Cryptococcal meningitis

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Abstract

HIV-associated cryptococcal meningitis is by far the commonest cause of adult meningitis in many areas of the world with high HIV seroprevalence, and in most centres in Sub-Saharan Africa cases are not decreasing despite availability of antiretroviral therapy because of the challenges of retention in care and adherence. In addition, non HIV-associated cryptococcal meningitis is a significant problem in transplant recipients and others with defects in cell-mediated immunity, and in the apparently immunocompetent - with high mortality rates in these groups, despite therapy. Recent advances have been made in rapid point-of-care diagnosis, and early detection of cryptococcal antigen in blood has enabled the development of a screen and pre-emptive treatment strategy to prevent the development of clinical infection in late stage HIV-infected patients. Progress in optimising antifungal combinations has been aided by evaluation of rate of clearance of infection derived from serial quantitative cultures of cerebrospinal fluid (CSF). Measurement and management of raised CSF pressure, a common complication, is a vital component of care. In addition, there is an improved understanding of protective immune responses in HIV-associated cases, immune-genetic pre-disposition to infection in non HIV-infected patients, and the role of immune-mediated pathology – in non HIV-infected patients, as well as in the context of HIV-associated immune reconstitution reactions.

Key Points

HIV-associated cryptococcal cases in most centres in Africa are not decreasing despite access to antiretrovirals due to challenges with retention in care and adherence.

Non-HIV-associated cryptococcal meningitis, although relatively rare needs to be considered in all cases of lymphocytic meningitis, even in the apparently immunocompetent, and carries a mortality at least as high as HIV-associated disease.

A point-of-care lateral flow “dipstick” test to detect cryptococcal antigen in blood or CSF is highly specific and a significant advance in terms of sensitivity as well as ease of use in the diagnosis of cryptococcal disease.

Amphotericin B, in conventional or liposomal formulation, plus flucytosine remains the induction therapy of choice and is associated with a survival advantage over amphotericin B alone.

Measurement of CSF opening pressure and appropriate management of raised CSF pressure is associated with reduced mortality

Any future attempts at adjunctive immunotherapies will need to be closely guided by the specific immune status, and/or defect, of the host at the time of any intervention.

Introduction:

Cryptococcal meningitis (CM) is the commonest cause of adult meningitis in large parts of the world with high rates of HIV infection [3-5]. In addition, it occurs in an increasing number of patients with other forms of natural and iatrogenic immunosuppression, and in the apparently immunocompetent, particularly in the Far East [6]. In the U.S., deaths from non-HIV-related CM now account for approximately a quarter of CM-related hospitalizations and a third of deaths [9]. Furthermore, an outbreak of cryptococcal disease in Pacific Northwest North America caused by a novel lineage [10], highlights the threat posed by *Cryptococcus sp* [see BOX, [12]]. *Cryptococcus sp* are currently the only significant human fungal pathogens from the large group of basidiomycete fungi, but they share some of the properties - high virulence, generalist pathogens, with long-lived environmental stages, and the potential for wide dispersal and rapid evolutionary change, that have made fungal organisms in general such a growing threat not only to human, but also to animal and plant health [14].

Herein, we review recent advances in the epidemiology, diagnosis, and clinical management of cryptococcal meningitis, including the optimisation of antifungal therapy and the management of neurological complications. We discuss patient and situation specific adjunctive immuno-suppressive or -augmenting therapies – based on

advances in our understanding of susceptibility to infection, immunopathological mechanisms, and protective immune responses, in specific patient groups; and strategies for the prevention of HIV-associated cases through screening for sub-clinical infection and pre-emptive therapy.

Epidemiology

HIV-associated Disease

In a landmark 2009 study, the CDC estimated the global burden of HIV-associated cryptococcal meningitis, based on available incidence data within HIV-infected cohorts in different regions. They estimated that there were close to one million cases per year, with at least 100,000 and perhaps 500,000 deaths per year in Sub-Saharan Africa alone [18]. Other data suggesting that in some populations 10-15% of AIDS deaths (which peaked at 2.3 million per year in 2005 according WHO figures) are due to cryptococcal disease [19], also put the number of deaths in the hundreds of thousands. An updated analysis by Boulware and colleagues, including the CDC group, has used a different approach, based on the number of patients at risk, with CD4 cell counts <100 and not on effective antiretroviral therapy (ART), and the prevalence of cryptococcal antigenaemia and risk of progression. This still puts the number of deaths at 180,000 per year, 132,000 in Africa, and accounting for 17% of AIDS mortality [Boulware DR, et al. Update on the Global Burden of Disease of HIV-Associated Cryptococcal Meningitis. Oral Abstract presented at: 9th International Conference on Cryptococcus and Cryptococcosis; 2014 May; Amsterdam]

In Europe and North America the numbers of cryptococcal cases fell dramatically after introduction of effective ART, with hospitalizations in one US study falling by half [9]; and in South Africa, where surveillance has been carried out since the early 2000s, there has been a modest reduction in cases from a peak between 2005 and 2009 [http://www.nicd.ac.za/assets/files/GERMS-SA%20AR%202014.pdf, p8-11]. But importantly, there is as yet no evidence of a decrease in cases in many high incidence African countries despite increased access to ART [20]. In Botswana, despite a relatively well-resourced and functioning ART programme, nationwide

surveillance shows that the numbers of cryptococcal cases have actually increased since 2011, likely driven by the numbers of patients defaulting from care [Tenforde et al. HIV-associated cryptococcal meningitis in Botswana: national incidence and temporal trends following ART rollout. IAS Meeting Durban 2016, Abtract WEPEB036]. Thus, while total numbers of cases are static, in many centres half of all patients are now presenting having been prescribed ART, but with persisting low CD4 cell counts due to loss to follow up, non-adherence, and/or the development of ART resistance [unpublished enrolment data ACTA trial, ISRCTN 45035509; [21, 22].

Predisposition to infection in non-HIV-infected individuals

The numbers of HIV-associated cases dwarf those in non-HIV infected populations in many low and middle income countries. Nevertheless, cryptococcal meningitis is a significant problem in transplant recipients and other patients with defects in cell-mediated immunity, with high rates of death despite therapy [9]. In a series of over 300 HIV-negative patients with cryptococcal infection from the USA, half had CNS involvement and of these, 25% had had steroid therapy, 24% chronic liver, kidney or lung disease, 16% malignancy, and 15% solid organ transplants [23]. Hematopoietic malignancies are associated although stem cell transplant patients are typically not at increased risk because of widespread use of azole prophylaxis in this population. In addition, there is a well-recognised but poorly understood association with sarcoidosis, as well as other autoimmune diseases such as ankylosing spondylitis, dermatomyositis, SLE, and autoimmune hepatitis, although some of these associations could be due to steroid therapy [24]. Interestingly, even in the developed world, mycobacterial disease is an associated co-morbidity [9], which could be due to a common susceptibility to these two intracellular pathogens as this relationship is seen in HIV-related cases as well [25]. Of note, in the USA series, 30% had no apparent underlying condition. *C. neoformans* cases occur in apparently immunocompetent patients across the world, with large numbers reported in the Far East [6]. In addition meningitis caused by *C. gattii*, occurs throughout the tropics, including Australasia [26] and South America, in apparently immunocompetent patients, and notably in the outbreak in Pacific Northwest of North America [10].

CM in previously healthy individuals is a relatively rare disease, with approximately 3000 cases annually in the U.S, thus affecting approximately 1 in 100,000 individuals per year. This incidence suggests that these apparently 'normal hosts', may actually harbour rare primary immune defects or uncommon autoimmune diseases. As shown in Table 1, a number of immune deficiencies have been associated with cryptococcal meningitis. Idiopathic CD4 lymphopenia (ICL) is the best known risk factor, defined as the repeated presence of a CD4+ T-lymphocyte count of fewer than 300 cells per cubic millimetre without a predisposing cause. In a recent meta-analysis of ICL cases, cryptococcosis was the most common infection seen, in 27% of reported cases [27]. ICL is a heterogeneous disease of unknown cause, although several monogenic mutations have been reported including a recent association of defects in T-cell receptor signalling and a mutation in the UNC119 gene [28]. Autoantibodies to granulocyte-macrophage colony stimulating factor (GMCSF) and its associated syndrome, pulmonary alveolar proteinosis has been associated with intracellular infections including *Cryptococcus* [29]. More recently, the antibody has been characterized as a functional antibody blocking STAT5 phosphorylation involved in macrophage signalling, and has been found in patients with *C. gattii* infection in the Pacific northwest [30, 31]. Autoantibodies against IFN-γ have also been associated with cryptococcal disease [32]. In addition, cryptococcal disease has been associated with syndromes caused by monogenic mutations including an autosomal dominant and sporadic monocytopenia caused by mutations in GATA2 zinc finger transcription factor essential for lymphatic angiogenesis [33-35], hyperimmunoglobulin E-recurrent infection (Job's) syndrome, caused by mutations in the signal transducer and activator of transcription, STAT3 [36, 37], as well as X-linked hyper-IgM immunodeficiency associated with a number of mutations [38, 39]. In addition, while CM in previously healthy patients is a rare disease, common genetic polymorphisms have been associated with disease in this population such as FCy receptor IIB polymorphisms and could represent disease modifier genes [40].

Outcomes and Prognostic factors

Outcomes for patients with HIV-associated cryptococcal meningitis in Africa remain poor, with overall best estimates suggesting a 70% 3 month mortality, driven by late

presentation, and lack of access to drugs, manometers, and optimal monitoring. Fluconazole therapy is associated with 10-week mortality of 50-60% in prospective research studies [41-43]. Amphotericin B-based treatment, in trial settings, is still associated with 10-week mortalities of 24-42% [21, 44-46]. In resource rich settings, studies from the USA and France suggest that 10-week mortality is still between 15 and 26%, and has not changed since the availability of ART [47-49]. In a US study, 90 day mortality for non-HIV infected patients was 27%, higher than for HIV-infected [50], which may be due to both delays in diagnosis and associated immune dysregulations [51]. In non-HIV-infected individuals, *C. gattii* tends to cause more granulomatous lesions in both lung and brain with more neurological sequelae [10, 52, 53], although in the context of HIV-infection, the presentation and outcomes of *C. gattii* and *C. neoformans* infections are not readily distinguishable [54].

A recent analysis of over 500 patients, confirmed and extended our understanding of the factors in HIV-related cryptococcal meningitis associated with poor outcome: above all, altered mental status at presentation, and baseline fungal burden (assessed by colony forming unit (CFU) count), and also, older age, and low weight [55]. In addition, the rate of clearance of infection, dependent on antifungal therapy and host immune response, is independently associated with outcome [55, 56], making this a clinically relevant endpoint for phase 2 studies of novel antifungal regimens (see below).

In non-HIV cases, in addition to altered mental status and fungal burden, markers of a poor inflammatory response, low CSF white cell count, and absence of headache, and underlying haematological malignancy and chronic renal and liver disease have been linked with poor prognosis [23, 53, 57, 58].

Protective immune responses in HIV-infected patients

Jarvis et al have carried out detailed analyses of local and systemic immune responses in patients with HIV-associated cryptococcal meningitis, at baseline and over time on treatment, from cytokine and chemokine profiling of CSF, and from determination of the pattern of intracellular cytokine production by CD4 memory cells when peripheral blood mononuclear cells (PBMC) are stimulated ex vivo with cryptococcal

mannoproteins [59, 60]. Linkage of immune response data to clinical parameters and outcomes allows an investigation of responses associated with survival. Variation in CSF parameters at baseline could be largely explained by 2 principal components. PC-1 was driven by higher levels of IL-6 and IFN-γ, and also IL-8, IL-10, RANTES, TNF-α, and IL-17. Consistent with prior work [61] this pro-inflammatory response was correlated with higher peripheral CD4 cell and CSF white cell counts, markers of CSF macrophage activation, lower fungal burden, more rapid clearance of infection, and survival [60] (Figure 1A). Analysis of systemic responses showed a similar pattern – patients who survived had a significantly higher proportion of CD4 memory cells producing IFN-γ and TNF-α and polyfunctional cells producing both cytokines, than patients who died, whose cryptococcal-specific CD4 memory cells were dominated by cells producing only MIP-1α [60] (□□□□□□□2). The results are consistent with recent independent work relating poor survival to decreased monocyte production of TNF-α; and reduced IFN-γ responses in whole blood stimulated with LPS [62].

Clinical features, pathology, and radiology

Cryptococcal meningitis is a sub-acute meningo-encephalitis. The organism is acquired by inhalation, but can then disseminate, probably in many cases after a latent period contained within lung lymph nodes [63]. Whilst involvement of almost all organs and tissues has been reported, there is a very significant predilection for the CNS. This strong neurotropism has been linked to a number of cryptococcal-specific virulence factors that facilitate penetration of the blood brain barrier such as specific metalloproteinases and ureases; enzymes that cause neuro-immunomodulation such as a dopamine-utilizing laccase; and mechanisms that facilitate survival within the nutrient deprived environment of the brain such as autophagy and high affinity sugar transporters (reviewed in [64]).

Thus, patients present with neurologic symptoms of headache and mental status changes as well as fever, nausea and vomiting, with a median duration in HIV-associated cases of 2 weeks and 6-12 weeks in non-HIV cases. Many will develop

visual symptoms, of diplopia and then reduced acuity secondary to high CSF pressure (see below), and /or involvement of the optic nerve and tracts [65]. Without treatment, patients progress with confusion, seizures, reduced conscious level, and finally coma. Many cases may also have concomitant lung involvement, although in HIV-associated CM, this may often be overlooked, or misdiagnosed as TB [66]. Indeed in HIV-infected patients, while meningo-encephalitis dominates the clinical presentation, disseminated infection is probably common. In non-HIV-infected patients, presentations may be determined as much by host immune responses as by fungal species/lineage differences, and exhibit marked heterogeneity. For example, organ transplant recipients undergoing intensive conditioning may have more acute presentations with limited inflammatory sequelae or may present with an IRIS-like picture after reductions of immunosuppression [67]. Previously healthy non-HIV infected patients infected with either *C. neoformans* or *C. gattii* present with a more chronic course often without fevers that delays diagnosis. Inflammatory sequelae, including hydrocephalus, may be present at diagnosis or occur during therapy and are reported more frequently with *C. gattii* [52], perhaps due to induction of higher concentrations of pro-inflammatory cytokines [68].

In autopsy series, HIV-associated CM is characterised by many predominantly extracellular organisms, throughout the parenchyma and meninges, sometimes in grossly visible accumulations, with little inflammatory response [69]. In non-HIV cases, there are fewer organisms, largely confined to the meninges and large perivascular Virchow-Robin spaces, associated with a diverse inflammatory response ranging from granulomatous to a more disorganized macrophage infiltration [69, 70]. Immunohistochemistry studies also demonstrate cryptococcal capsule polysaccharide throughout the brain, localized in macrophages and microglia, especially in HIV-associated cases [71].

Many of these pathological features can be seen on brain MRI scans, including dilated Virchow-Robin spaces, pseudocysts, cryptococcomas, and cortical and lacunar infarcts [72, 73]. In HIV-negative cases, a larger proportion of patients have large space-occupying cryptococcomas with a marked surrounding inflammatory response, and hydrocephalus.

Diagnosis

Characteristic CSF parameters are a raised white cell count, with lymphocyte predominance, elevated CSF protein, and low CSF glucose. In HIV-associated cryptococcal meningitis, the CSF white cell count is lower (median 15 x 10^6/L [55]) and frequently normal. Diagnosis should not be an issue in HIV-associated cryptococcal meningitis, given the high fungal burden. Simple India Ink examination of CSF has a sensitivity in the order of 70-90% and cases that are India Ink negative will be reliably diagnosed by detection of cryptococcal antigen (glucuronoxylomannan, the dominant capsular polysaccharide) and culture. However, until recently, antigen detection was based on latex agglutination tests, which though sensitive and specific, were never widely available in high burden, resource-limited settings. In this context, development of a lateral flow assay (LFA) has been a major advance. The test meets the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free, Delivered) criteria [74] for point-of-care tests, has a 2-year shelf life at room temperature and requires no specimen preparation. In addition, it is in the order of 100-fold more sensitive in ng/ml of polysaccharide detected, than the older latex agglutination tests, across the 4 cryptococcal serotypes [75, 76]. Availability of this test makes the screen and pre-emptive therapy prevention strategy, discussed below, feasible. It also enables earlier diagnosis prior to lumbar puncture, through testing of serum, plasma, or whole blood finger prick samples [77], in primary care settings, and through screening of medical in-patients in high incidence areas. Urine samples although containing antigen, are prone to false positive results with the current test [78, 79]. More studies are needed, however, to demonstrate better outcomes through earlier, LFA-based diagnosis. A second, semi-quantitative, lateral flow test is now in development by Biosynex in collaboration with Francoise Dromer at Institut Pasteur [www.biosynex.com/fr/test-cryptops/], with two positive bands giving a high or low level antigen result, which may prove useful in individualising treatment, based on fungal burden, especially in the setting of screening.

Fungal burden may be much lower in some cases of HIV-associated unmasking cryptococcal meningitis, presenting after initiation of antiretroviral therapy, in which immune reconstitution may play a more dominant role than active infection (see

below). In these cases, CSF cultures may be negative. Similarly, in HIV-negative cryptococcal meningitis, cultures and latex agglutination tests, especially for *C. gattii* infections, may be negative and repeat large volume CSF samples have sometimes been required in the past [80]. In these settings, the greater sensitivity of the new LFA test for both species is a major advantage [81, 82], and means the diagnosis of cryptococcal meningitis should not be delayed so long as the diagnosis is considered in all patients with a lymphocytic meningitis, even those who are apparently immunocompetent.

Antifungal therapy

Current recommendations for first-line antifungal treatment have not changed significantly for a decade [83-85] (Table 2), and are based on an induction, consolidation and then maintenance therapy approach first used in the landmark 1997 Mycoses Study Group trial [86], and subsequent studies showing that Amphotericin B plus flucytosine led to the most rapid clearance of infection [87], and, more recently, that demonstrated a survival benefit with this combination over amphotericin alone [88]. In fact, the latter study found a reduction of almost 40% in the relative risk of death at 10 weeks with addition of flucytosine. Of note, flucytosine at currently used dosage, for 2 weeks in HIV-associated CM, with monitoring of FBC, rather than serum levels, has been found to be well-tolerated, [88-90]; and in PK/PD studies in patients, levels usually associated with bone marrow suppression were not found [91]. Care must be taken, however, to adjust the dose if renal impairment secondary to amphotericin develops.

Importantly however, Amphotericin B deoxycholate (D-AmB) is associated with renal impairment, hypokalaemia and hypomagnesaemia, and anaemia, especially during the second week of induction therapy [90]. Saline and fluid loading equivalent to 1L Normal saline, in addition to usual fluid requirements, has been shown to reduce renal impairment [92, 93], and potassium and magnesium supplements are recommended, with careful monitoring [83]. In a recent combined cohort, the average fall in haemoglobin with 14 days D-AmB, was 2.3 g/dl [90], which is challenging in centres where transfusion capacity is limited. Liposomal amphotericin B (L-AmB) is

equally effective and better tolerated [94]. Optimal dosing and schedules are still not defined, and it may be that one or few large intermittent doses together with oral therapy, could provide a safe and effective induction regimen, which would be more cost-effective and sustainable in resource limited settings compared with current daily dosing. This approach is being tested in the ongoing Ambition-CM trial [95].

The rate of clearance of infection derived from quantitative cultures of CSF from serial lumbar punctures over the first 2 weeks of treatment provides a statistically powerful and clinically relevant endpoint to explore the activity of new dosages and drug combinations. Such early fungicidal activity (EFA) studies have influenced recommendations where resources are limited. Higher doses of fluconazole, up to 1200 mg/d, are more fungicidal than previously used lower doses and safe [43], and the combination of high dose fluconazole plus flucytosine has an EFA that approaches that measured for amphotericin B alone [96]. This oral combination may also prevent emergence of secondary fluconazole resistance . In addition shorter, 7-day induction with D-AmB does not appear to lead to reduced EFA, perhaps due to the prolonged half-life of D-AmB in brain tissues, and is much better tolerated [97-99]. Based on these phase II studies, high dose fluconazole plus flucytosine and one week D-AmB-based induction are being compared against the standard of 2 weeks of D-AmB-based therapy in African centres in the phase III ACTA trial that will report next year (ISRCTN 45035509). Both these new regimens, if as effective as 2 weeks D-AmB, would be much more readily and safely sustained in resource-limited settings than 2 weeks D-AmB.

EFA has also been used to explore the in vivo efficacy of established drugs, known from pre-clinical studies to have anticryptococcal activity, for possible repurposing for cryptococcal meningitis. Addition of sertraline to AmB plus fluconazole was associated with an increase in EFA in a Ugandan cohort, compared with historical controls treated without sertraline at the same centre [22], and is being tested now in phase III (results expected in 2018); and there are other candidate agents, suitable for similar testing [100]. At least one new agent, Viamet 1129, is being developed specifically for cryptococcal meningitis. It is a novel oral azole-like compound that is concentrated in brain tissue and shows impressive cidal activity in animal models

[http://www.viamet.com/pipeline/vt-1129.php]. Phase I studies have started, with phase II EFA studies under development.

In non-HIV infected patients, clinical response is as much about controlling immune dysregulation as in microbiological control. Initial therapy with Amphotericin B and flucytosine is similar to that for HIV-related disease, but induction is for 4-6 weeks [10, 84, 101](Table 2) and lipid formulations have been favoured over the deoxycholate preparation because of reduced renal toxicity. Previously healthy individuals patients with idiopathic CD4 lymphopenia may take longer to respond microbiologically, but have less immune sequelae; whereas those with normal CD4 counts may have immune sequelae on presentation or, in a significant minority, such sequelae may develop despite microbiological control (discussed below).

Reduction of immunosuppression in solid organ transplant recipients is an intuitively logical approach after severe infections such as cryptococcosis and is well tolerated. However, discontinuation of agents such as calcineurin agents has been associated with clinical deterioration and IRIS, although discontinuation of corticosteroids was not (Sun HY, Forrest G, Alexander BD, et al. Predictors of immune reconstitution syndrome (IRS) in transplant recipients with cryptococococcosis [abstract #M-1138]. Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago IL, September 17–20, 2011.), [102]. Calcineurin inhibitors have an added benefit of being synergistic with antifungals against *Cryptococcus* and have been associated with better outcomes in post-transplant cryptococcosis [103].

Raised CSF pressure

Half of HIV-infected patients with cryptococcal meningitis have a CSF opening pressure of >25 cm and roughly a quarter >35 cm [55, 104]. High pressures are associated with worse symptoms, of headache, nausea, diplopia secondary to 6th nerve palsies, and finally altered mental status. Head scans show that hydrocephalus is rare in HIV-associated meningitis [72], and the pathophysiology is most likely due to blockage of CSF reabsorption by organisms, alive or dead, and /or shed cryptococcal polysaccharide at the level of the arachnoid granulations and elsewhere [105]. In a small post mortem series arachnoid granulation tissue contained many fungal cells, in

comparison with the rest of the brain, with higher numbers of organisms seen being associated with higher pre-morbid CSF pressure [105]. Interestingly, a high fungal burden appears necessary but not sufficient for the development of high pressure [106]. Based on this understanding of mechanism, careful therapeutic lumbar punctures are recommended to control high pressure. The safe maximum volume of CSF to drain at one LP is unclear, but up to 30 ml are frequently removed in patients with high pressure, with checking of pressure after each 10 ml removed. Increasing evidence points convincingly to their efficacy. In the original data from the 1997 MSG trial, high pressure was associated with increased acute mortality [104], whereas in a large cohort of patients in whom regular lumbar punctures were performed with additional therapeutic lumbar punctures if the pressure was high, there was no association of high baseline pressure with mortality. In fact, interestingly, the opposite trend was seen [55]. In addition, adherence to guidelines regarding CSF pressure measurement and management has been associated with improved outcome [107], and, in a Ugandan study, therapeutic LPs were associated with reduced mortality, irrespective of baseline pressure [108].

Importantly from a clinical standpoint, it is well recognised that high pressure, even if absent on presentation, may develop later, despite sterilization of CSF, typically in the second and third weeks [106]. Thus, it is vital that lumbar puncture is repeated and pressure measured in patients who fail to improve or in whom symptoms recur. Repeated daily therapeutic LPs are sufficient to control raised pressure in the majority of patients. Occasionally however, only a temporary lumbar drain, ideally managed on a neurosurgical facility, with which an order of magnitude more CSF per day can be safely drained, is sufficient [109, 110]. Our own and other's experience suggests around 7 days temporary drainage is usually required (Figure 3)[110]. Ventricular shunts are also effective, but can usually be avoided in HIV-related CM.

In contrast, a significant minority of non-HIV-infected individuals with CM have central obstruction of the choroid plexes within the foramen of Monroe or Luschka/Magendie with hydrocephalus or a superior arachoiditis and a communicating process of elevated pressures, both associated with robust inflammatory responses [70]. Thus, shunts are needed more often in non-HIV

associated meningitis because of hydrocephalus or persistent obstruction, and can be used safely provided antifungal therapy has been started [111].

Cryptococcal immune reconstitution inflammatory syndrome (CM-IRIS)

CM-IRIS is a second common and life-threatening complication. In the context of HIV, there are 2 forms: paradoxical IRIS in patients who respond to initial antifungal therapy and then suffer a relapse of symptoms after starting ART; and unmasking cases who present for the first time after ART is started [112]. While reported rates of paradoxical IRIS vary as this remains a diagnosis of exclusion, dependent on how thoroughly alternative diagnoses can be investigated, it probably occurs in 10 to 20% of those surviving to start ART, at a median of 1-2 months after ART [113-115]. Risk factors include a low baseline CD4 cell count, with rapid subsequent rise, a low initial CSF white cell count, lower markers of inflammation and IFN-γ responses on initial presentation, and a high fungal burden, at baseline and day 14 [116-118]. In the principal component analysis of baseline CSF cytokine profiles by Jarvis et al, described above, PC-2, driven by high concentrations of chemokines, MCP-1 and MIP-1a, and of GM-CSF, were associated with the later development of IRIS [60] (Figure 1B), consistent with the findings of Chang et al [119]. These chemokines may enhance T and myeloid cell trafficking into the CNS, resulting, once restoration of CD4 Th1 subsets occurs following initiation of ART, in the excessive Th1-type immune responses seen during CM-IRIS episodes [51, 114, 120, 121] (Figure 4).

As with other opportunistic infections, the question arises as to the best timing of ART, to avoid precipitating IRIS, while establishing HIV therapy as soon as possible. For cryptococcal disease, we know that within 3 days [122] and a median of 8 days [123] is too soon, and 6 weeks probably unnecessarily late [124]. In the COAT study [123], patients given early (at a median of 8 days) ART were found, as early as day 14, to have higher CSF white cell counts and CSF markers of macrophage/microglial activation than patients not yet started on ART, suggesting the excess deaths in the early ART arm may have been immune mediated [125]. Thus, current guidelines suggest 4-6 weeks, although cohort evidence suggests starting patients during the

fourth week is safe in the context of rapidly fungicidal induction therapy [55], which should per se also help prevent IRIS.

Patients who represent after the start of ART, should have an LP to check for ongoing active infection, and consideration of re-induction antifungal therapy (Table 2) pending culture results; and to measure and manage CSF pressure (high pressure again being an important feature of paradoxical CM-IRIS). Alternative diagnoses should be actively pursued. While CM-IRIS is clearly life-threatening, with awareness and early recognition, and short courses of corticosteroids for patients who are deteriorating, CM-IRIS-related mortality should be minimized [116]. In some cases, steroid weaning leads to recurrent IRIS, and case reports have described the use of thalidomide and adalimumab in refractory CM-IRIS in both HIV and transplant patients [126, 127].

Unmasking HIV-associated CM-IRIS cases are characterised by lower organism loads compared with ART-naïve cases, and some, although not conclusive evidence, for increased inflammatory responses [128]. The balance between active infection and immune pathology may vary depending on the interval between ART start and presentation. If this is just a few days, there may be little difference from ART-naïve cases, while cases presenting much later, may be culture negative, and diagnosed on antigen testing only. Patients presenting with unmasking CM-IRIS are treated with the same antifungal regimens, although care is needed in view of interactions and overlapping side effects between antifungals and ART. Careful assessment is also needed to try to distinguish between unmasking cases, and the increasing number of patients who are ART-experienced but present with CM with persisting low CD4 cell counts due to non-adherence and/or ART resistance. In these latter cases, any switches or re-initiation of ART are best postponed until 4 weeks into antifungal therapy.

Post-infectious Inflammatory Response Syndrome (PIIRS) in non-HIV patients

In the non-HIV infected host, paradoxical immune reactions are a significant cause of clinical failure. In patients previously immunosuppressed with transplant conditioning or chemotherapy for haematological malignancies, reductions in immune suppressing

medications to boost the immune response frequently result in an IRIS-like reconstitution syndrome [102]. In previously healthy individuals where no immune reconstitution occurs, clinical deterioration commonly occurs during therapy not from microbiological failure but from an aggravated immune response, PIIRS. Primary differences in the non-HIV, compared with the HIV, setting include compartmentalization, with only intrathecal responses measurable, and an alternatively activated M2-like skewing of CNS-tissue infiltrating macrophages, which is a non-protective response in animal models [129]. As shown in Figure 4, PIIRS shares features with CM-IRIS including activation of the dendritic cell-T-cell synapse and T-cell inflammatory responses evidenced by elevated cytokines IFN-γ and IL-6. Biomarkers of this inflammatory process are the CSF soluble T-cell activation marker sCD27, which is associated with release of the axonal damage protein, neurofilament light chain, NFL. However, the syndrome differs from CM-IRIS in that PIIRS lacks effective activation of macrophages, resulting in a "macrophage-T-cell dissociation," resulting clinically with persistent tissue antigen and inflammation. As shown in Figure 5, corticosteroids have been useful in these patients with inflammatory brain lesions, whether infected with *C. neoformans* or *C. gattii*, once microbiological clearance is documented by negative CSF cultures [70, 130].

Prospects for immunotherapies

As exemplified above, an increased understanding of the immunopathology in some non HIV cases, of immunological pre-dispositions to cryptococcal meningitis in the heterogeneous group of non HIV-infected CM patients, and of protective immune responses in patients with HIV-associated CM, should help to guide attempts to manipulate immune responses for patient benefit. Any such attempts will need to be host and situation specific, moving the damage-response framework [131] to an optimal balance of effective fungal clearance without immune pathology.

Since PIIRS may be quite persistent in non-HIV CM due to macrophage clearance defects, and given the side effects of prolonged corticosteroid use, active research is currently directed at identifying steroid sparing immunosuppressants that are effective

in neuroinflammation. In non HIV-infected previously healthy patients, identification of genetic or immunological defects could be useful to guide attempts to augment microbiological control and to identify family members at risk. In addition, diseases such as pulmonary alveolar proteinosis are important co-morbidities to identify in non-HIV CM because pulmonary pathology may be problematic after resolution of meningitis and a number of therapies such as aerosolized GM-CSF have proven effective for pulmonary pathology [132, 133].

In HIV-associated CM, clinical trial data are consistent with the immune data from patients showing the importance of Th1-type immunity in controlling infection. In a large randomised trial, adjunctive steroids at the time of CM diagnosis were associated with a significantly increased risk of disability or death, increased adverse events, and a substantially reduced rate of fungal clearance [21]. In contrast, in two smaller trials, adjunctive IFN-γ appeared to be safe and augmented clearance without any indication of adverse effects on HIV viral control or IRIS [46, 134]. Unsurprisingly perhaps, the greatest effect was seen in patients with poor baseline Th1-type responses. Further studies are needed, and rapid assessment of immune status could enable targeting IFN-γ to those likely to benefit most. Even in HIV-associated CM though, there are exceptions, in late unmasking cases, and those with paradoxical CM-IRIS, in whom immune pathology plays a significant role, and in whom such augmentation of immune responses should be avoided.

Prevention

Cryptococcal infection can be diagnosed through detection of cryptococcal polysaccharide antigen in blood, at a median in one study of 22 days prior to CNS symptoms developing [19], providing an opportunity for preventing the development of meningitis in very high-risk groups, such as those with late-stage HIV. In a retrospective study, Jarvis et al tested stored blood samples taken prior to initiation of ART from a cohort of over 700 prospectively monitored patients in Cape Town, South Africa [135]. None of 661 patients testing antigen negative (93% of the total), had gone on to develop CM in the first year of ART. In contrast, at least 7/25 (28%), of those testing antigen positive with no prior history of CM, had developed CM. This

100% negative predictive value supported the utility of antigen detection in a screen and pre-emptive therapy strategy, whereby patients at risk, with CD4 cell counts <100, would be tested for antigen and those testing positive given pre-emptive therapy with widely available and safe oral fluconazole. Several modelling studies suggested such a strategy would be highly cost-effective, saving lives and money, in light of the fact that the costs of admission and care of prevented cases would be avoided, even at very low prevalences of antigenaemia [136, 137]. On this basis, screening was endorsed in WHO guidelines [83], and programmes initiated in South Africa [138] and elsewhere, and by MSF.

Some prospective data is now available that supports the strategy: in a randomised controlled trial in Tanzania and Zambia (REMSTART), screening, as described above of patients presenting for ART with a CD4 cell count <200, and simple lay-worker ART adherence support for the first month, led to a 28% reduction in overall mortality in the first year of ART [139]. Still, questions remain regarding how best to implement screening and in particular, over the best therapy for antigen positive patients. Data from REMSTART, and studies in Uganda and Cape Town all suggest that the mortality of antigen-positive patients remains significantly higher, at 25-30%, than that of those testing antigen negative, at around 10-12% [79, 139]; also, that a very significant proportion, around 40%, of antigen positive patients even if asymptomatic, are found, if they agree to LP, to have evidence of meningitis, defined by CSF antigenaemia [79]. Furthermore, higher blood antigen titres can predict those with meningitis [79], and are associated with poor outcome [Bozena et al CROI 2016]. While fluconazole may be enough for many antigen positive patients, patients with evidence of meningitis may need more aggressive antifungal therapy, and a follow up trial is planned that will compare fluconazole with fluconazole plus flucytosine for those testing antigen positive. New semi-quantitative antigen tests may be able to rapidly identify those who would benefit from more intensive treatment.

Conclusions

Cryptococcal meningitis is a leading fungal cause of human disease and death worldwide. However, expanding access to current antifungal drugs [140], and optimising their use in regimens that are sustainable in resource-limited settings,

together with earlier diagnosis, enabled by a new point-of-care immunodiagnostic test, and therapeutic lumbar punctures to manage the common complication of raised cerebrospinal fluid pressure, have the potential to significantly reduce the global disease burden. Drug discovery aimed specifically at *Cryptococcus* species is limited, but one promising new agent, Viamet-1129, is now entering clinical evaluation. Immunomodulatory adjunctive therapies hold promise, but need to be carefully tailored to the patient and clinical scenario, based on advances in our understanding host immunity in different patient populations.

BOX: Ecology, evolution and virulence of Cryptococcus

Cryptococcus is a genus within the Tremellales, an order of fungi that are commonly found growing on rotting wood as saprophytes and called 'jelly fungi' due to their gelatinous fruiting bodies. There are over 30 recognised species of *Cryptococcus* of which two, *C. neoformans* and *C. gattii*, cause the majority of human infections. Both species are readily recovered from the environment where they can be isolated from the bark of a wide variety of tree species and other organic matter, notably bird faeces. *Cryptococcus* are therefore members of a growing class of pathogens, sapronoses [1], that are accidental parasites on primarily immunocompromised individuals, and which gain no evolutionary advantage from infecting the human host [2]. The virulence of *Cryptococcus* therefore most likely owes to adaptations that allow it to survive in the environment [7]. An array of recognised 'dual-use' virulence factors are known [8], including a thick polysaccharide capsule [11] which, in the environment defends against parasitic amoeba, however in the human allows the fungus to survive as macrophages assault and to disseminate as an intracellular parasite. Genetic analysis has shown that *C. neoformans* and *C. gattii* have had independent evolutionary histories for an estimated 30-40 million years [13]. This separation has resulted in subtle variation in their virulence with *C. neoformans* being the predominating infection in immunocompromised HIV/AIDS individuals compared to *C. gattii* which is a rarer infection and associated with putatively immunocompetent individuals. Both species are highly diverse and contain within them a number of phylogenetically distinct lineages that likely represent cryptic species; the two species are currently undergoing taxonomic revision into potentially seven species [15]. Genomic analysis of *C. gattii* has described four main major lineages (VGI-VGIV) [16] with population genetic analyses suggesting a center of origin of the hypervirulent VGII lineage in South America [17]. *C. neoformans* is similarly diverse, with two recognised cryptic species each containing evolutionary distinct lineages; *C. neoformans* var. *grubii* (lineages VNI, VNII, VNB) which appears to have a center of diversity in Africa, and *C. neoformans* var. *neoformans* (lineage VNIV). Both *C. gattii* and *C. neoformans* are facultatively sexual with a bipolar mating system, and hybrids can form both between species and lineages.

Table 1
Summary of data re genetic pre-disposition in non HIV CM

Syndromes, Autoantibodies	**Reference**
Idiopathic CD4 lymphopenia	27,28
Pulmonary alveolar proteinosis, autoantibodies to GMCSF	29-31
and IFN-γ	32
Monogenic Disorders	
GATA2	33-35
CGD	
Job's syndrome	36,37
X-linked CD40L deficiency (hyper IgM syndrome)	38,39
Polygenetic modifiers	
FCg receptor II	40
Co-morbidities	
Sarcoidosis, autoimmune disease, steroid treatment	
Hepatic disease	
Solid organ transplant conditioning	

Table 2: Antifungal therapy

Induction therapy	**Duration**
L-AmB 3-6 mg/kg/day *or* D-AmB 0.7–1.0 mg/kg/day; **plus** 5-FC 100 mg/kg/day (75 mg/kg/day if intravenous formulation used) L-AmB preferred in transplant patients; and when induction for >2 weeks	2 weeks (HIV-CM) ≥2 weeks (transplant-CM)* 4-6 weeks (non-HIV non transplant, including *C. gatti*)*
Consolidation	
Fluconazole 400-800 mg/day**	8 weeks
Maintenance therapy	
Fluconazole 200 mg/day, in HIV-CM start ART at 4 weeks. Consider discontinuing maintenance after a minimum of 1 year if $CD4^+$ cell count >100 /μL and HIV viral load suppressed	≥1 year
Induction therapy in resource limited settings	
If 5-FC is not available: D-AmB 0.7–1 mg/kg/day intravenously plus fluconazole 800-1200 mg/day	2 weeks (1 week better than no D-AmB)
If D-AmB is not available: Fluconazole 1200 mg/day† **plus** flucytosine 100 mg/kg/day orally (if available)	2 weeks

HIV-CM: HIV-associated cryptococcal meningitis; L-AmB: Liposomal amphotericin B; D-AmB: amphotericin B deoxycholate; 5-FC: flucytosine; ART: antiretroviral therapy.
* see IDSA guidelines [84]
** 800 mg/d preferred if second line induction regimens used
†Fluconazole increases nevirapine levels, and safety of high-dose fluconazole with nevirapine is unknown. Alternative antiretrovirals are preferred.

Figure legends

Figure 1

Associations between baseline cerebrospinal fluid immune response profiles and clinical outcome. Baseline CSF cytokine and chemokine concentrations were measured in 90 patients with HIV-associated cryptococcal meningitis. Principal component analysis was used to identify co-correlated cytokine and chemokine measurements that accounted for the variance across the data set. A majority of this variance was accounted by two components, PC1 and PC2 (see text). A. PC1 score was associated with 2 week survival. B. In those who survived and were started on ART, PC2 score was associated with the development of CM-IRIS. The points represent the mean values, with standard errors denoted by the error bars. From Jarvis et al [60]

Figure 2

Differences in functional phenotype of the cryptococcal antigen (CRAG)–specific responses at baseline between subjects who survived and subjects who died. Flow cytometry results show the proportion of cryptococcal antigen (mannoprotein fraction) -specific CD4 positive memory T cells producing interferon γ (IFN-γ), interleukin 2 (IL-2), macrophage inflammatory protein 1α (MIP-1α), tumor necrosis factor α (TNF-α), and combinations of these cytokines, at baseline, for patients who survived to 2 weeks and those who died. From Jarvis et al [59].

Figure 3. Raised CSF pressure. Time course of CSF pressure, visual acuity, and volume of CSF drained through a temporary lumbar drain in situ for 11 days, in a patient developing severely raised pressure, unresponsive to repeated daily lumbar punctures, during the third week of antifungal therapy, despite sterilization of CSF. ↓ Indicates time of lumbar puncture. From Macsween et al [109].

Figure 4

Host damage from infection-related inflammatory syndromes. CM patients with immune reconstitution syndrome (HIV-IRIS) after effective anti-retroviral therapy present with clinical deterioration with negative CSF cultures, but an aggravated T-cell and pro-inflammatory macrophage response (top panel). Previously healthy

patients with CM having clinical deterioration on effective therapy develop a similar post-infectious immune response syndrome (PIIRS) consisting of a similar T-cell response but a defective M2 macrophage polarization that is ineffective in clearing fungal antigen. From Panackal et al [51].

Figure 5

Magnetic resonance imaging demonstrating reductions in brain edema after treatment with corticosteroid therapy. T1 and FLAIR-weighted Magnetic resonance images of a patient with *C. gattii* infection and anti-GMCSF autoantibody treated with Amphotericin B from day 1-66 + adjunctive prednisone (50 mg/d) between day 1 and day 24. Therapy was then stopped on day 24 but then re-instituted at days 34-66 after clinical deterioration. (Figure from Panackal et al [70])

Figure 1

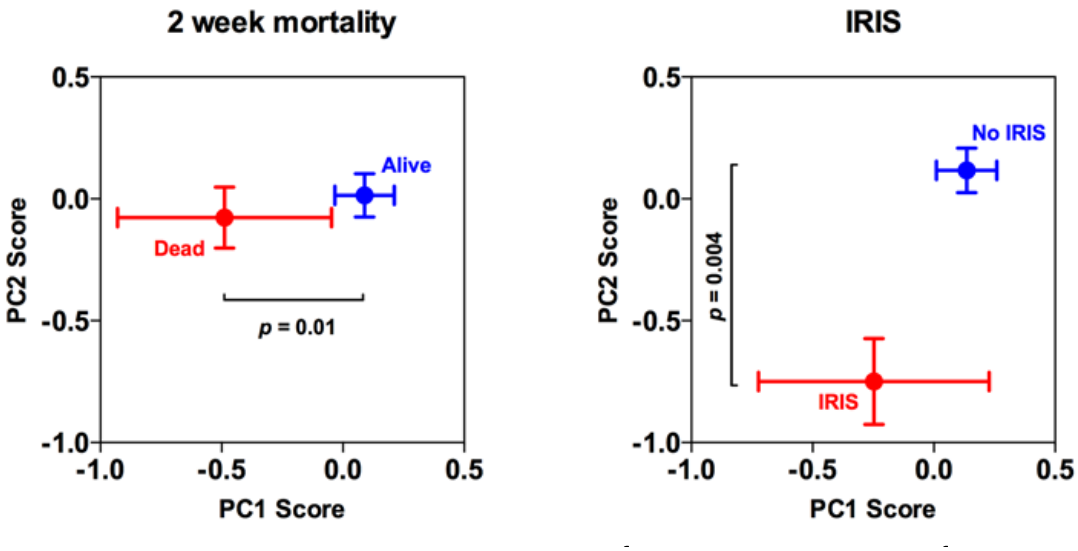


Figure 2

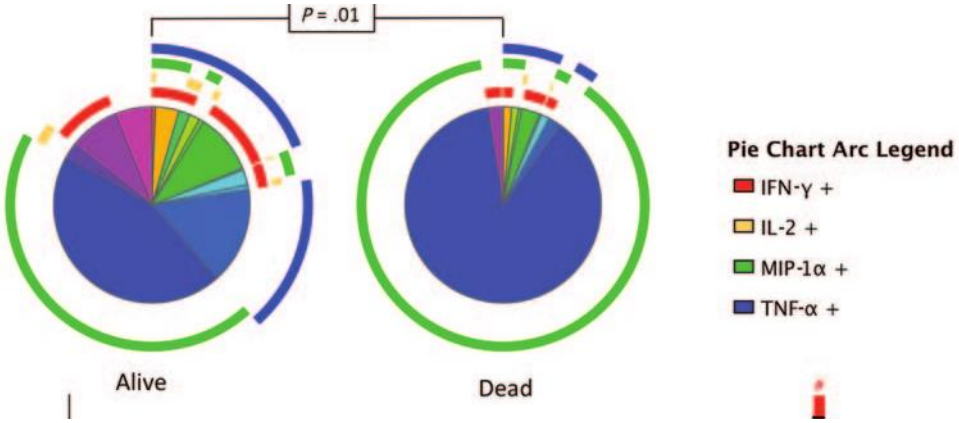
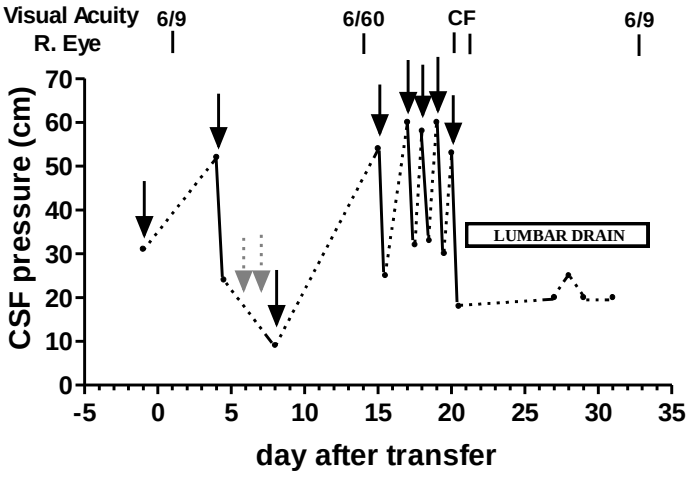


Figure 3:

A



B

Day after transfer	21	22	23	24	25	26	27	28	29	30	31	32	33
CSF drained (ml)	250	231	227	205	174	185	143	113	170	172	51		

drain clamped overnight

drain clamped, then removed

Figure 4

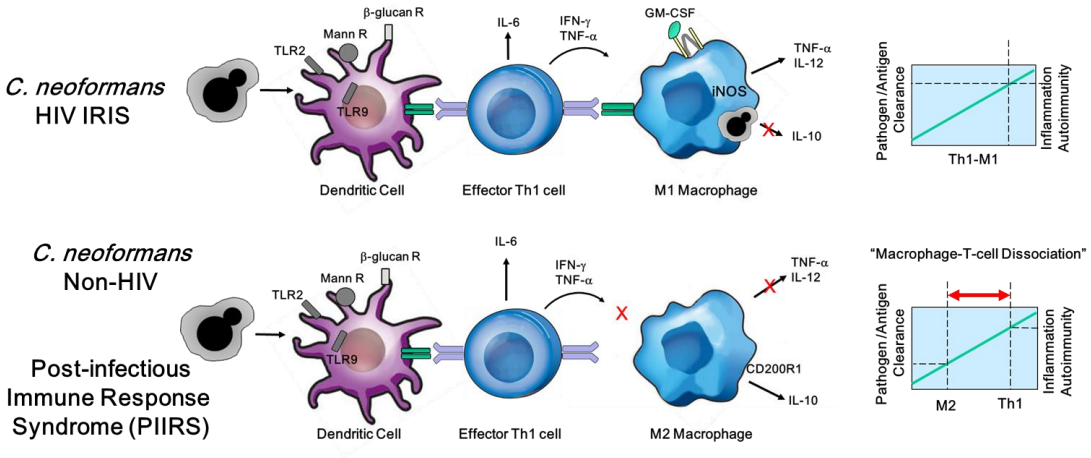
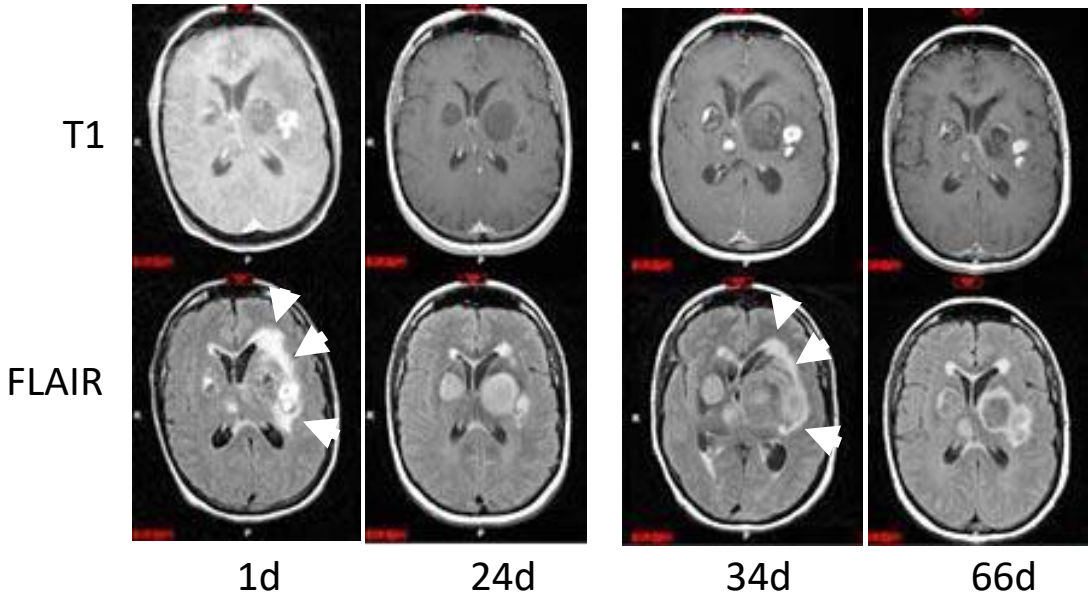


Figure 5.



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