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Review

Cryptococcal immune reconstitution inflammatory syndrome: from blood and cerebrospinal fluid biomarkers to treatment approaches.

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Abstract: Immune reconstitution inflammatory syndrome (IRIS) presents as an exaggerated immune reaction that occurs during dysregulated immune restoration in immunocompromised patients in late-stage HIV infection who commenced antiretroviral treatments. Virtually, any opportunistic pathogen can provoke this type of immune restoration disorders. In this review, we focus on recent development in the identification of risk factors for Cryptococcal IRIS and on advancements in our understanding of C-IRIS immunopathogenesis. We overview new findings in blood and cerebrospinal fluid which can potentially be useful in the diagnosis of cryptococcal meningitis IRIS. We assess the utility of these biomarkers to identify putative host-based targets, which may justify a clinical need for improvement in monitoring a patient's laboratory results and adjusting treatment modalities in AIDS patients co-infected with *Cryptococcus*.

Keywords: immune reconstitution inflammatory syndrome (IRIS); AIDS/HIV; antiretroviral therapy (ART); cryptococcal meningitis (CM); blood biomarkers; cerebrospinal fluid biomarkers.

1. Introduction

Cryptococcus species are the most common cause of meningitis in adults and one of the leading causes of HIV-related mortality in the world, with a global incidence estimated at 223,100 cases per year [1,2]. In countries with limited resources, cryptococcosis drives up to 40% of all hospitalizations and deaths associated with the advanced stage of HIV infection [3–5]. Immune reconstitution inflammatory syndrome (IRIS) associated with cryptococcosis is a common complication that manifests after the initiation of antiretroviral therapy (ART) [6,7]. Cryptococcal IRIS presents as an exaggerated and deregulated proinflammatory immune reaction, which accompanies the reduction of peripheral blood HIV viral load and the initiation of CD4+ T cell recovery [7]. Approximately 25% of HIV and *Cryptococcus* co-infected patients develop cryptococcal meningitis IRIS (CM-IRIS) within the first four months of ART treatment, with an averaged mortality rate of around 20 +/- 10% [8].

There are two recognized forms of CM-IRIS. The first form is "unmasking" IRIS, in which the individual manifests an inflammatory response to *Cryptococcus spp.* revealing previously undiagnosed cryptococcal meningitis (CM) in ART-naïve individuals after starting ART [7,9]. Unmasking CM-IRIS may include neurological symptoms driven by high intracranial pressure and

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inflammation, such as severe headache, vomiting, visual impairment (diplopia, photophobia, blindness), hearing loss, seizures, ataxia, or aphasia [10–12]. Altered mental status, including personality and behavioral changes, confusion, hallucinations, and in rare cases, lethargy, are attributable to unmasking CM-IRIS. Unmasking IRIS is usually diagnosed within 2 to 6 weeks on antiretroviral treatment and it is the deadliest [13]. Thus, the diagnosis of cryptococcal infection is essential for the prevention of unmasking CM-IRIS. The second form is "paradoxical" IRIS, which occurs in the settings of induction antifungal therapy [6,14]. Paradoxical CM-IRIS became the most common due to significant improvements in the diagnosis of cryptococcal meningitis and the introduction of antifungal therapy regimens prior to ART commencement [6,15]. The paradoxical form also presents itself as neuro-cryptococcosis, with clinical symptoms of worsening neurological function impairments and altered mental status due to raised intracranial/cerebrospinal fluid (CSF) pressure. Paradoxical CM-IRIS can be clinically assessed according to Glasgow Coma Scores (GCS) and via radiological and imaging findings [16,17]. Paradoxical C-IRIS manifests on an average, from 1 to 6 months after the initiation of ART, and it occurs on the background of initial clinical and microbiological response to antifungal treatment, as well as virologic response to ART [2,14,18]. As described below, periodic examination of cerebrospinal fluid found to be helpful to diagnose and predict paradoxical CM-IRIS [19].

Pulmonary C-IRIS has been described primarily in *Cryptococcus neoformans* infection. Clinical manifestations include cough, dyspnea due to pneumonitis, pulmonary infiltrates, lymphadenopathy, cavitation, and nodular lesions [9,20,21]. Several components of the immune system such as T cells and macrophages, pro-inflammatory cytokines and chemokines, are thought to be involved in the pathology of pulmonary IRIS, although not studied systematically (reviewed in [22]).

In this review, we focus on recent advances in mitigating risk factors for C-IRIS, and the prognostic and diagnostic molecular biomarkers for better understanding CM-IRIS immunopathogenesis. New biomarkers may help to identify putative host-based targets to justify a clinical need for improvement of laboratory monitoring and adjusting treatment modalities in AIDS patients co-infected with *Cryptococcus*.

2. Conventional risk factors for CM-IRIS

The conventional risk factors of CM-IRIS can be divided into 3 categories (Figures 1, 2, and 3).

2.1. Host-related factors

Because the variety of opportunistic pathogens have been linked to ART-associated IRIS in people with acquired immunodeficiency syndrome (AIDS), host-related risk factors are considered most important and universal for several types of IRIS [23]. Improvements in immune status in patients who had been severely immunocompromised often accompanied by disbalanced immune reconstitution, which represents a host risk factor for the development of IRIS. Patients as such, have very low pre-ART $CD4^+$ T cell count in the blood (<100 CD4+ cells/uL) [24,25] and high HIV viral load (>100,000 copies/mL of blood) [26]. They include low baseline antibody responses to *Cryptococcus spp.*, e.g. decreased levels of total plasma IgM and specific antifungal IgM (GXM-IgM or β-glucan-binding IgM) [27], and lack of pro-inflammatory cytokines in the serum, or CSF, as will be described below. More recently, transcriptomic profiles had been assessed, and several molecular

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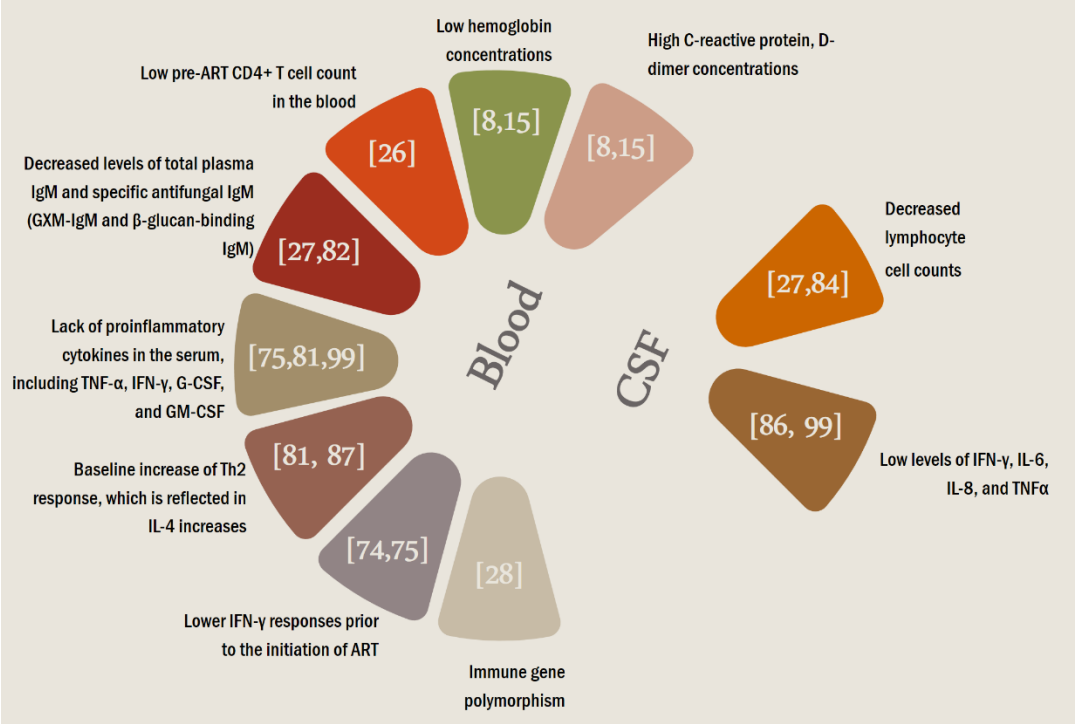
pathways proposed as potential baseline biomarkers, as described below [28]. Genetic factors, such as single nucleotide polymorphism Interleukin 7 receptor subunit alpha (IL7RA) may affect predisposition to developing of IRIS [29]. Allelic polymorphisms (e.g. in CYP2C19 gene) can be considered as patient-specific factors affecting fungicidal drug activities, toxicity, and the level of inflammation (e.g. C-reactive protein or albumin levels) [30]. Among readily available baseline biomarkers, low hemoglobin concentrations (<8.5 g/dL) and high C-reactive protein levels (CRP >32 mg/L) or D-dimers (>3.89 ug/mL) are predictive of IRIS events [8,15].

Figure 1. Schematic representation of different studies assessing host-related risk factors for CM-IRIS and the CSF and blood profiles in HIV-positive patients with cryptococcal meningitis (CM).



2.2. Pathogen-related factors

The genomic differences in clinical isolates may underlay differential drug susceptibility and virulence of *Cryptococcal spps*., which play important role in the severity of CM and CM-IRIS [30]. Genetic make-up allowed some *Cryptococcal spps*. advantaged metabolic fitness, as was tested in pre-clinical models [32–34]. Mutated species of HIV may also play role in the pre-treatment drug resistance to non-nucleoside reverse transcriptase inhibitors ART, and in alterations of immune responses after ART initiation [35].

In recent years, there has been an international collaborative effort focused on the development of point-of-care assays in resource-limited laboratory settings [36,37]. The lateral flow assay (LFA, IMMY, USA) utilizes gold-conjugated monoclonal antibodies that targeted capsular polysaccharide glucuronoxylomannan (GXM), a primary cryptococcal antigen of 4 serotypes [38]. LFA is fast and able to quantify cryptococcal antigen (CrAg) titers, with high sensitivity and specificity in CSF: 100% and 99.8%, respectively (reviewed in [39]). Cryptococcal antigenemia (CrAg+) in serum, CSF or other biofluids is often detected in asymptomatic CM patients who

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subsequently develop CM-IRIS [40–43]. Mortality also remains higher in CrAg+ immunocompromised patients after initiation of ART, and the levels of CD4+ T cells are inversely correlated with CrAg titers [44,45]. The FDA-approved FilmArray meningitis/encephalitis nested multiplex PCR panel (BioFire Diagnostics, USA) had recently been introduced into routine clinical practice, which can detect and differentiate DNA from *C. neoformans* and *C. gattii* (among other pathogens) [46].

With the development of highly sensitive and specific molecular tests, great advances have been made in the diagnostic procedures of CM caused by various Cryptococcal species. Isothermal molecular techniques, such as LAMP (Loop-mediated isothermal AMPlification), have also contributed to improving the diagnosis of fungal diseases. LAMP technique is based on the principle of isothermal loop amplification to identify the species of *Cryptococci* from the CSF culture isolates targeting the internal transcribed spacer (ITS) region and CAP59 gene. LAMP assay has high specificity for molecular genotypes VNI, VNII, and VNIII of *Cryptococcus neoformans*, and able to differentiate from *C. gattii* and other fungal species [47]. LAMP does not require expensive laboratory instrumentation to perform, thus in the future can be introduced as a point-of-care assay.

Culture remains a gold standard to assess live pathogen in the CSF or blood, by measuring colony-forming units (CFU/mL) growth on Sabouraud dextrose agar, for 48 hours, at 30°C. An important prognostic parameter, such as early fungicidal activity (EFA), can be calculated from recurrent cultures during induction regimens (described below). Microbiological clearance is measured as $\log_{10}$ clearance of *Cryptococcus* yeasts per mL of CSF and serves as an important predictor of increased mortality, including that from IRIS [48]. Cultured isolates can subsequently be serotyped by real-time PCR assay, and mating type can be determined by amplified fragment length polymorphism (AFLP) or PCR-restriction fragment length polymorphism (RFLP) [33,49]. Molecular typing revealed that genotypes, drug susceptibility, and virulence of *Cryptococcus species* varied between different continents and in different countries [50]. However, recent studies found no correlation between antifungal drug susceptibility and hazards of death for therapeutic outcomes in the cohort of severely immunosuppressed AIDS patients [51].

Figure 2. Schematic representation of different studies assessing pathogen-related risk factors for CM-IRIS in the CSF and blood of HIV-infected patients with cryptococcal meningitis (CM).

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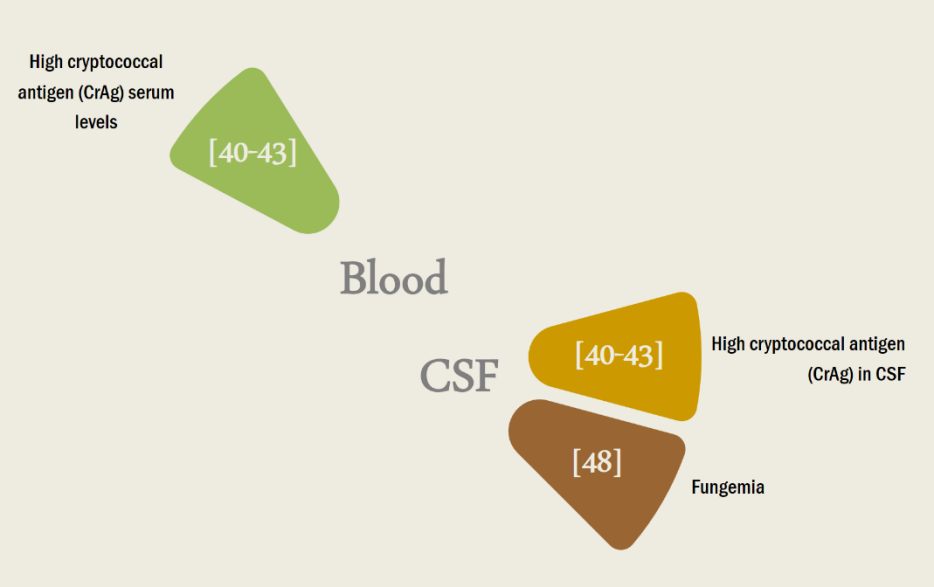

2.2. Treatment-related risk factors

Inadequacy of high-dose antifungal drug monotherapy or combination therapy is the number one treatment-related factor for IRIS, as mortality from CM is the highest during the first 6 months under routine protocols [52,53]. A combination of various antifungal regimens had been tested and compared, in the procurement of the best antifungal activity in the shortest period of time [54,55]. However, a shorter duration between induction antifungal treatment and the initiation of ART may predispose patients to fatal C-IRIS events. The proposed explanation is an insignificant time for the achievement of microbiological clearance [53,56].

A combination of highly active antiretroviral drugs seems to have immunomodulatory effects, yet in some cases increase IRIS incidence, depending upon patient-specific factors [57]. The rapid decrease in HIV viral load on ART (> 2.5 log reduction in IRIS patients, over 4 weeks, when compared to pre-ART viral load) has also been identified as a risk factor for activation of the host immune system and IRIS [26,58,59]. On the other hand, interruption of ART or development of ART resistance slows down the immune reconstitution and prolongs chronic inflammation [60,61]. After ART initiation, confirmation of the virologic response is highly recommended for diagnosis of treatment failure or suboptimum responses to combination ART, but not essential for the prediction of IRIS [62].

Thus, rapid cellular immune activation, which drives the symptoms of CM-IRIS, is predicated by a combination of several factors such as the hosts' immunological predisposition, the microbial antigen burden, and effectiveness of the drugs (ART or antifungals).

Figure 3. CM-IRIS risk factors related to treatment. Schematic representation of different studies assessing treatment-related risk factors for CM-IRIS in HIV-infected patients with cryptococcal meningitis (CM).

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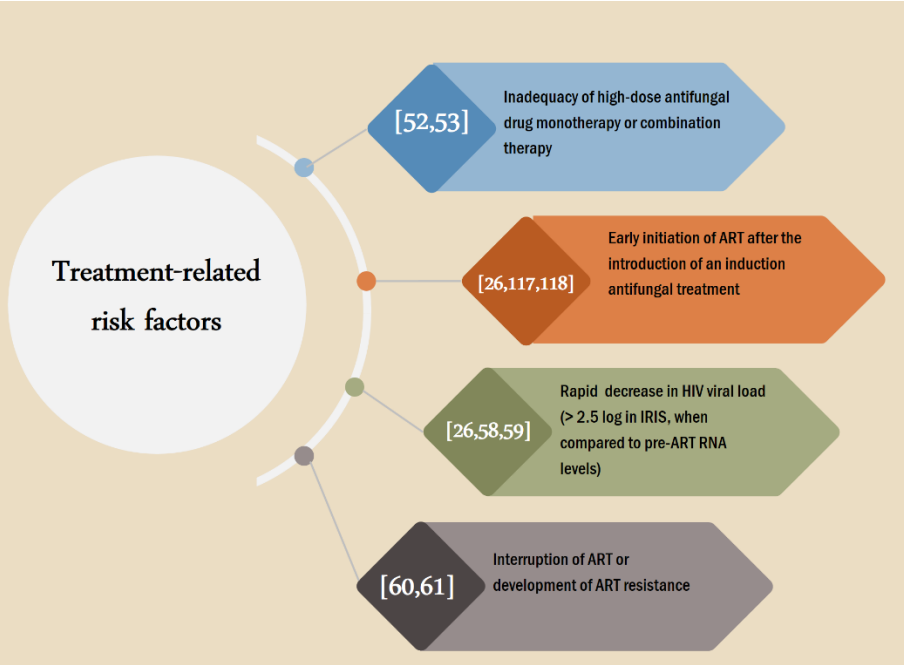

3. Immunopathogenesis of C-IRIS

Human immunodeficiency virus infects and persists in CD4+ T cells, astrocytes, microglial cells, and less frequently, in cells of monocyte-macrophage lineage [63,64]. During the first few weeks on ART the rate of HIV viral load decreases, T cell population stabilizes, however, the recovery initiates very slowly in severely immunodeficient individuals. The initiation of ART at the advanced stage of HIV infection (at CD4+ T cells ≤ 200/mm3) is associated with long-term immune and metabolic abnormalities (up to 3 years after ART initiation) [65]. In fact, the thymic output of naïve CD4 T cells (detected as T cell receptor excision circles (TRECs)) exhibits an augmenting trend only after 12 months on ART in adults and young children [66,67]. Pre- and post-ART, CD4+, and CD8+ T cells express immune checkpoint molecules (e.g. PD-1, TIGIT, and LAG-3), which is associated with long-term HIV persistence and immune exhaustion [68]. During IRIS episodes, higher frequencies of PD-1^+/$CD4^+$ T cells expressing LAG-3, CTLA-4, and ICOS have also been detected when compared with patients without IRIS [69]. HIV-AIDS patients, who subsequently die from CM-IRIS or CM after ART initiation also exhibit upregulation of immune checkpoint gene expression (PD-1, PDL-1) in peripheral blood at the time of ART (Vlasova-St. Louis, unpublished). The persistence of expression of immune checkpoint molecules and T cell immune exhaustion correlates with the increased size of the HIV reservoir, which seems to play important role in the subsequent ART-driven activation of cytotoxic CD8+ T cells and antigen-presenting cells [70,71]. This may lead to unproductive and paradoxical immune reactions to a reservoir of antigens, secretion of pro-inflammatory mediators by activated monocytes and macrophages, and tissue damage [72]. Thus, the transition from the state of immune exhaustion and poor macrophage function to ART-related immune reconstitution, often accompanied by exaggerated innate inflammatory responses which lead to IRIS.

3.1. Blood plasma and serum biomarkers in CM-IRIS

Blood transcriptomic profiles have been used to characterize changes in ART-induced gene expression between HIV-infected patients with CM who developed paradoxical CM-IRIS and those

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who did not [28,73]. The analysis of these profiles showed that before starting ART, patients who eventually developed CM-IRIS exhibit a significant decrease in the expression of transcripts encoding type I interferons (IFN I) and antiviral defense proteins, particularly those that lead to the downregulation of viral replication, while antimicrobial defense genes are upregulated [28]. Earlier observation of high plasma levels of soluble CD40L, which is known to suppress IFN-α production, may provide an explanation for INF type I deficiency in C-IRIS patients [74]. Prior ART, patients with CM-IRIS also showed a reduction in interferon-gamma (IFN-γ) gene expression and IFN-γ secretion by the stimulated mononuclear cells in response to cryptococcal mannoproteins [75]. Interestingly, the most recent study led by Vlasova-St. Louis, which specifically focused on fatal CM-IRIS cases, identified that baseline IFN-γ expression had been elevated in patients who subsequently died from CM-IRIS (the manuscript is under review by BMC Medical Genomics). Thus, the expression of components of antiviral defense pathways, such as type I/II interferons and IFN-induced genes, could be used as a predictive biomarker for fatal and non-fatal CM-IRIS ([28] and unpublished).

Blood granulocyte activation status, particularly neutrophils', had been correlated with high mortality, including that from CM-IRIS, perhaps reflecting systemic oxidative stress generated by these short-lived effector cells of the innate immune system [76]. Transcriptomic biomarkers for activated granulocytes (e.g. oxidases, arginase, integrins, etc.) had been found to precede CM-IRIS events, which accompanied by markers of tissue destruction (e.g. matrix metalloproteinases) [28]. Markers of monocyte activation (e.g., soluble CD14) in plasma positively correlate with levels of IL-6, C-reactive protein, serum amyloid A and D-dimer, and associated with the mortality rate [77]. Studies also showed that a higher percentage of activated CD14+CD16++ monocytes produce high amounts of TNF-α and IL-6, independently from IFN-γ co-stimulation [78]. Higher frequencies of activated CD14+CD86+ or CD14+HLA-DR blood monocytes have also been observed in C-IRIS patients who failed to clear *Cryptococci* from CSF pre-ART [79]. In addition, inappropriately polarized and activated macrophages may persistently harbor a residual viral replication during ART due to enhanced expression of efflux transporters [80]. Altogether, this may represent a source of pro-inflammatory cytokines like IL-6 which can be measured in abundance in patients' blood during CM-IRIS events [81].

Excessive expression of chemokines and integrins in peripheral blood precedes CM-IRIS and can be assessed by monitoring patients for pathological immune reconstitution [28]. Plasma levels of cytokines, such as IL-6, IL-18, TNF-α, IL-5, IFN-α, IL-7, IL-17, GM-CSF, CCL11, and CXCL10 are also increased in patients who develop CM-IRIS [82–84]. The association between cytokines and CM-IRIS reflects a systemic immune response to events that occur in the central nervous system (CNS) [84]. To mitigate the cytokine storm during CM-IRIS, several antibody-based biologics had been studied, as described below. Additionally, CXCR7 has recently been proposed as a potential therapeutic target receptor on CD14+CD16+ monocytes to limit neuroinflammation by controlling activated patrolling monocyte entry to CNS [85].

The baseline (pre-ART) absolute number of B cells in the peripheral blood are not different between patients who subsequently developed CM-IRIS and those who have not. However, low plasma levels of IgM antibodies secreted toward the cryptococcal polysaccharide antigens, glucuronoxylomannan (GXM), laminarin, and pustulan represent poor B cell function and may put patients at high risk of CM-IRIS [27]. Poor antibody response to cryptococcal antigens indicate an

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important role of antibody-mediated cryptococcal antigen clearance. A longitudinal study using blood leucocytes from patients with HIV and CM (with and without IRIS) showed disproportionate cellular expansion, with the increase in neutrophil counts ($CD66^{+}CD16^{+/-}$), and the heightened percentage of deactivated monocyte subpopulations, but no increases in T-helper cell type 1 populations, which confer the inability to clear *Cryptococcal* antigens [82,86]. Peripheral blood from patients who had fatal outcomes revealed low GXM- and LPS-driven monocyte responses ($CD16^{+/-}$ $HLA\text{-}DR^{low}$), reduced TNF-α, but increased IL-6, IL-10, CXCL10 production [86]. In the setting of severe lymphopenia, the decreased phagocytosis and antigen-presentation, and a failure to properly activate T lymphocytes and clear antigenic load results in an exaggerated response during the early phase of CD4+ T-cell reconstitution [87]. IL-7/IL-7R interaction plays a homeostatic role in T cell survival and repopulation during the initial phase of immune recovery, and abnormal IL-7 plasma levels are strongly associated with CM-IRIS [79]. Dysbalanced of cytokines milieu may inappropriately differentiate T helper 0 cells into Th1 and Th2 types, which in turn, impairs the adaptive immune response to *Cryptococcus spp.* [81,88]. Recent genetic studies revealed that the T allele homozygosity at rs6897932 of the L-7Rα gene (encodes CD127) links to a faster CD4+ T cell recovery after ART initiation, as opposed to CC genotypes in HIV- infected individuals [29,89]. Future genotyping studies of cytokine and cytokine receptor genes may reveal the link between the single nucleotide polymorphism in AIDS patients and predisposition to immune reconstitution disorders, such as IRIS [90–93].

3.2. Cerebrospinal fluid biomarkers of CM-IRIS

CNS injury is a hallmark of CM and CM-IRIS. Accumulating evidence suggests the involvement of microglia inflammation and recruitment of naïve T cells in the brain during immune restoration, causing CNS pathology [94]. During CM-IRIS episode, the phenotype of mononuclear cells in cerebrospinal fluid (CSF) demonstrates a CNS-compartmentalized shift from classic ($CD14^{++}CD16^{-}$) to an intermediate/pro-inflammatory phenotype ($CD14^{++}CD16^{+}$) [95]. However, immunosuppressive ligand PD-L1 expression is also high on both, the CSF monocytes and $CD56^{dim}$ and $CD56^{bright}$ NK cells subsets, reflecting CNS immunopathology behind C-IRIS [95].

Paradoxically, weak cellular inflammatory responses in the CSF at the time of ART initiation are observed to be predictive of CM-IRIS [96]. In patients with HIV and CM, low CSF IFN-γ, IL-5, IL-6, and G-CSF concentrations are associated with higher fungal load, double-negative $CD4^{-}CD8^{-}$ T cells, and increased mortality [97]. In severe cases of CM, the infected monocytes/macrophages often express alternative activation markers (e.g., CD206, CD163, CD200) are unable to eradicate *Cryptococcus spp.* from CSF spreading the pathogen to the central nervous system [98]. Examination of associations between immune phenotype and clinical outcomes, particularly death and CM-IRIS, the CSF immune responses showed two divergent routes. The first is the protective immune route that is driven by increased pre-ART levels of IL-6, IL-8, IL-10, IL-17, TNF-α, and IFN-γ [99]. These cytokines can increase the traffic of CD4+ T cells and myeloid cells to the CNS and CSF. This route aims to decrease the fungal burden and improve microbial clearance. The second route is represented by high levels of cytokines CCL2 (MCP-1), CCL3 (MIP-1α), and GM-CSF that are secreted by CNS-resident monocytes and are associated with the subsequent development of CM-IRIS [99]. It has been demonstrated that after initiation of effective antifungal therapy, there is an enrichment for CD8+ T cells that co-express CXCR3 and CCR5 receptors and the increase in CCL2/CXCL10 and

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CCL3/CXCL10 ratios in the CSF CM patients with neurological deteriorations, who subsequently developed CM-IRIS on ART [100]. The trafficking of CD8+T cells and chemokines into the CSF is probably expedited through the damaged blood-brain barrier caused by ongoing chronic inflammation [100]. Soluble macrophage-specific activation biomarkers (e.g., sCD163, sCD14, CCL3) that are abundant in CSF of patients, who are at a higher risk of mortality from CM-IRIS, also suggest macrophage/microglial involvement in recruiting cytotoxic cells to CNS during CM-IRIS pathogenesis [101].

In addition to opportunistic infections, patients with advanced HIV infections suffer from HIV associated neurocognitive disorders (HAND). CM exacerbates neurocognitive impairment in these patients. HAND patients already exhibit a high percentage of TNFα- and IFNγ- expressing T cells, increased levels of sCD163, sCD14, and have low CD8+CD107+ T cell degranulation capacity. Thus, the additional cryptococcal burden may exaggerate CSF immune responses, the influx and retention of activated immune cells and proinflammatory mediators, which contribute to high intracranial pressure during CM-IRIS pathogenesis [102].

Based on the assessment of 21 biomarkers, it had been suggested that patrolling monocytes and CNS residing monocyte have distinct chemokine expression and cytokine production [103]. The macrophages can also be present at different polarization states in peripheral blood and CNS. Although not readily available in human subjects, the CNS-resident macrophages may be the most relevant source of pro-inflammatory biomarkers and the main drivers of immunopathology [104]. Considering that the data on the pathogenesis of CM-IRIS is derived from analyses of few immunological parameters, further comprehensive studies are needed to understand whether the baseline and the kinetics of inflammatory response before and during C-IRIS, in different body compartments, may help to identify biomarker panels that may be useful in clinical settings.

4. Treatment advances for management cryptococcal infection and CM-IRIS

With respect to therapy, there are still no effective treatments for IRIS**,** however targeting the risk factors described above, may decrease IRIS incidence and severity. The CM treatment regimens are composed of three pharmaceuticals: amphotericin B deoxycholate (AmB) or liposomal AmB (L-Amb), flucytosine (5-FC), and fluconazole (FLU). The treatment of CM is divided into three phases: induction, consolidation, and maintenance (reviewed in [105]).

4.1. The induction phase aims to drastically decrease the fungal burden in the patient's cerebrospinal fluid in the first 2 weeks and is fundamental for survival. AmB has high toxicity, at standard doses 1 mg/kg/day, such as hepatic and renal toxicity, anemia, electrolytic abnormalities, or reactions at the site of infusion. For this reason, a recent formulation of liposomal amphotericin B (L-AMB) is recommended as it is less toxic at a single dose (10 mg/kg), has a longer half-life, and can more effectively penetrate the brain tissues [106–108]. Presently, the combination AmB/5-FC provides the most effective fungicidal activity and cryptococcal clearance [109]. Novel formulation of oral AmB is currently being tested for safety and tolerability in the EnACT Trial [110].

Voriconazole (VCZ) is an antifungal agent that is used to treat invasive fungal infections, such as cryptococcosis, aspergillosis, and candidiasis. A study conducted in South Africa assessed its efficacy in treating CM and showed no statistically significant difference between the use of AmB/FLU or AmB/voriconazole and the standard AmB/5-FC therapy [111]. VCZ has good bioavailability but

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owing to a higher cost, scarcity of studies on CNS penetration, and altered pharmacokinetics on the background of inflammation, its use is limited [112]. Although other triazoles such as itraconazole, posaconazole, and prodrug isavuconazole [113,114] exhibit anticryptococcal activity, they are used only as a second-line agent or in combination with AmB, due to drug-drug interactions and toxicity (reviewed in [115]).

4.2. In the consolidation phase (2-6 weeks of induction therapy), doses of antifungal agents are decreased, and antiretroviral therapy is initiated. The initiation of the consolidation phase and ART must be considered carefully [116]. The introduction of ART is recommended 4-6 weeks after starting induction antifungal therapy, to improved survival rates and achieve sustained clinical responses [117]. A pragmatic approach to the management of patients with HIV-associated cryptococcal meningitis has been outlined in the recent study [118]. Recently, a new tetrazole compound VT-1129 (Viamet Pharmaceuticals Inc.) had been shown to exhibit potent *in vitro* activity against *Cryptococcus spp*. [119]. VT-1129 is highly selective for fungal CYP51, has minimal effect on human cytochrome P450 enzyme metabolism, and may potentially be used as preemptive or consolidation therapy for fluconazole-resistant cryptococcal meningitis [120].

4.3. The maintenance phase is introduced to maintain the sterility of CSF culture and to prevent a relapse of cryptococcal disease. The fluconazole maintenance therapy (200 mg/day) is extremely important, however, this phase is the most vulnerable to non-compliance and loss to follow-up [121,122].

4.4. The symptoms of paradoxical CM-IRIS are accompanied by abnormal radiological and magnetic resonance imaging findings (e.g. enhanced radiographic characteristics of leptomeningeal enhancement or cystic lesions) and high intracranial pressure (>250 mm H2O) [14]. Thus, therapeutic lumbar punctures and in some cases, shunting, are recommended for those suspected of CM-IRIS [123,124]. The addition of nonsteroidal anti-inflammatory drugs (NSAIDs) to ART and antifungal regimens is also advised in mild and self-contained forms of CM-IRIS. In cases of severe symptoms of CM-IRIS, the administration of corticosteroids (dexamethasone particularly) may be useful to decrease inflammation, although had been shown to associate with higher mortality [104,125,126]. In cases of pulmonary cryptococcal IRIS, corticosteroid treatment may be considered in the event of the development of respiratory distress, but the antifungal regimen should be continued [119,120]. Immunosuppressive drug hydroxychloroquine reduces lipopolysaccharide/TLR-mediated immune activation, which might be important for CM-IRIS prevention, as early CM-IRIS is solely driven by innate immune activation pathways, especially in immunological non-responders (patients with CD4 increase < 5% in the last 12 months on ART) [127].

Other immunomodulatory agents (thalidomide or adalimumab) had been tested. There have been reports documenting the neurological improvement in severe cases of CM-IRIS after the use of thalidomide and adalimumab, human monoclonal antibodies that bind to TNFα and block its anti-inflammatory activity [128,129]. Another biologic, a recombinant IFNγ, had been shown to expedite CSF fungal clearance by increasing Th1 cell responses and depolarizing macrophages, although it has failed to exhibit evident benefit to patient survival [130,131]. In all cases of IRIS, ART should be continued unless there is a risk of fatal outcomes [132,133].

5. Conclusions

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Herein, we discuss immune reconstitution inflammatory syndrome in patients co-infected with HIV and cryptococcosis, with particular attention to clinical presentation, risk factors, immunopathology, and treatment. Mortality from CM-IRIS can be reduced significantly if patients initiated antiretroviral therapy during the phase of moderate immunosuppression, and before significant CD4+ T cell count loss [134,135]. The morbidity and costs associated with IRIS in people living with HIV continue to decline in the USA since 2012 (< 8%), with no fatality [3]. Although much research is being done on the mechanisms that modulate the immunopathogenesis of CM-IRIS, no biomarkers for this condition have been validated to produce a sufficient level of evidence to enter clinical practice. Recently proposed EQUAL Cryptococcus score, as a guideline for the optimal management of cryptococcosis includes intervention steps in CM-IRIS cases [136]. Future frontiers for more effective therapy appear to be to: 1) improve the time to HIV diagnosis; and 2) identify and manage associated immune-inflammatory conditions. The development of effective antifungal medications, companion immunomodulatory therapies, and improvement of patients' healthcare in resource-limited countries should be a priority for the next few decades.

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Abbreviations

ART	antiretroviral therapy
HIV	human immunodeficiency virus
IRIS	immune reconstitution inflammatory syndrome
CM IRIS	cryptococcal meningitis IRIS
CSF	cerebrospinal fluid
AIDS	acquired immunodeficiency syndrome
GXM	glucuronoxylomannan
IFN	interferon
IL	interleukin
Th	T helper cells
LFA	lateral flow assay
CrAg	Cryptococcal antigen
LAMP	Loop mediated isothermal AMPlification
PD	programmed death
PD-L1	programmed death ligand 1
TIGIT	T Cell Immunoreceptor With Ig And ITIM Domains
LAG3	Lymphocyte Activating 3
ICOS	inducible T cell costimulatory
TNF-α	tumor necrosis factor
LPS	lipopolysaccharide
CNS	central nervous system
TLR	toll-like receptor
GM-CSF	granulocyte-macrophage colony-stimulating factor
G-CSF	granulocyte colony-stimulating factor
AmB	amphotericin B
LAmB	liposomal amphotericin B
FC	flucytosine

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FLU fluconazole
VCZ voriconazole
NSAIDs nonsteroidal anti-inflammatory drugs

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