcineurin-inhibitor agents on mortality. *J Infect Dis*. 2007;195(5):756-764. doi:10.1086/511438
3. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in solid organ transplant recipients: current state of the science. *Clin Infect Dis*. 2008;47(10):1321-1327. doi:10.1086/592690
4. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis*. 2001;7(3):375-381. doi:10.3201/eid0703.010302
5. Rhein J, Bahr NC, Hemmert AC, et al. Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis*. 2016;84(3):268-273. doi:10.1016/j.diagmicrobio.2015.11.017
6. Tanner DC, Weinstein MP, Fedorciw B, Joho KL, Thorpe JJ, Reller L. Comparison of commercial kits for detection of cryptococcal antigen. *J Clin Microbiol*. 1994;32(7):1680-1684. doi:10.1128/jcm.32.7.1680-1684.1994
7. Kozel TR, Bauman SK. CrAg lateral flow assay for cryptococcosis. *Expert Opin Med Diagn*. 2012;6(3):245-251. doi:10.1517/17530059.2012.681300
8. Ponzio V, Chen Y, Rodrigues AM, et al. Genotypic diversity and clinical outcome of cryptococcosis in renal transplant recipients in Brazil. *Emerg Microbes Infect*. 2019;8(1):119-129. doi:10.1080/22221751.2018.1562849
9. Day JN, Chau TT, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med*. 2013;368(14):1291-1302. doi:10.1056/NEJMoa1110404
10. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis*. 2010;50(3):338-344. doi:10.1086/649861
11. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: feasibility and power of quantitative CSF cultures to determine fungicidal activity. *Lancet*. 2004;363(9423):1764-1767. doi:10.1016/S0140-6736(04)16301-0
12. Bicanic T, Wood R, Meintjes G, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. *Clin Infect Dis*. 2008;47(1):123-130. doi:10.1086/588792
13. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal combinations for treatment of cryptococcal meningitis in Africa. *N Engl J Med*. 2018;378(11):1004-1017. doi:10.1056/NEJMoa1710922
14. Witt MD, Lewis RJ, Larsen RA, et al. Identification of patients with acute AIDS-associated cryptococcal meningitis who can be effectively treated with fluconazole: the role of antifungal susceptibility testing. *Clin Infect Dis*. 1996;22(2):322-328. doi:10.1093/clinids/22.2.322
15. Vena A, Munoz P, Guinea J, et al. Fluconazole resistance is not a predictor of poor outcome in patients

CLINICAL MYCOLOGY COURSE

- with cryptococcosis. *Mycoses*. 2019;62(5):441-449. doi:10.1111/myc.12847
16. Stone NR, Rhodes J, Fisher MC, et al. Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. *J Clin Invest*. 2019;129(3):999-1014. doi:10.1172/JCI124516
 17. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2010;50(3):291-322. doi:10.1086/649858
 18. Singh N, Lortholary O, Alexander BD, et al. An immune reconstitution syndrome-like illness associated with *Cryptococcus neoformans* infection in organ transplant recipients. *Clin Infect Dis*. 2005;40(12):1756-1761. doi:10.1086/430606
 19. Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. *Lancet Infect Dis*. 2007;7(6):395-401. doi:10.1016/S1473-3099(07)70085-3
 20. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups. *Clin Infect Dis*. 2000;30(1):47-54. doi:10.1086/313603
 21. Rolfes MA, Hullsiek KH, Rhein J, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. *Clin Infect Dis*. 2014;59(11):1607-1614. doi:10.1093/cid/ciu596
 22. Fessler RD, Sobel J, Guyot L, et al. Management of elevated intracranial pressure in patients with cryptococcal meningitis. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;17(2):137-42. doi:10.1097/00042560-199802010-00006
 23. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Clinical Trials Group. *New Engl J Med*. 1992;326(2):83-89. doi:10.1056/NEJM199201093260202
 24. Milefchik E, Leal MA, Haubrich R, et al. Fluconazole alone or combined with flucytosine for the treatment of AIDS-associated cryptococcal meningitis. *Med Mycol*. 2008;46(4):393-395. doi:10.1080/13693780701851695
 25. Gutschik E, Lippert S. Dental procedures and endocarditis prophylaxis: experiences from 108 dental practices. *Scand J Infect Dis*. 1990;98(2):144-148. doi:10.1111/j.1600-0722.1990.tb00953.x
 26. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. *N Engl J Med*. 1997;337(1):15-21. doi:10.1056/NEJM199707033370103
 27. Bennett JE, Dismukes WE, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med*. 1979;301(3):126-131. doi:10.1056/NEJM19790719301030
 28. Dismukes WE, Cloud G, Gallis HA, et al. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. *New Engl J Med*. 1987;317(6):334-341. doi:10.1056/NEJM198708063170602
 29. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS*. 1997;11(12):1463-1471. doi:10.1097/00002030-199712000-00010
 30. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and

CLINICAL MYCOLOGY COURSE

- conventional amphotericin B deoxycholate for treatment of AIDS- associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis*. 2010;51(2):225-232. doi:10.1086/653606
31. Coker RJ, Viviani M, Gazzard BG, et al. Treatment of cryptococcosis with liposomal amphotericin B (AmBisome) in 23 patients with AIDS. *AIDS*. 1993;7(6):829-835. doi:10.1097/00002030-199306000-00011
 32. Baddour LM, Perfect JR, Ostrosky-Zeichner L. Successful use of amphotericin B lipid complex in the treatment of cryptococcosis. *Clin Infect Dis*. 2005;40 Suppl 6:S409-S413. doi:10.1086/429337
 33. Jarvis JN, Lawrence DS, Meya DB, et al. Single-dose liposomal amphotericin B treatment for cryptococcal meningitis. *N Engl J Med*. 2022;386:1109-1120. doi:10.1056/NEJMoa2111904
 34. Beardsley J, Wolbers M, Kibengo FM, et al. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. *N Engl J Med*. 2016;374:542-54. doi:10.1056/NEJMoa1509024
 35. Neal LM, Xing E, Xu J, et al. CD4+ T cells orchestrate lethal immune pathology despite fungal clearance during *Cryptococcus neoformans* meningoencephalitis. *mBio*. 2017;8(6):e01415-17. doi:10.1128/mBio.01415-17
 36. Thwaites GE, Bang ND, Dung NH, et al. Dexamethasone for the treatment of tuberculosis meningitis in adolescents and adults. *N Engl J Med*. 2004;352:1741-1751. doi:10.1056/NEJMoa040573
 37. Siddiqui AA, Brouwer AE, Wuthiekanun V, et al. IFN-gamma at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J Immunol*. 2005;174(3):1746-1750. doi:10.4049/jimmunol.174.3.1746
 38. Jarvis JN, Meintjes G, Rebe K, et al. Adjunctive interferon- γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS*. 2012;26(9):1105-1113. doi:10.1097/QAD.0b013e3283536a93
 39. Pappas PG, Bustamante B, Ticona E, et al. Recombinant interferon- gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J Infect Dis*. 2004;189(12):2185-2191. doi:10.1086/420829
 40. Pitisuttithum P, Negroni R, Graybill JR, et al. Activity of posaconazole in the treatment of central nervous system fungal infections. *J Antimicrob Chemother*. 2005;56(4):745-755. doi:10.1093/jac/dki288
 41. de Gans J, Portegies P, Tiessens G, et al. Itraconazole compared with amphotericin B plus flucytosine in AIDS patients with cryptococcal meningitis. *AIDS*. 1992;6(2):185-190. doi:10.1097/00002030-199202000-00007
 42. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, et al. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. *Clin Infect Dis*. 2016;63(3):356-362. doi:10.1093/cid/ciw305
 43. Yao Y, Zhang JT, Yan B, et al. Voriconazole: a novel treatment option for cryptococcal meningitis. *Infect Dis (Lond)*. 2015;47(10):694-700. doi:10.3109/23744235.2015.1044260
 44. Rollot F, Bossi P, Tubiana R, et al. Discontinuation of secondary prophylaxis against cryptococcosis in patients with AIDS receiving highly active antiretroviral therapy. *AIDS*. 2001;15(11):1448-1449. doi:10.1097/00002030-200107270-00019
 45. Vibhagool A, Sungkanuparph S, Mootsikapun P, et al. Discontinuation of secondary prophylaxis for Cryptococcal meningitis in human immunodeficiency virus-infected patients treated with highly active antiretroviral therapy: a prospective, multicenter randomized study. *Clin Infect Dis*. 2003;36(10):1329-

CLINICAL MYCOLOGY COURSE

1331. doi:10.1086/374849

46. Pappas P, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis*. 2010;50(8):1101-1111. doi:10.1086/651262
47. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010;50(8):1091-1100. doi:10.1086/651263
48. Silveira FP, Husain S, Kwak EJ, et al. Cryptococcosis in liver and kidney transplant recipients receiving anti-thymocyte globulin or alemtuzumab. *Transpl Infect Dis*. 2007;9(1):22-27. doi:10.1111/j.1399-3062.2006.00149.x
49. Vilchez R, Shapiro R, McCurry K, et al. Longitudinal study of cryptococcosis in adult solid-organ transplant recipients. *Transpl Int*. 2003;16(5):336-40. doi:10.1007/s00147-002-0541-7