The CD4 cell count at which to initiate HIV-associated cryptococcal antigen (CrAg) screening and preemptive antifungal treatment among CrAg positive persons may need to be raised to 200 cells/μL. Evidence based on a meta-analysis

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Abstract
Background: Current WHO guidelines (2018) recommend screening for cryptococcal antigen (CrAg) in HIV-infected persons with CD4<100 cells/µL, followed by pre-emptive antifungal therapy among CrAg positive (CrAg+) persons, to prevent Cryptococcal meningitis related deaths. The strategy may also be considered for those persons with a CD4 count of<200 cells/µL according the WHO guidelines. However, there remains little evidence for doing so in those HIV-infected persons with this CD4 cell count.

Objective: We aimed to assess the necessity of CrAg screening and the efficacy of pre-emptive antifungal therapy in CrAg+ persons with CD4<200 cells/µL.

Methods: We conducted a meta-analysis using data obtained from randomized controlled studies (RCTs) and cohort studies found in Pubmed, Web of Science, Cochrane Library and EMBASE/MEDLINE.

Results: The pooled prevalence of CrAg positivity in HIV-infected persons with CD4<200 cells/µL was 5% (95%CI: 3-6). The incidence of CM in CrAg+ persons was 7- fold (7%, 95%CI: 4-10) that of CrAg negative (CrAg-) persons (1%, 95%CI: 0-1). Among CrAg+ persons who did not receive any treatment or only received placebo, the incidence of CM was 9% (95%CI: 5-13), whereas the incidence of CM among those who received antifungal therapy was 2% (95%CI: 0-3), a highly statistically significant reduction of 78% (RR: 6.03, 95%CI: 2.74-13.24, p<0.00001).

Conclusions: In our meta-analysis, the incidence of CM in CrAg+ persons were significantly higher than in CrAg- persons with CD4<200 cells/µL. Furthermore, the incidence of CM was significantly reduced by pre-emptive antifungal therapy in CrAg+ persons with CD4<200 cells/µL.

Introduction
Cryptococcal meningitis (CM) continues to cause significant mortality in HIV-infected individuals (1, 2) resulting in 181100 deaths globally each year (3). In resource-limited regions such as sub-Saharan Africa, 75% of HIV-related deaths are due to CM (3). However, cryptococcal antigen (CrAg) can be detected in blood several weeks to months (22 days on average) before the onset of signs and symptoms of meningitis (4, 5) and therefore, can be used as a trigger for pre-emptive antifungal therapy in HIV-infected individuals with low CD4 cell counts. According to previous studies, pre-
emptive antifungal therapy in CrAg+ persons is imperative to preventing death (6-8). Firstly, the prevalence of CrAg positivity among HIV-infected individuals can be considerable, ranging between 1% to 16% in several African and Southeast Asian countries (9), and among persons with CD4<100 cells/µL, the prevalence of CrAg positivity averages 7% with regional variation (3). Secondly, CrAg positivity resulted in a 20% increase in mortality at the time of antiretroviral therapy (ART) initiation (10), and the risk of CM in CrAg+ persons was as high as 25% during the first year of ART if fluconazole pre-emptive therapy was not prescribed (11, 12).

According to the 2018 version of the WHO guidelines, routine CrAg screening and pre-emptive antifungal therapy are recommended in treatment-naive HIV persons with CD4<100 cells/µL (13). The guidelines also state that these strategies may also be considered for HIV-infected persons with CD4<200 cells/µL (13), suggesting that the evidence for the necessity of CrAg screening and the efficacy of implementing pre-emptive antifungal therapy in HIV persons with a higher CD4 cell count level is not sufficient enough to include them deterministically in these recommendations. Therefore, we conducted a meta-analysis to investigate whether routine CrAg screening and pre-emptive antifungal treatment in HIV-infected persons with <200 cells/µL would be beneficial.

**Method**

**Search strategy and article screening**

We searched relevant English articles in Pubmed, Cochrane Library, MEDLINE/EMBASE and Web of Science from inception until the end of September 15th 2018. The search terms we used were as follows: ‘acquired immunodeficiency syndrome’, “HIV”, “AIDS”, “cryptococcosis”, and “prophylaxis”. We combined these terms by using “and” or “or”. To avoid missing significant articles, we also screened references of previous meta-analyses and their included studies for eligibility.

Two reviewers (Yao Li, Yuanyuan Qin) independently screened all obtained articles by titles and abstracts. After removing ineligible articles by inclusion and exclusion criteria, the remaining articles were further selected by full-text reviewing.

**Inclusion and exclusion criteria**

**Inclusion criteria**
Randomized-controlled studies (RCTs) or cohort studies were included with the following criteria:

1. Study subjects had baseline CD4 cell counts < 200 cells/μL.
2. CrAg was tested for study subjects.
3. Fluconazole or otherazole medications were used as the intervention.

**Exclusion criteria**
We excluded articles if: (1) all of the study subjects were diagnosed with CM or asymptomatic CM; (2) sample size was less than 50; or (3) the incidence of CM and all-cause mortality was unreported.

**Data extraction and quality assessment**
The data we extracted included first author, publication year, type of study, study duration, location, total number of persons, number of CrAg+ or CrAg- persons, baseline CD4 cell counts, age, CrAg screening methods, diagnostic methods of CM, CM events, death events, adverse effects, other opportunistic infections. The JBI (Joanna Briggs Institute) Critical Appraisal Checklist for Cohort Studies was used as a quality assessment tool for cohort studies (14). The potential bias risk of the RCT was assessed using the Cochrane “risk of bias” tool (15).

**Data analysis**
The proportion of CrAg positivity, the incidence of CM, and all-cause mortality were performed by STATA 14 (Statacorp, Texas, USA) with a 95% confidence interval (95%CI). We used random-effects or fixed-effects models in Review manager 5.3 (The Nordic Cochrane Center, Copenhagen) to compare the incidence of CM and all-cause mortality in CrAg+ persons.

We evaluated statistical heterogeneity through visual inspection of forest plots. Statistical heterogeneity was also assessed by $I^2$ statistics (16), which was considered non-negligible if $I^2 > 50\%$.

Herein, random-model was applied if $I^2 > 50\%$ and fixed-model was used when $I^2 < 50\%$ (17). Reporting bias was assessed by examining asymmetry of funnel plots (16).

The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO), and the registration number is CRD42018110980.

**Results**
In total, 490 articles were obtained from 4 databases, among which 276 were from Pubmed, 106 were
from Web of Science, 12 were from Cochrane Library, and 96 were from MEDLINE/EMBASE. 82 of the 490 articles were RCTs or cohort studies. Additional 12 articles (RCTs or cohort studies) were extracted from references of previous meta-analyses and their included studies.

All the 94 RCTs or cohort studies were included for screening. Firstly, 9 articles (6 from Web of Science and 3 from MEDLINE/EMBASE) were reduplicative and were therefore excluded from the 94 articles. Next, after screening titles and abstracts, 43 of the remaining 85 articles were excluded. Then, 25 articles were excluded from the remaining 42 articles after screening full-text, among which 1 article was news report, 3 articles reported patients with cryptococcal disease, 2 articles reported HIV-negative patients with cryptococcal antigenemia, 4 articles reported patients with CM or asymptomatic CM, 6 articles reported HIV-infected patients with negative CrAg, 1 article reported the epidemiology of cryptococcosis and 8 articles did not report CD4 cell counts or primary outcomes. Finally, 17 articles were included in our meta-analysis.

The characteristics of the included 17 studies were shown in Table 1. The assessment of quality and potential risk bias showed that the following may contribute to clinical and methodological heterogeneity, including: (1) the confounding factors or subject recruiting or incomplete follow-up in 7 of the 16 cohort studies, (2) the unclear risk of attrition in the RCT, and (3) the unclear risk of reporting and other bias in the RCT, as shown in Supplementary Table 1 and Supplementary Table 2.

Twelve of the 17 included studies reported the prevalence of CrAg positivity (1735 persons with CD<200 cells/μL in 3 studies; 783 persons with CD<150 cells/μL in 1 study; 3876 persons with CD<100 cells/μL in 8 studies). The pooled CrAg positivity prevalence in 6394 HIV infected persons with CD<200 cells/μL was 5% (95%CI: 3-6, I²=85.5%) (Figure 2).

Thirteen studies reported the incidence of CM among CrAg+ persons (1789 persons with CD<200 cells/μL in 3 studies; 312 persons with CD<150 cells/μL in 2 study; 248 persons with CD<100 cells/μL in 8 studies), and 4 studies reported the incidence of CM among CrAg- persons (54 persons with CD<200 cells/μL in 1 studies; 2107 persons with CD<100 cells/μL in 3 studies). The incidence of CM in 2349 CrAg+ persons was 7% (95%CI: 4-10; P=0.000; I²=74.6%), whereas the incidence of CM in 2161
CrAg- persons was 1% (95%CI: 0-1; $P=0.343$; $I^2=10.0\%$). The former is 7 times that of the latter (Figure 3 A and Table 2).

Nine studies reported the incidence of CM among persons who received antifungal therapy (909 persons with CD<200 cells/μL in 3 studies; 166 persons with CD<200 cells/μL in 2 studies; 88 persons with CD<100 cells/μL in 2 studies), and 9 studies reported the incidence of CM among persons who received placebo or no intervention (946 persons with CD<200 cells/μL in 3 studies; 146 persons with CD<150 cells/μL in 1 study; 160 persons with CD<100 cells/μL in 5 studies). The incidence of CM of 1163 persons receiving antifungal therapy was 2% (95%CI: 0-3; $P=0.008$; $I^2=61.1\%$), whereas the incidence of CM of 1252 persons in 9 studies who received placebo or no intervention was 9%, a 78% reduction (95%CI: 5-13; $P=0.000$; $I^2=73.7\%$) (Figure 3 B and Table 2).

Four studies compared the incidence of CM between 1081 persons receiving azoles and 1072 persons receiving placebo or no intervention (1768 persons with CD<200 cells/μL in 2 studies; 295 persons with CD<150 cells/μL in 1 study; 90 persons with CD<100 cells/μL in 1 study). We found that the risk ratio of CM events among persons who received placebo or no intervention was 6.03 times of those who received antifungal therapy (95%CI: 2.74-13.24; $P<0.00001$; $I^2=17\%$).

Thirteen of the 17 included studies reported all-cause mortality among CrAg+ persons (1936 persons with CD<200 cells/μL in 5 studies; 312 persons with CD<150 cells/μL in 2 studies; 255 persons with CD<100 cells/μL in 6 studies), 6 studies reported all-cause mortality in CrAg- persons (54 persons with CD<200 cells/μL in 1 study; 2087 persons with CD<100 cells/μL in 5 studies). The all-cause mortality of 2503 CrAg+ persons was 18% (95%CI: 11-25; $P=0.000$; $I^2=94.0\%$), 1.06 times that of 2141 CrAg- persons (17%, 95%CI: 3-31; $P=0.000$; $I^2=99.0\%$), which is shown in Figure 4A and Table 2.

Nine studies reported all-cause mortality in persons who received antifungal therapy (230 persons with CD<200 cells/μL in 4 studies; 166 persons with CD<150 cells/μ in 2 studies L; 88 persons with CD<100 cells/μL in 3 studies), 7 studies reported all-cause mortality in persons receiving placebo or no intervention (946 persons with CD<200 cells/μL in 3 studies; 146 persons with CD<150 cells/μL in 1 study; 110 persons with CD<100 cells/μL in 3 studies). The all-cause mortality of 484 persons
receiving antifungal therapy was 16% (95%CI: 10-22; \(P=0.002; I^2=67.3\%\)), which was same to that of 1202 CrAg- persons receiving placebo or no intervention (16%, 95%CI: 7-24; \(P=0.000; I^2=92.4\%\)) (Figure 4A and Table 2).

Five studies (1897 persons with CD<200 cells/\(\mu\)L in 3 studies; 295 persons with CD<150 cells/\(\mu\)L in 1 study; 90 persons with CD<100 cells/\(\mu\)L in 1 study) compared all-cause mortality between persons who received azole antifungal therapy and persons who received placebo or no intervention. No significant difference was found in all-cause mortality (risk ratio: 0.82, 95%CI: 0.42-1.60; \(P=0.55; I^2=61\%\)) between 1144 CrAg+ persons who received an azole drug and 1138 CrAg+ persons who received placebo or no intervention (Figure 5).

In addition, we estimated and compared the prevalence of CrAg positivity, the incidence of CM and all-cause mortality between persons with CD4<100 and persons with CD4 100-200 cells/\(\mu\)L. The results showed that the risk ratio of CrAg positivity prevalence among HIV-infected persons with CD4<100 cells/\(\mu\)L was 2.69 times that of those with 100-200 cells/\(\mu\)L (95%CI: 1.48-4.88; \(P=0.001, I^2=34\%\); 2 studies; 1672 persons) (Table 3). The risk ratio of the incidence of CM among HIV-infected persons with CD4<100 cells/\(\mu\)L was 4.96 times that of those with CD4 100-200 cells/\(\mu\)L (95%CI: 1.94-12.68, \(P=0.23, I^2=31\%\); 3 studies, 1943 persons). The risk ratio of the all-cause mortality among HIV-infected persons with CD4<100 cells/\(\mu\)L was 4.15 times that of those with CD4 100-200 cells/\(\mu\)L (95%CI: 0.89-19.42, \(P=0.07, I^2=0\%\); 2 studies, 1552 persons) (Table 3). Further, the risk ratio of the incidence of CM among persons with CD4 100-200 cells/\(\mu\)L receiving antifungal treatment was 0.90 compared to those receiving placebo or no intervention (95%CI: 0.06-13.89, \(P\) and \(I^2\) not applicable; 2 studies; 135 persons) (Table 3). The risk ratio of the all-cause mortality among persons with CD4 100-200 cells/\(\mu\)L receiving antifungal treatment was 0.27 compared to those receiving placebo or no intervention (95%CI: 0.01-4.93, \(P\) and \(I^2\) not applicable; 1 study; 7 persons) (Table 3).

**Discussion**

Several meta-analyses have been conducted to evaluate the necessity of CrAg screening and preemptive antifungal treatment among HIV-infected CrAg+ persons with varying CD4 levels. For
example, Temfack et al investigated the effectiveness of CrAg detection and the initiation of pre-emptive fluconazole treatment in HIV-infected persons with cryptococcal antigenemia and CD4<100 cells/µL (16). Their results suggested that offering fluconazole pre-emptive therapy to CrAg+ persons greatly reduced the risk of incident CM and may have survival benefits (16). Another meta-analysis conducted by Ssekitojelo et al also suggested that CrAg+ persons, in resource-limited settings, should routinely receive primary antifungal prophylaxis (18), but they did not clarify at which CD4 cell count levels antifungal prophylaxis should be initiated. Ford et al’s (19) meta-analysis only reported the combined prevalence of cryptococcal antigenemia among HIV-infected persons with CD4≤100 and with CD4 101~200 cells/µL. Importantly, their study did not mention whether pre-emptive antifungal treatment was necessary or effective among HIV-infected persons with cryptococcal antigenemia at these two CD4 strata. From the above studies, the necessity and benefits of CrAg screening and pre-emptive antifungal therapy remain unknown at higher CD4 counts. The purpose of our meta-analysis was to investigate the necessity and benefits of CrAg screening andazole pre-emptive antifungal therapy among HIV-infected persons with cryptococcal antigenemia and CD4<200 cells/µL.

The pooled prevalence of CrAg positivity in HIV-infected persons was 5% (12 studies) in our meta-analysis, 6% (31 studies with) in Temfack’s meta-analysis (16) and 6.5% (60 studies) in Ford’s meta-analysis (19). The incidence of CM in CrAg+ persons was 7 times that of in CrAg- persons with CD4<200 cells/µL and all-cause mortality in CrAg+ persons was 1.06 times that of CrAg- persons. The incidence of CM in Temfack’s study was 21.4% among CrAg+ persons and 0.4% in CrAg- persons (16). All-cause mortality in Temfack’s study among CrAg+ persons was significantly higher than in CrAg-persons: 39.7% vs 13.9%, respectively (16). From the above results, it is clear that regardless of whether the CD4 count is less than 200 or 100 cells/µL, the incidence of CM is higher in CrAg+ persons than in CrAg- persons. Therefore, antifungal prophylaxis seems necessary for HIV-infected persons with cryptococcal antigenemia and CD4<200 cells/µL.

Our results showed that the risk ratio of CM events among persons who received placebo or no intervention was significant higher than those who received antifungal therapy, suggesting that
antifungal prophylaxis still significantly reduced the risk of CM events in CrAg+ persons with a higher CD4 cell count level. However, the limited number of studies and included participants contributed to the insignificance of risk ratio in the CM incidence between persons with CD4 100-200 cells/μL receiving antifungal treatment and those receiving placebo or no intervention. More trials are still needed to verify the benefits of antifungal treatment in HIV-infected persons with CD4 100-200 cells/μL.

As for all-cause mortality, no significant difference was found in our meta-analysis among CrAg+ persons when receiving pre-emptive antifungal therapy versus receiving placebo or no intervention. The reason of this may be associated with the discrepant sample sizes in the two groups (493 vs. 1259). In addition, no difference was found among persons with CD4 100-200 cells/μL receiving antifungal therapy versus receiving placebo or no intervention, which could be explained by the same reason (4 vs. 3).

We considered the following possible reasons for clinical and methodological heterogeneity: follow-up time of reporting CM events and death events, dosing or course or type of antifungal therapy, ART status of subjects, and risk of bias. For example, the study durations ranged from 1 year to 6 years, and the dosing ofazole antifungal treatments ranged from 100 mg to 800 mg. As for reporting bias, the uninformed funnel plot for all-cause mortality may be associated with the different ART status of study participants, different dosage and duration of treatment and the different follow-up time in each of the studies.

There are some limitations in our study. Firstly, the data supporting the association between prevalence of CrAg positivity and adverse outcomes in HIV-infected persons with CD4 100-200 cells/μL was quite limited. Secondly, there has been a lack of fresh data of CrAg positivity prevalence, CM incidence and all-cause mortality in HIV-infected persons with CD4<200 cells/μL since 2015 (33), and our pooled outcome analyses heavily relied on older studies which may be less applicable to the modern test-and-treat era. And thirdly, the dosage and durations ofazole therapy was not assessed in our meta-analysis. All of the above limitations may contribute to the clinical and methodological heterogeneity in our study.
In conclusion, our meta-analysis found that in HIV-infected persons with CD4<200 cells/μL, the incidence of CM was higher in those CrAg+ than in those CrAg-, and thatazole pre-emptive treatment significantly reduced the incidence of CM in CrAg+ persons with CD4<200 cells/μL. Our results support that the CD4 cell count at which to initiate HIV-associated CrAg screening and pre-emptive antifungal treatment among CrAg+ persons may need to be raised to 200 cells/μL.

Declarations

- Ethics approval and consent to participate

Not applicable.

- Consent to publish

Not applicable.

- Availability of data and materials

All the data and materials were available from Pubmed, Cochrane Library, MEDLINE/EMBASE and Web of Science.

- Competing interests

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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- Authors’ Contributions

YL, XJH, HW, XFY and YKC conceived and designed the protocol and study. YL, YYQ, JHH and AXL identified studies to be screened. XJH and HC identified studies for eligibility, extracted data and assessed the methodology quality of included studies. YL performed the analysis with assistance from
XJH, and YKC. All authors read and approved the final manuscript.

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Tables

Table 1. Characteristics of the 17 included studies.

| Author, year (reference) | Number of participants | Study type | Study duration | Age (years) | CD4 (cells/µL) | CrAg screening methods | CM diagnostic methods |
|--------------------------|------------------------|------------|----------------|-------------|---------------|------------------------|----------------------|
| Chariyalertsa, 2002 (20) | 129                    | Prospective study | 104 weeks     | 18~60       | 200           | Not report             | Fungal culture, a histopathological examination, oruffy coat smear |
| Manfredi, 1997 (21)      | 249                    | Retrospective study | 6 years       | 22~59       | 200           | Not report             | Specific polysaccharide antigen detection from body fluids |
| Parkes-Ratanshi, 2011 (22) | 1519                   | Prospective study | 42 months     | Not report  | 200           | Not report             | CrAg titre>1:8 on two occasions, or a positive CSF CrAg or Cryptococcus neoformans grown from blood or CSF culture |
| McKinsey, 1999 (23)      | 295                    | Randomized, placebo-Controlled study | Not report | ≥13          | 150           | Not report             | Fungal culture |
| Chetchotisakd, 2004 (24) | 90                     | Prospective study | 18 months     | >14         | 100           | Not report             | Not report |
| Meya, 2010               | 584                    | Prospective    | 30 months     | ≥18         | 200           | Not report             | Not report |
| Study Reference | Study Type | Study Duration | Age | Cut-off | Test | Diagnosis |
|-----------------|------------|----------------|-----|---------|------|-----------|
| Kapoor, 2015    | Retrospective | 15 months | ≥18 | 200 | LFA | Positive CSF India ink |
| Govender, 2015* (1) | Retrospective | 19 months | Not report | <200 | LA or the Latex-Cryptococcus antigen detection system | CrAg detected in CSF |
| Beyene, 2017 (10) | Prospective | 18 months | >14 | ≤150 | LFA | CSF CrAg |
| Vallabhaneni, 2016 (27) | Retrospective | 1 year | ≥18 | 100 | LA | Laboratory-confirmed |
| Rick, 2017 (28) | Observational | 1 year | Not report | 100 | LFA | Lumbar puncture and CSF testing using the CrAg LFA |
| Kwan, 2014 (29) | Observational | 48 weeks | 31 (median) | <100 | LA | India ink stain of CSF |
| Longley, 2016 (2) | Prospective | 3 years | >18 | ≤100 | LFA and LA | CSF CrAg-LFA |
| Ganiem, 2014 (30) | Prospective | 4 years | Not report | 100 | Semiquantitative LFA | Cryptococcus found in CSF, either with direct India ink staining or positive CrAg in CSF |
| Jarvis, 2009 (11) | Retrospective | 1 year | 34 (mean) | 19~77 | LA | Microbiologically confirmed |
| Liechty, 2007 (31) | Retrospective | 18 months | ≥18 | ≤100 | LA | Not report |
| Linares, 2012 (32) | Retrospective | 12 months | Not report ≤100 | LFA | Not report |

“No” means “no data”; “Yes” means “data exist”; OIs means: other opportunity infections.
* “Govender, 2015” study was included for evaluating the prevalence of CrAg positivity. Only the data in persons with CD4<200 was used. “LFA”: lateral flow assay; “LA”: Latex Agglutination; LP lumbar puncture

Table 2. The incidence of CM and all-cause mortality among CrAg+ and CrAg- persons, and among persons with and without antifungal therapy.
| Incidence of CM among CrAg+ and CrAg- persons | Number of reported studies | Number of persons |
|---------------------------------------------|---------------------------|------------------|
| 1.1 Incidence of CM among CrAg+ persons     | 13                        | 2349             |
| 1.2 Incidence of CM among CrAg- persons     | 4                         | 2161             |
| Incidence of CM among persons with and without antifungal therapy | Number of reported studies | Number of persons |
| 2.1 Incidence of CM among persons with antifungal therapy | 9                         | 1163             |
| 2.2 Incidence of CM among persons without antifungal therapy | 9                         | 1252             |
| All-cause mortality in CrAg+ and CrAg-persons | Number of reported studies | Number of persons |
| 1.1 All-cause mortality among CrAg+ persons  | 13                        | 2503             |
| 1.2 All-cause mortality among CrAg- persons  | 6                         | 2141             |
| All-cause mortality among persons who with and without antifungal therapy | Number of reported studies | Number of persons |
| 2.1 All-cause mortality among persons with antifungal therapy | 9                         | 484              |
| 2.2 All-cause mortality among persons without antifungal therapy | 7                         | 1202             |

Table 3. Comparisons of CrAg positivity, incidence of CM and all-cause mortality among HIV-infected persons stratified by CD4
| Author, year       | Prevalence of CrAg positivity | Incidence of CM |
|--------------------|-------------------------------|-----------------|
|                    | CD4100 (cells/µL) | CD4 100~200 (cells/µL) | CD4100 (cells/µL) |
|                    | with antifungal treatment | without antifungal treatment | with antifungal treatment |
| Chariyalertsak, 2002 (18) | Not reported | 0 of 40 | 6 of 45 | 0 of 40 |
| Parkes-Ratanshi, 2011 (20) | Not reported | 17 of 698 | | |
| McKinsey, 1999 (21) | Not reported | 0 of 101 | 7 of 103 | 1 of 11 |
| Meya, 2010 (23) | 26 of 295 | 7 of 298 | 3 of 21 | 2 of 5 |
| Govender, 2015 (1) | 20 of 708 | 6 of 371 | | Not reported |

The pooled CrAg+ prevalence: 0.06 [-0.00, 0.11], \( p=0.001, \text{i}^2=91.3\% \)

Risk ratio with 95%CI: 2.69 [1.48, 4.88], \( p=0.001, \text{i}^2=34\% \)

The pooled CrAg+ prevalence: 0.02 [0.01, 0.03], \( p=0.504, \text{i}^2=0\% \)

Risk ratio with 95%CI: 4.96 [1.94, 12.68], \( p=0.2 \)

*: Died within four weeks

Figures
Figure 1

Flow chart of the study selection process.
Prevalence of CrAg positivity in HIV-infected persons with CD4<200 cells/µL. Abbreviations:

ES, effect size.
Figure 3

Incidence of CM among CrAg+ and CrAg- persons with CD4<200 cells/μL. Incidence of CM among CrAg+ and CrAg- persons (A) and incidence of CM among CrAg+ persons with antifungal therapy and without antifungal therapy (B).
### A. All cause mortality among CrAg+ and CrAg- persons

1. All cause mortality among CrAg+ persons

| Study ID | ES (95% CI) | Weight |
|----------|-------------|--------|
| Baiya (2017) | 0.24 (0.03, 0.46) | 5.51 |
| Rin (2017) | 0.31 (0.09, 0.53) | 5.19 |
| Kapoo (2015) | 0.06 (0.00, 0.36) | 7.06 |
| Lomlay (2016) | 0.46 (0.05, 0.87) | 6.13 |
| Maya (2010) | 0.30 (0.05, 0.54) | 3.23 |
| Jains (2008) | 0.22 (0.05, 0.40) | 7.19 |
| Layley (2007) | 0.34 (0.07, 0.61) | 7.52 |
| Gennari (2014) | 0.19 (0.05, 0.34) | 9.02 |
| Marshafi (2007) | 0.05 (0.00, 0.13) | 10.20 |
| Parkes-Ratman (2011) | 0.11 (0.02, 0.21) | 8.82 |
| Cheek (2012) | 0.15 (0.04, 0.26) | 8.10 |
| Coughlin (2022) | 0.12 (0.05, 0.19) | 9.35 |
| Overall (I-squared = 94.9%, p = 0.000) | 0.18 (0.01, 0.35) | 100.00 |

**NOTE:** Weights are from random effects analysis.

### B. All cause mortality among persons with and without antifungal therapy

1. All cause mortality among persons with antifungal therapy

| Study ID | ES (95% CI) | Weight |
|----------|-------------|--------|
| Baiya (2017) | 0.24 (0.06, 0.44) | 8.68 |
| Rin (2017) | 0.31 (0.09, 0.54) | 9.05 |
| Kapoo (2015) | 0.11 (0.05, 0.13) | 9.24 |
| Lomlay (2016) | 0.37 (0.15, 0.59) | 8.29 |
| Maya (2010) | 0.29 (0.09, 0.49) | 5.13 |
| Jains (2008) | 0.56 (0.44, 0.68) | 17.66 |
| Layley (2007) | 0.31 (0.25, 0.37) | 16.24 |
| Gennari (2014) | 0.15 (0.19, 0.22) | 12.24 |
| Marshafi (2007) | 0.18 (0.12, 0.23) | 10.70 |
| Parkes-Ratman (2011) | 0.11 (0.06, 0.17) | 8.13 |
| Cheek (2012) | 0.16 (0.12, 0.19) | 9.34 |
| Overall (I-squared = 87.2%, p = 0.000) | 0.16 (0.12, 0.20) | 100.00 |

**NOTE:** Weights are from random effects analysis.

2. All cause mortality among persons without antifungal therapy

| Study ID | ES (95% CI) | Weight |
|----------|-------------|--------|
| Jains (2008) | 0.32 (0.26, 0.38) | 10.91 |
| Layley (2007) | 0.32 (0.25, 0.39) | 9.37 |
| Gennari (2014) | 0.30 (0.24, 0.36) | 10.39 |
| Overall (I-squared = 98.5%, p = 0.000) | 0.30 (0.25, 0.35) | 100.00 |

**NOTE:** Weights are from random effects analysis.

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**Figure 4**

All-cause mortality among CrAg+ and CrAg- persons with CD4<200 cells/μL. All-cause mortality among CrAg+ and CrAg- persons (A) and all-cause mortality among CrAg+ persons with antifungal therapy and without antifungal therapy (B).
1. Incidence of CM among CrAg + persons receiving azole vs. no intervention or placebo

| Study or Subgroup       | No inter or plac | Azole inter | Risk Ratio M-H, Fixed, 95% CI |
|-------------------------|------------------|-------------|-------------------------------|
| Chethobidsook 2004      | 7                | 46          | 44 43.8% 2.23 [0.62, 8.09]    |
| Muthele 1997            | 9                | 1          | 120 27.8% 4.76 [0.39, 61.59]  |
| McKenzie 1999           | 8                | 148        | 1 149 14.1% 0.19 [0.03, 64.48]|
| Parkes-Ratshini 2011    | 18               | 759        | 1 760 14.3% 19.02 [2.41, 134.67]|
| Total (95% CI)          | 1072             | 1081       | 100.0% 6.03 [2,74, 13.24]     |
| Total events            | 42               | 7          |                              |
| Heterogeneity: Ch² = 3.60, df = 3 (P = 0.31); I² = 17% |
| Test for overall effect: Z = 4.47 (P < 0.00001) |

2. All-cause mortality among CrAg + persons receiving azole vs. no intervention or placebo

| Study or Subgroup       | No inter or plac | Azole inter | Risk Ratio M-H, Random, 95% CI |
|-------------------------|------------------|-------------|-------------------------------|
| Chethobidsook 2002      | 12               | 93          | 11 26.6% 1.34 [0.54, 2.40]    |
| Chethobidsook 2004      | 2                | 44          | 9 46 13.1% 0.29 [0.05, 1.32]  |
| Manfred 1997            | 12               | 120         | 13 121 25.5% 0.87 [0.41, 1.94]|
| McKenzie 1999           | 32               | 149         | 21 146 31.0% 1.40 [0.60, 2.46]|
| Parkes-Ratshini 2011    | 0                | 780         | 7 759 4.7% 0.07 [0.00, 1.16]  |
| Total (95% CI)          | 1144             | 1138        | 100.0% 0.82 [0.42, 1.66]      |
| Total events            | 56               | 61          |                              |
| Heterogeneity: Tau² = 0.30; Ch² = 10.29, df = 4 (P = 0.04); I² = 61% |
| Test for overall effect: Z = 0.58 (P = 0.56) |

Figure 5

Forest plots of incidence of CM and all-cause mortality. Forest plots of incidence of CM and all-cause mortality among CrAg + persons receiving azole vs. no intervention or placebo.

Abbreviations: M-H, Mantel Haenszel; CI, confidence interval. (“Azole inter” means “Azole drug intervention”, “No inter or plac” mean “No intervention or placebo”).

Supplementary Files

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(3) Revised supplementary materials.doc