CRYPTOCOCCAL ANTIGEN SCREENING SURVEILLANCE REPORT, SOUTH AFRICA, FEBRUARY 2017 – JULY 2019

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Executive summary

Thousands of South Africans with advanced HIV die each year from cryptococcal meningitis. Even with ideal antifungal treatment, mortality from this disease remains high. However, screening for cryptococcal antigenaemia among persons with CD4 counts below 100 cells/µL offers an opportunity to detect cryptococcal disease early and pre-emptively treat patients to prevent progression from antigenaemia to meningitis. Following a recommendation by the World Health Organization in 2011, South Africa piloted reflex laboratory-based cryptococcal antigen (CrAg) screening in 2012 and scaled this up to a national screening programme in 2016. During the period February 2017 to July 2019, over 600 000 patients were screened of which almost 35 000 (5.8%) were identified with cryptococcal antigenaemia, most of whom are working-age men with CD4 counts below 50 cells/µL. CrAg screening numbers remained steady over the reporting period, although a slight decrease in CrAg-positive cases was observed. Case burden was highest in urban areas, although prevalence nearing 10% has been observed in several rural areas of KwaZulu-Natal, Western Cape, and Eastern Cape provinces. Reflex testing has achieved a coverage of 99%, but questions remain as to the proportion of patients who receive adequate care following positive test results. Programmatic data collected by the CAST-NET study are expected to fill this knowledge gap and guide future screening programme improvements. This will hopefully ensure that South Africa's CrAg screening programme will prevent deaths caused by this HIV-related disease.

Introduction

Cryptococcal meningitis (CM) is the second leading cause of AIDS-related deaths worldwide and is responsible for nearly 200,000 deaths per year, the majority of which occur within sub-Saharan Africa.[1] Persons with very advanced HIV disease (CD4 count <100 cells/µL) are at highest risk for development of CM and, if left untreated, face certain death.[2]

Cryptococcal meningitis is caused by the ubiquitous environmental fungi *Cryptococcus neoformans* and *Cryptococcus gattii*. Infection can be diagnosed by lumbar puncture and antigen testing or by culturing of cerebrospinal fluid. Subsequent treatment requires hospitalisation, therapeutic lumbar punctures, and combination therapy with amphotericin B and either fluconazole or flucytosine. Even with optimal combination antifungal treatment, mortality is still nearly 40%. With fluconazole monotherapy, the only available treatment in much of sub-Saharan Africa, mortality can be in excess of 80%.[3–5] A recent clinical trial showed that 1-week combination therapy with amphotericin B and flucytosine for the induction phase of treatment can reduce mortality by 38%.[6]

An increase in antiretroviral treatment (ART coverage) was associated with a decline in CM incidence in resource-rich settings.[7] However, late presentation to care still persists in resource-limited countries, and a growing number of people identified with advanced HIV are now ART-experienced.[8] In South Africa, reductions in the numbers of people with CD4 counts <100 or <200 cells/µL (reductions that were previously realised following the expansion of ART coverage) have recently stalled, with over 30% of people seeking care having advanced HIV disease (CD4 < 200 cells/µL), and over 15% having very advanced disease (CD4 <100 cells/µL).[9]

Early detection of cryptococcal disease in persons with CD4 counts <100 cells/µL offers the opportunity to pre-emptively treat antigenaemia and possibly prevent progression to CM. Cryptococcal antigen (CrAg), a highly-specific biomarker of cryptococcal disease, can be found in the blood weeks to months prior to the development of CM, and is strongly associated with CM and CM-related mortality.[10,11] Previously detected by cryptococcal latex agglutination tests (CLAT), the development of inexpensive and highly accurate (>99% sensitivity and specificity) CrAg lateral flow assays (LFA) have made routine screening for early detection simpler.[12] A randomised-controlled trial conducted among outpatients in Tanzania and Zambia found that, along with ART adherence support, screening for CrAg and pre-emptively treating antigenaemia with fluconazole reduced

all-cause mortality by 28%.[13] CrAg screening is also cost-effective, and the World Health Organization (WHO) has recommended routine CrAg screening for all individuals with CD4 <100 cells/µL since 2011. This recommendation has recently expanded to include those with a CD4 count <200 cells/µL.[14]

Cryptococcal antigen screening in South Africa

Following the WHO's recommendation for routine CrAg screening, laboratory-based reflexive screening was first piloted in South Africa in 2012. In this reflex approach, all CD4 samples with results below 100 cells/µL were automatically, or *reflexively*, tested for CrAg using remnant plasma, allowing for CD4 count and CrAg test results to be returned to clinicians simultaneously. A subsequent cost-effectiveness analysis found this approach to be superior in terms of coverage and the potential for saving lives as compared to traditional provider-initiated CrAg screening.[15] These findings led to the inclusion of CrAg screening in South Africa's national ART guideline in 2015.[16] The programme was later scaled up to cover all provinces in 2016, making South Africa the first country in the world to implement a national routine screening programme for cryptococcal disease. Currently, a network of 45 National Health Laboratory Service (NHLS) CD4 laboratories reflexively screen all samples with CD4 <100 cells/µL nationwide.

Cryptococcal antigenaemia in South Africa, 2017 - 2019

Data extraction

CrAg test results are stored along with CD4 results in the NHLS laboratory information system, TrakCare. The NICD Surveillance Data Warehouse (SDW) routinely extracts and processes data stored in the TrakCare system for use in NICD surveillance. To obtain surveillance results for this report, epidemiologists from the NICD's Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) extracted all CD4 tests and accompanying CrAg results for all NHLS laboratories from 1 February 2017 through to 31 July 2019. February 2017 was chosen as the beginning of the reporting period due to a CrAg LFA kit recall that was in effect from October 2016 through to January 2017. The dataset obtained was subsequently restricted to the above-mentioned period and to include only tests coded as reflex CrAg tests performed by CD4 laboratories, excluding provider-ordered tests processed by NHLS microbiology laboratories. For reporting of patient-level data, deduplication was performed using a NHLS-developed algorithm and unique identifier based

on names and birth dates. Although not providing perfect deduplication, this algorithm has demonstrated >80% matching accuracy.[17]

Summary of the CrAg screening programme

From 1 February 2017 through to 31 July 2019, 721 323 CrAg tests were performed through the NHLS reflex screening programme. As these tests were performed automatically on eligible samples, patients with multiple CD4 tests below 100 cells/µL were retested for CrAg. Deduplication of test data revealed that 604 558 patients were screened through the programme, achieving 99% coverage of eligible patients. Of these, 34 534 were found to be CrAg-positive, giving a prevalence of 5.8%.

Demographics of people with cryptococcal antigenaemia

CrAg-positive patients were predominately men of working age, although more women in the late teens to late 20s age range ($p<0.001$) tested positive (Figure 1). The largest difference in the number of CrAg-positive test results between men and women occurred between the ages of 35 and 54 years. Across all age categories, the odds ratio of a CrAg-positive test for men to women was 1.26, adjusted for CD4 count (95% confidence interval (CI) = 1.23-1.29).

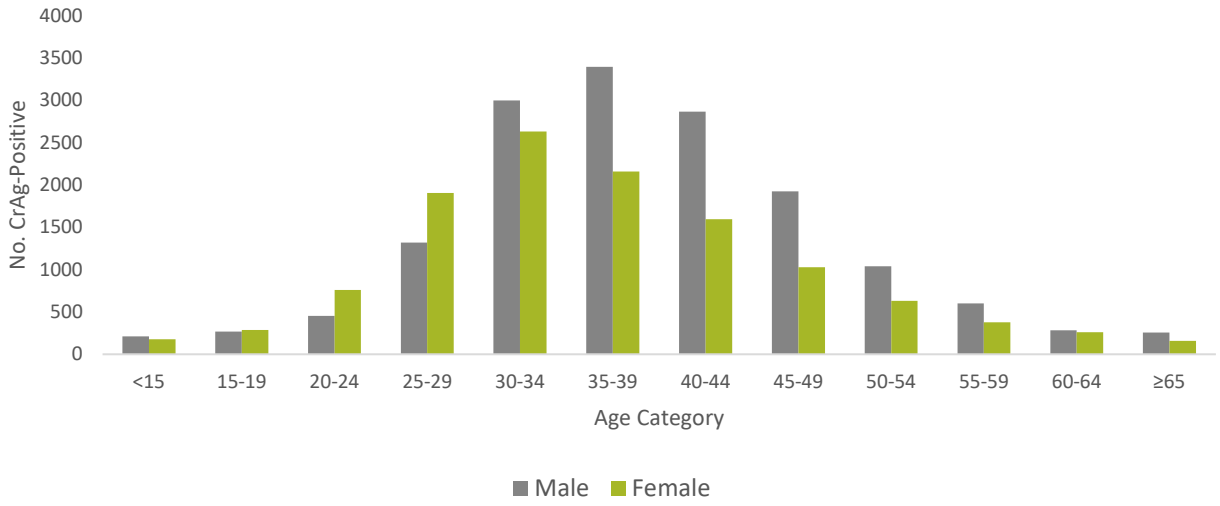

Figure1. Age and sex of cryptococcal antigen (CrAg)-positive patients, February 2017 – July 2019, South Africa.

CrAg and CD4 count

The number of CrAg-positive test results was inversely correlated to CD4 count, with most antigenaemia-positive persons detected in the lower CD4 count ranges. Almost half of the CrAg-positive patients had a CD4 count <30 cells/µL, and over 20% had a CD4 count <10 cells/µL at time of screening (Figure 2). Although most cases fell within the lower CD4 strata, over 8 800 CrAg-positive patients had CD4 counts between 50 and 99 cells/µL. Persons with CD4 counts ≤50 cells/µL were 1.95 (95% CI = 1.91-1.99) times more likely to be CrAg-positive, and were classified as *very* advanced HIV disease.

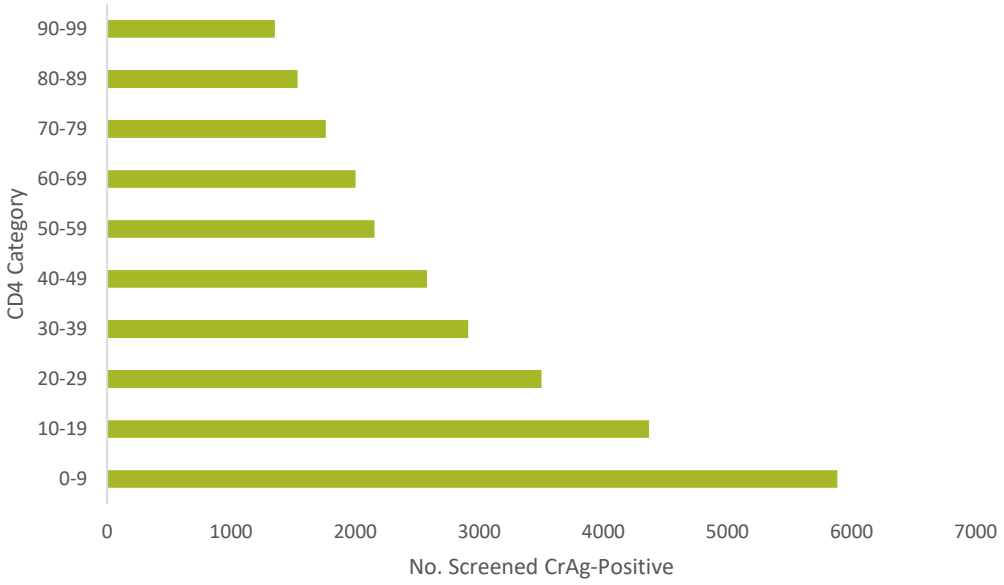

Figure 2. Distribution of CD4 count (cells/µL) categories (amongst cryptococcal antigen (CrAg)-positive patients, February 2017 – July 2019, South Africa.

CrAg screening and prevalence over time

On average, almost 24 000 tests were performed per month across South Africa. Yearly CrAg prevalence remained relatively constant across the survey period, ranging from 5.6% in 2017 to 5.8% in 2019. There was no significant trend in CrAg positivity over the 30-month surveillance period (p=0.11).

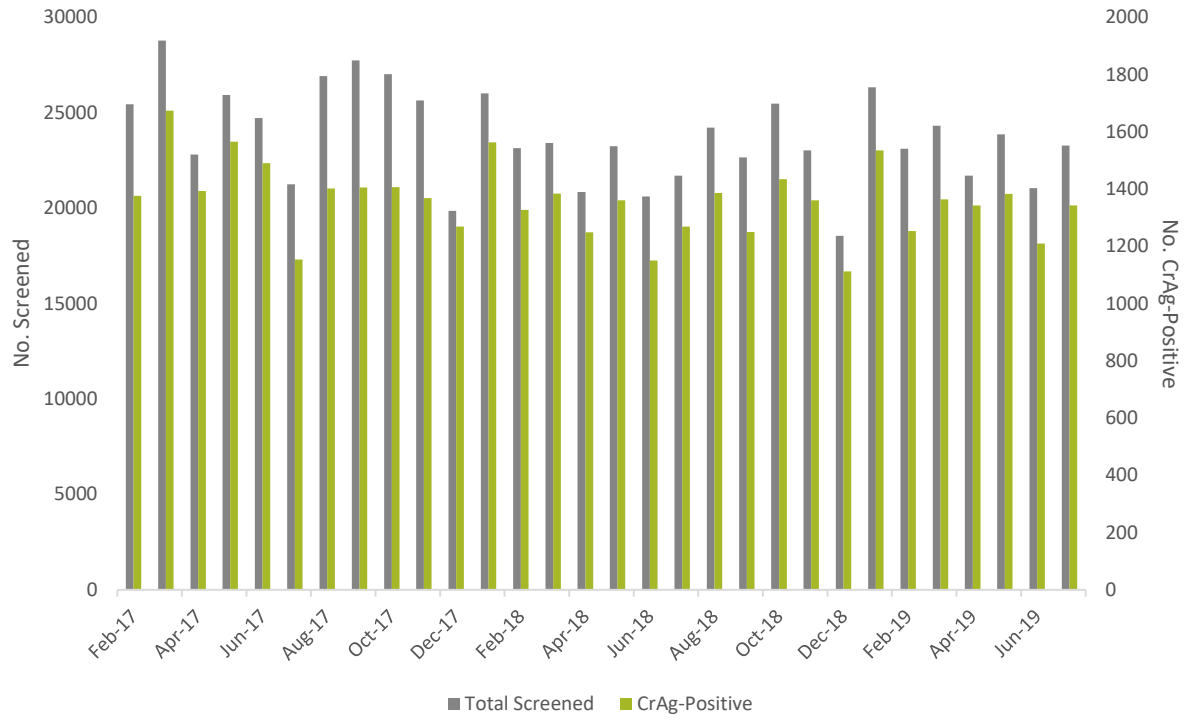

Figure 1. Numbers of patients screened for cryptococcal antigen (CrAg) and total positive by month, February 2017 – July 2019, South Africa.

Geographic distribution

CrAg prevalence and total number of CrAg-positive cases differed vastly by district (Figure 4). The highest prevalence and absolute case load was found in northern and central KwaZulu-Natal Province, while the sparsely-populated Northern Cape Province had the lowest prevalence and case burden. As expected, the metropoles had the largest absolute number of CrAg-positive cases. However, prevalence in Johannesburg, Pretoria, and Cape Town was lower than in Durban, East London and Port Elizabeth where the prevalence was in excess of 7.5%. Although few cases were detected in the northern districts of the Western Cape Province, the proportion of CrAg-positive patients mirrored that of the high-prevalence urban areas.

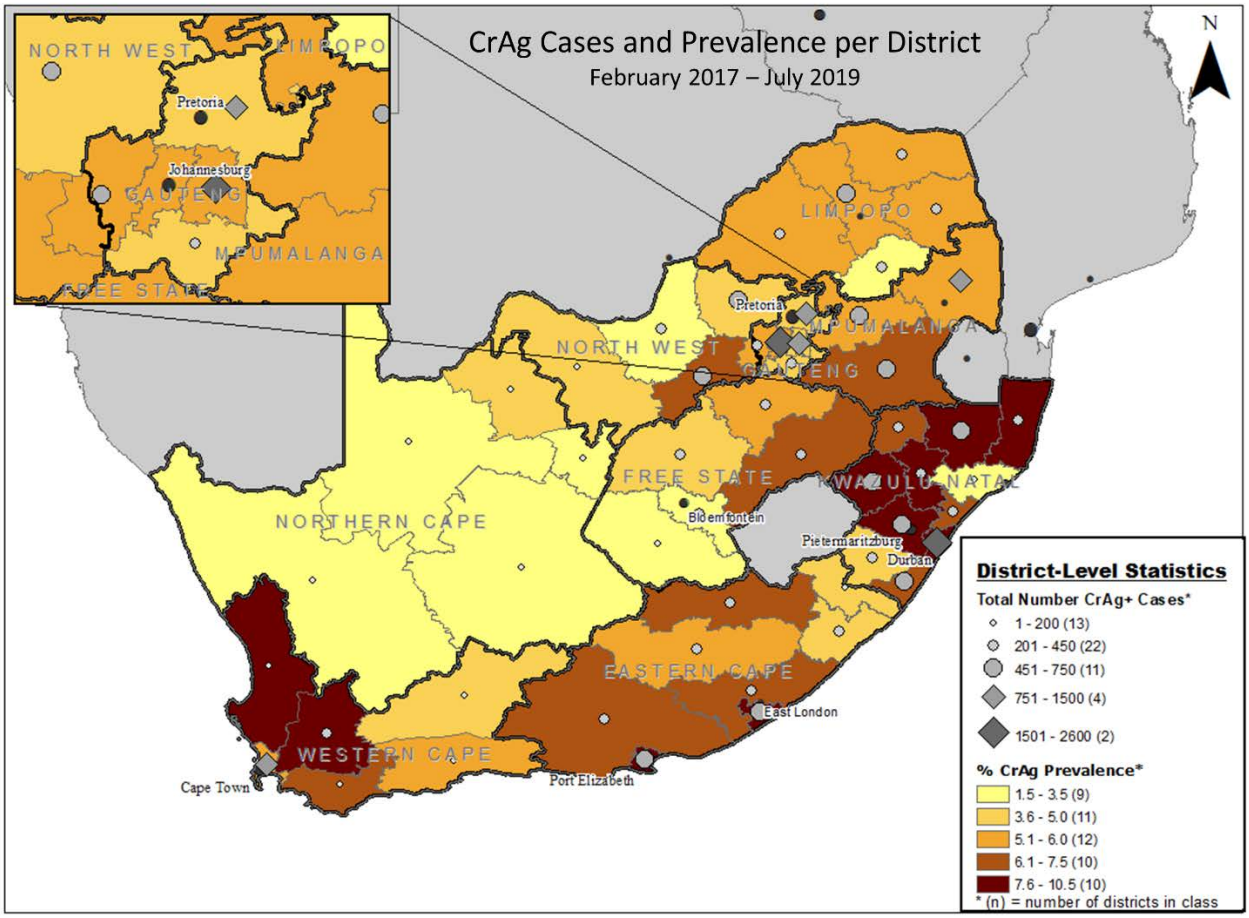

Figure 4. Cryptococcal antigen (CrAg) prevalence and total case counts by district and province, February 2017 - July 2019, South Africa.

Discussion

South Africa's national reflex CrAg screening programme has achieved successes in terms of its laboratory implementation and comprehensive coverage. Within the next year, >1 million patients will have been screened by this programme, making it the largest of its kind in the world. Reflexive screening coupled with functional information systems reveals important information about the epidemiology of cryptococcal disease in South Africa, and highlights the disproportionate disease burden in working age men in specific geographic areas. Such findings can be used to direct resources to key demographics and regions as well as to guide further investigation into the causes behind these trends.

Screening also provides the basis for clinical action to prevent or, at the very least, sooner detect CM in people with advanced HIV disease. Such results give healthcare workers the power to make

informed decisions regarding the management of these patients without the need to order the tests themselves. Test results are currently delivered by NHLS couriers to healthcare facilities and are also available on the NHLS's online TrakCare portal, making the results directly available to clinicians. Additional efforts have been undertaken to promote the receipt of and action on positive CrAg tests, such as the NICD's Results for Action (RFA) email and web portal delivery service for subscribed healthcare providers. Such a test result delivery system is critical to the functioning of South Africa's CrAg screening programme.

Although this programme's reflex approach maximises coverage and reduces the turnaround time of CrAg test results, it does not guarantee action on the part of healthcare providers. Clinical information and data related to patient outcomes is not routinely captured in laboratory-based surveillance and is not freely available for integration into such systems, making it difficult to ascertain the clinical successes or gaps of the screening programme on the ground. The CAST-NET study, a 5-year National Institutes of Health (NIH)-funded programme evaluation, seeks to fill this knowledge gap through collection of data from medical records and clinical outcomes of CrAg-positive patients in 27 sampled sub-districts covering all of South Africa's provinces. In this retrospective evaluation, the CAST-NET study seeks to collect data on over 5000 CrAg-positive patients at over 400 health facilities to determine whether clinical action was taken on CrAg test results and to assess the benefit realised by CrAg screening. Programmatic findings are expected in mid- to late-2020 and will provide insight into the functioning of this CrAg screening programme in the various contexts of South Africa's complex health system.

Conclusions

South Africa is the first country to fully implement a routine CrAg screening programme through its extensive CD4 laboratory network. Hundreds of thousands of patients were screened in two-and-a-half years, and tens of thousands identified with cryptococcal antigenaemia. CrAg prevalence varied by age, sex, CD4 count and geographic region, highlighting key groups and areas for future focus. More work needs to be done to assess and improve clinical action following receipt of positive CrAg results.

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