Impact of routine cryptococcal antigen screening and targeted pre-emptive fluconazole therapy in antiretroviral naive HIV-infected adults with less than 100 CD_4 cells/µL: a systematic review and meta-analysis

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Summary: Targeted pre-emptive fluconazole initiated at 800 mg/day following post-screening lumbar puncture to exclude underlying cryptococcal meningitis in blood cryptococcal antigen (CrAg)-positive asymptomatic patients starting antiretrovirals at less than 100 CD_4 cells/µL, significantly reduces incidence of CM and has some survival benefits.

Abstract

Cryptococcal antigen (CrAg) screening and targeted pre-emptive fluconazole in antiretroviral naive HIV-infected adults with less than 100 CD_4 cells/μL seems promising to reduce the burden of cryptococcal meningitis (CM). We searched MEDLINE, EMBASE, and Web of Science and used random-effect meta-analysis to assess the prevalence of blood CrAg-positivity (31 studies; 35,644 participants) and asymptomatic CM in CrAg-positives, incidence of CM and all-cause mortality in screened participants. Pooled prevalence of blood CrAg-positivity was 6% (95%CI: 5 – 7) and asymptomatic CM in CrAg-positives was 33% (95%CI: 21 – 45). Incidence of CM without pre-emptive fluconazole was 21.4% (95%CI: 11.6 – 34.4) and 5.7% (95%CI: 3.0 – 9.7) with pre-emptive fluconazole initiated at 800 mg/day. In CrAg-positives, post-screening lumbar puncture prior to initiating pre-emptive fluconazole at 800 mg/day further reduced incidence of CM to null and showed some survival benefits. However, all-cause mortality remained significantly higher in CrAg-positives than CrAg-negatives: RR: 2.2 (95%CI: 1.7 – 2.9, $p<0.001$).

INTRODUCTION

Cryptococcal meningitis (CM) is due to a ubiquitous environmental encapsulated yeast, *Cryptococcus* spp, and occurs primarily in patients with advanced defective cell-mediated immunity [1, 2]. Consequent to the HIV pandemic, there has been a remarkable surge in the incidence of CM, especially in Sub-Saharan Africa [3, 4]. In such settings, over 90% of CM occur in HIV-infected patients [5, 6]. With the introduction of antiretroviral therapy (ART) in the 1990s, the incidence of CM has declined in high-income countries (HIC) [7, 8]. However, in low- and middle-income countries (LMIC), around 20% of patients still present to HIV care with less than 100 CD_4 cells/μL, a major risk factor for developing CM [4, 9]. In LMIC settings, CM accounts for around 15% of HIV-related mortality [4] with in-hospital case fatality rates ranging between 30 – 60% in recent Sub-Saharan African cohorts [6, 10-13].
There is therefore urgent need for effective preventive strategies to reduce the burden of CM [14]. The "blanket" strategy no longer recommended, relied on fluconazole-based primary prophylaxis in all patients with less than 100 CD_4 cells/μL [15]. Though this strategy was shown to reduce the incidence of CM [16], it was not widely implemented because of lack of evidence on survival benefits, potential for inducing resistance to fluconazole and high cost. This prompted experts to suggest targeted pre-emptive fluconazole therapy to patients identified at higher risk of CM who are more likely to benefit from this pre-emptive treatment [17].

Cryptococcus contains a capsular polysaccharide, known as cryptococcal antigen (CrAg), which can be detected in blood weeks to months prior to onset of CM [18]. Evidence suggests that without fluconazole therapy, CrAg-positive patients have up to 25% risk of CM in the first year of ART [14, 19]. Thus, in 2011, the World Health Organisation (WHO) suggested routine CrAg screening in ART-naïve HIV-infected adults with less than 100 CD_4 cells/μL, using either latex agglutination (LA) or lateral flow assay (LFA) procedures [20] (LFA easier and results obtained within ten minutes [21]). Following WHO's advice, CrAg-positive patients without meningitis should be offered pre-emptive oral fluconazole at a tapering dose of 800 mg/day for two weeks, then 400 mg/day for eight weeks, followed by 200 mg/day until control CD_4 is above 200 cells/μL [20]. Nonetheless, this recommended dosage remains provisional because the optimal antifungal regimen for this population is not clearly established [22].

CrAg screening with targeted pre-emptive fluconazole therapy seems attractive and cost-effective [23-26], but how best to implement it in overstretched, under-resourced high disease burdened health care settings remains a challenge. Nevertheless, it is incorporated into several national HIV care guidelines, both in LMIC (Botswana, Kenya, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Uganda) and HIC settings (USA and France) [4, 27]. Though promising, a systematic assessment of the impact of this strategy is lacking. We therefore performed a systematic review and meta-analysis (SRMA) to assess four key clinical outcomes of routine CrAg screening and targeted pre-emptive fluconazole therapy in ART-naïve HIV-infected adults with less than 100 CD_4 cells/μL: the prevalence of CrAg positivity, the prevalence of asymptomatic CM in CrAg-positives, the incidence of CM and all-cause mortality during follow-up in screened participants.

METHODS

Search strategy and study selection

A medical information specialist (RS) developed a comprehensive search strategy to identify published and unpublished studies in MEDLINE, EMBASE, and Web of Science. Medical subject headings (MeSH) and keywords included: "cryptococcal antigen", "cryptococcal surface polysaccharides", "cryptococcal meningitis", "HIV", "screening", "detection", "latex agglutination", "lateral flow assay" (Supplementary Table 1). To avoid missing relevant studies, we did not use methodological filters. Searches were run from January 1981 (year of first HIV case) through April 2018. References of included studies and previous reviews on the subject were screened for eligibility. Reports that cited included studies were also searched on Google Scholar. Conference proceedings of the Conference on Retroviruses and Opportunistic Infections (CROI), the International Conference on Cryptococcus and Cryptococcosis (ICCC), and the International AIDS Society (IAS) conference were screened from 2010 onwards.

Two review authors (ET, JJB) independently screened studies by title and abstract and assessed full texts of potentially relevant studies. Discrepancies were discussed and when consensus was not reached, study inclusion was further discussed with a third author (JFC). Study selection was done using Rayyan systematic reviews online application (http://rayyan.qcri.org).

We included cross-sectional studies, randomised controlled trials (RCT), and cohort studies (retrospective and prospective) in which study participants were screened for CrAg using LA or LFA procedures. Case-control studies and case reports were excluded. Study participants had to be HIV-infected adults (age >18 years) presenting to HIV-care programs with less than 100 CD_4 cells/μL, naïve to ART, with no symptoms suggestive of CM, in whom serum CrAg screening was done prior to ART initiation. There was no country restriction. Only studies published in English, French and Spanish were included.

In this review, the main intervention of interest was pre-emptive fluconazole therapy in CrAg-positive patients. However, to the best of our knowledge, there is no RCT evaluating the effectiveness of this intervention. A placebo-controlled trial would be unethical because there is enough clinical evidence to suggest that fluconazole therapy may reduce the risk of CM in severely immunosuppressed HIV-infected patients [16]. Consequently, in the present review, the impact of this intervention was evaluated based on observational studies.

Our clinical outcomes of interest were: (i) the prevalence of blood (serum/plasma) CrAg positivity in screened participants, (ii) the prevalence of asymptomatic CM (ascertained by positive fungal culture and/or Indian ink staining and/or CrAg in cerebrospinal fluid [CSF]) in blood CrAg-positive patients, (iii) the incidence of CM during follow-up, and (iv) all-cause mortality during follow-up.

Data extraction and quality assessment

For each study, we extracted:

- Study characteristics: first author, publication year, design (RCT, cohort, cross-sectional), country;
- Participant characteristics: total number, proportion of ART-naïve, number with less than 100 CD_4 cells/μL;
- CrAg screening test procedure: LA or LFA;
- CrAg screening outcome: number screened, number and proportion of CrAg-positive
- Interventions offered to CrAg-positive patients: lumbar puncture (number of confirmed asymptomatic CM), pre-emptive fluconazole therapy (offered or not, number of participants offered fluconazole, initial dose offered, duration), ART (median time to initiation if available);
- Clinical outcomes within follow-up: incidence of CM (number and proportion in CrAg-

positive and CrAg-negatives), all-cause mortality (number and proportion in CrAg-positive and CrAg-negatives), number lost to follow-up within each group, if reported;

We assessed risk of bias only in studies where screened patients were subsequently followed up. For this, we adapted a quality assessment tool (Supplementary Table 2) based on the Joanna Briggs Institute checklist for cohort studies [28]. The main components of the review question considered were: study population (HIV-infected adults with less than 100 CD_4 cells/μL), exposure (CrAg status and the method used to determine it), intervention (targeted pre-emptive fluconazole therapy or not, ART to screened patients), and the outcomes of interest during follow-up (incidence of CM and all-cause mortality). For each study, we assessed patient selection bias, treatment allocation bias, outcome assessment bias and completeness of outcome data bias. Where insufficient information was reported we contacted study authors for clarification.

Data analysis

Data were pooled using standard random-effects meta-analysis for proportions using the Freeman-Tukey double arcsine transformation and the *metaprop* command [29] in STATA 15.0 (Statacorp, Texas, USA) to estimate the prevalence of CrAg positivity in screened participants and the prevalence of asymptomatic CM in CrAg-positive participants, and reported with their 95% confidence interval (95%CI).

In studies where screened participants were subsequently followed up, random-effects models were used in Review manager (Revman) version 5.3 [30] to estimate the incidence of CM and all-cause mortality during follow-up as well as risk ratios (RR) comparing CrAg-positive to CrAg-negative participants. This analysis was stratified by the type of interventions offered to CrAg-positive participants (i.e., no pre-emptive fluconazole, pre-emptive fluconazole initiated at <800 mg/day or 800 mg/day or initiated at 800 mg/day following post-screening lumbar puncture).

Heterogeneity was evaluated graphically by observing forest plots and by calculating I^2 statistics. Additional stratified analysis was performed to explore heterogeneity when I^2 was greater than 50%.

The protocol was registered in the PROSPERO international prospective register of systematic reviews, registration number CRD42018087608.

RESULTS

The electronic search ran on April 20th, 2018 identified 2,115 citations (314 duplicates). Based on title and abstract screening, 1,741 citations were excluded (Figure 1). On further assessment of 60 citations, 29 more were excluded. A total of 31 studies were included for estimating the prevalence of CrAg positivity [14, 22, 23, 26, 31-57], of which ten to evaluate the prevalence of asymptomatic CM in CrAg-positive participants [31, 33, 40, 41, 45, 48, 50, 51, 55, 57], four to evaluate the incidence of CM and all-cause mortality in the context of no fluconazole pre-emptive therapy [14, 32, 35, 49], and twenty to evaluate the incidence of CM and/or all-cause mortality in the context of pre-emptive fluconazole therapy [14, 22, 23, 32, 33, 35, 39-45, 48-51, 53, 55, 56]. The quality of included studies is summarised in Supplementary text and Supplementary Figure 1.

Prevalence of blood CrAg positivity and asymptomatic CM in CrAg-positives

Thirty-one studies from 22 countries (67.7% Sub-Saharan African) were included [14, 22, 23, 26, 31-57] of which 22 (71%) cohorts, 3 (9.7%) randomised trials, and 6 (19.4%) cross-sectional (Table 1). In these, 38,383 participants underwent CrAg screening irrespective of CD_4 count, of whom 35,644 (92.9%) had less than 100 CD_4 cells/μL (our target population). Screening was done with LFA in 20 (64.5%) studies, and LA in the rest. Screening was performed in real-time on fresh sera in 20 (64.5%) studies and retrospectively on stored sera in 11 (35.5%). In participants with >100 CD_4 cells/μL, the median prevalence of CrAg positivity was 2% (Interquartile range [IQR]: 1 – 3). In those with <100 CD_4 cells/μL, CrAg positivity ranged from 0 - 21% and pooled prevalence was 6% (95%CI: 5 – 7; I^2 = 89.3%) (Figure 2). Pooled CrAg prevalence was slightly higher with LA than LFA: 8% (95%CI: 5 - 11; I^2 = 90.34%) vs 5% (95%CI: 4 – 6, I^2 = 88.9%), p = 0.13, respectively; in prospective than retrospective cohorts: 6% (95%CI: 5 – 8, I^2 = 83.8%) vs 5% (95%CI: 3 – 8, I^2 = 87.6%), p = 0.78 and in fresh than stored sera: 7% (95%CI: 5 – 9, I^2 = 89.9%) vs 6% (95%CI: 5 – 7, I^2 = 76.4%), p = 0.02, respectively (Supplementary Figures 2 and 3).

Following CrAg screening, lumbar puncture (LP) was offered to CrAg-positive participants (who presented no symptoms of CM) in 10 studies [31, 33, 40, 41, 45, 48, 50, 51, 55, 57]. Among the 403 participants eligible for LP, 276 (68.5%) accepted and the pooled prevalence of confirmed asymptomatic CM in CrAg-positives was 33% (95%CI: 21 – 45; I^2 = 76.1%); Figure 3.

Incidence of cryptococcal meningitis

During the median follow up of 1-year (IQR: 0.5 – 1), when CrAg-positive participants were not offered pre-emptive fluconazole, incidence of CM was 21.4% (95%CI: 11.6 – 34.4) vs 0.4% (95%CI: 0.1 – 1) in CrAg-negatives (Table 2, Figure 4.1).

When pre-emptive fluconazole was offered to CrAg-positives, stratifying by initial dose, less than 800 mg/day was associated with more incident cases of CM than 800 mg/day: 9.1% (95%CI: 2.5 – 21.7) vs 5.7% (95%CI: 3.0 – 9.7), Figure 4.2. In these analyses, incidence was consistently less than 1% in CrAg-negatives (Table 2). Moreover, performing LP to CrAg-positive participants to exclude those with confirmed asymptomatic CM prior to initiating pre-emptive fluconazole at 800 mg/day significantly reduced the incidence of CM to similar levels in CrAg-negatives: 0% (95%CI: 0 – 0.8) and 0.4% (95%CI: 0 – 1), p = 0.12, respectively and this was independent of CrAg test used (Supplementary figure 4.1).

Incidence of all-cause mortality

Following CrAg screening, when no pre-emptive fluconazole was offered to CrAg-positives, incidence of all-cause mortality during follow-up was significantly higher than in CrAg-negatives: 39.7% (95%CI: 28.8 – 51.5) vs 13.9% (95%CI: 11.8 – 16.2), respectively (Table 3, Figure 5.1). Offering pre-emptive fluconazole at 800 mg/day was associated with decreased mortality risk in CrAg-positives compared to no fluconazole: 17.4% (95%CI: 13.9 – 21.4). Nevertheless, incidence of all-cause mortality remained significantly higher in CrAg-positives than in CrAg-negatives even after excluding CrAg-positives with asymptomatic CM prior to initiating fluconazole at 800 mg/day, RR: 2.2 (95%CI: 1.7 – 2.9, p<0.001) (Table 3, Figure 5.3), independent of CrAg test used (Supplementary figure 4.2)

DISCUSSION

Main findings

This SRMA shows that (i) the prevalence of CrAg positivity in asymptomatic HIV-infected patients with less than 100 CD_4 cells/μL is around 6% [4, 58], (ii) among CrAg-positives, the prevalence of asymptomatic CM is approximately 30%, (iii) the incidence of CM in CrAg-positives drops from around 20% without pre-emptive fluconazole to 5% with pre-emptive fluconazole initiated at 800 mg/day, (iv) initiating pre-emptive fluconazole at 800 mg/day after excluding asymptomatic CM reduced overall mortality in CrAg-positives from around 40% to around 20%, but CrAg-positives still had more than two-fold risk of death than CrAg-negatives.

Implications for practice

Our findings show that targeted pre-emptive fluconazole initiated at 800 mg/day may reduce the incidence of CM from around 20% to around 5%, thus strong evidence of its effectiveness. Furthermore, when CrAg-positive patients were offered post-screening lumbar puncture, the incidence of CM even reduced further to less than 1%, which is comparable to that observed in CrAg-negatives. This supports systematically offering LP to CrAg-positives to prevent clinically asymptomatic patients with CSF evidence of meningitis from receiving sub-optimal induction antifungal treatment with fluconazole monotherapy, known to be less effective in CM even at highest dosages [59, 60]. In other words, the observed incident CM cases during follow-up despite pre-emptive fluconazole therapy might be a resultant of insufficient treatment and unmasking secondary to immune reconstitution inflammatory syndrome [61]. We therefore suggest that the objective is not only to identify CrAg-positive patients, but also, among them, those who have asymptomatic CM. Patients with asymptomatic CM should be treated with recommended induction antifungal combination therapy: one-week Amphotericin B plus flucytosine or oral high dose fluconazole plus flucytosine [62], while fluconazole pre-emptive therapy should be restricted to those without CSF evidence of CM.

In studies reporting the experience of routine CrAg screening and targeted fluconazole therapy in LMIC settings, we found little heterogeneity, suggesting similarities across these studies in the overall implementation of the CrAg screen-and-treat strategy: tests used, classification of patients as CrAg-positives or -negatives, fluconazole to CrAg-positive patients, post-screening ART initiation, follow-up and reporting of ascertained CM cases over time. However, there was much variability in the way fluconazole was offered to CrAg-positive patients in terms of dosage and duration. Few studies provided fluconazole at the WHO-suggested tapering dose and duration [40, 42, 43, 45, 48, 51-53, 55]. In some, fluconazole was initiated at 800 mg/day and provided for four weeks only [41] or for two weeks then 400 mg/day for another two weeks and stopped [22]; these short courses seemed to be due to local realities of insufficient fluconazole availability. This shows that for targeted pre-emptive therapy to be effective as a preventive strategy for CM, readily available and sustainable fluconazole is a prerequisite, especially as CrAg point-of-care tests are becoming more available [21, 63] and accepted by clinicians and patients.

Implications for research

Given that most studies show moderate lumbar puncture feasibility and acceptance (68.5%), there is critical need for more acceptable methods for identifying those with asymptomatic CM among CrAg-positives. With existing evidence of association between serum CrAg titres and asymptomatic CM [43, 45, 55, 57, 64], systematic per-screening CrAg quantification can be done, and a threshold defined beyond which patients could be considered for recommended inductive combination antifungal therapy [62]. Available evidence suggests such a threshold is around 1:160 [45, 55, 57, 64] and a recent Ugandan study [43] showed strong association between this titre level and incident CM within weeks of ART initiation. Future research should aim at evaluating whether semi-quantitative point-of-care CrAg tests [55, 63] capable of identifying patients with high titres [65] would increase the effectiveness of pre-emptive therapy.

With regards to the effect of targeted pre-emptive therapy on all-cause mortality, we found some evidence that initiating fluconazole at 800 mg/day in CrAg-positive patients exempt of asymptomatic CM may have some benefits on mortality during the first year of ART initiation. However, mortality was still significantly higher than in CrAg-negative patients suggesting the existence of poorly understood non-CM CrAg status-related mortality predispositions worthy of further exploration. Perhaps, following ART initiation, CrAg positivity may affect immune response to other opportunistic infections leading to death. Further research could therefore focus on quality of immune responses following ART initiation, comparing CrAg-positives to CrAg-negatives.

Study limitations

Our study has some limitations. The effect of pre-emptive fluconazole on the incidence of CM and all-cause mortality in CrAg-positive patients was indirectly evaluated because most of the included studies were observational with very few RCTs. Even the included RCTs, none was randomised to compare pre-emptive fluconazole to no fluconazole or to an alternative pre-emptive therapy in CrAg-positive patients. Consequently, we report only indirect evidence for the effectiveness of the WHO CrAg screen-and-treat strategy. Furthermore, not all studies evaluated our predefined main outcomes of interest and this resulted in variable denominators (number of studies and number of participants) across the outcomes. Also, the data were scarce for several outcomes, with zero cells leading to unstable estimates and wide confidence intervals. We acknowledge that effects on incidental CM cases and mortality rates during follow-up would have been better assessed through more

reliable survival methods that account for censoring, but these data were not available for analysis. None of the studies addressed CrAg screening and pre-emptive therapy in ART-experienced patients though growing evidence suggests a considerable proportion of patients with advance HIV due to failing ART.

Authors' conclusion

Offering fluconazole pre-emptive therapy at presently recommended doses to CrAg-positive patients compared to no fluconazole, substantially reduces the risk of incident CM and may have survival benefits. The high prevalence of asymptomatic CM in CrAg-positive patients together with low uptake of lumbar puncture, justifies the development of reliable point-of-care tests capable at point of screening, of identifying CrAg-positive patients at higher risk of underlying asymptomatic CM. The availability of sustainable fluconazole in ART programs is essential for effective pre-emptive strategies.

Contributors

ET, JFC and OL designed the study. ET, JFC and OL wrote the study protocol. RS did the literature search. ET, JJB and JFC did data extraction and analysis. ET, FD, TH and OL drafted the manuscript which was proofread and edited by HNL, GM, JNJ, FD, TH and JFC. All co-authors agreed on the final manuscript to be submitted.

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Conflict of interest

FD has produced a monoclonal antibody that is used in the Pastorex CrAg test and has also been involved in the development of the new Biosynex CryptoPS LFA test. OL is a consultant with Gilead and member of speaker bureau of Pfizer, Merck, Astellas and Gilead and has also been involved in the development of the new Biosynex CryptoPS LFA test. JNJ has received grants from Gilead. TH reports grants from Gilead Sciences, personal fees from Pfizer, personal fees from Gilead Sciences, personal fees from Viamet, non-financial support from Immuno-Mycologics. However, the above declarations are outside of this work. The other authors declare no competing interest.

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TABLES AND LEGEND OF FIGURES

Table 1. Characteristics of included studies and outcomes assessed per study

Author, Year	Study design (screening)	CrAg test	Median follow-up	Country	N*	Fluconazole pre-emptive therapy	Outcomes assessed			
							CrAg positivity	Asymptomatic CM in CrAg+	CM during follow-up	Mortality during follow-up
Desmet *et al* (1989) [31]	Prospective	LA	None	Democratic Republic of Congo	450	No	Yes	Yes	No	No
Liechty *et al* (2007) [32]	Retrospective	LA	> 3 months	Uganda	377	No	Yes	No	Yes	Yes
Jarvis *et al* (2009) [14]	Retrospective	LA	1 year	South Africa	707	No	Yes	No	Yes	Yes
Meya *et al* (2010) [23]	Prospective	LA	47 months	Uganda	295	200 - 400 mg/day for 2 - 4 weeks	Yes	No	Yes	Yes
Pongsai *et al* (2010) [33]	Retrospective	LA	1 year	Thailand	85	Yes (dose not reported)	Yes	Yes	Yes	No
Mamoojee *et al* (2011) [34]	Retrospective	LA	Not reported	Ghana	92	No	Yes	No	No	No

Linares *et al* (2012) [35]	Retrospective	LFA	1 year	Peru	365	No	Yes	No	Yes	Yes
Osazuwa *et al* (2012) [36]	Cross-sectional	LA	None	Nigeria	81	No	Yes	No	No	No
Smith *et al* (2013) [26]	Retrospective	LFA	None	Vietnam	226	No	Yes	No	No	No
Ganiem *et al* (2014) [37]	Retrospective	LFA	HIV diagnosis till incidence of death	Indonesia	810	No (primary prophylaxis: < 200 CD_4)	Yes	No	No	No
Mckenney *et al* (2014) [38]	Retrospective	LFA	Not reported	USA	1,872	Not reported	Yes	No	No	No
Manabe *et al* (2015) [39]	Prospective	LA	> 1 year	USA	117	Yes (at physician's discretion)	Yes	No	Yes	Yes
Pac *et al* (2015) [41]	Prospective	LA	6 months	Uganda	177	800 mg/day for four weeks	Yes	Yes	Yes	Yes
Kapoor *et al* (2015) [22]	Prospective	LFA	6 months	Tanzania	216	800 mg/day for two weeks, then 400mg/day for two weeks	Yes	No	Yes	Yes
Mfinanga *et al*	Prospective	LFA	1 year	Tanzania and Zambia	717	**WHO recommended dose	Yes	Yes	No	Yes

(2015) [40]										
Chipungu *et al* (2015) [42]	Prospective	LFA	6 months	Malawi	113	**WHO recommended dose	Yes	No	Yes	No
Vallabhaneni *et al* (2015) [44]	Retrospective	LA	1 year	South Africa	1,170	Yes (at physician's discretion)	Yes	No	Yes	No
Ezeanolue *et al* (2016) [46]	Retrospective	LFA	Not reported	Nigeria	2,752	No	Yes	No	No	No
Longley *et al* (2016) [45]	Prospective	LFA	1 year	South Africa	645	**WHO recommended dose	Yes	Yes	Yes	Yes
Morawski *et al* (2016) [43]	Prospective	LFA	1 year	Uganda	2,135	**WHO recommended dose	Yes	No	Yes	Yes
Ogouyemi-Hounto *et al* (2016) [47]	Cross-sectional	LFA	None	Benin	155	No	Yes	No	No	No
Frola *et al* (2017) [48]	Prospective	LFA	9 months	Argentina	123	**WHO recommended dose	Yes	Yes	Yes	No
Hajiabdolbaghi *et al* (2017) [49]	Prospective	LFA	6 months	Iran	86	No	Yes	No	Yes	No
Kadam *et al* (2017) [50]	Prospective	LA	6 months	India	208	No	Yes	Yes	No	Yes

Makadzange *et al* (2017) [51]	Cross-sectional	LFA	1 year	Zimbabwe	1336	**WHO recommended dose	Yes	Yes	No	Yes
Rick *et al* (2017) [52]	Prospective	LFA	5 months	Lesotho	128	**WHO recommended dose	Yes	No	No	No
Vu *et al* (2017) [53]	Prospective	LFA	6 months	Vietnam	944	**WHO recommended dose	Yes	No	No	Yes
Nalintya *et al* (2017) [54]	Prospective	LFA	6 months	Uganda	1,440	**WHO recommended dose	Yes	No	No	No
Temfack *et al* (2018) [55]	Prospective	LFA	1 year	Cameroon	186	**WHO recommended dose	Yes	Yes	Yes	Yes
Thomsen *et al* (2018) [56]	Retrospective	LFA	1 year	Guinea Bissau	200	No	Yes	No	No	Yes
Wake *et al* (2018) [57]	Cross-sectional	LFA	None	South Africa	19,233	**WHO recommended dose	Yes	Yes	No	No

Abbreviations: CrAg, cryptococcal antigen; LA, latex agglutination; LFA, lateral flow assay; CM, cryptococcal meningitis

*N is number of patients except for Mckenney and Ezeanolue (number of stored samples).

**WHO recommended dose: 800 mg/day for two weeks, then 400 mg/day for 8 weeks followed by 200 mg/day till CD4 above 200 cells/μL

Table 2. Incidence of cryptococcal meningitis during follow-up

			Incidence of CM during follow-up, % (95%CI)		Risk ratio (95% CI)	p-value
Interventions offered to CrAg-positive participants	Number of studies	N	CrAg-positives	CrAg-negatives		
No pre-emptive fluconazole	4	1,143	21.4 (11.6 – 34.4)	0.4 (0.1 – 0.9)	52.7 (6.4 – 431.2)	0.0002
Any pre-emptive fluconazole	11	5,006	6.3 (3.6 – 9.9)	0.3 (0.2 – 0.5)	15.6 (4.5 – 53.8)	<0.0001
Stratified analysis						
Pre-emptive fluconazole initiated at < 800 mg/day	4	1,635	9.1 (2.5 – 21.7)	0.6 (0.3 – 1.0)	15.9 (3.3 – 75.7)	0.0005
Pre-emptive fluconazole initiated at 800 mg/day	7	3,371	5.7 (3.0 – 9.7)	0.1 (0 – 0.3)	14.9 (1.9 – 111.7)	0.009
Pre-emptive fluconazole initiated at 800 mg/day following post-screening lumbar puncture	4	1108	0 (0 – 0.8)	0.3 (0 – 0.8)	5.7 (0.7 – 49.8)	0.12

Abbreviations: CrAg, cryptococcal antigen; 95%CI, 95% confidence interval; N, number of participants; CM, cryptococcal meningitis.

Table 3. All-cause mortality rates during follow-up

			All-cause mortality during follow-up, % (95%CI)		Risk ratio (95% CI)	p-value
Interventions offered to CrAg-positive participants	Number of studies	N	CrAg-positives	CrAg-negatives		
No pre-emptive fluconazole	4	1099	39.7 (28.8 – 51.5)	13.9 (11.8 – 16.2)	2.6 (1.8 – 3.6)	<0.00001
Any pre-emptive fluconazole	10	6605	17.9 (14.4 – 21.8)	14.1 (13.2 – 15.0)	1.7 (1.0 – 3.0)	0.06
Stratified analysis						
Pre-emptive fluconazole initiated at <800 mg/day	2	395	25.9 (11.1 – 46.3)	6.5 (4.2 – 9.5)	9.4 (0.04 – 2069)	0.42
Pre-emptive fluconazole initiated at 800 mg/day	8	6,210	17.4 (13.9 – 21.4)	14.6 (13.7 – 15.5)	1.6 (0.9 – 2.7)	0.11
Pre-emptive fluconazole initiated at 800 mg/day following post screening lumbar puncture	5	3060	21.3 (16.1 – 27.3)	10.7 (9.6 – 11.9)	2.2 (1.7 – 2.9)	<0.00001

Abbreviations: CrAg, cryptococcal antigen; 95%CI, 95% confidence interval; N, number of participants; CM, cryptococcal meningitis.

Legend of figures.

Legends of figures:

Figure 1. Flow diagram of the study selection process. Abbreviations: N, number of studies; CrAg, cryptococcal antigen; CM, cryptococcal meningitis

Figure 2. Prevalence of CrAg positivity in patients with less than 100 CD_4 cells/µL. Abbreviations: ES, effect size; CI, confidence interval

Figure 3. Prevalence of asymptomatic cryptococcal meningitis among CrAg-positive patients with less than 100 CD_4 cells/µL. Abbreviations: ES, effect size; CI, confidence interval

Figure 4. Forest plots of incidence of cryptococcal meningitis during follow-up. Abbreviations: M-H, Mantel-Haenszel; CI, confidence interval, LP, lumbar puncture

Figure 5. Forest plots of incidence of all-cause mortality during follow-up. Abbreviations: M-H, Mantel-Haenszel; CI, confidence interval, LP, lumbar puncture

Figure 1.

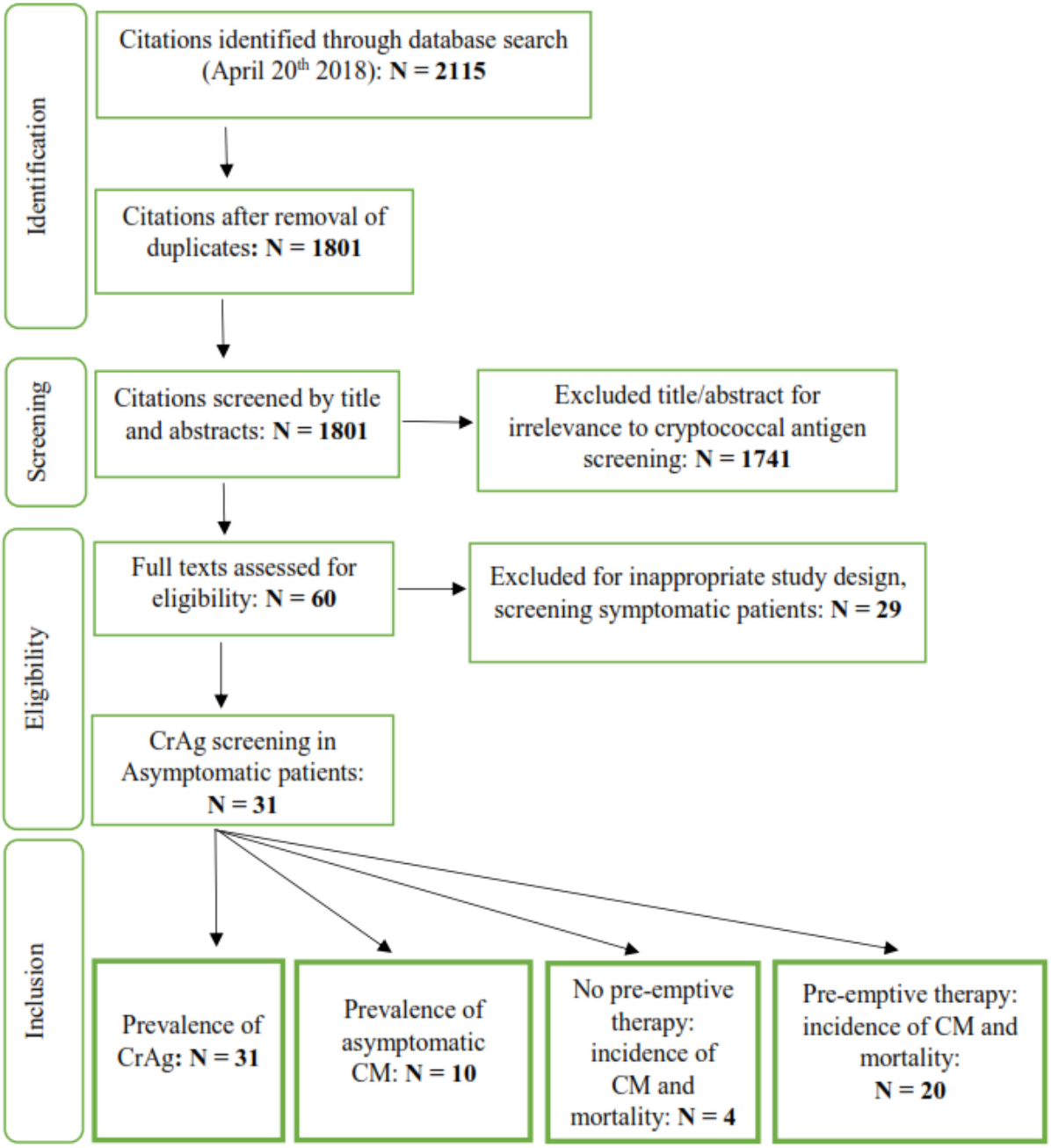


Figure 2.

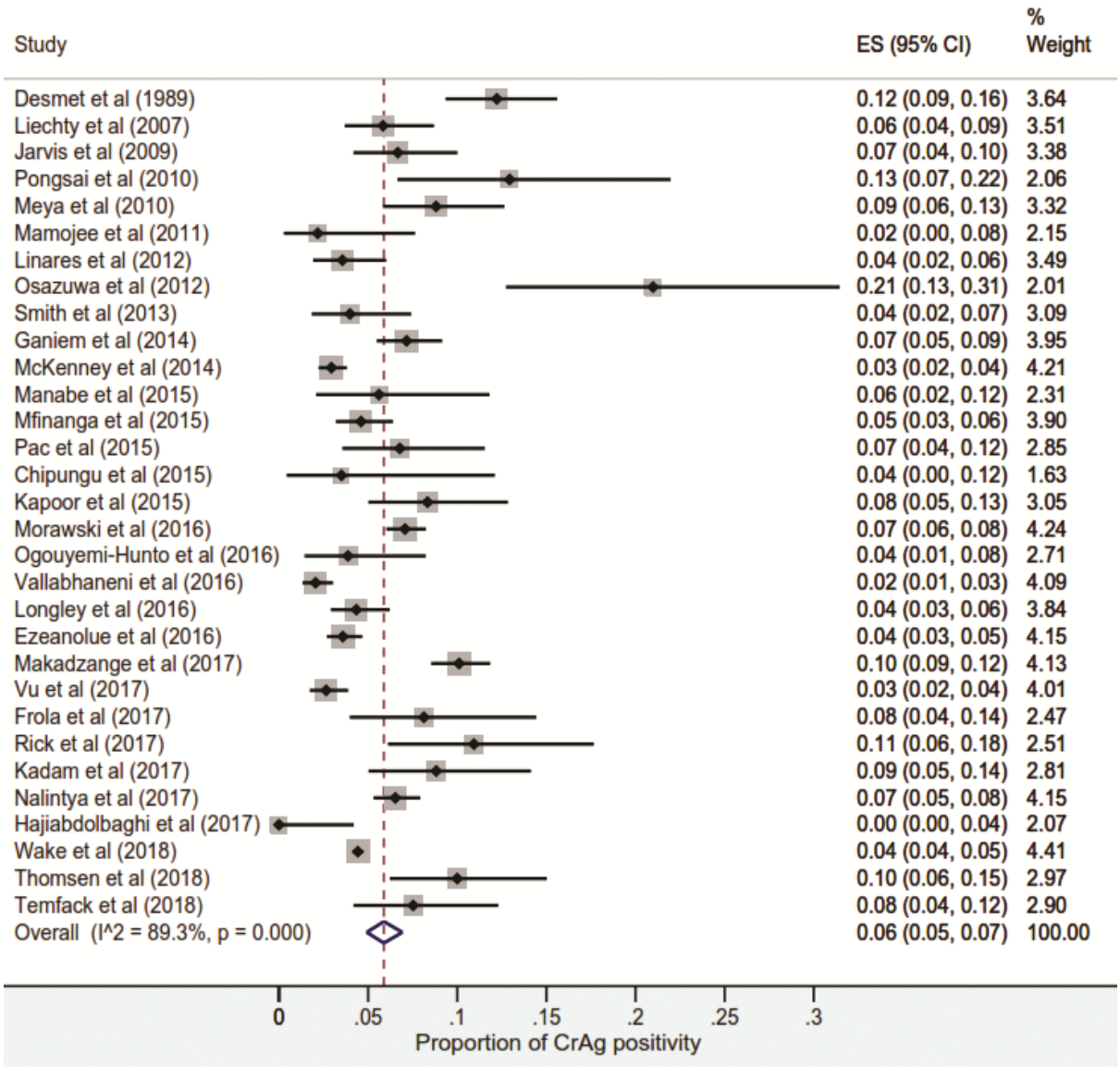
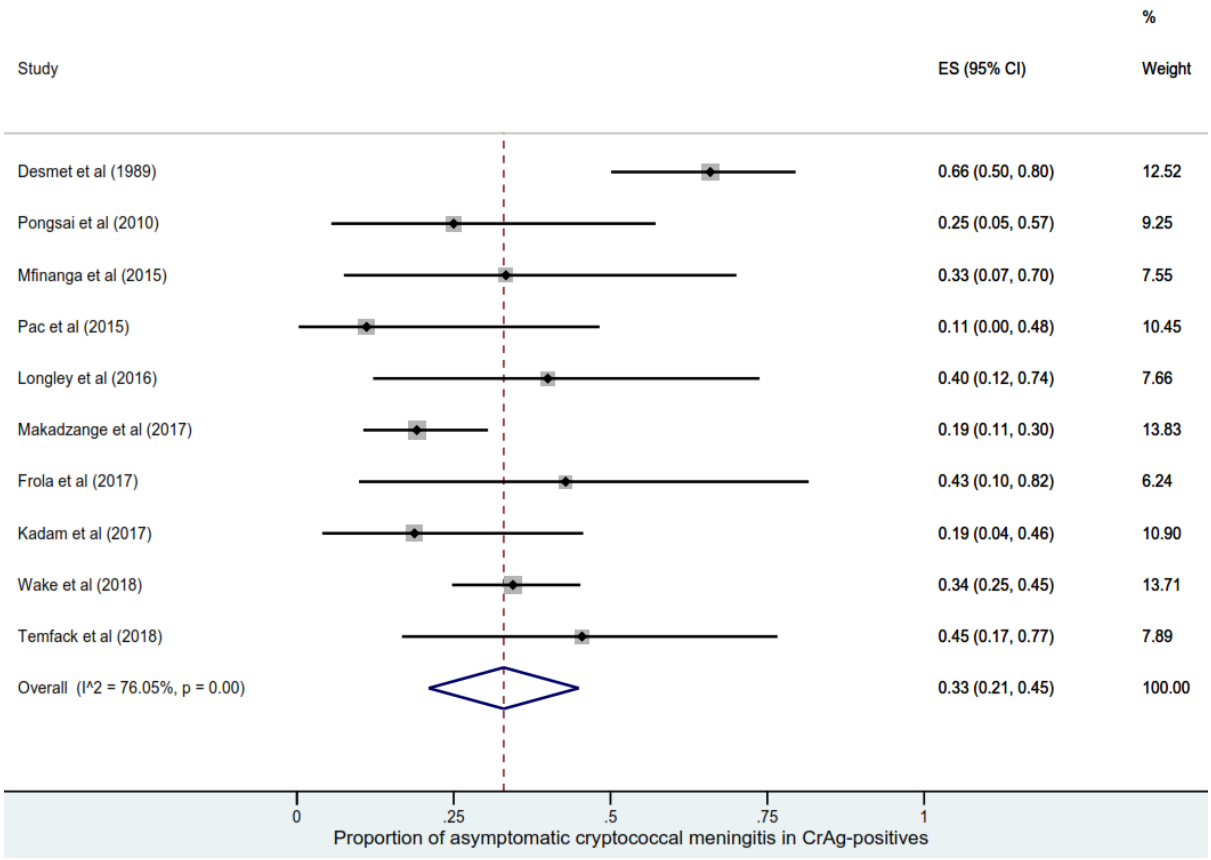


Figure 3.



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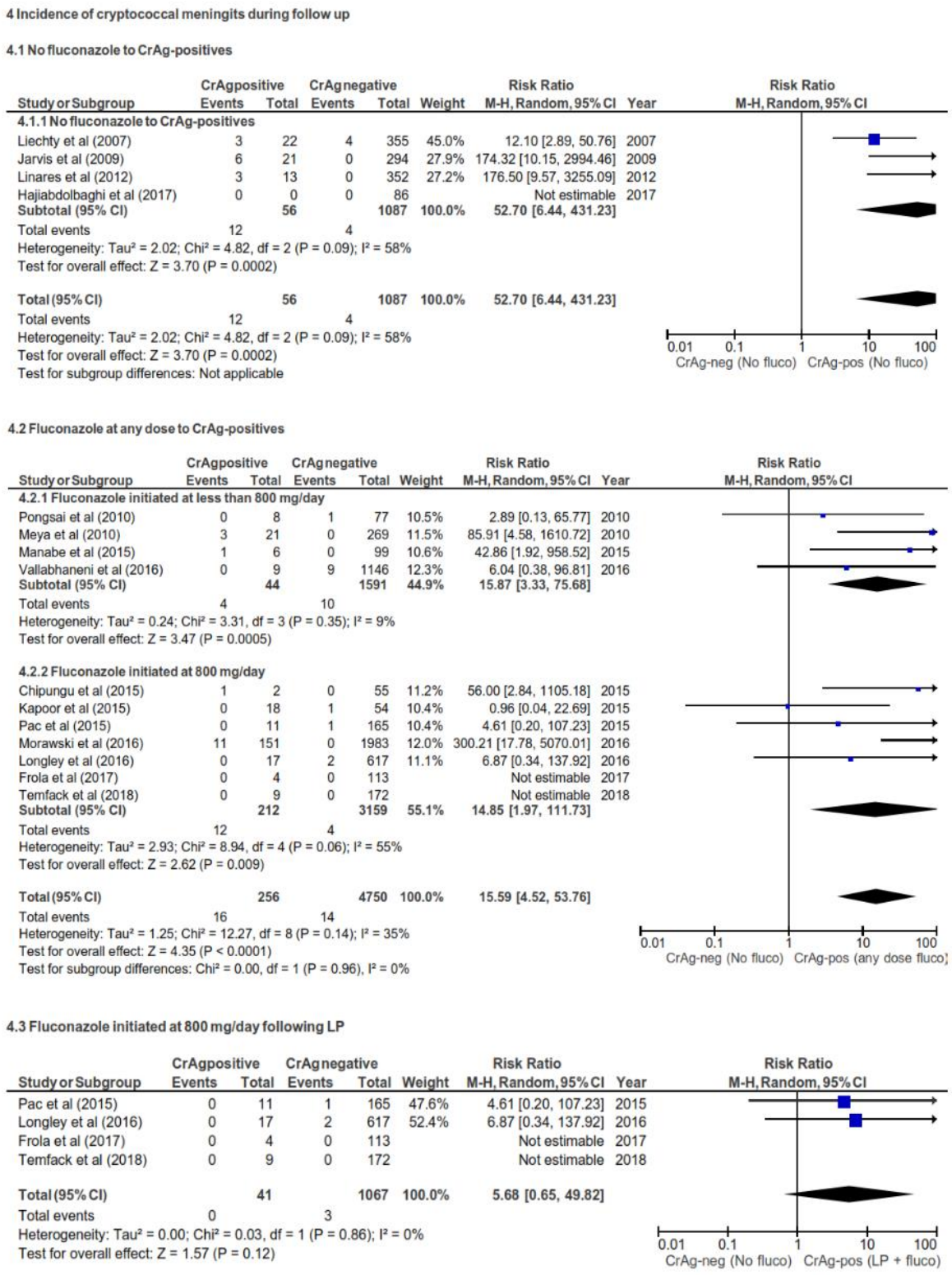

Figure 5.